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Molecular dissection of complex traits in autopolyploids: mapping QTLs affecting sugar yield and related traits in sugarcane

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Abstract Mapping quantitative trait loci (QTLs) for sugar yield and related traits will provide essential information for sugarcane improvement through marker-assisted selection. Two sugarcane segregating populations derived from interspecific crosses between *Saccharum offinarum* and *Saccharum spontaneum* with 264 and 239 individuals, respectively, were evaluated in three replications each for field performance from 1994 to 1996 at Weslaco, Texas. These two populations were analyzed for a total of 735 DNA marker loci to seek QTLs for sugar yield, pol, stalk weight, stalk number, fiber content and ash content. Among the 102 significant associations found between these six traits and DNA markers, 61 could be located on sugarcane linkage maps, while the other 41 were associated with unlinked DNA markers. Fifty of the 61 mapped QTLs were clustered in 12 genomic regions of seven sugarcane homologous groups. Many cases in which QTLs from different genotypes mapped to corresponding locations suggested that at least some of the QTLs on the same cluster might be different allelic forms of the same genes. With a few exceptions that explained part of the transgressive segregation observed for particular traits, the allele effects of most QTLs were consistent with the parental phenotype from which the allele was derived. Plants with a high sugar yield possessed a large number of positive QTLs for sugar yield components and a minimal number of negative QTLs. This indicates the potential effectiveness of marker-assisted selection for sugar yield in sugarcane.

Keywords Sugar yield · DNA markers · Quantitative trait loci · Selection · Correlation

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Introduction

Obtaining higher sugar yield is a major focus of sugarcane variety improvement programs. The efficiency of selection for sugar yield (tons of sugar per hectare) relies on an understanding of the relationship among sugar yield components in a particular environment. The components of sugar yield are stalk weight, stalk number and sugar content. Increases in sugar yield have been achieved primarily by increasing the biomass yield as opposed to increasing the percentage of fixed carbon allocated to sucrose (Moore et al. 1997). Stalk weight has been identified as the most-important predictor in some studies (Sunil and Lawrence 1996), while stalk number was the primary determinant in other studies (Rosario and Musgrave 1974; Kang et al. 1989; Milligan et al. 1990).

The success of a sugarcane variety usually requires a balance between sugar yield and its related traits, as well as stress and disease tolerance. For example, fiber content affects both sugar yield and milling efficiency. High fiber content reduces the juice extracted from cane and

requires more energy to crush the cane. Low fiber content is associated with lodging and with increased fuel cost because of insufficient energy recovered from burning bagasse (Hogarth and Cross 1987). QTL mapping can improve our understanding of the relationships among genes influencing sugar yield and related traits, and facilitate deterministic manipulation of these traits towards the development of superior sugarcane varieties.

The average sugar yield in sugarcane has more than doubled over the past century due to genetic improvement through breeding and optimization of cultural practices. Although the field record yield reached 23.6 tons per hectare per year in Hawaii, this is only 65% of the theoretical physiological maximum (Moore et al. 1997). However, in the past decade sugar yield has reached a plateau, and selection for new higher yielding varieties has proven to be difficult (K.K. Wu, personal communication). Current and emerging molecular techniques may one day help to realize the full physiological potential for sugar yield in sugarcane.

Economically important traits such as yield have been dissected with molecular markers in tomato, maize and rice (Stuber et al. 1987, 1992; Paterson et al. 1988, 1991; Xiao et al. 1995, 1996). Seven linkage maps have been constructed in sugarcane with the number of linkage groups ranging from 64 to 96 (Da Silva et al. 1995; Grivet et al. 1996; Mudge et al. 1996; Ming et al. 1998). Sugar content, as a major component of sugar yield and measured by pounds of sugar per ton of stalk, was analyzed with DNA markers, and QTLs have been mapped and compared with genes involved in sucrose metabolism in maize (Ming et al. 2001). We report here the mapping of QTLs for sugar yield and related traits in two interspecific sugarcane populations.

Materials and methods

Mapping populations

Two interspecific segregating populations, each made by P. Tai, USDA-ARS, Canal Pt., Fla., were evaluated for field performance and analyzed with DNA markers. The first population consisted of 264 plants from *Saccharum officinarum* 'Green German' (GG, 2n = 97–117) x *Saccharum spontaneum* 'IND 81-146' (IND, 2n = 52–56) (GG x IND), and the second of 239 plants from *S. spontaneum* 'PIN 84-1' (PIN, 2n = 96) (PIN x MJ) x *S. officinarum* 'Muntok Java' (MJ, 2n = 140). The taxonomic classification of these parental varieties has been discussed previously (Ming et al. 2001, 2002). In sugarcane, 2n + n transmission predominates in *S. officinarum* (2n = 80) x *S. spontaneum* F_1 and BC_1 crosses, a phenomenon known as "female restitution," (Bermer 1923; Price 1957). However, the chromosome numbers of a sampling of the progenies from these two crosses were 2n = 73–85 for GG x IND and 2n = 99–121 for PIN x MJ, indicating n + n transmission (Burner 1997). Both populations were grown at Texas A & M Agricultural Research and Extension Center, Weslaco, Tex., from November 1994 to February 1996, in three replications as randomized complete block designs with rows 1.5-m apart and plants 0.6-m apart in the row. The average phenotypic values of the three replications for each trait were used for QTL mapping.

Phenotyping

Sugar yield is the product of stalk weight x stalk number x sugar content and is expressed in units of tons per hectare. Fiber content is the percentage of dry weight of the shredded and pressed stalk tissues after the juice is expressed (dry weight/fresh weight). Pol is a measurement made on the expressed juice to calculate the level of sucrose in stalk juice determined by polarimetry. To measure pol, a "clarified" juice sample from which optically active non-sugar compounds have been removed is placed in a standard optical cylinder and polarized light is passed through the cylinder (Birkett and Seip 1975). The degree of rotation of the plane of light exiting the tube is the product of the optical properties of the sugars the juice contains. Sucrose and glucose are dextro-rotatory, while fructose is levo-rotatory. In sugarcane juice, glucose and fructose levels are usually similar and small, so cancel each other out. Ash is measured in juice in units of mMhos/cm with a conductivity meter.

Sugar-content QTLs were reported in a separate paper (Ming et al. 2001). Sugar content is pounds of sugar per ton of cane, equivalent to the content of sucrose at 96% purity, calculated based on brix and pol values as described by Legendre and Henderson, (1972). Brix is the percentage of all soluble solids, mostly sugars, minerals, and organic acids, in the sugarcane juice. If the ratio of pol to brix is lower than 35% (varies slightly at different factories), the calculated sugar content will be negative, indicating sucrose can not be separated from other soluble solids in cane juice. Fresh and dry weights (after drying at 70 °C) of the pressed stalk tissues were used to calculate fiber content (the percentage of dry weight to fresh weight). Stalk weight was calculated based on an average of ten stalks per plot, or all of the stalks available if there were fewer than ten.

Genotyping and data analyses

DNA extractions were carried out as previously described (Chittenden et al. 1994). DNA probes used for QTL mapping were selected based on preliminary analysis of 1,255 single-dose RFLP markers on 85 plants; additional probes were picked at 20 cM or smaller intervals for a more comprehensive search of the genome. A total of 186 probes were mapped in both populations using methods previously described (Ming et al. 1998). These probes generated 243, 232, 122 and 138 single-dose markers for GG, IND, MJ and PIN, respectively. SAS programs (SAS Institute 1989) were used to calculate correlations (CORR) among traits and to perform analysis of variance (GLM). When flanking markers were available, MAPMAKER/QTL version 1.1 was used to calculate LOD scores by interval mapping. Significance thresholds of LOD > 2.5 (interval mapping) or $P < 0.003$ (analysis of variance) were used to declare QTLs. The QTL with the largest effect (if $R^2 > 0.1$) on each trait was fixed and the genome was rescanned (Lander and Botstein 1989). The allele effect of each single-dose QTL was the average difference in phenotype of individuals differing by one copy of the indicated allele (single dose versus zero dose).

Results

Sugar yield was highly correlated with components of yield and other related traits. This inter-relationship was reflected in the finding that some QTLs for different traits showed clear patterns of association. Sugar yield was positively correlated with pol, sugar content, stalk number and stalk weight, but negatively correlated with ash content except that sugar yield and stalk number were not correlated in the PM population (Table 1). Other positively correlated traits include stalk weight with

Table 1 Correlation coefficients among sugar yield and related traits in GG x IND and PIN x MJ populations

Trait	Sugar content	Ash	Fiber	Stalk number	Stalk weight	Sugar yield
GG x IND						
Pol	0.6390***	–0.6131***	0.0243	0.1342	0.3331***	0.3737***
Sugar content		–0.6568***	–0.3593**	0.0039	0.4959***	0.4501***
Ash			0.1977*	–0.0607	–0.3970***	–0.3537***
Fiber				0.2522***	–0.4418***	–0.0936
Stalk number					0.1487*	0.6816***
Stalk weight						0.6481***
PIN x MJ						
Pol	0.9212***	–0.5153***	0.0369	–0.1065	0.4361***	0.7434***
Sugar content		–0.5462***	0.1060	0.0860	0.5319***	0.8259***
Ash			0.0893	0.0328	–0.4480***	–0.3892***
Fiber				0.0842	0.0993	0.0117
Stalk number					–0.0450	0.1301
Stalk weight						0.4313***

*$P < 0.05$ **$P < 0.01$ ***$P < 0.001$

pol, stalk weight with sugar content and pol with sugar content, while negatively correlated traits were stalk weight with ash content, pol with ash content, and sugar content with ash content in both GI and PM populations. In the GI population only stalk weight was negatively correlated with fiber content, and fiber content was positively correlated with stalk number and ash content.

Sugar yield (SUYD) QTLs

The pol values of GG x IND progeny values ranged from 0.07 to 31.9 tons per hectare, a range that was about 39.8% wider than the albeit large difference between the parents (IND = 1.14, GG = 20.3) (Fig. 1). A full model that comprised three QTLs, two from GG and one from IND, explained 18.4% of the phenotypic variation (PV). The two GG QTLs alone explained 11.5% of PV, while the one IND QTL alone explained 6.1%. The allele effects of the two GG QTLs were positive, while the allele effect of the IND QTL was negative, consistent with the parental phenotypes (Table 2, Fig. 2).

Sugar yield of PIN x MJ progeny ranged from –1.5 to 4.59 tons per hectare, a range about 20.2% wider than the difference between the parents (PIN = –1.3, MJ = 3.96). Negative sugar yield values reflect a low pol to brix ratio. A full model comprised of seven QTLs from MJ explained 30.2% of PV. Allele effects of all QTLs were consistent with the parental phenotypes. No QTL was mapped for sugar yield in PIN (Fig. 2).

Pol QTLs

GG x IND progeny values ranged from 8 to 22, a range that was about 50% wider than the difference between the parents (IND = 12, GG = 19). A full-model comprised of two QTLs, one from GG and one from IND, explained 18.5% of PV. The allele effect of the GG QTL was negative, while the allele effect of the IND QTL was positive, accounting for part of the progeny transgression of parental phenotypes (Table 2, Fig. 2).

Pol values of PIN x MJ progeny ranged from 0.1 to 7.1, a range about 20.0% wider than the difference between the parents (PIN = 0.0, MJ = 5.6). A full model comprised of 12 QTLs, seven from MJ and five from PIN, explained 39.9% of PV. The seven MJ QTLs alone explained 24.8% of PV, while the five PIN QTLs alone explained 23.3%. Allele effects of all MJ QTLs were positive, while all five PIN QTLs were negative, consistent with the parental phenotypes (Fig. 2).

Stalk weight QTLs

The stalk weight of GG x IND progeny ranged from 0.1 to 2.9 lb, a range that was about 81% wider than the difference between the parents (IND = 0.22, GG = 1.8). A full model comprised of ten QTLs, three from GG and seven from IND, explained 62.7% of PV. The three GG QTLs alone explained 14.9% of PV, while the seven IND QTLs alone explained 49.2%. The allele effects of all GG QTLs were positive, while the IND QTLs were negative, consistent with the parental phenotypes.

The stalk weight of PIN x MJ progeny ranged from 0.1 to 1.46 lbs, a range somewhat below the range of parental values (PIN = 0.22, MJ = 2.02). A full model comprised of 24 QTLs, 14 from MJ and ten from PIN, explained 71.6% of PV. The 14 MJ QTLs alone explained 53.1% of PV, while the ten PIN QTLs alone explained 37.8%. Allele effects of all MJ QTLs were positive, while all PIN QTLs were negative, consistent with the parental phenotypes.

Stalk number QTLs

The stalk number of GG x IND progeny ranged from 1 to 54, a range that was about 253% wider than the difference between the parents (GG = 20, IND = 35). A full model comprised of two QTLs, one from GG and one from IND, explained 13.9% of PV. The allele effect of the GG QTL was negative, while the allele effect of the IND QTL was positive, consistent with the parental phenotypes.

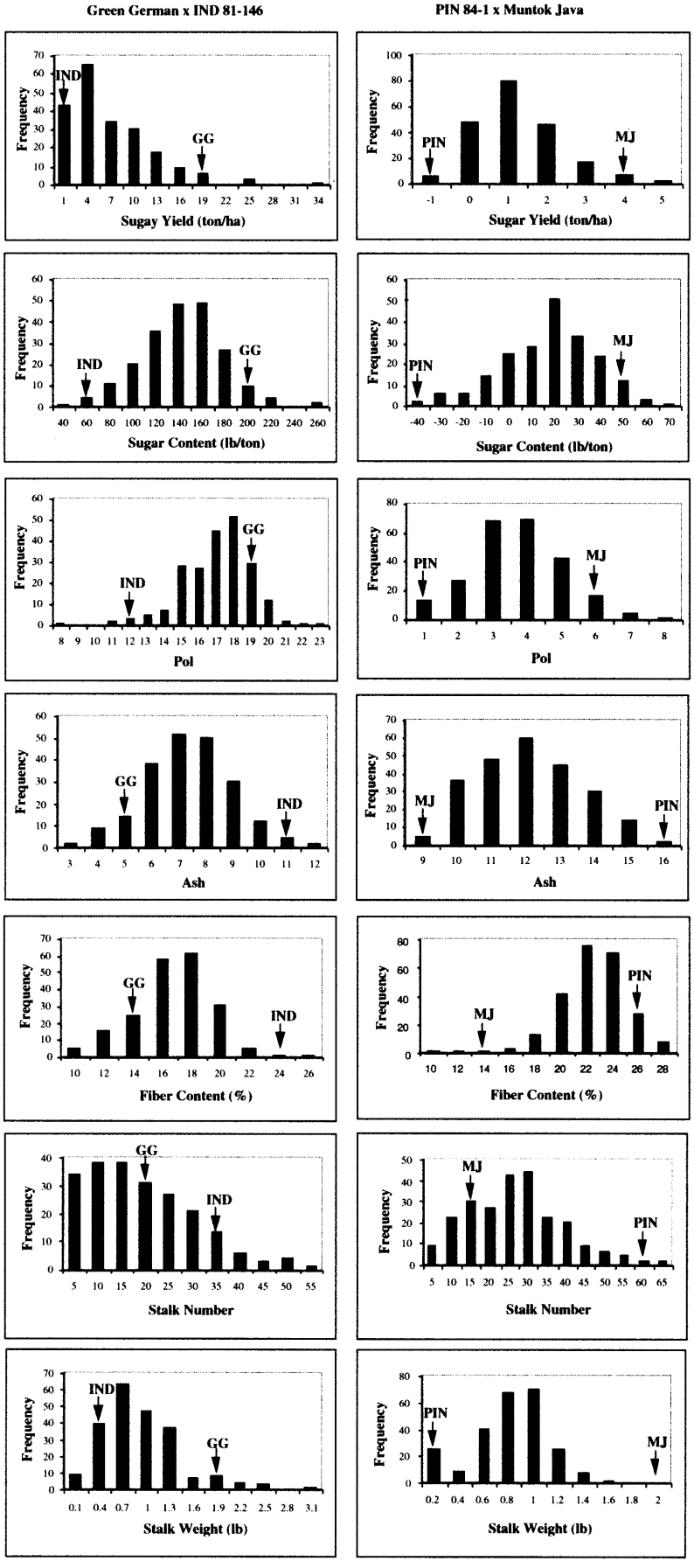

Fig. 1 Frequency distribution of phenotypes for each trait in two sugarcane segregating populations derived from interspecific crosses Green German x IND 81-146 and PIN 84-1 x Muntok Java

Table 2 Biometrical parameters of QTLs associated with sugar yield and related traits

Marker	Trait	LG	HG	S-LG	*P* (LOD)	PVE[a] (%)	A effect[b]
CDSR35eG	ASH	40	5	D	0.0001	11.1	–1.05
CSU450aG–pSB121iG	ASH	26	3	C	(2.72)	9.2	–0.63
CDSB31dG	FIB	13	15	G	0.0021	7.6	–1.61
CDSR46fG	FIB	2	3	C	0.0010	7.7	–1.51
CDSR66aG	FIB	7	3	C	0.0006	8.6	–1.58
CDSR91iG	FIB				0.0008	6.7	–1.36
CSU440aG	FIB				0.0001	8.3	–1.53
5C04H05bG–pSB 173dG	FIB	69	3	C	(2.83)	10.1	–1.57
CSU537aG	POL	63	3	C	0.0001	8.8	–1.27
CDSR33cG	SN	35	3	C	0.0012	5.3	–5.05
CDSB53fG	SW				0.0002	7.0	2.62
pSB121hG	SW	58	5	D	0.0009	5.9	2.39
CSU450aG–pSB121iG	SW	26	3	C	(3.62)	10.3	2.66
CDSB53fG	SUYD				0.0024	5.0	0.44
CDSR91eG–CDSR91gG	SUYD	28	4	B	(3.78)	10.7	0.55
CDSB22aI	ASH				0.0029	5.2	0.76
CDSR160bI–CDSC24aI	ASH	23	9	H	(3.33)	11.2	0.83
CDSR78dI	ASH	53	4	B	0.0020	5.2	0.73
CDSR94aI	ASH				0.0006	7.1	0.85
RZ508jI	ASH				0.0003	9.4	1.04
CDSB22cI–pSB341dI	FIB	11	6	F	(2.61)	7.7	2.31
CDSB31hI	FIB				0.0005	6.1	1.29
CDSC52dI–CDSR87aI	FIB	36	2	A	(5.59)	12.1	1.8
CDSR133cI–pSB302dI	FIB	31	10	J	(3.27)	8.2	1.56
CDSR17bI	FIB	47	6	F	0.0001	8.9	1.58
CDSR88eI–CSU469bI	FIB	35	2	A	(3.26)	9.5	1.62
pSB146cI–CSU415dI	FIB	59		I	(2.62)	6.7	1.31
pSB1652cI–pSB581aI	FIB	4	2	A	(2.93)	8.1	1.89
pSB188bI	FIB	65		D	0.0011	6.1	1.28
UMC114hI–CSU395eI	FIB	64	3	C	(3.97)	13.2	2.67
UMC147dI–SG305fI	FIB	22	3	C	(4.46)	10.1	1.69
UMC44aI–CDSR125cI	FIB	41	5	D	(4.16)	12.9	1.85
SG305iI	FIB	20	3	C	0.0017	5.6	1.24
pSB44dI	POL				0.0024	5.1	0.92
BCD1107aI–CDSB44dI	SN	1	3	C	(4.31)	15.0	7.38
CDSB22cI	SW	11	6	F	0.0002	13.3	–4.14
pSB341cI–CDSR17cI	SW	10	6	F	(2.51)	6.9	–2.32
CDSR94aI–CDSC49eI	SW	29	4	B	(2.51)	10.6	–3.29
CDSR87aI–CDSR88eI	SW	36	2	A	(3.24)	9.4	–2.77
CDSR133cI–pSB302dI	SW	31	10	J	(3.21)	7.4	–2.64
pSB188bI–pSB189hI	SW	65		D	(3.45)	9.0	–2.59
UMC114hI–CSU395eI	SW	64	3	C	(4.06)	10.0	–4.45
CDSC52eI–pSB289bI	SUYD	70	3	C	(2.71)	6.1	–0.46
CDSC5kM	ASH				0.0025	4.1	–0.6
CDSR95hM	ASH				0.0003	6.2	–0.75
CDSR96fM–CDSR35hM	ASH	74		C	(2.73)	12.4	–0.78
CSU449aM	ASH	39		J	0.0004	6.3	–0.75
pSB103cM	ASH				0.0015	7.1	–0.77
pSB142cM	ASH				0.0018	4.5	–0.62
pSB188lM	ASH	59	5	D	0.0020	4.2	–0.62
pSB82eM	ASH				0.0023	4.0	–0.6
CDSC42cM	POL				0.0026	4.3	0.57
CDSR15fM	POL	42	2	A	0.0023	4.1	0.55
CDSR46dM	POL				0.0028	7.6	0.71
CDSR96fM–CDSR35hM	POL	74		C	(4.32)	15.4	0.98
CSU449aM	POL	39		J	0.0024	4.6	0.57
pSB103cM	POL				0.0013	7.2	0.7
UMC147eM	POL	67	3	C	0.0001	7.9	0.75
CSU440aM	SN			F	0.0006	6.1	6.08
CDSB35eM	SW				0.0001	11.4	2
CDSB44fM	SW				0.0002	7.0	1.6
CDSC42gM	SW	8		F	0.0013	4.9	1.33
CDSC46fM	SW				0.0005	5.4	1.39
CDSC49bM	SW	31	4	B	0.0001	9.6	1.88
CDSC52cM–CDSR128cM	SW	32	2	A	(4.14)	9.7	1.68
CDSR15fM	SW	42	2	A	0.0001	8.5	1.77
CDSR70gM	SW	27	9	H	0.0001	7.6	1.63
CDSR96fM–CDSR35hM	SW	74		C	(5.28)	16.2	2.36
CSU39cM	SW				0.0006	7.3	1.52

Table 2 (continued)

Marker	Trait	LG	HG	S-LG	*P* (LOD)	PVE[a] (%)	A effect[b]
CSU428dM	SW				0.0001	7.0	1.59
CSU449aM	SW	39		J	0.0001	9.4	1.81
CSU453cM	SW				0.0001	10.3	1.95
pSB142cM	SW				0.0006	5.4	1.35
pSB289dM	SW	13	2	A	0.0002	6.0	1.45
CDSB35eM	SUYD				0.0010	7.8	0.11
CDSR15fM	SUYD	42	2	A	0.0002	7.2	0.1
CDSR46dM	SUYD				0.0022	9.8	0.1
CDSR96fM–CDSR35hM	SUYD	74		C	(4.40)	15.4	0.14
CSU440aM	SUYD				0.0019	6.0	0.1
pSB82eM	SUYD				0.0027	4.6	0.08
UMC147eM	SUYD	67	3	C	0.0001	10.7	0.12
CDSB32cP	ASH	4	1	G	0.0030	3.8	0.58
CDSB32fP	ASH	5	1	G	0.0014	4.4	0.63
CDSR88eP	ASH				0.0005	5.8	0.71
pSB101bP	ASH				0.0011	5.4	0.67
RZ508bP	ASH	13	7	I	0.0023	4.6	0.64
SHO87eP	FIB	69	3	C	0.0001	7.0	–1.28
CDSB32cP–CDO202bP	POL	4	1	G	(3.36)	10.1	–0.57
CDSB7eP	POL	25	4	B	0.0001	7.2	–0.72
CDSR160aP	POL	20	9	H	0.0018	4.6	–0.57
CDSR29aP–CDSB67hP	POL	24		F	(2.63)	5.5	–0.64
CDSR35bP	POL	43		C	0.0009	8.2	–0.78
CDSB32cP	SW	4	1	G	0.0004	5.4	–1.37
CDSB32eP	SW	11	1	G	0.0006	5.0	–1.33
CDSB32fP	SW	5	1	G	0.0009	4.7	–1.29
CDSB7eP	SW	25	4	B	0.0002	6.6	–1.49
CDSC46eP	SW				0.0001	7.5	–1.67
CDSC53fP–CDSR133eP	SW	32		J	2.5500	6.7	–1.49
CDSR25cP	SW	40	5	D	0.0011	5.0	–1.35
CDSR94bP	SW	50	2	A	0.0007	5.6	1.42
pSB124bP	SW				0.0009	5.1	–1.34
SG302hP	SW				0.0024	7.8	–1.91

[a] PVE: percentage of variance explained
[b] A effect: allele effect

Stalk number of PIN x MJ progeny ranged from 1 to 61, a range about 36% wider than the difference between the parents (MJ = 14, PIN = 58). Only one QTL from MJ was mapped and this explained 6.1% of PV. The allele effect of this MJ QTL was positive, consistent with the parental phenotype.

Fiber content QTLs

Fiber content of GG x IND progeny ranged from 38.5% to 62.4%, a range that was about 83.3% wider than the difference between the parents (IND = 52.6%, GG = 48.6%). A full model comprised of 19 QTLs, six from GG and 13 from IND, explained 60.6% of PV. The six GG QTLs alone explained 27.3% of PV, while the 13 IND QTLs alone explained 49.3%. The allele effects of all six GG QTLs were negative, while the allele effects of all 13 IND QTLs were positive, consistent with the parental phenotypes.

Fiber content of PIN x MJ progeny ranged from 53.2% to 66.3%, a range about 45.8% wider than the difference between the parents (PIN = 60.1%, MJ = 53.0%). Only one QTL could be detected from PIN, explaining 7.0% of PV. The allele effect of this PIN QTL was negative, which might explain part of the transgressive segregation observed in this population.

Ash QTLs

GG x IND progeny values ranged from 2.1 to 12, a range that was about 37.4% wider than the difference between the parents (IND = 11, GG = 4.8). A full model comprised of seven QTLs, two from GG and five from IND, explained 39.1% of PV. The two GG QTLs alone explained 16.7% of PV, while the five IND QTLs alone explained 25.6%. The allele effects of the two GG QTLs were negative, while allele effects of the five IND QTLs were positive, consistent with the parental phenotypes.

Ash values of PIN x MJ progeny ranged from 8.6 to 15.6%, a range about 16.7% narrower than the difference between the parents (PIN = 16.5, MJ = 8.1). A full model comprised of 13 QTLs, eight from MJ and five from PIN, explained 41.4% of PV. The eight MJ QTLs alone explained 28.7% of PV, while the five PIN QTLs alone explained 22.2% of PV. Allele effects of all MJ QTLs were negative, while the allele effect of the PIN QTL were positive, consistent with the parental phenotypes.

Comparative analysis of QTLs

Since the sugarcane linkage maps are incomplete (Ming et al. 1998) it is difficult to compare the genomic locations of some QTLs controlling the same traits in differ-

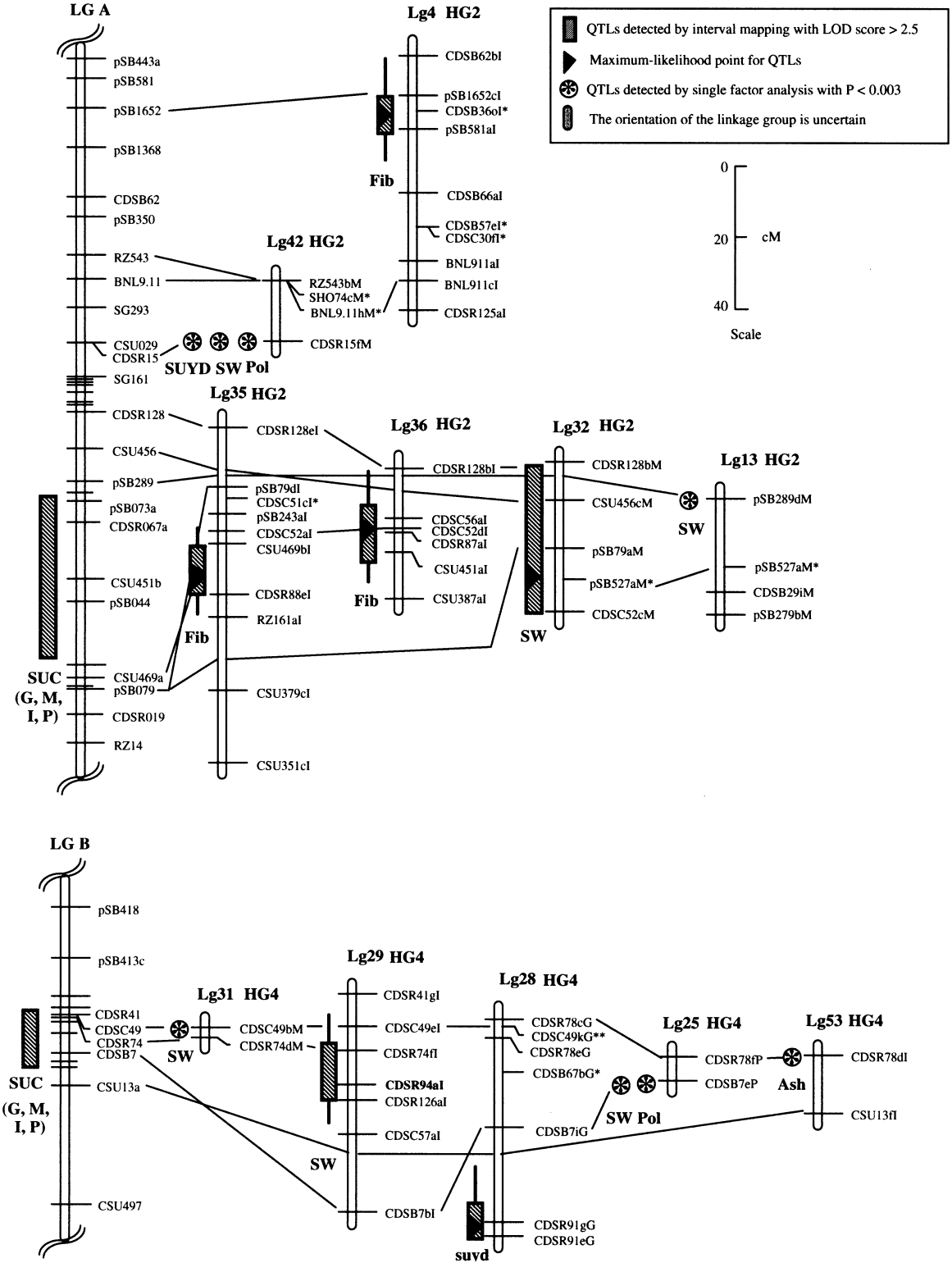

Fig. 2 Comparative mapping of sugar yield-related QTLs. *Solid lines* connect homologous loci on different sugarcane and sorghum linkage groups. Individual sorghum linkage groups (*LGs*) are represented by LGs *A to J*. Sugarcane linkage groups (Lgs, to be distinguished from sorghum LGs) from four parental varieties are indicated by the *last letter* of the marker name: *G* (Green German); *M* (Muntok Java); *I* (IND 81-146); *P* (PIN 84-1). Approximate map positions of double-dose (#) markers are inferred by the method of Da Silva (1995). *The letters in parenthesis* following the marker name represent the sorghum linkage groups where the marker was mapped, if different from the corresponding location shown. Only regions that contain, or are homologous to, QTLs are shown. *Bars and whiskers* indicate 1 and 2 LOD-likelihood intervals. Sugar-content QTLs (Ming et al. 2001) are shown to the left of the sorghum linkage groups

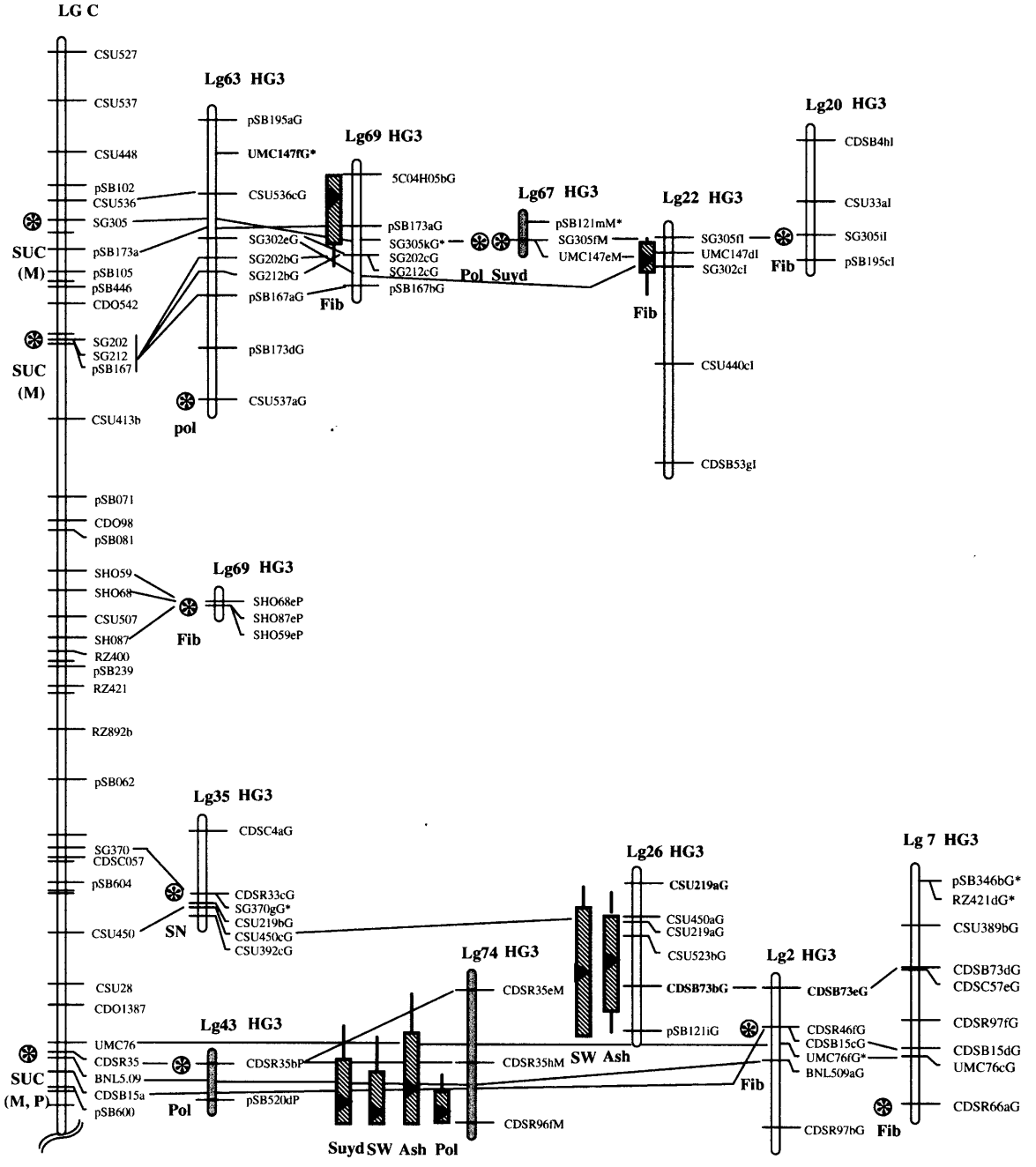

Fig. 2 Legend see page 338

ent sugarcane populations, and some QTLs controlling related traits within and between populations. Alignment between the high-density sorghum linkage map and sugarcane linkage maps helped us to evaluate QTLs affecting sugar yield and related traits from different sugarcane maps. The previously reported sugar-content QTLs (Ming et al. 2001) were placed on the left of sorghum linkage groups (LG A–J), while QTLs for sugar yield and related traits were aligned on the right. Comparative QTL analyses among these traits in two populations were summarized in reference to sorghum linkage groups as follows:

Sorghum linkage group A

Four QTLs controlling fiber content and stalk weight corresponded to a genomic region between markers pSB289 and pSB79 containing sugar-content QTLs (Fig. 2). Among these four QTLs, two were for fiber content in IND and the other two for stalk weight in MJ. These four QTLs were aligned to a genomic region spanning about 30 cM. One QTL each for pol, stalk weight and sugar yield in MJ, and one for fiber content in IND, corresponded to a genomic region between pSB1632 and BNL9.11. These eight QTLs were all located on sugarcane homologous group (HG) 2.

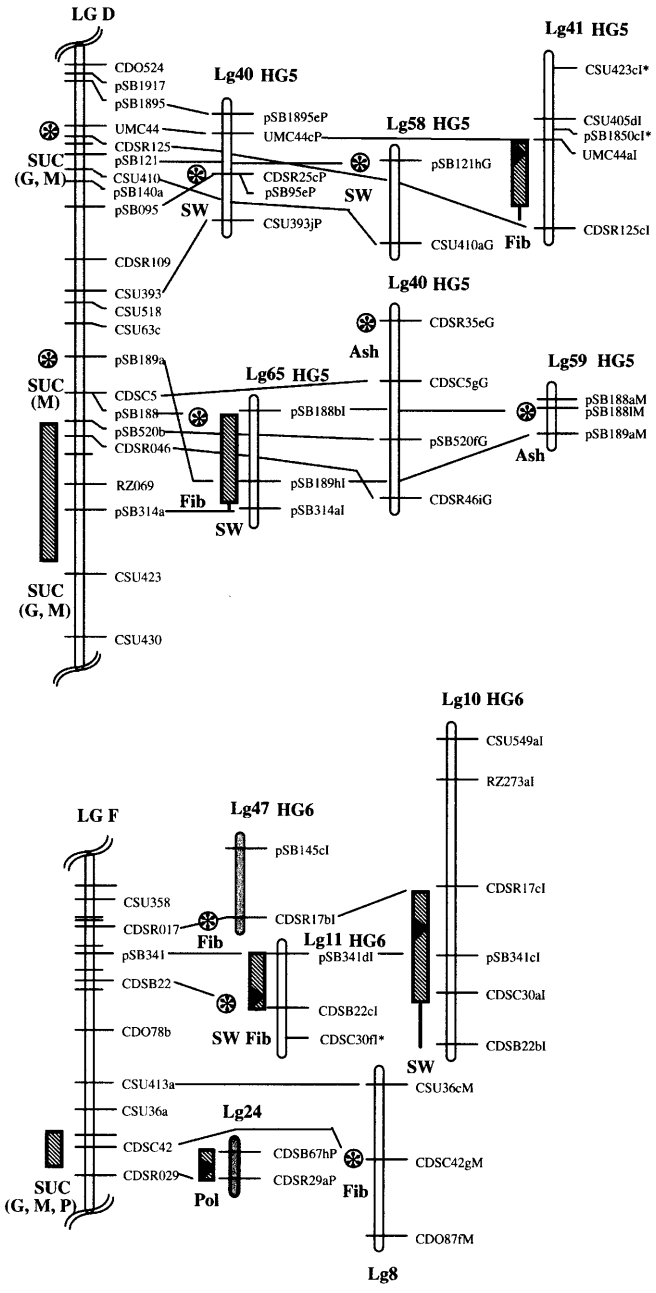

Fig. 2 Legend see page 338

Sorghum linkage group B

Five QTLs, one for pol from PIN, three for stalk weight from MJ, IND and PIN, and one for ash from IND, corresponded to a genomic region between markers CDSC49 and CDSB7 containing sugar-content QTLs. Another QTL controlling sugar yield in GG was mapped to an adjacent genomic region. These six QTLs were located on sugarcane HG 4.

Sorghum linkage group C

Five QTLs controlling fiber content, pol, and sugar yield in GG, MJ and IND corresponded to a genomic region between markers CSU536 and pSB167 containing sugar-content QTLs. Among these five QTLs, three were for fiber content in GG and IND, one each for pol and sugar yield in MJ. One pol QTL in GG was located on an adjacent region. Nine QTLs, two each for fiber content, stalk weight, ash content, and pol in GG, MJ and PIN, and one for sugar yield in MJ, corresponded to a genomic region between markers CSU450 and pSB600 containing a sugar-content QTL. Another two QTLs for fiber content and stalk number in GG and PIN were scattered on the genomic regions corresponding to sorghum linkage group C. Sixteen of the seventeen QTLs were located on HG 3. The PIN Lg43 containing a pol QTL could not be assigned to any sugarcane HG.

Sorghum linkage group D

Three QTLs controlling fiber content and stalk weight corresponded to a genomic region between markers UMC44 and pSB95 containing a sugar-content QTL. Among these three QTLs, two were for stalk weight in GG and PIN, one for fiber content in IND. Four QTLs, one each for fiber content and stalk weight in IND and two for ash in GG and MJ, corresponded to a region between markers CSU63 and pSB340 containing two sugar-content QTLs. These seven QTLs were located on sugarcane HG 5.

Sorghum linkage group F

Two QTLs, one for pol from PIN and one for fiber content from MJ corresponded to a genomic region between markers CDSC42 and CDSR29 containing sugar-content QTLs. Four QTLs, two each for fiber content and stalk weight in IND, corresponded to the region between markers CDSR17 and CDSB22. Four of the six QTLs were mapped on sugarcane HG 6.

Sorghum linkage group G

Five QTLs in PIN controlling stalk weight, ash content and pol corresponded to a narrow genomic region (<5 cM) between markers CDO202 and CDSB32. These five QTLs were mapped on sugarcane HG 1. Another QTL for fiber content in GG was associated with CDSB31 on HG 15. A sugar-content QTL was mapped to a different location between markers BCD454 and CSU63.

Sorghum linkage group H

Two QTLs controlling pol and ash from IND and PIN corresponded to a genomic region near marker CDSR160. A third QTL was associated with marker CDSR70.

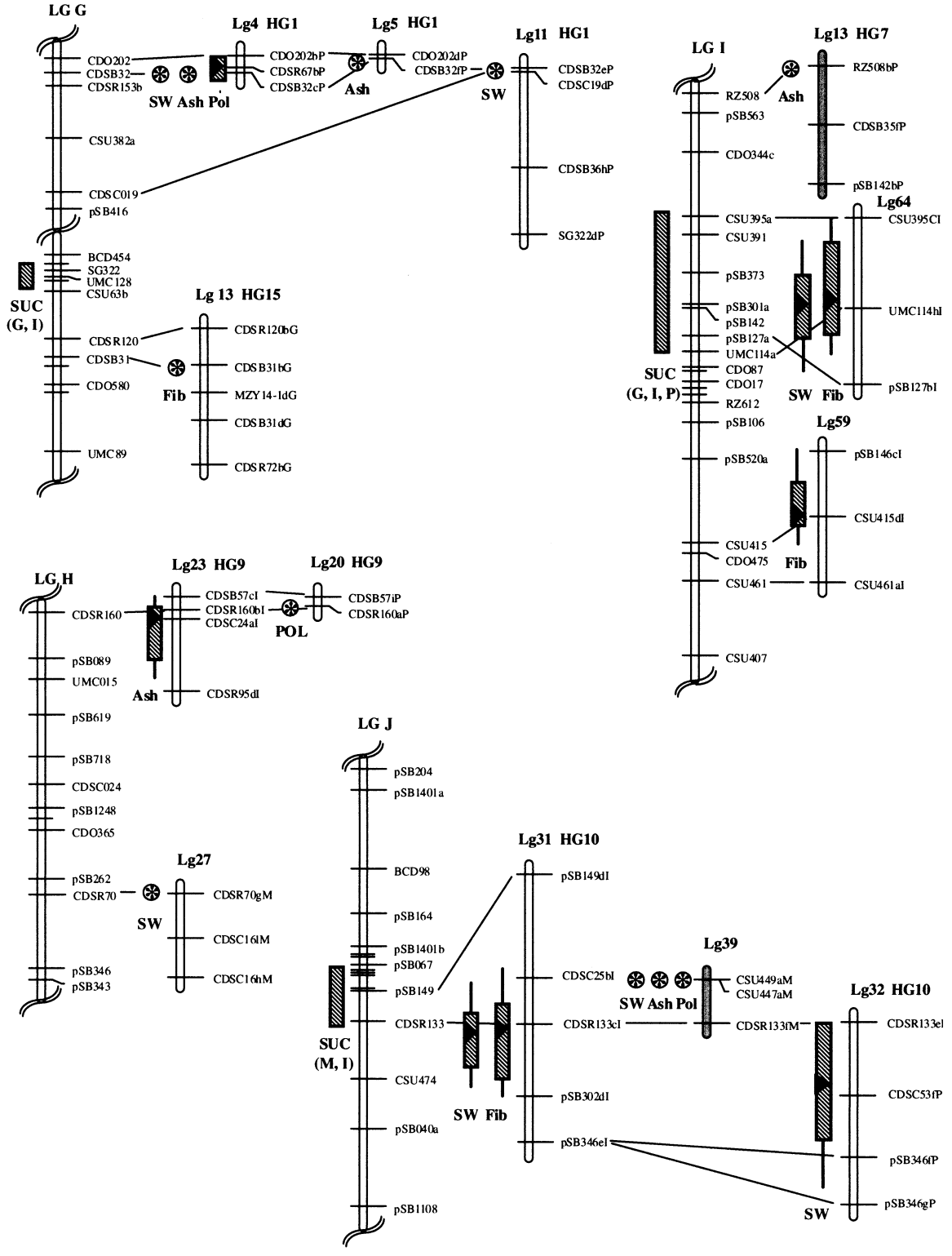

Fig. 2 Legend see page 338

Sorghum linkage group I

Two QTLs controlling fiber content and stalk weight corresponded to a genomic region between markers CSU395 and UMC114 containing sugar-content QTLs. One QTL for ash content in PIN was associated with marker RZ508. Another QTL for fiber content in IND corresponded to a region between markers pSB520 and CSU461.

Sorghum linkage group J

Six QTLs, three for stalk weight in MJ, IND and PIN, and one each for fiber content, ash content and pol in

IND and MJ, corresponded to a region between markers pSB149 and CDSR133 containing sugar-content QTLs. Three of the six QTLs were mapped on sugarcane HG 10.

A total of 102 QTLs were mapped for the six sugar yield-related traits. Among the total of 61 QTLs placed on the map affecting sugar yield and related traits, 50 were located in 12 genomic regions, corresponding to other QTLs within and between mapping populations (Fig. 2). These 50 QTLs can be categorized into the following five groups:

(1) QTLs mapped in the same species that corresponded to each other. Four sets of QTLs were mapped in the wild species *S. spontaneum* corresponding to sorghum genomic regions between markers CDSR17 and CDSB22 on LG F, CDO202 and CDSB32 on LG G, CSU395 and UMC114 on LG I, and near marker CDSR160 on LG H.

(2) QTLs mapped in the same population that corresponded across species. Two QTLs controlling pol in PIN and fiber content in MJ corresponded to regions between markers CDSC42 and CDSR29 on LG F.

(3) QTLs controlling related traits that corresponded across species and mapping populations. Eight of the twelve clusters of QTLs corresponded across species and mapping populations. These eight clusters of QTLs were located on genomic regions between markers pSB289 and pSB79 on LG A, CDSC49 and CSU13 on LG B, CSU536 and pSB167 on LG C, CSU450 and pSB600 on LG C, UMC44 and pSB95 on LG D, CSU63c and pSB314 on LG D, and pSB149 and CDSR133 on LG J.

(4) QTLs controlling the same trait and mapped on different linkage groups that corresponded to homologous locations. Two QTLs controlling fiber content in IND corresponded to a region between markers pSB289 and CSU469 on LG A, and SG305 and pSB167 on LG C. Two QTLs controlling stalk weight in MJ and IND corresponded to a region between markers pSB289 and CSU469 on LG A, and CDSR17 and CDSB22 on LG F, respectively.

(5) QTLs mapped to corresponding locations of a sugarcane LG affecting different traits. Two QTLs mapped on MJ LG 67 controlled both pol and sugar yield, corresponding to sorghum LG C. Four QTLs on MJ LG 74 controlled stalk weight, ash content, pol and sugar yield, corresponding to sorghum LG C. Two QTLs on LG 26 in GG controlled stalk weight and ash content, corresponding to sorghum LG C. Three QTLs on PIN LG 4 controlled stalk weight, ash content and pol, corresponding to sorghum LG G. Two QTLs on ING LG 64 controlled both stalk weight and fiber content, corresponding to sorghum LG I. Two QTLs on IND LG 31 controlled both stalk weight and fiber content, corresponding to sorghum LG J. Three QTLs on MJ LG 39 controlled both stalk weight ash content and pol, corresponding to sorghum LG J.

The phenotypic value of an individual plant is the aggregate product of QTLs with positive and negative effects in the plants. Stalk weight in GI and PM was examined to show the relationship between the phenotype and the number of QTLs with positive or negative allele effects. The individual plants were grouped into eight (GI) and ten (PM) classes based on their stalk weight and plotted with the average numbers of positive (GG or MJ) or negative (IND or PIN) QTLs (Fig. 3). The total numbers of positive and negative QTLs were three and seven in GI and 15 and 10 in PM, respectively. The number of negative QTLs was much greater than that of positive QTLs in plants with low stalk weight, while the opposite was true in high stalk-weight plants. On the highest stalk-weight class no negative QTLs were present. However, all three positive QTLs were present in plants with the highest stalk weight in GI, but only six (40%) of the 15 positive QTLs were present in MJ. This might explain why the highest stalk weight (1.46 lb) of the PM progeny fell far short of the high parental value of 2.02 lb (MJ).

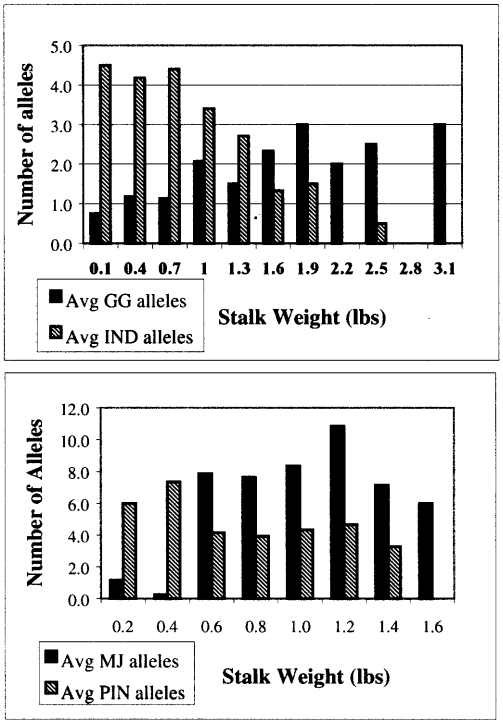

Fig. 3 Allele frequency distribution based on stalk weight values in two sugarcane populations derived from Green German x IND 81-146 and PIN 84-1 x Muntok Java

Discussion

Variations of quantitative traits are affected by many loci, and each gene replacement may have effects on many traits (Wright 1968). Since sugar yield and related traits are highly correlated, either positively or negatively, pleiotropic effects of a QTL on related traits were often observed. If the effects were not sufficiently large to reach significance, the influence of these QTLs on different traits might be reflected by the alignment of QTLs affecting different traits in the same genomic region. For example, in the present work there are 12 genomic regions containing 50 QTLs. If the effects of a QTL on dif-

ferent traits reached significance, that QTL would be mapped for two or more traits simultaneously, such as the QTL affecting sugar yield, stalk weight, ash content and pol mapped on MJ LG 74. However, not every QTL showed pleiotropic effects on related traits. For example, the traits with the highest correlation coefficient in PM was sugar yield and sugar content at 0.83 (Table 1), but only four of the seven (57%) MJ sugar yield QTLs controlled sugar content as well (Ming et al. 2001). Selection based on a correlated trait, while is more easily selected, is frequently practiced in conventional breeding programs (Skinner 1972; Skinner et al. 1987; Ram and Hemaprabha 1998).

Among the 102 QTLs mapped for six sugar yield-related traits in these two mapping populations, 61 were placed on sugarcane linkage maps and 50 of them were clustered in 12 genomic regions and seven HGs (Fig. 2). The number of QTLs in a cluster ranged from two to nine, and the QTLs on each cluster belong to a single homologous group except those that could not be assigned to a homologous group due to lack of common probes (Ming et al. 2002). Since these clustered QTLs were located on the same genomic region of different homologs, it is possible that the QTLs on the same cluster are different alleles of the same locus. The polymorphisms among these homologs might be caused by chromosomal rearrangements through the course of evolution. Considering the polyploid nature of sugarcane, the number of QTLs controlling sugar yield-related traits could be significantly fewer than what we have detected in these two populations.

The identification of particular genomic regions that segergate for QTLs that increase sugar yield in *S. officinarum*, and also QTLs that reduce sugar yield in *S. spontaneum*, suggests that the genes or gene clusters in these regions may be especially mutable. Genomic regions containing QTLs controlling sugar yield-related traits within each species may reflect functional divergence of the two species towards high and low sugar content, in response to selection for different fitness criteria. Confirmation of QTLs influencing related traits from different varieties and/or species increased the level of confidence that QTLs exist in the region, and also increase the likelihood that DNA markers linked to these QTLs would be useful in other germplasms.

Among the six traits reported here, five traits were directly measured while sugar yield was derived from sugar content, stalk weight and stalk number. None of these traits were simply inherited and many genes would be expected to control each of them. Although the differences between parental phenotypic values were large and transgressive segregation was observed in these six traits, the number of QTLs mapped ranged from one (stalk number and fiber content in PM) to 24 (stalk weight in PM). A single fiber-content QTL in PM explained 7% of the PV; most of the QTLs affecting fiber content were not detectable in this population due to a possibly higher dosage of these loci (Wu et al. 1992; Da Silva et al. 1995). However, 19 fiber-content QTLs were mapped in GI that explained 60.6% PV. The difference in the number of QTLs in these two populations might be due to differences in dosage of the loci where fiber-content QTLs reside, and/or to a higher polymorphism rate in the GI population. There were only two and one stalk-number QTLs mapped with 13.9% and 6.1% PVE in GI and PM, respectively, showing that most of the stalk-number QTLs were not single dose in these two mapping populations. The highest number of QTLs mapped among these six traits was stalk weight with a total of 25 in PM. Among these 25 loci, two MJ loci were mapped on LGs 13 and 32 of homologous group (HG) 2 corresponding to the same genomic region on sorghum LG A; three PIN loci were mapped on LGs 4, 5 and 11 of HG 1 corresponding to the same region on sorghum LG G; one MJ locus and one PIN locus were mapped on MJ LG 31 and PIN LG 25 of HG 4, respectively, corresponding to the same region on sorghum LG B. These three groups of loci could be just three loci with different allelic forms on HGs 1, 2 and 4. The total of 25 stalk-weight QTLs might be an overestimate of the underlying number of unique genes. On the other hand, other QTLs controlling stalk weight may not have been mapped due to either lack of polymorphism in this particular population (PM) or different dosage forms that were not detectable with the current population size (Wu et al. 1992).

Fiber content was negatively correlated with sugar content and stalk weight in GI, consistent with Gravois and Milligan's (1992) observations on 22 randomly selected clones derived from 14 parents. In the PM population, since sugar contents were low in both parents (50.4 lb/t for MJ, and –37 lb/t for PIN), the correlation coefficients were not significant between fiber content and the other sugar yield-related traits.

Allele effects of QTLs for sugar yield, stalk weight, stalk number and ash content were positive for QTL alleles from high-value parents and negative for QTL alleles from low-value parents in both populations. However, the allele effects of QTLs for pol in the GI population and fiber content in the PM population were opposite to their parental values, contributing to the transgressive segregation observed for these two traits.

The correlation coefficients among the measured traits were mostly consistent with well-established expectations. Sugar content, stalk number and stalk weight are components of sugar yield, and this was confirmed by the positive correlation among them. Stalk weight and sugar content had been identified previously as important predictors of sugar yield (Sunil and Lawrence 1996) and the correlation coefficients obtained from these two sugarcane interspecific populations support this conclusion. Stalk number of the GI population segregated with a bias towards the high sugar yield parent Green German with an average of 17, while the stalk number of the PM population was normally distributed with an average of 25 (Fig. 1). The difference in these distribution patterns resulted in a significant correlation between stalk number and sugar yield in GI, but no correlation between

these two traits in PM. That is because some individuals of the PM population were more like the *S. spontaneum* parent with many small stalks and virtually no sugar. The stalk number of commercial cane is always positively correlated with sugar yield since the variation is low for stalk diameter and sugar content (K.K. Wu, personal communication). It was suggested that increased sugar yield is more likely from increasing the biomass yield (i.e.), stalk weight x stalk number rather than from increasing sugar content (Hogarth et al. 1981).

Segregation distortion was observed for sugar yield and stalk number in GI and fiber content in PM (Fig. 1), and contributed to fewer QTLs being detected with single-dose markers for each of these traits (3, 2 and 1, respectively). A different set of QTLs, which were not inherited in simplex ratios, might control these traits and this could produce the segregation distortion. For example, 19 QTLs for fiber content were mapped in GI, but only one was mapped in PM. Fiber content has been known to have a heritability as high as 86% (Kang et al. 1990) to 91% (Gravois and Milligan 1992), and was due to predominantly additive gene action (Hogarth and Cross 1987). This single-fiber-content QTL in PIN explained only 7% of the PV, indicating that additional QTLs should be involved but they were not detectable with single-dose markers and the current population size.

Detecting QTLs for components of sugar yield has provided a valuable set of markers having potential for breeders to use in the selection of improved sugarcane genotypes (Wu et al. 2000). Active breeding activities using plant materials derived from these two mapping populations are in progress, and markers linked to the QTLs could be used directly to incorporate positive alleles and eliminate negative alleles of the sugar yield components. To balance the high sugar content with reasonable stalk-strength, fiber-content and ash-content QTLs would also be useful in selection. The use of DNA markers in selection would allow the identification of potentially superior materials and the elimination of undesirable ones in the early stages of a breeding program.

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