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Genetic mapping in sugarcane, a high polyploid, using bi-parental progeny: identification of a gene controlling stalk colour and a new rust resistance gene

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Abstract Modern sugarcane cultivars (*Saccharum* spp) are highly polyploïd and aneuploid interspecific hybrids ($2n = 100$–130). Two genetic maps were constructed using a population of 198 progeny from a cross between R570, a modern cultivar, and MQ76-53, an old Australian clone derived from a cross between Trojan (a modern cultivar) and SES528 (a wild *Saccharum spontaneum* clone). A total of 1,666 polymorphic markers were produced using 37 AFLP primer combinations, 46 SSRs and 9 RFLP probes. Linkage analysis led to the construction of 86 cosegregation groups for R570 and 105 cosegregation groups for MQ76-53 encompassing 424 and 536 single dose markers, respectively. The cumulative length of the R570 map was 3,144 cM, while that of the MQ76-53 map was 4,329 cM. Here, we integrated mapping information obtained on R570 in this study with that derived from a previous map based on a selfed R570 population. Two new genes controlling Mendelian traits were localized on the MQ76-53 map: a gene controlling the red stalk colour was linked at 6.5 cM to an AFLP marker and a new brown rust resistance gene was linked at 23 cM to an AFLP marker. Besides another previously identified brown rust resistance gene (*Bru*1), these two genes are the only other major genes to be identified in sugarcane so far.

Keywords Sugarcane · Genetic mapping · AFLP · Rust resistance gene · Colour gene · Polyploid

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Introduction

Modern sugarcane cultivars have one of the most complex genomes among important crops (Grivet and Arruda 2001). They are polyploid and aneuploid hybrid derivatives from two highly polyploid species, i.e. the domesticated sugar-producing species *Saccharum officinarum* ($x = 10$, $2n = 8x = 80$) and the wild species *Saccharum spontaneum* ($x = 8$, $2n = 40$–128). The first interspecific hybrids produced in the early twentieth century were backcrossed with *S. officinarum*. In both interspecific F1 and BC1 crosses, *S. officinarum* transmitted its somatic chromosome number ($2n$) to the progeny (Bhat and Gill 1985). Modern cultivars thus have chromosome numbers in the 100–130 range, around 15 to 25% of which are derived from *S. spontaneum* (D'Hont 1993). Despite this complicated picture, the meiosis of modern sugarcane cultivars is fairly regular, mainly involving bivalent pairing (Price 1963; Burner and Legendre 1993, 1994), and the chromosome assortment is the result of a combination of preferential and random pairing (Jannoo et al. 2004).

Genetic maps have been produced, based on single dose markers (Wu et al. (1992), for the two ancestral species *S. spontaneum* (Al Janabi et al. 1993; Da Silva et al. 1993, 1995; Ming et al. 1998, 2000b) and *S. officinarum* (Mudge et al. 1996; Guimaraes et al. 1997;

Ming et al. 1998, 2000b). Genetic maps have also been constructed for two modern sugarcane cultivars, i.e. Q165 (Aitken et al. 2005) and R570. For the latter cultivar, a first RFLP map was constructed using a selfed population derived from R570 (Grivet et al. 1996). AFLPs (Hoarau et al. 2001), SSRs and resistance gene analogs (Rossi et al. 2003) were later mapped using a larger number of selfed R570 progeny. This AFLP-based map encompassed more than 1,100 markers and reached a total length of 7,800 cM, which represents a coverage of around 46% of the anticipated genome size (17,000 cM). The species origins of the markers and the results of molecular cytogenetic studies (D'Hont et al. 1996) revealed that about 10% of the R570 chromosomes were inherited from *S. spontaneum* and that another 10% were recombinants between *S. spontaneum* and *S. officinarum*. The marker coverage on the map of the interspecific cv. R570 is uneven, with *S. spontaneum* chromosomes being covered more densely than those of *S. officinarum*. This discrepancy results from the lower polymorphism rate in the highly polyploid *S. officinarum*—the main component of the modern sugarcane cultivar genome—as compared to *S. spontaneum* (D'Hont et al. 1994; Grivet et al. 1996).

A saturated map must be developed to be able to efficiently localize major genes or Mendelian factors involved in quantitative trait loci (QTL). Moreover, QTL mapping in sugarcane is a real challenge, since many alleles coexist at each locus due to the high polyploidy. At a particular locus, the effect of an allele should be perceptible only if it exceeds the average effect of all other segregating alleles in the background but not, as in diploids, if its effect simply exceeds that of a single alternative allele (D'Hont and Glaszmann 2001; Hoarau et al. 2002).

Ming et al. (2001, 2000a) investigated the genetic basis of traits related to sugar content, plant height and flowering in interspecific crosses between *S. officinarum* and *S. spontaneum*. Numerous QTLs were detected that could be localized in a few genomic regions, suggesting that substantially fewer genes may actually be involved in the genetic control of these traits. Within an interspecific cross, wide phenotype segregation can provide a favourable setting for QTL detection. By comparison, a study of yield components (plant height, stalk diameter, stalk number and Brix) in the selfed progeny of the modern cultivar R570 revealed numerous QTLs with smaller individual effects (Hoarau et al. 2002). Similarly, Jordan et al. (2004) detected numerous small QTLs for stalk number in sugarcane and found sorghum QTLs for tillering in syntenic positions. The only major gene that has been localized so far in the sugarcane genome is a rust resistance gene (Daugrois et al. 1996). This gene (called *Bru*1 for brown rust) is currently the focus of a map-based cloning project (D'Hont et al. 2001; Asnaghi et al. 2004).

In this paper, we describe the identification and mapping of two other major genes, including a new potential rust resistance gene and a gene controlling stalk colour. These genes were identified in the sugarcane clone MQ76-53 through an extensive genetic mapping study of a bi-parental R570 × MQ76-53 cross. We discuss the constraints and advantages of working in a bi-parental cross in comparison with a selfed progeny population.

Materials and methods

Plant and DNA material

The mapping population consisted of 198 progeny derived from a R570 × MQ76-53 cross. R570 is a modern cultivar that was developed at CERF [Centre d'Essai de Recherche et de Formation, Réunion] and is derived from a cross between two modern cultivars H32-8560 and R445. MQ76-53 is an old Australian sugarcane clone that comes from a cross between the old cultivar Trojan (Co 270 × *S. officinarum*) and the *S. spontaneum* clone SES528. Its genetic structure should therefore be close to that of an interspecific F1 hybrid. R570 is rust resistant and has green stalks. MQ76-53 is also rust resistant but has red stalks. Total genomic DNA of mapping progenies was extracted from fresh leaves according to the method described by Hoisington (1992).

In addition, 133 progeny from a cross between the cultivars B63-758 (rust susceptible) and MQ76–53 (rust resistant) were evaluated for rust resistance.

Field evaluation and statistical analysis of rust resistance

A subset of 166 out of the 198 mapping progenies was evaluated for rust resistance, under natural infestation conditions, in a randomized complete block design with three replicates at the Ligne Paradis CIRAD research station (Saint Pierre, Reunion). Each plot included four distinct stools. The trial was planted in mid-November 2002 and evaluated for rust resistance in early September 2003, i.e. at the end of the winter season which is the most favourable period for rust development in Reunion (cool humid conditions). Rust resistance was scored on each plot on a 1 (the most resistant:) to 9 (the most susceptible) scale according to Tai et al. (1981). A score of 1 indicated the absence of sporulating pustules (uredospores). Susceptible plants could have scores ranging from two (a few sporulating pustules) to nine (many pustules even on younger leaves and necrosis of older ones). Natural rust infestation during the experiment was heavy. Indeed, B34-104, a moderately susceptible clone that is usually rated between 3 and 4, planted as control, was rated 6. The two other controls, R570 and MQ76-53, were rated 1. Analysis of variance of the rust score variable was performed using the SAS mixed procedure (SAS version 6.12, SAS Institute, Inc., NC, USA). The genotype (clone) factor was considered as random and replication as fixed. Broad-sense heritabilities were calculated at the experimental design

level from the ratio between genetic variance (σ_g^2) and phenotypic variance (σ_p^2), with $\sigma_p^2 = \sigma_g^2 + \sigma_e^2/j$, where σ_e^2 is the error variance and j the number of replications. Segregation analysis was performed on mean scores computed for all replications for each progeny. One way ANOVA was performed to calculate the proportion of phenotypic variance (R^2) explained by markers associated with rust resistance. These analyses were performed using all available markers and mean rust scores for each genotype (clone). The proportion of total phenotypic variance explained by a marker (R^2) was calculated using the sums of squares obtained by ANOVA.

Progeny from the B63-758 × MQ76-53 cross were studied in the field, with seedlings planted randomly 50 cm apart. In this cross, rust resistance was scored for each individual seedling on the basis of the presence/absence of sporulations. Seedlings bearing sporulating pustules were classified as susceptible, and otherwise they were classified as resistant.

Field evaluation and statistical analysis of stalk colour

Stalk colour of the 198 mapping progenies was scored in three different environments on the basis of the discrete classification red versus non-red colour (mostly green or yellow stalks);(1) in the collection used to conserve the population colour was scored in August 2002, a few weeks after leaves had been stripped from the first stool in each conservation plot; (2) in a duplicate of this collection, colour was scored in June 2003, without previous stripping of the stalks (3) finally, colour was scored in a separate trial where two cuttings of each of the 198 clones were planted in 10 L pots that were well separated from each other in order to favour sunlight exposure. Stalks were regularly stripped before colour evaluation in July 2004. The consistency of the three different stalk colour evaluations was checked using Fisher's exact test (Mehta and Patel 1983; SAS Institute 1990, FREQ procedure) between scoring of environments (1) and (2), environments (2) and (3), and environments (1) and (3).

AFLP markers

AFLP analysis (Vos et al. 1995) was performed using the Gibco BRL genome I kit according to the manufacturer's instructions, except for slight modifications as described in Hoarau et al. (2001). Each AFLP marker was identified by the primer combination consisting of six letters plus a band number indicated as a suffix. The first three letters represent *Eco*RI selective nucleotides and the last three *Mse*I selective nucleotides.We used the same 37 combinations as those used to build the R570 map of Hoarau et al. (2001) and Rossi et al. (2003), except for the three *Eco*RI/*Mse*I primer combinations aag/caa, aag/cat and aag/cta, which were replaced by the combinations aag/ctg, aac/ctg and acc/cta. Most of the bands inherited from R570 could thus be labelled as in the previously published maps.

Microsatellite markers

Thirty-three SSRs developed at CIRAD in collaboration with Génoscope (Evry, France), in addition to 13 SSRs mined from the Brazilian sugarcane EST database (Pinto et al. 2004) were analysed using the protocol described in Rossi et al. (2003). CIRAD SSR bands inherited from R570 were coded according to this previously published map using the following nomenclature: m (microsatellite) followed by the number of the SSR, and then the letter m (as for marker) followed by a number identifying the band. CIRAD SSRs (complete nomenclature = mSSCIRxx) are described at http://www.tropgenedb.cirad.fr/en/sugarcane.html. The nomenclature of EST SSR was m (microsatellite) followed by a letter and a two digit code number and then the letter m (as for marker) followed by a number identifying the band. SSR bands were scored as dominant markers (presence vs absence).

RFLP markers

Nine candidate genes differentially expressed in response to challenge by smut (Heinze et al. 2001) were kindly provided by the South African Sugarcane Research Institute (SASRI) and used as RFLP probes (Table 1). The nomenclature used for these RFLP markers was 'ADS' followed by a figure between 1 and 9, and then a code for the restriction enzyme used (Eco for EcoRV and Hin for HindIII) followed by a number identifying the band. DNA preparation, Southern blotting and hybridization were performed as described previously by Grivet et al. (1996). RFLP bands were scored as dominant markers (presence vs. absence).

Map construction

The genetic maps were constructed using linkages in coupling phase between single dose markers only

Table 1 Description of candidate genes used as RFLP probes provided by SASRI

Probe name	Putative gene function
ADS1	Thaumatin
ADS2	Flavonoid pathway transcription factor
ADS3	Pto ser/thr protein kinase
ADS4	Pathogen induced nucleotide binding site
ADS5	Phosphoprotein phosphatase
ADS6	Isoflavone reductase
ADS7	G protein receptor
ADS8	Cellulose synthase
ADS9	Cell wall associated kinase

Table 2 Synthesis of mapping information on R570: alignment of the present map with maps of Hoarau et al. (2001) and Rossi et al. (2003). Locus-specific markers (SSRs and RFLPs) highlighted in bold characters represent newly localised markers. CGs preferentially pairing at meiosis are associated within boxes (bold boxes indicate newly detected repulsion linkages) When a CG is involved in repulsion linkage with two CGs a vertical line is drawn between each pair of CGs in repulsion). When part of a former CG was presumably wrongly linked in coupling phase to its remaining part, it is shown as crossed out in bold characters. When two former CGs could be linked together to form a new CG, they are shown in bold characters with a + sign. Detailed information on the different genetic maps of R570 is available at http://www.tropgenedb.cirad.fr/en/sugarcane.html

HG[4]	Cons[5]	Rossi[1]	JYH[2]	LMR[3]	SSR loci	RFLP loci
I					m2, m13, m14, m19, m27 m42*, m52, m53	R024Sst$, R057hin, R083Sst, R125Sst, R183Sst, R313eco, R371Dra
I	I a	I a	51	54	m52m8	R057Hinc, R024Sstf
I	I b	**I b+U 62**	**52+38**	30	m14m3, 27ml	R057Hinf
I	I c	I c	105	29	m27m2	R083Sstc
I	I 4	I 4	1	43	m19m1, m42m7, m52m3, m53m2	R083Sste, R125Ssta
I	I 5	I 5	3	154	m2m2, **m14m6**	R024Sstc, R024Sstg,R183Sstg
I	I 6	**I 6+U 3**	**37+72**	111	m13m2, m27m3, m52m6	R024Ssta, R183Ssta, R313Ecob, R313Ecoe,R371Draa
I	I 7	I 7	74	40	m19m5, m53m9	R024Sste, R083Sstd
I	I 8	I 8	68	-	m52m1	R125Sstc
I	I 9	I 9	2	108	m13m1, m14m1	R057Hine, R057Hind, R125Sstb, R313Ecoa, R313Ecod
I	I 10	I 10	10	42	**m19m2,** m53m1	
I	I 17	U 8	82	114+143	**m52m2**	
II					mA03, mA16*,mB01, m25, m34, m35, m39, m41, m48, m50, m56, m69*	R012Eco, R145Eco, R169Hin, R258Dra, R275Eco, R366Hin, R386Dra
II	II a	II a	90	128		R012Ecof, R145Ecoe, R275Ecoe
II	II b	II b	71	166		R012Ecoc, R275Ecoc
II	II 4	U 35	84	-	m39m1	
II	II u21	U 21	85	107		
II	II 6	II 6	92+5	21	**mA03m5**, m25m2, m34m1	R012Ecoa, R386Drac
II	II u29	U 29	88	-	m25m3	
II	II 7	II 7	62	-	m39m2	R275Ecof
II	II 8	II 8	59	23	m25m1, m34m3, m48m2, m50m1,m69m2	R169Hina, R386Draa
II	II 9	II 9	4	69	**mA03m6, mB01m1,** m34m2, m35m1, m41m1	R169Hinc, R258Draa, R275Ecod, R366Hina
II	II 10	II 10	7	148	**mA16m1**, m35m2, m41m2	R169Hinf, R258Drab, R366Hinc
II	II 11	II 11	-	-	m48m3, m56m4	
II	II 12	II 12	-	-	m35m3	R366Hind
II	II 14	U 61	6	115		
III					mC03, m33, m40, m42*, m55, m64, m70	R149Eco, R523Eco
III	III a	III a	-	189	m40m6, **m64m2**	R523Ecoc
III	III b	III b	-	-	m64m6, m70m4	R523Ecoa
III	III 2a	U 57	~~24~~	-		
III	III 3	III 3	23	53	m42m4, **m55m2**, m70m2	
III	III 2	III 2	24	126	m55m8,m70m6	
III	III 4	III 4	22	68+191	m40m1	R149Ecoa
III	III 7	III 7	83	82	m40m4, m55m9, m64m8, m70m7	
III	III 8	III 8	107	27	m33m5, m55m5, m64m5, m70m3	
III	III u	~~VI 3~~	~~29~~	59	**mC03m3,** m40m2	R149Ecoe
III	III u54	U54	34	-	m33m2, m42m1	
III	III 10	III 10	114	57	m64m1, m70m1	
IV					mA06, m74	R142Eco*, R184Eco, R372Eco, R441Sst, R526Eco*, R533Hin
IV	IV 1	IV 1	8	81+109	**mA06m4**	R142Ecod, R184Ecog, R184Ecof, R372Ecoe, R372Ecoa, R441Sstd, R441Ssta, R526Ecob, R533Hinb
IV	IV 2	IV 2	9	106	m74m2**, mA06m2**	R 441Sstf, R441Sste, R533Hina
VI					m7, m9, m10, m23 , m31, m37, m47, m54, m57, m58, m60, m68, m73*	ADS1Eco, ADS3Hin, ADS4Eco, ADS6Hin, ADS8Hin, R087Hin, R152Hin, R162Eco, R196Eco, R272Eco, R367Dra, R396Hin, R482Eco
VI	VI 1a	VI 1a	26	-	m10m1	R196Ecoc, R196Ecod
VI	VI 1b	VI 1b	33	-	m47m4, m60m4	
VI	VI 2	VI 2	28	8+173	m10m7, m54m7	**ADS3Hin10, ADS6Hin4, ADS8Hin3b,** R272Ecoa, R482Ecod
VI	VI 3	**VI 3+VI 8**	**29+70**	7	m10m3, m31m6, m37m1, m54m6, m60m3, m73m3	**ADS6Hin8, ADS8Hin3h**, R272Ecod
VI	VI 4	VI 4	31	125	m9m2, m68m1	
VI	VI 9	VI 9	32	32	m37m5,m47m5, m57m3	
VI	VI 5	VI 5	94	-	m10m2, m58m2	
VI	VI 10	VI 10	25	66	m37m4, m57m1, m60m2	
VI	VI 11	VI 11	27	-	m60m1, m73m1	R367Draa
VI	VI 12	VI 12	30	113	m37m3, m57m2	
VI	VI 15	VI 15	109	169	m10m5	

Table 2 (Contd.)

HG[4]	Cons[5]	Rossi[1]	JYH[2]	LMR[3]	SSR	RFLP
VI	VI 20	U 11	77+104	164+178	**m54m8**	R087Hinb, R152 Hinb, R162Ecoa, R162Ecod, R162Ecoc
VI	VI a	VI a	-	-	m10m6	R196Ecof, R272Ecob
VI	VI b	VI b	-	-	m58m1	R196Ecoa, R272Ecog
VI	VI c	VI c	57	11		**ADS4Eco6**, R396Hinb
VI	VI u52	U 52	56	12	**m23m2, m54m2**, m7m1	**ADS4Eco5**
VI	VI d	VI d	61	65+110		R367Drac, R396Hinc
VI	VI u19	U 19	73	19		**ADS3Hin4, ADS8hin7**
VI	VI u36	U 36	95	16		**ADS1Eco4**, R272Ecoc
VI	VI u60	U 60	75	72	m23m1, m54m1	
VI	VI u51	U 51	54	-		R152Hinc, R396Hina
VI	VI u45	U 45	117	-	m31m1	
VII					mB41, m4, m8, m21, m29, m36, m43, m67, m69*	R019Sst, R137Sst
VII	VII a	VII a	80		m 4m4	R019Sstc, R 137Ssta, R 137Sstb, R 137Sstf
VII	VII 1a	VII 1a	60	146	m4m5, **m43m1**	R137Sstd, (Rust resistance gene)
VII	VII 1b	VII 1b	13	48	**m8m3**, m67m1, m69m1	
VII	VII 2	U 12	53	-		
VII	VII 3	U 14	11	-		
VII	VII 4	U 50	67	49	m8m4	
VII	VII 5	U 13	12	-		
VII	VII 8	VII 8	63	-	m21m1, m36m2, m67m8	
VII	VII 9	VII 9	-	-	m4m8, m43m3	
VII	VII 14	VII 14	-	-	m4m3	R019Sste
VII	VII 18	U 2	-	-	m21m3, m29m3	
VII	VII 20	U 40	39	129	**mB41m2**, m29m1	
VIII					mA16*, m1, m12, m17, m18, m26, m28, m38, m44, m46, m61, m65, m73*	ADS7Hin, ADS9Eco, R039Sst, R082Dra, R118Eco, R131Eco, R142Eco*, R 173Sst, R185Sst, R267Sst, R281Hin, R326Sst, R335Eco, R342Sst,
VIII						R406Sst, R488Hin, R526Eco*, R542Hin
VIII	VIII a	VIII a	108	184	**mA16m3**	R118Ecoa, R185Ssth, R281Hind, R281Hing, R326Ssta, R326Sstf
VIII	VIII b	VIII b	65	55		R185Sstb, R267Sste, R326Sstb
VIII	VIII d	VIII d	55	156	m61m2	R542Hinb
VIII	VIII u16	U 16	58	-		
VIII	VIII e	VIII e	78	71		R542Hina
VIII	VIII f	VIII f	45	-	m17m4, m46m1	
VIII	VIII g	VIII g	102	-		R039Sstf, R488Hinc
VIII	VIII u58	U 58	101	-		R488Hind
VIII	VIII 1	VIII 1	14	67	m1m2, m12m4, m18m1, m38m1	R082Draa, R082Drab, R185Ssti, R281Hinb, R326Ssti
VIII	VIII 2	VIII 2	15	5	m1m1, m12m5, m17m3, m28m6, m44m1, m46m2, m65m3	**ADS7Hin2**, R039Sstd, R118Ecoc, R131Ecoe, R173Sstc, R185Sstg, R281Hine, R326Sste, R335Ecog, R342Sstb, R406Ssta
VIII	VIII 11	VIII 11	16	56	m17m2, m26m3, m65m2	R039Ssta, R335Ecoa, R335Ecob, R342Sstc, R342Sstd, R488Hinb
VIII	VIII u49	U 49	18	-		
VIII	VIII 3	VIII 3	-	-	m12m1	R142Ecoe, R526Ecoc
VIII	VIII 4	VIII 4	43	155	m12m8, m28m2	R142Ecoc, R526Ecoa
VIII	VIII 5	VIII 5	20	1	m38m2, m73m2	**ADS9Eco2**, R118Ecob, R 173Sstf, R173Sstd, R173Ssta, R185Sstf, R267Sstc, R281Hina, R326Sstd
VIII	VIII 16	VIII 16	21	-	m61m3	R173Sste
VIII	VIII 8	U 53	17	-		
VIII	VIII 10	VIII 10	19	-	m18m3	
VIII	VIII 15	VIII 15	106	-	m38m4	R118Ecoe, R118Ecod, R173Sstb, R173Sstg, R281Hinf
-	U 1	U 1	99+110	-	m49m6	
-	U 4	U 4	69	172	m23m3	R016Drae
-	U 5	U 5	-	-		R482Ecob
-	U 15	U 15	-	-		R149Ecod
-	U 27	U 27	81	176		R012Ecoe
-	U 42	U 42	120	181	**m4m2**	
-	U 43	U 43	-	-		R031Ecoa
-	U 46	U 46	-	-		R129Ecod, R371Drab
-	U 47	U 47	100	112		R088Ecoa
-	U 55	U 55	118	-	R129Ecoc	
-	-	-	-	175	**m54m4**	
-	-	-	-	186	**m54m5**	
-	-	-	-	46	**m16m1**	
-	-	-	-	58	**m4m6**	

[1] Rossi *et al.* (2003) ; [2] Hoarau *et al.* (2001), [3] This study, [4] proposed homology groups, [5] Consensus map integrating information from [1,2,3]
* indicates probe/enzyme combinations or SSR primers producing alleles (bands) attributed to CGs belonging to different HGs
$ Resistance gene analogs mapped by Rossi et al. (2003)

(Wu et al. 1992). In order to distinguish single dose markers (1:1) from bi-parental single dose markers (3:1), we used a segregation ratio threshold of 1.73:1 since this ratio gives equal χ^2 values for both 1:1 and 3:1 hypotheses (Mather 1957). Two-point analyses between single dose markers were performed at a LOD score threshold of five and a recombination fraction threshold of 0.35 using Mapmaker 3.0 (Lander et al. 1987). Markers within each cosegregation group (CG) were then ordered by multipoint analysis using the Haldane mapping function. CGs were pooled in the same homology groups (HG) when (1) they had at least two RFLP probes or SSR in common, or when (2) they were linked in repulsion. Moreover, some R570 CGs could be assigned to HG on the basis of anchor markers (AFLP markers or SSR markers) they had in common with CGs from a previous R570 RFLP-based map (Grivet et al. 1996 and unpublished results). Chromosome pairing behaviour was investigated using the procedure described in Hoarau et al. (2001).

Results

Parental linkage maps

A total of 1,666 polymorphic markers were produced in the progeny using 37 AFLP primer pair combinations, 46 SSRs and 9 RFLP probes. Among these markers, 1,057 were single dose markers and used to build the map, with 584 (55%) specific to MQ76-53 and 473 (45%) specific to R570. Linkage analysis of all of these single dose markers resulted in 191 CGs encompassing a total of 960 markers, while 97 single dose markers remained unlinked: 424 R570-specific markers formed 86 CGs and 536 MQ76-53-specific markers formed 105 CGs. CG lengths ranged from 1.6 to 179.4 cM in MQ76-53 and from 1.1 to 158.3 cM in R570. The cumulative length of the MQ76-53 map was 4,329 cM. The cumulative length of the R570 map was 3,144 cM. The detailed maps are available at http://www.tropgenedb.cirad.fr/en/sugarcane.html (Ruiz et al. 2004). Only 17 out of the 105 MQ76-53 CGs could be assigned to the homology group framework defined for the R570 map on the basis of at least two common RFLP probes or SSR loci. Out of the 86 CGs on the R570 map, 60 CGs (70%) could be assigned to an HG. No preferential pairing between MQ76-53 CGs was detected at LOD = 3. In contrast, preferential pairing was observed between 18 pairs of CGs in R570, 14 of which had been previously detected by Hoarau et al. (2001).

Alignment of different R570 AFLP-based maps

The present R570 map was aligned with the former AFLP-based map of the same cultivar (Hoarau et al. 2001, Rossi et al. 2003). This was possible because most of the single dose AFLP markers specific to R570 in the present map (based on a bi-parental progeny derived from the R570 × MQ76-53 cross) were common to the former map (based on a selfed R570 progeny). Localisation of 32 new locus-specific markers (19 SSRs and 13 RFLPs) and detection of four new pairs of CGs in repulsion improved the assignment of the CGs of the previously published map to the homology group (HG) framework. With these new data, 72% of the CG could be assigned to a HG as compared to 52% in the R570 map of Rossi et al. (2003). When we integrated the information of all three studies, we obtained a tentative consensus map organized in seven HGs encompassing a total of 90 assigned CGs. The number of CGs per HG ranged from 2 (HG IV) to 22 (HG VI). The present mapping data also allowed reorganizing several previous published CGs (Table 2). In particular, the basal part of CG VI3 in Rossi et al. (2003) may have been erroneously attached to the upper part of this CG, assigned to HG VI. This basal part, which corresponds to CG 59 in this study, carries two locus-specific markers (m40 and R149Eco), thus suggesting that it belongs to HG III. In three cases, based on the new data, two formerly separate CGs could be pooled into a single CG, i.e. CG30 = Ib + U62 (Rossi et al. 2003); CG7 = VI3 + VI8 (Rossi et al. 2003); and CG111 = I6 + U3 (Rossi et al. 2003). In addition to the CGs presented in Table 2, the R570 genetic map encompassed 30 small CGs (average size = 21.8 cM) bearing no locus-specific markers. These CGs thus cannot yet be assigned to homology groups.

Identification and mapping of a major rust resistance gene in MQ76-53

The distribution of rust resistance scores for 166 progeny clones is presented Fig. 1. Since the broad sense heritability of rust resistance scores at the experimental design level was very high ($h^2 = 0.96$), the distribution was based on mean scores for three replications. The results showed clear segregation between resistant clones (mean score < 2) and susceptible clones (mean score ≥ 2) with a segregation ratio of between 1:1 and 1:3 (Table 3). We already know that R570 has one copy of a rust resistance gene (*Bru*1) that has been mapped, and flanked with AFLP markers (Asnaghi et al. 2004). Two of these AFLP markers, i.e. aaccac6 mapped at 1 cM on the distal side of the gene and actctg9R at 2.2 cM on the proximal side of the gene, were also mapped in the present study. This gave us the opportunity to select clones that do not carry the *Bru*1 gene among the 166 clones evaluated for rust. We thus only selected clones without the two AFLP markers flanking *Bru1*. This left us with a subpopulation of 90 clones that did not bear the *Bru*1 gene—note that we assumed that the probability of having a double recombination (one recombination on each side of *Bru*1) within the 3.2 cM defined by the flanking AFLP markers was almost nil. The distribution of the rust resistance scores of these 90 clones presented in Fig. 1 shows a clear segregation

Table 3 Observed ratios of resistant and susceptible clones in three different populations and the Chi-square test of two hypotheses: one major gene is segregating in the population (1:1 ratio is expected) or two major genes are segregating (3:1 ratio is expected)

Studied populations	Parental rust resistance status		Number of progenies			χ^2	
	Female (R/S)	Male (R/S)	Total	Resistant	Susceptible	2 doses[a]	1 dose[rm a]
Full population of clones evaluated for rust resistance	R570 (R)	MQ76-53 (R)	166	105	61	11.66*** ↔	12.22***
Subpopulation of clones without markers flanking the R570 resistance gene *Bru*1	R570 (R)	MQ76-53 (R)	90	36	54	36.75***	3.60 NS
Control cross	B63-758 (S)	MQ76–53 (R)	133	68	65	6.61***	0.07 NS

[a] $\chi^2 = 3.84$ at the 5% level

between resistant clones (mean score <2) and susceptible clones (mean score ≥ 2). The segregation ratio was skewed but not significantly different from 1:1 (Table 3). This led us to hypothesize that a second rust resistance gene inherited from MQ76-53 was present. This hypothesis was confirmed by the 1:1 segregation ratio (68 resistant:65 susceptible clones) observed within 133 progenies derived from a cross between cultivars B63-758 (susceptible to rust) and MQ76-53 (Table 3).

For mapping purposes, rust resistance, in the subpopulation of 90 clones not bearing *Bru*1, was used as a morphological marker that was ranked 1 for resistant clones and 0 for susceptible clones. This marker was localized on CG3 of the MQ76-53 map (Fig. 2), which was assigned to HGVIII on the basis of two locus-specific markers (ADS7 and m38). A Fisher's exact test performed to assess associations between rust resistance and each of the 1666 segregating markers at the conservative threshold of $P = 3\times10^{-5}$ ($P = 0.05/1666$) revealed that the only markers significantly associated to the rust resistance gene belonged to CG3, except for an unmapped bi-parental single dose marker (Table 4). This test confirmed the localisation of the rust resistance gene on CG3 and showed that the most tightly associated marker was acgcta16 (Table 4), which was located 23.1 cM from the gene (Fig. 2).

The QTL analysis revealed two strong QTLs corresponding to the two genes described above. Marker aaccac6, which was closest to *Bru1*, explained 26% of the phenotypic variance whereas marker acgcta16, which was closest to the new rust resistance gene from MQ76-53, only explained 13% of the phenotypic variance. Both markers explain 38% of the phenotypic variation after multiple regression. However, in the subset of 90 clones not carrying *Bru1*, marker acgcta16 explained 35% of the phenotypic variance. Both genes probably have an effect of the same magnitude, but the closest marker associated with the new rust resistance gene detected in MQ76-53 was still located far from the gene. Moreover, as already observed by Daugrois et al. (1996) for *Bru1*, an important rust susceptibility level variation still exist in the susceptibility progeny class (Fig. 1) that can not be explained by the segregation of the two resistance genes.

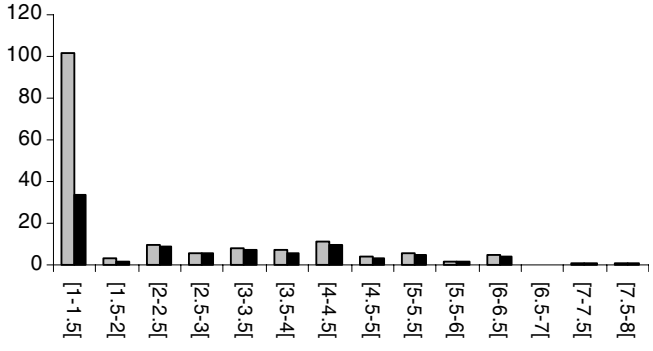

Fig. 1 Distribution of mean rust resistance scores. In *gray* (166 unselected clones of the progeny) and in *black* (90 clones of the progeny selected because they did not have any AFLP markers flanking the R570 rust resistance gene)

Identification and mapping of a major gene controlling stalk colour in MQ76-53

Stalk colour of 198 progeny clones was scored in three different environments (see Materials and methods). The

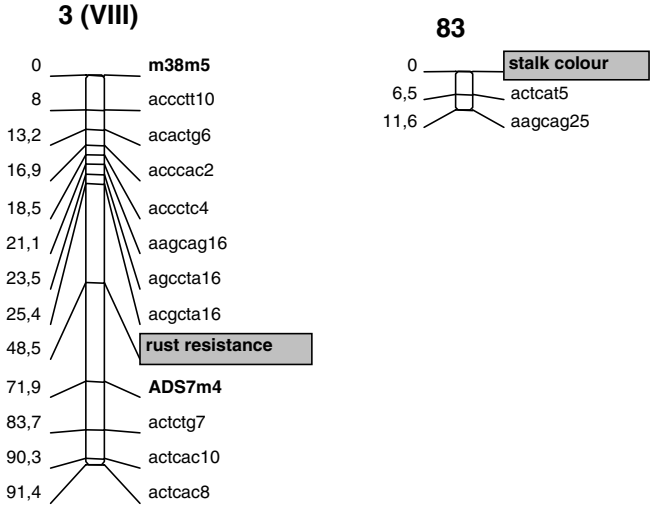

Fig. 2 Representation of cosegregation groups of the MQ76-53 genetic map bearing the putative new rust resistance gene (CG 3, HG VIII) and the putative gene controlling stalk colour (CG 83, unassigned to an HG). The detailed genetic map of MQ76-53 is available at http://www.tropgenedb.cirad.fr/en/sugarcane.html

Table 4 Significant associations between markers and rust resistance according to a Fisher exact test at $P < 3\times10^{-5}$, proportion of phenotypic variance (R^2) explained by these markers in the subset of 90 clones not carrying *Bru1*

Marker[a]	$P < F$	HG	CG	Position	R^2 (%)
actctg7	1.08×10^{-05}	VIII	3	83.7	29
ADS7_r4[a]	NS[a]	VIII	3	71.9[a]	37[a]
Putative gene		**VIII**	**3**	**48.5**	–
acgcta16	6.98×10^{-09}	VIII	3	25.4	35
agccta16	5.37×10^{-08}	VIII	3	23.5	29
aagcag16	3.77×10^{-07}	VIII	3	21.1	24
accctc4	3.77×10^{-07}	VIII	3	18.5	24
acccac2	2.36×10^{-06}	VIII	3	16.9	22
acactg6	8.30×10^{-06}	VIII	3	13.2	18
accctt10	1.47×10^{-05}	VIII	3	8	25
actctt26	1.73×10-05	Unlinked	–	–	7

[a]A lot of missing data for this RFLP marker

three evaluations were highly consistent, as indicated by the Fisher's exact test probabilities [$P = 1.15\times10^{-34}$ between environments (1) and (2); $P = 1.6\ 10^{-34}$ between (2) and (3); $P = 1.27\ 10^{-39}$ between (1) and (3)]. The evaluation was more difficult in environment (2) since the leaves had not been stripped off and since sunlight could not readily penetrate through the closed canopy in this field. Eighteen clones were not classified because of difficulties in deciding to which of the two categories they belonged, e.g. some clones appeared to be slightly red in some environments and not red in others, and some clones presented a peculiar striped colour pattern. These few ambiguous clones were not considered in the further analysis. Finally, a clear segregation was observed between 91 red stalk clones and 89 non-red stalk clones (this subpopulation of clones had green or yellow stalks). The resulting observed segregation ratio was not significantly different from 1:1 ($\chi^2 = 0.02$), suggesting that red stalk colour is under the control of a major gene inherited from MQ76-53. Stalk colour was therefore used as a morphological marker that, for mapping purposes, was ranked 1 for red stalk clones and 0 for non-red stalk clones. The major gene was localized on cosegregation group 83 on the MQ76-53 map, closely linked to an AFLP marker (actcat5) at 6.5 cM (see Fig. 2).

Discussion

The cumulative lengths of the genetic maps developed from our bi-parental population were 3,144 and 4,329 cM for R570 and MQ76-53, respectively. For R570, the map coverage (3,144 cM) was less than that of the map built from selfed R570 progeny (5,849 cM in Hoarau et al. 2001), although the number of AFLP primer combinations used was nearly the same. As expected, the same genotyping effort yielded less informative markers for mapping a given parent when comparing a bi-parental population to a selfed population. This is because it is not possible to use markers common to both parents for mapping. In our study, 168 bi-parental single dose markers common to R570 and MQ76-53 (identical alleles or homoplasic bands) could not be used for mapping, whereas most of them had been mapped in the selfed R570 progeny. These non-mappable, bi-parental single dose markers represented at least 26% of the single dose markers in the R570 genome in this study. Moreover, this percentage marker loss is underestimated since it does not take into account single dose R570 markers not coded due their presence in multiple doses in MQ76-53.

MQ76-53 is an F1 hybrid between an old cultivar and a wild *S. spontaneum* clone (SES528) that is not found in the recorded pedigree of any international cultivars (Machado 2001). Therefore this clone is presumably quite genetically distant from R570 and from other present-day cultivars. This was confirmed by an AFLP-based diversity study that included R570, MQ76-53 and 72 modern cultivars from various breeding programs. This study revealed that 40 to 65% of the single dose AFLP bands mapped in R570 are common with the other cultivars (unpublished data). These figures provide an interesting estimation of the extent of informative (= single dose) marker loss that could occur in a bi-parental mapping project. In the present study, we loosed 40% of single dose markers that were common to R570 and MQ76-53. However, in a cross between two standard modern cultivars this proportion should be higher. Despite the lower yield of mappable markers, a bi-parental mapping population seems more suitable than a selfed population when QTL detection is the ultimate objective, especially if the two parents have highly contrasted phenotypes for the trait of interest. Indeed, in bi-parental progeny: (1) the two populations to be compared (the one with the marker to be tested and the one without it) are of much more balanced size (1:1) than in a selfed progeny context (3:1); (2) the buffering effect of the background of alternative alleles should be less strong since half of this background is inherited from the "contrasted parent". QTL detection power is therefore likely to be greater in a bi-parental context, with other parameters being equal (population size, type 1 error, etc.), and should help to more accurately detect the most favourable alleles.

After alignment of the different R570 AFLP-based maps, cosegregation groups of R570 were grouped into 7 HGs containing between 2 (HG IV) and 22 (HG VI) CGs. Structural differences between *S. spontaneum* and *S. officinarum* are expected since they have different basic numbers, i.e. $x = 8$ and $x = 10$, respectively (D'Hont et al. 1998). These differences may result from simple fusion or fission events, as suggested for HG VIII in which two sets of homologous *S. officinarum* chromosomes are assigned to the same HG (VIII) because of their homology to the same *S. spontaneum* CG (Grivet et al. 1996; D'Hont et al. 1996, this study). Considering the basic chromosome number of *S. spontaneum*, we would have expected 8 HGs instead of the seven found. Tentative explanations could be proposed: (1) genome

coverage with locus-specific markers is still insufficient for identifying all HGs (some unassigned CGs may belong to missing HGs); (2) duplication of some SSR and RFLP locus within the basic chromosome set may lead to misassembly of sets of homologous CGs into the same HG (Butterfield et al. 2001); (3) complex structural differences between the basic chromosome sets of *S. officinarum* and *S. spontaneum* may also lead to misassembly of sets of homologous or homeologous CGs. Mapping additional locus-specific markers (SSRs and RFLPs) should help to resolve this question.

No linkage in repulsion was detected between CGs of MQ76-53, while preferential pairing was observed for 18 CG pairs in R570. This contrasting situation may in part be explained by the differences in genome constitution between these two clones. The MQ76-53 clone is the result of a cross between a cultivar (Trojan) and a *S. spontaneum* clone (SES528) with $2n = 64$ chromosomes (Panje and Babu 1960). The *S. spontaneum* component of the MQ76-53 genome is therefore composed of the *S. spontaneum* chromosome inherited from SES528 plus the *S. spontaneum* chromosomes inherited from Trojan. By comparison, R570 is composed of only one or two *S. spontaneum* chromosomes per homologous class. In MQ76-53 no preferential pairing could be detected within *S. spontaneum* at LOD = 3. This picture is in agreement with the lack of preferential pairing noted in *S. spontaneum* SES208, which suggests that there is polysomic inheritance and autopolyploidy in this species (Al Janabi et al. 1993; Ming et al. 1998). However, the absence of preferential pairing among MQ76-53 *S. officinarum* chromosomes is out of line with the incomplete polysomy observed in *S. officinarum* (Mudge et al. 1996; Guimaraes et al. 1997). This could be due to a dramatic deficiency in the coverage of the *S. officinarum* component of the MQ76-53 genome which would make it almost invisible. Chromosome pairing behaviour is complex in R570. Preferential pairing involves CGs of *S. officinarum* origin, CGs of *S. spontaneum* origin, as well as *S. spontaneum* × *S.officinarum* recombinant CGs (Hoarau et al. 2001; Grivet et al. 1996). In-depth analysis of all pairing frequencies between homologous and homeologous chromosomes of a single homology group (HGI) in R570 revealed pairing affinities between chromosomes ranging from 0 to 40%. These chromosome affinities are only partly explained by the species origin of the chromosomes (Jannoo et al. 2004).

A Mendelian factor governing stalk colour has been identified in the MQ76-53 genome. This gene is linked to two AFLP markers in a cosegregation group (CG 83). Although cane colour varies considerably according to age of the stalk and to the amount of sunlight received (Stevenson 1965), a rather clear 1:1 segregation ratio between red stalk cane and non-red stalk cane was noted. This finding may not be relevant from an agronomic standpoint but is another indication that even in the complex polyploid background of sugarcane it is possible to identify traits controlled by a single gene, and that genetic mapping in sugarcane should be continued to identify new major genes. In addition, a new rust resistance gene has been located in the MQ76-53 genome. This gene clearly differs from the R570 rust resistance gene *Bru*1 (Asnaghi et al. 2004). Indeed, none of the AFLP markers flanking *Bru1* in R570 is present in MQ76-53, indicating that this new resistance gene belongs to a different haplotype. Moreover, this gene is located on CG3, which should belong to HG VIII, whereas *Bru*1 is located on a R570 CG that belongs to HG VII. Rossi et al. (2003) identified three resistance gene analog clusters, including RGA of the NBS-LRR and S/T kinase types, in HG VIII. These RGAs could be used as candidate genes and mapped in our population to determine whether they are located in the vicinity of this new rust resistance gene. This approach could help to more rapidly pinpoint the exact location of the gene (Pflieger et al. 2001). In addition, it would be interesting to trace the origin of this resistance gene. It could represent a yet untapped alternative source of rust resistance if it turns out to have been inherited from the *S. spontaneum* SES528 clone.

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References

Aitken KS, Jackson PA, McIntyre CL (2005) A combination of AFLP and SSR markers provides extensive map coverage and identification of homo(eo)logous linkage groups in a sugarcane cultivar. Theor Appl Genet 110:789–801

Al-Janabi SM, Honeycutt RJ, McClelland M, Sobral BWS (1993) A genetic linkage map of *Saccharum spontaneum* L 'SES 208'. Genetics 134:1249–1260

Asnaghi C, Roques D, Ruffel S, Kaye C, Hoarau JY, Telismart H, Girard JC, Raboin LM, Risterucci AM, Grivet L, D'Hont A (2004) Targeted mapping of a sugarcane rust resistance gene (*Bru*1) using bulked segregant analysis and AFLP markers. Theor Appl Genet 108:759–764

Bhat SR, Gill BS (1985) The implication of 2*n* egg gametes in nobilisation and breeding of sugarcane. Euphytica 34:377–384

Burner DM, Legendre BL (1993) Chromosome transmission and meiotic stability of sugarcane (Saccharum spp) hybrid derivatives. Crop Sci 33:600–606

Burner DM, Legendre BL (1994) Cytogenetic and fertility characteristics of elite sugarcane clones. Sugar Cane 1:6–10

Butterfield MK, D'Hont A, Berding N (2001) The sugarcane genome: a synthesis of current understanding, and lessons for breeding and biotechnology. Proc S Afr Sug Technol Ass 75:1–5

Da Silva JAG, Burnquist WL, Tanksley SD (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum*. Genome 36:782–791

Da Silva J, Honeycutt RJ, Burnquist W, Al-Janabi SM, Sorrells ME, Tanksley SD, Sobral BWS (1995) *Saccharum spontaneum* L 'SES 208'genetic linkage map combining RFLP- and PCR-based markers. Mol Breed 1:165–179

Daugrois JH, Grivet L, Roques D, Hoarau JY, Lombard H, Glaszmann JC, D'Hont A (1996) A putative major gene for rust resistance linked with an RFLP marker in sugarcane cultivar R570. Theor Appl Genet 92:1059–1064

D'Hont A (2005) Unravelling the genome structure of polyploids using FISH and GISH; examples of sugarcane and banana. Cytogenet Genome Res 109(1–3):27–33

D'Hont A, Glaszmann JC (2001) Sugarcane genome analysis with molecular markers, a first decade of research. Proc Int Soc Sugarcane Technol 24: 556–559

D'Hont A, Lu YH, Gonzàlez de Leòn D, Grivet L, Feldmann P, Lanaud C, Glaszmann JC (1994) A molecular approach to unraveling the genetics of sugarcane, a complex polyploid of the *Andropogoneae* tribe. Genome 37:222–230

D'Hont A, Grivet L, Feldmann P, Rao S, Berding N, Glaszmann JC (1996) Characterisation of the double genome structure of modern sugarcane cultivars (*Saccharum* spp) by molecular cytogenetics. Mol Gen Genet 250:405–413

D'Hont A, Ison D, Alix K, Roux C, Glaszmann JC (1998) Determination of basic chromosome numbers in the genus Saccharum by physical mapping of ribosomal RNA genes. Genome 41:221–225

D'Hont A, Garsmeur O, Raboin LM, Paulet F, Begum D, Wing R, Glaszmann JC (2001) Chromosome walking toward a major resistance gene for common rust of sugarcane. Proc Int Soc Sugarcane Technol 24: 315–317

Grivet L, Arruda P (2001) Sugarcane genomics: depicting the complex genome of an important tropical crop. Curr Opin Plant Biol 5:122–127

Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann JC (1996) RFLP mapping in a highly polyploid and aneuploid interspecific hybrid. Genetics 142:987–1000

Guimaraes CT, Sills GR, Sobral BWS (1997) Comparative mapping of Andropogoneae: *Saccharum* L (sugarcane) and its relation to sorghum and maize. Proc Natl Acad Sci USA 94:14261–14266

Heinze BS, Thokoane LN, Williams NJ, Barnes JM, Rutherford RS (2001) The smut–sugarcane interaction as a model system for the integration of marker discovery and gene isolation. Proc S Afr Sug Technol Ass 75:88–93

Hoarau JY, Offmann B, D'Hont A, Risterucci AM, Roques D, Glaszmann JC, Grivet L (2001) Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp) I Genome mapping with AFLP markers. Theor Appl Genet 103:84–97

Hoarau JY, Grivet L, Offmann B, Raboin LM, Diorflar JP, Payet J, Hellmann M, D'Hont A, Glaszmann JC (2002) Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp) II Detection of QTLs for yield components. Theor Appl Genet 105:1027–1037

Hoisington D (1992) Laboratory protocols. CIMMYT applied molecular genetics laboratory, Mexico

Jannoo N, Grivet L, D'Hont A, Glaszmann JC (2004) Differential chromosome pairing affinities at meiosis in polyploid sugarcane revealed by molecular markers. Heredity 93:460–467

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

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Machado GR (2001) Sugarcane variety notes—an international directory seventh revision, Piracicaba, Int. Soc. Sugar Cane Techn. Februray 2001, 132p

Mather K (1957) The measurement of linkage in heredity. Methuen, London

Mehta CR, Patel NR (1983) A network algorithm for performing Fisher's exact test in rxc contingency tables. J Am Statist Assoc 78:427–434

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

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

Ming R, Del Monte TA, Hernandez E, Moore PH, Irvine JE, Paterson AH (2002a) Comparative analysis of QTLs affecting plant height and flowering among closely-related diploid and polyploid genomes. Genome 45:794–803

Ming R, Liu SC, Bowers JE, Moore PH, Irvine JE, Paterson AH (2002b) Construction of a Saccharum consensus genetic map from two interspecific crosses. Crop Sci 42:570–583

Mudge J, Andersen WR, Kehrer RL, Fairbanks DJ (1996) A RAPD genetic map of *Saccharum officinarum*. Crop Sci 36: 1362–1363

Panje RR, Babu CN (1960) Studies in *Saccharum spontaneum* distribution and geographical association of chromosome numbers. Cytologia 25:152–172

Pflieger S, Lefebvre V, Causse M (2001) The candidate gene approach in plant genetics: a review. Mol Breed 7:275–291

Pinto LR, Oliveira KM, Ulian EC, Garcia AA, de Souza AP (2004) Survey in the sugarcane expressed sequence tag database (SUCEST) for simple sequence repeats. Genome 47(5):795–804

Price S (1963) Cytogenetics of modern sugar canes. Econ Bot 17:97–105

Rossi M, Araujo PG, Paulet F, Garsmeur O, Dias VM, Chen H, Van Sluys MA, D'hont A (2003) Genomic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. Mol Gen Genet 269:406–419

Ruiz M, Rouard M, Raboin LM, Lartaud M, Lagoda P, Courtois B (2004) Tropgene-DB, a multitropical crop information system. Nucleic Acids Res 32:D364-D367

SAS Institute (1990)SAS procedures guide, ver 6, 3rd edn. SAS Institute Inc, Cary

Stevenson GC (1965) Genetics and breeding of Sugar cane. Longmans, London, pp 284

Tai PYP, Miller JD, Dean JL (1981) Inheritance of resistance to rust in the sugarcane field. Crops Res 4:261–268

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wu KK, Burnquist W, Sorrels ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor Appl Genet 83:294–300