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Identification of a major quantitative trait locus (QTL) for yellow spot (*Mycovellosiella koepkei*) disease resistance in sugarcane

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Abstract In this study we used amplified fragment length polymorphism (AFLP) and microsatellite (short sequence repeat or SSR) markers to identify a major quantitative trail locus (QTL) for yellow spot (*Mycovellosiella koepkei*) disease resistance in sugarcane. A bi-parental cross between a resistant variety, M 134/75, and a susceptible parent, R 570, generated a segregating population of 227 individuals. These clones were evaluated for yellow spot infection in replicated field trials in two locations across two consecutive years. A χ^2-test (χ^2 at 98% confidence level) of the observed segregation pattern for yellow spot infection indicated a putative monogenic dominant inheritance for the trait with a 3 (resistant):1(susceptible) ratio. The AFLP and SSR markers identified 666 polymorphisms as being present in the resistant parent and absent in the susceptible one. A genetic map of M 134/75 was constructed using 557 single-dose polymorphisms, resulting in 95 linkage groups containing at least two markers based on linkages in coupling. QTL analysis using QTLCARTOGRAPHER v1.17d and MAPMAKER/QTL v1.1 identified a single major QTL located on LG87, flanked by an AFLP marker, actctc10, and an SSR marker, CIR12284. This major QTL, which was found to be linked at 14 cM to an AFLP marker, was detected with LOD 8.7, had an additive effect of –10.05% and explained 23.8% of the phenotypic variation of yellow spot resistance.

Keywords Linkage mapping · Molecular markers · *Mycovellosiella koepkei* · Polyploidy · Quantitative resistance · Sugarcane

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Introduction

Modern cultivated sugarcane is the result of the hybridization between mainly *Saccharum officinarum* species and the wild relative, namely *Saccharum spontaneum*. The *Saccharum* complex has long been accepted as alloploids because of their overlapping geographic ranges and promiscuity (Brandes et al. 1939; Daniels and Roach 1987). In fact, the entire *Saccharinae* subtribe, to which *Saccharum* belongs, forms a closely-knit interbreeding group according to one modern view (Clayton and Renvoize 1986). Other than chromosome counts, the genomic composition of the *Saccharum* complex has been largely

speculative, and its proposed taxonomic complexity has largely defied a detailed systematic and evolutionary understanding of the genera within the complex. Variation in chromosome number is also common within the complex, thereby further complicating our knowledge on the origin and relationship of the species within the *Saccharinae*. Modern sugarcane varieties have approximately 100–140 chromosomes, comprising 8–18 copies of a basic $x = 8$ or $x = 10$ (D'Hont et al. 1995, 1998; Ha et al. 1999; Irvine 1999). Most chromosomes of cultivated sugarcane appear to be largely derived from *S. officinarum* (Irvine 1999), with a 10% contribution from *S. spontaneum* (D'Hont et al. 1996). The high numbers of chromosome duplications and autogamous chromosome pairing in sugarcane preclude genetic mapping based on co-dominant ''alleles''. Unlike many other crop species in which diploid relatives are clearly defined, no known diploid progenitor exists for sugarcane, and even postulated polyploid ''progenitors'' have unknown origins and ploidy numbers, thus rendering the understanding of phylogenetic relationships difficult.

The lack of morphological markers showing disomic inheritance in *Saccharum* is one of the reasons underlying the incomplete understanding of its genetics. However, the existence of DNA-based markers has provided sugarcane geneticists with the means necessary for unraveling the genetic complexity of such polyploids and allowed many hypotheses to be tested experimentally (Wu et al. 1992; Aljanabi et al. 1993, 1994; da Silva et al. 1995; Ming et al. 1998, 2002; Hoarau et al. 2001). DNA markers showing simplex (single-dose) segregation (Wu et al. 1992), which represent 70% of the detectable polymorphic loci in sugarcane, have been utilized to construct genetic linkage maps of at least five sugarcane populations (Aljanabi et al. 1993; da Silva et al. 1993, 1995; Mudge et al. 1996; Ming et al. 1998; Hoarau et al. 2001). The number of loci in these maps ranges from 160 random amplified polymorphic DNA (RAPD; Mudge et al. 1996) to 887 amplified fragment length polymorphism (AFLP) loci (Hoarau et al. 2001).

Genetic tools for sugarcane have only recently become adequate to quantify the effect of many genomic regions on a trait. Only a few earlier studies on sugarcane genetics have reported the association of DNA markers with agronomic traits in sugarcane (Sills et al. 1995; Daugrois et al. 1996; Guimarães et al. 1997; Ming et al. 2001; Hoarau et al. 2002).

Yellow spot disease of sugarcane is caused by the imperfect fungus *Mycovellosiella koepkei* (Kruger) Deighton. It has a high incidence in the wet uplands of Mauritius. Under high relative humidity, the disease is severe and causes low juice purity, a high reducing sugar/sucrose ratio and sucrose losses at early harvest (Ricaud 1974). At late harvest, cane yield may also be affected (Ricaud 1974; Autrey et al. 1983). Little information on the mode of inheritance of resistance to yellow spot is presently available since it is relatively unimportant in most sugarcane-producing countries except in some parts of Australia, Barbados, Guyana, India, Trinidad and Indonesia where the environmental conditions favor infection in susceptible varieties (Autrey et al. 1983). Ramdoyal et al. (1996) reported that crosses between resistant and slightly susceptible parents produced relatively high frequencies of resistant clones and, to a lesser extent, crosses between two susceptible parents produced a number of resistant clones, indicating that resistance to yellow spot is inherited in a dominant manner. They also suggested that a few genes might be involved in the inheritance of resistance to yellow spot disease with dominance for resistance.

The selection of resistant varieties based on disease reaction in the field can be time-consuming, and the strong influence of environmental factors on the reaction can actually mask the genetic potential of a plant (Moore and Irvine 1991). The complexity of sugarcane genetics and the lack of a Mendelian inheritance of traits introduce other obstacles in identifying a truly resistant plant. The fundamental complexity of autopolyploid genetics resulting from heterozygosity and the lack of preferential pairing is further complicated by the fact that disease resistance, in our case yellow spot of sugarcane, is a complex trait. The trait is influenced not only by the genetic background of the plant but also by environmental factors, such as variation in temperature, relative humidity and the fungal inoculum (Ramdoyal et al. 1996).

In the investigation reported here, our primary objectives were to construct a genetic linkage map of sugarcane variety M 134/75 and to determine the number and location of quantitative trait loci (QTLs) for resistance to yellow spot in sugarcane.

Materials and methods

Plant material and disease scoring

A population of 227 individuals derived from a bi-parental cross between a yellow spot-resistant female parent (M 134/75) and a susceptible male one (R 570) was planted in 1999 at Union Park Sugar Experimental Station (UPSES), in Mauritius, in order to provide sufficient planting material for replicated trials. Two experiments were then established in 2000, one in mid-July and the other in mid-September, at two locations, UPSES and Britannia, where the environmental conditions are conducive for the development of yellow spot disease. Three 3-eye (three internodes with buds) cuttings of each progeny were planted in 1 m-long rows in three replications in a randomized complete block design. The two parental clones, M 134/75 and R 570, as well as four control varieties (M 596/56-resistant, M 377/56-highly susceptible, B 3337-highly susceptible and S 17-highly susceptible) were also included in the trials. In order to increase natural fungal inoculum in the trial, the two highly susceptible control varieties, B 3337 and S 17, were planted in alternate rows between the progeny lines, and the whole experimental plot was surrounded by three rows of variety B 3337. Yellow spot susceptibility was evaluated in the plant cane crop in 2001 and in first-ratoon crop in 2002. Four disease categories, resistant (R), slightly susceptible (SS), susceptible (S), highly susceptible (HS), were assigned, as described by Ricaud (1970). Each progeny, parents and control varieties were rated twice in each year at the time of peak infection in April and towards the end of the peak infection in May.

DNA extraction, primer screening and marker analysis

Total genomic DNA was extracted from the meristematic tissue using the method described by Aljanabi et al. (1999). AFLP analysis was performed according to the method described by Vos et al. (1995). Sixty-four AFLP oligonucleotide combinations (eight *Eco*RI and eight *Mse*I) were initially tested on DNA extracted from parental clones M 134/75 and R 570 using both the fluorescent and radioactive labeling techniques. Sugarcane microsatellite (simple sequence repeat; SSR) primers were obtained through the International Consortium for Sugarcane Biotechnology (ICSB, http://www.icsb.org). Two sugarcane microsatellite libraries were developed at the Centre for Plant Conservation Genetics, Southern Cross University, Australia (Cordeiro et al. 2001) and at the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France (D'Hont, personal communication). SSR analysis was performed according to Cordeiro et al. (2001). A total of 260 SSR primers were first screened against the mapping parents.

Map construction

The names of AFLP and microsatellite markers were limited to a maximum of eight characters. Each AFLP marker was assigned as follows: six letters corresponding to two sets of the three selective nucleotides (*Eco*RI and *Mse*I) followed by one or two numbers representing the order of the bands from the top of an AFLP autoradiograph downwards. The original names of the microsatellite markers developed at Southern Cross University consisted of the letters Sugarcane Microsatellite Consortium (SMC), followed by the sequence number and a two- to three-letter abbreviation of the institution to which the marker belongs. These were modified and limited to eight characters. SMC was replaced with M, the sequence number was kept the same and the abbreviation letters were replaced with one letter followed by the size of the allele in base pairs. The primers developed at CIRAD originally consisted of the prefix mSSCIR followed by a primer number. These were modified as CIR followed first by the sequence number (1–77) and then by the size of the allele in base pairs. Thirty-five AFLP primer combinations and 65 SSR

primers were used to construct a genetic linkage map of variety M 134/75. Polymorphic markers were scored as present in M 134/75, absent in R 570 and segregating in the mapping progeny. Each marker was tested against the expected ratios for single-dose (SD) or simplex (1:1), double-dose (DD) or duplex (7:2), and triple-dose (TD) or triplex segregation (11:1) (da Silva et al. 1993; da Silva and Sorrells 1996). A χ^2 threshold of 2% was used to keep both type-I and type II errors below 5% (Wu et al. 1992). Only single-dose markers (SDMs) were used to construct the genetic linkage map of M 134/57. The linkage relationships of SDMs (Wu et al. 1992) were determined using MAPMAKER/EXP v. 3.0b for PC (Lander et al. 1987). Two-point analysis was performed at a minimum LOD score of 5.0 and a maximum distance of 35 cM. The Kosambi mapping function was used to convert the percentage of recombination into map units. Groups with linked markers at this stage were referred to as linkage groups (LGs). The order of the markers in each LG was determined using the ''order'' command of the MAPMAKER algorithm. The chromosome graphs were obtained with WINQTLCARTOGRAPHER v.2.0 (Wang et al. 2003). Homologous groups (HGs) were assembled on the basis of SSR markers common between LGs.

Chromosome pairing

The type of chromosome pairing was investigated by comparing the proportion of markers in coupling phase versus repulsion phase in all the linkage groups by testing all pairwise linkages in repulsions between markers. This was achieved by copying the segregation data matrix at the end of the original set, inverting the scores for each of the polymorphisms (called recoding in the MAPMAKER v. 3.0b manual), running MAPMAKER again (LOD = 5, $\theta = 0.35$, two-point analysis) on the combined data set and finally looking for linkages between the first set and the newly created second set (Aljanabi et al. 1993). Markers in repulsion phase should appear on the same linkage group when this is done. Chromosome assortment was also investigated by comparing the number of SDMs to the number of non-SDMs (da Silva et al. 1993).

Data analysis

Single-factor ANOVA (SAS Institute 1990) was used to determine the association between markers and resistance to yellow spot in sugarcane. All SD and multiple-dose (duplex, triplex and multiplex) markers were used for the analysis. The coefficient of determination, R^2, was calculated for each marker or QTL as the percentage of variation in disease resistance explained by each marker. The analysis was based on individuals that had and those that did not have the marker. The total phenotypic variance explained was estimated by including all significant single- and multiple-dose QTLs in a full model for multiple regression analysis. The allele effect of each SD QTL was the average difference in phenotype of individuals differing by one copy of the indicated allele (SD versus zero-dose). When flanking markers were available, QTLCARTOGRAPHER v1.17d for Windows (Basten et al. 2001) and MAPMAKERQTL v1.1 were used to calculate LOD scores by composite interval mapping (CIM) and simple interval mapping, respectively. A set of 1000 permutations was performed using Zmapqtl of QTLCARTOGRAPHER in order to determine the experimentwise significance level of LOD = 3.3 for $\alpha = 0.05$. CIM increases the precision of the QTL location and the R^2 evaluation, and allows more than one QTL to be mapped on the same chromosome (Zeng 1994).

Results

Disease field evaluation and data analysis for yellow spot infection level in plant cane and first-ratoon crops in two locations and over a period of 2 years allowed four different assessments for yellow spot resistance. These data permitted evaluation of environmental (years and locations) and physiological (plant cane and first ratoon) factors affecting the trait. The test of normality (Shapiro and Wilk 1965) indicated that field data for yellow spot resistance did not follow a normal distribution. The distribution of disease infection rate over the 2-year period deviated from a normal distribution. The distribution of clones was ''L shaped'' with a high

number of resistant clones on the left tail and a small minority of susceptible clones dispersed along the right tail (Fig. 1). According to this distribution, the segregating progeny could be assigned into two groups: one homogeneous group comprised of resistant clones and one heterogeneous group with clones having a wide range of susceptibility. The segregation pattern observed for disease resistance, calculated with the data of the first observation year, the second observation year and the average data of both years, indicated a monogenic inheritance of the target trait. Despite the fact that yellow spot resistance might look like a quantitative trait, the segregation data tested by χ^2 fitted an expected 3:1 ratio for monogenic dominant inheritance (χ^2 at the 98% confidence level). To confirm this segregation ratio in each environment as well, χ^2 values were calculated for each of the four different environments. The number of observed clones showing resistance or susceptibility was 164(R):63(S) ($\chi^2 = 1.03$) for year 1/location 1 (Y1L1), 176:50 ($\chi^2 = 1.05$) for year 1/location 2 (Y1L2), 161:66 ($\chi^2 = 2.54$) for year 2/location 1 (Y2L1) and 175:51 ($\chi^2 = 0.74$) for year 2/location 2 (Y2L2). The calculated χ^2 values confirmed the 3:1 segregation ratio. There was a high significant correlation ($P = 0.0001$) in infection level measured in both trials (locations) in plant cane and first-ratoon crops (Table 1). Heritability (h^2) estimation, based on the estimates of the genetic variance component σ^2g, that was calculated on plot and entry bases was 0.87 and 0.94, respectively. The analysis of variance of the phenotypic data of the 227 clones showed that the genotypic variance was significant ($P = 0.02$), environment variance was significant ($P = 0.0001$) and genotype × environment interaction (G × E) variance was not significant ($P < 0.73$).

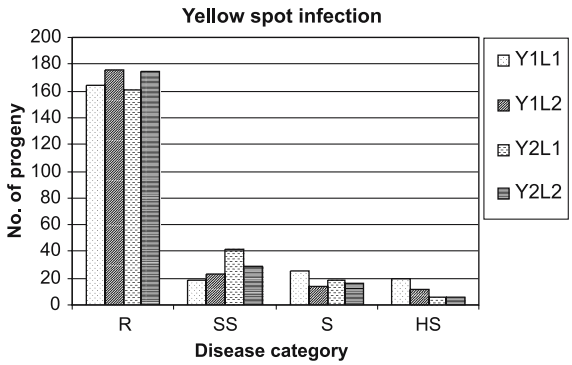

Fig. 1 Distribution of yellow spot infection in the field in the population of M 134/75 × R 570 in 2001 and 2002 at Union Park Sugar Experimental Station and Britannia. Four infection categories were assigned based on infection expressed on the ten uppermost leaves on a sample of one stalk per stool. *R* Resistant, *SS* slightly susceptible), *S* susceptible, *HS* highly susceptible

Linkage mapping

Of the 64 AFLP primer combinations (eight *Eco*RI and eight *Mse*I) evaluated on parental DNAs, 58 gave reliable and reproducible amplification products that could be scored using the ABI-310 Gene Scan software (Applied Biosystems, Foster City, Calif.) or directly from autoradiographs. A total of 666 AFLP and SSR polymorphisms were generated and then scored as present in M 134/75 alone and absent in R 570 using either fluorescent or radiolabeled primers. Thirty-five AFLP primer combinations screened against the 227 progeny generated 500 polymorphisms, and 65 SSR primers screened against these same progeny added an additional 166 polymorphic alleles. The 666 polymorphisms were used to calculate SDMs, which were then utilized to construct a genetic linkage map of cultivar M 134/75, DD and TD markers. Of the 666 polymorphic markers, 557 were SDMs segregating 1:1, 79 were DD and 30 were TD. Duplex and multiplex markers were not considered for the map construction but were used for QTL statistical analysis. Due to the high number

Table 1 Correlation coefficient values calculated between markers score and average yellow spot infection rate scored in two locations (L1, Union Park Sugar Experimental Station; L2, Britannia) and 2 years (Y1, 2001; Y2, 2002) ($P = 0.0001$)

QTL experiment	Correlations			
	Y1L1	Y1L2	Y2L1	Y2L2
Y1L1	–	0.87	0.88	0.88
Y1L2	0.87	–	0.73	0.75
Y2L1	0.88	0.73	–	0.89
Y2L2	0.88	0.75	0.89	–

of markers, a stringent LOD score of 5.0 was used to map SDMs in order to avoid false linkage when building LGs. Of the 500 AFLP markers, 423 (84.6%) were SDMs, 354 (69%) were assigned to LGs and 69 (13.8%) were unlinked, whereas of 166 SSR markers, 134 (80.7%) were SDMs, 120 (72.3%) were assigned to LGs and 14 (8.4%) remained unlinked (Fig. 2). The 474 SDMs (354 AFLP and 120 SSRs) were assigned to 95 LGs, each containing at least two linked markers, with a total of 83 markers remained unlinked (Fig. 2). Markers were not uniformly distributed across LGs. The number of SDMs per linkage group varied from 2 to 18. The length of these LGs ranged from 2.8 to 262.4 cM. The total map coverage represented by the cumulative length of all LGs was 6200 cM, with an average distance of 11.1 cM between two markers. Six LGs were built entirely with SSR markers, while 41 LGs were exclusively built with AFLP markers; the remaining 48 LGs contained mixed markers. The distribution of markers tested by χ^2 goodness of fit showed significant deviation from random distribution for both types of markers.

Chromosomes preferential pairing

Thirteen pairs of the 95 LGs had markers showing evidence for preferential pairing, including three pairs in HG II, two pairs in HG IV, one pair in HG VI and seven pairs not yet linked to HGs. SDMs linked in repulsion phase with a sample of 227 individuals strongly suggested probable complete disomic behavior for these pairs of chromosomes and that the M 134/75 genome is incompletely polysomic (Aljanabi et al. 1994;

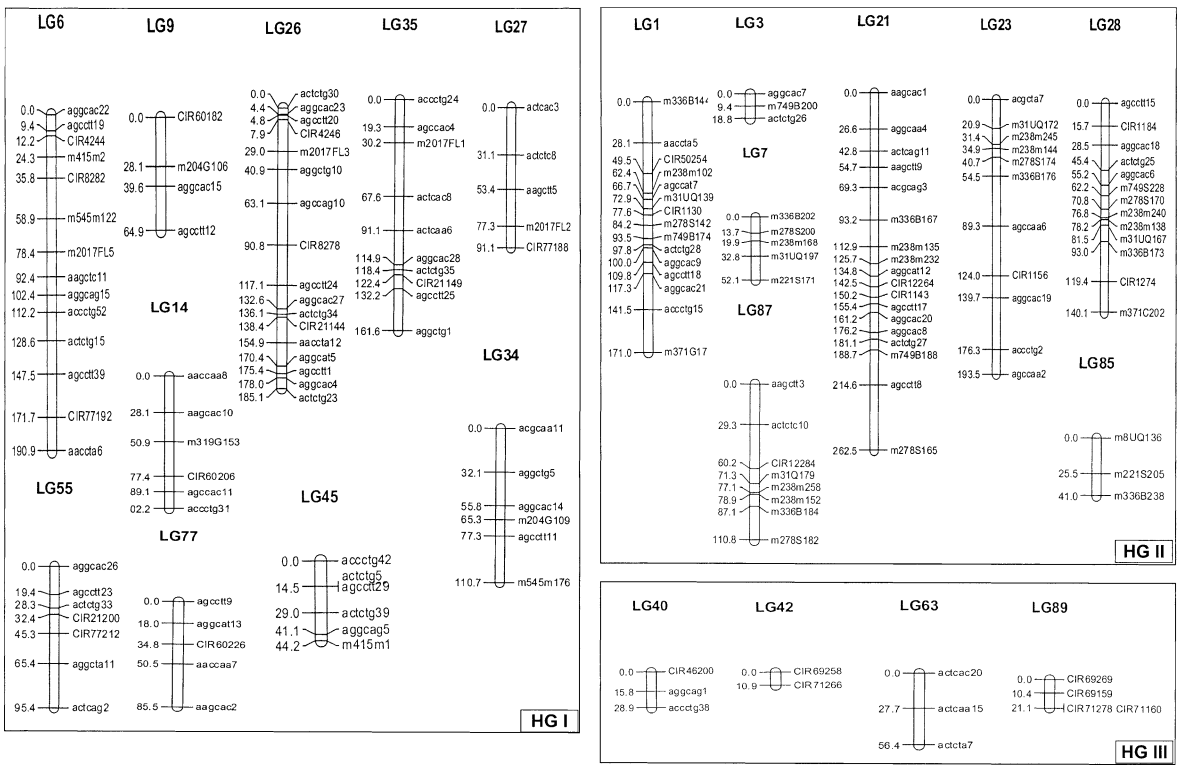

Fig. 2 A genetic linkage map of variety M 134/75 obtained with LOD = 5.0 and a maximum distance of 35 cM. *Numbers to the left* of the linkage groups represent accumulative genetic distance in CentiMorgans (Kosambi function); the names of the markers are on the *right*. The original names of the SSR markers were modified. For SSRs from Southern Cross University, SMC was replaced by *M*, the sequence number was unchanged, the abbreviation letters for the institutions replaced by *one letter* followed by the size of the allele in base pairs. For CIRAD, the prefix mSSCIR was changed to *CIR*, followed by the primer number (*1–77*) and the size of the allele in base pairs. Ninety-five LGs were defined by at least two linked markers. Homologous groups (HGs) were placed in a *box* identified by Roman numbers *I–XI*

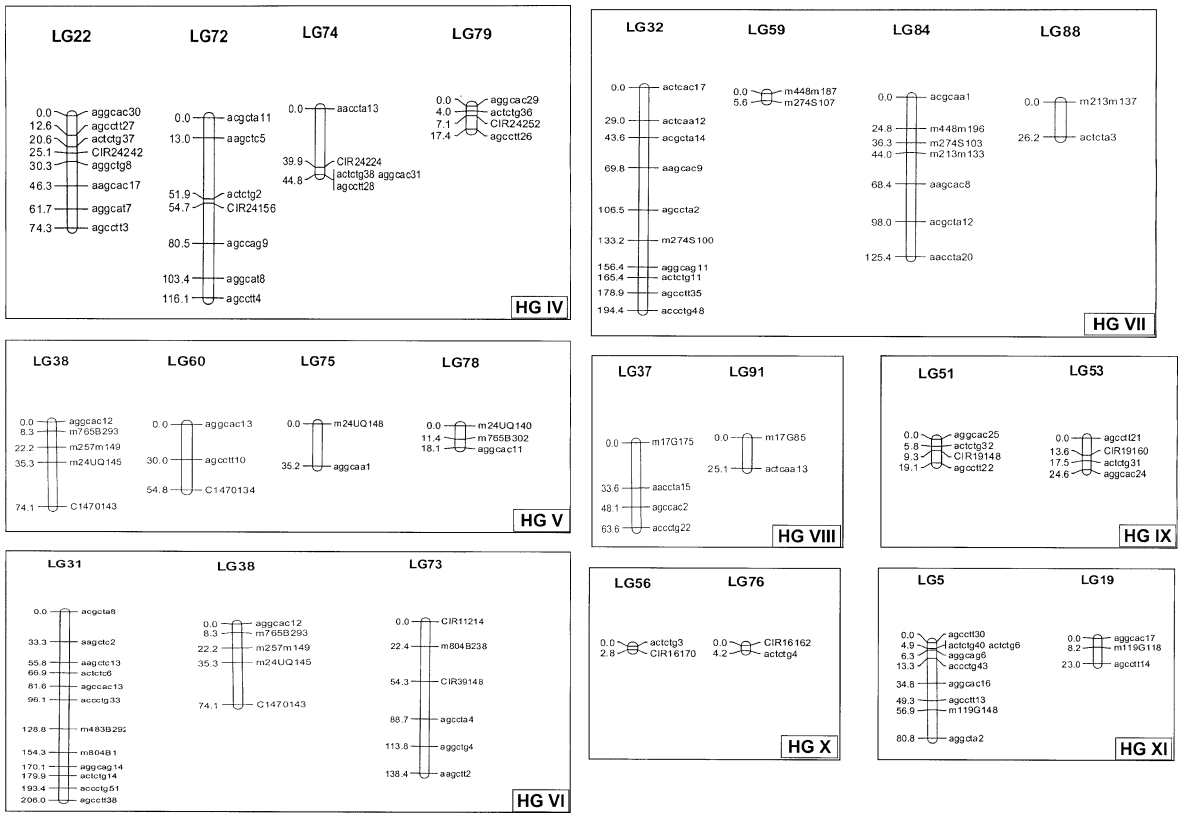

Fig. 2 continued

da Silva et al. 1995). A high LOD score of 5.0 for linkages in repulsion was applied in order to avoid false positives, indicating a strong linkage between markers in the LGs and evidence for preferential pairing.

QTL analysis

The set of 1000 permutations determined the experimentwise significance level of LOD = 3.3 for $\alpha = 0.05$. The most important QTL identified in this study using single-marker ANOVA, QTLCARTOGRAPHER and MAPMAKERQTL was the one located on HG II (LG 87), position 43 cM, between the AFLP marker actctc10 and the SSR marker CIR12284. This probable major QTL was linked at 14 cM to the AFLP marker actctc10. It was detected at a high LOD of 8.7, with an additive effect of –10.05% and a R^2 value of 23.8%, indicating that this allele reduced susceptibility to yellow spot by about 10% and explained 23.8% of the phenotypic variation (Table 2). The resistant parental clone M 134/75 contributed to this genetic effect. The LOD significance threshold of 3.3 used to infer the presence of QTLs in the large sugarcane genome assured that the likelihood of even a single false positive in the population remained below 1%.

Single-marker ANOVA confirmed the association between markers and yellow spot resistance or susceptibility for the QTL on LG 87 and for the minor QTL on LG 4, respectively (Table 2). Three other markers on LG 87 (mCIR12284, m238258 and m238152) showed significant association ($P > 0.0001$) with the yellow spot resistance. In contrast to other QTLs, the AFLP marker agcac5, linked at 4 cM from a QTL controlling susceptibility to yellow spot, was detected with an LOD of 3.3 (Table 2). As the region on LG 4 is associated with susceptibility it is likely that there is a corresponding region on R 570 associated with resistance.

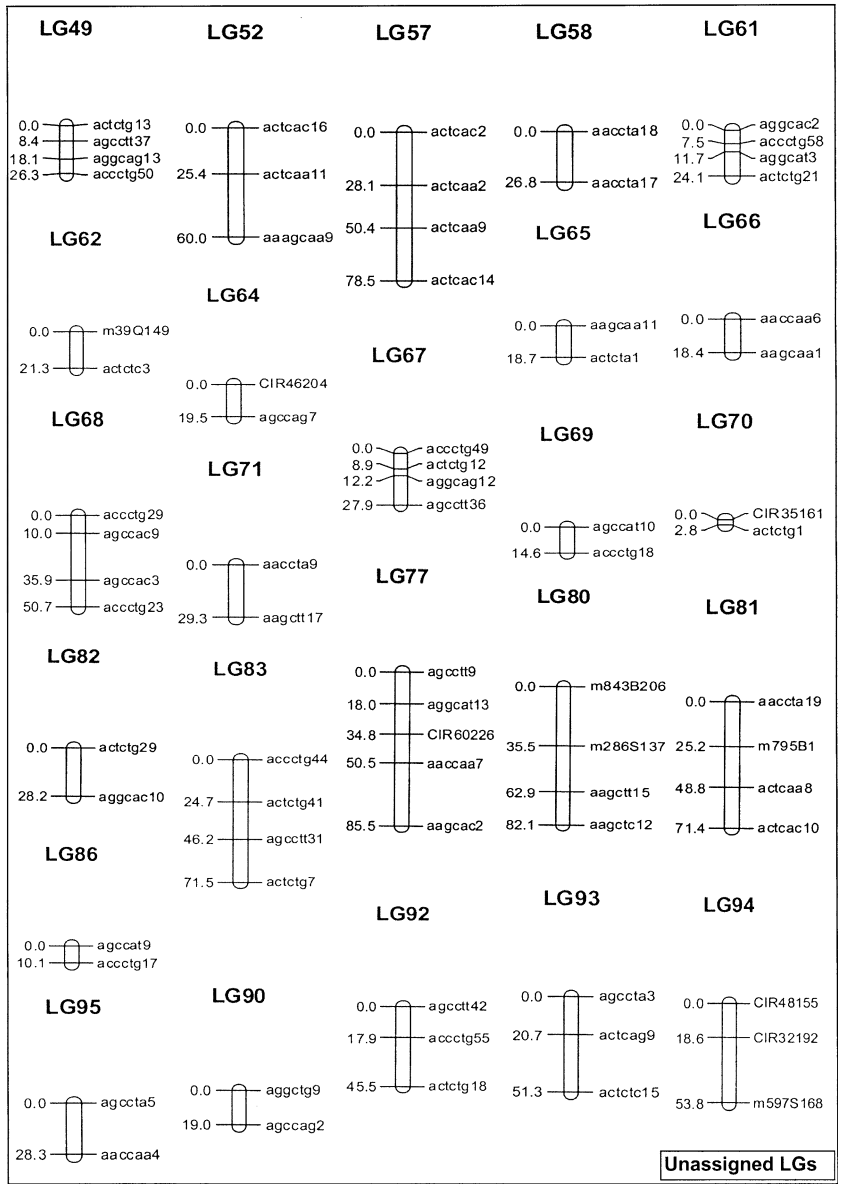

Fig. 2 continued (unassigned linkage groups)

To determine whether the QTL effect was consistent across years, trait value for the first year, second year and the average of the 2 years was analyzed with MAPMAKERQTL using the SCAN command. The QTL effect was significant in both years and detected with a high LOD value of 8.5 and 7.1 in year 1 and year 2, respectively. Furthermore, despite the fact that no significant variation was observed between replications, QTL analysis was performed using the trait value for each replication in each environment, the average of three replications in each location and year; the effect of the QTL on LG 87 was found to be significant across all environments (data not shown).

Discussion

Although QTL detection in autopolyploids is complicated by the possibility of the segregation of more alleles at a locus and by a lack of prefer-

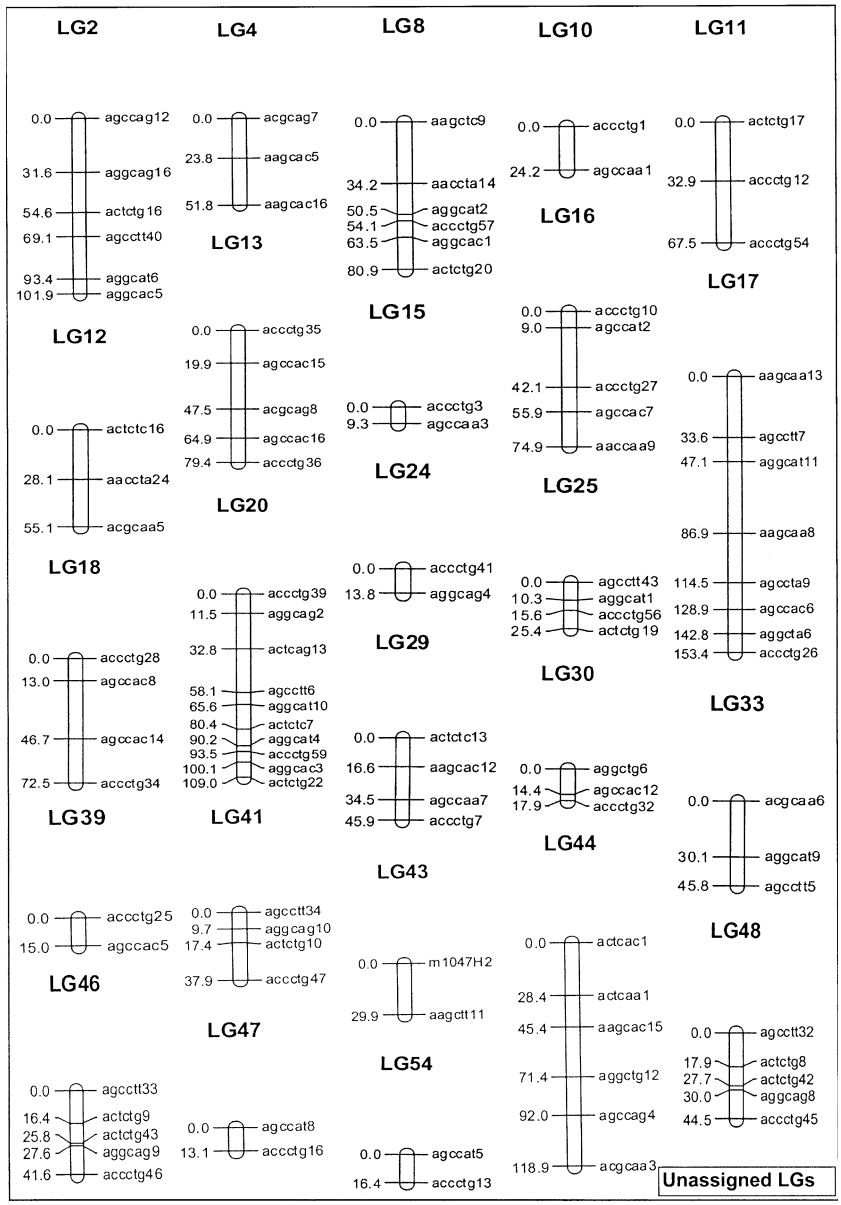

Fig. 2 continued (unassigned linkage groups)

ential pairing, a subset of polymorphic alleles that show simplex segregation ratios can be used to locate QTLs. While plant disease resistance is generally a polygenic characteristic, it is, however, governed by fewer genes compared to other traits such as yield. If resistance is inherited in a monogenic or an oligogenic manner, resistant and susceptible phenotypes occur in genetically segregating populations, and these phenotypes can be placed in discrete categories that can be fit to Mendelian ratios. The results of this study indicate that the QTL associated with resistance to yellow spot is a major genetic factor. This finding is consistent with the assumption based on the segregation data obtained on field resistance in the 227 individual plants in the two locations and across the 2-year experimental period. The detection of significant associations between markers in the same genomic region of sugarcane cultivar M 134/75 provided independent confirmation of the importance of this genomic region in the control of yellow spot resistance. In addition, based on

Table 2 Summary of QTLs associated with yellow spot resistance in M 134/75 × R 570 progeny using three different methods of analysis: composite interval mapping (CIM) using QTLCARTOGRAPHER; MAPMAKERQTL; single-marker ANOVA based on the trait value for year 1 (2001), year 2 (2002) and the average of 2 years (*LG* linkage group)

LGs	Position (cM)	Marker loci	Year 1			Year 2			Average		
			LOD	R^{2a}	a^b	LOD	R^2	a	LOD	R^2	a
CIM analysis											
LG87	43.3	actctc10-CIR12284	6.4	15.4	–6.8	6.1	14.9	–6.3	6.6	15.9	–7.1
MAPMAKERQTL											
LG4	4.0	aagcac5-agcac16	3.3	5.8	5.7	3.2	6.5	4.2	3.5	4.5	5.0
LG87	14	actctc10-CIR12284	8.5	23.7	–8.4	7.1	20.0	–8.9	8.7	23.8	–10.05
Single-marker ANOVA											
LGs	Marker	$b0^c$	$b1^d$	LOD	pr(F)	R^2 Y1	R^2 Y2	R^2 (average)			
LG4	aagcac5	9.5	–4.7	3.5	0.0001	7.3	6.6	7.7			
LG87	actctc10	4.6	6.1	6.5	0	18.6	17.3	17.6			
LG87	CIR12284	5.0	5.4	5.0	0	13.1	13.4	13.5			
LG87	m238m258	4.9	5.0	4.3	0	8.2	8.2	8.6			
LG87	m238m152	5.0	4.8	4.0	0	7.9	7.7	8.1			

[a] Coefficient of determination presented as percentage of explained variance

[b] Additive effect

[c,d] The value represented by the simple linear regression model $y = b0+b1x+e$

phenotypic segregation data and QTL analysis, this genomic region probably contains a putative major gene that controls resistance to yellow spot. Despite the significant association between three additional SSR markers – determined using single-factor ANOVA – and resistance to yellow spot on the LG 87, only marker actctc10 was significant by the CIM method on this linkage group. The CIM method allows more than one QTL to be mapped on the same chromosome (Zeng 1994) if they are relatively far apart. However, as all four significant markers fell within a distance of 80 cM on LG 87, the CIM peak area detecting a possible QTL narrowed and pinpointed one marker, which is actctc10. In this regard CIM is more reliable and accurate than single-marker ANOVA. MAPMAKERQTL is comparable to CIM in the sense that even though the peak area of detecting QTLs is wider than the CIM method (wider confidence level), the precise position of a QTL can be based on the highest LOD value. Daugrois et al. (1996) reported the first monogenic inheritance for rust disease (brown rust) in sugarcane. This gene was found linked at 10 cM with an restriction fragment length polymorphism (RFLP) marker. Other minor factors involved in brown rust resistance were also detected.

Hyne and Kearsey (1995) reported the presence of more than one QTL on one linkage group. However, it is more difficult to detect two QTLs that have the same effects on a linkage group. This is due to the fact that unless they are of unequal size or very far apart, they will appear as a single QTL located somewhere in the middle of the two or more actual QTLs (Hyne and Kearsey 1995). A survey study using sequence analysis of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA of several isolates of the fungal pathogen, *Mycovellosiella koepkei*, showed no difference in DNA sequences among the isolates, thereby suggesting the presence of a single strain of the pathogen in Mauritius (MSIRI 2002). Therefore, the detection of one major and a few minor effect QTLs might support the gene-for-gene concept of the presence of one resistant allele for each strain of the pathogen (Flor 1971). Resistance to bacterial speck disease in tomato occurs when the Pto kinase in the plant responds to the expression of the avirulence gene *avrPto* in the *Pseudomonas* pathogen. Transient expression of an *avrPto* transgene in plant cells containing *Pto* elicited a defense response. In the yeast two-hybrid system, the Pto kinase physically interacted with *AvrPto*.

Alterations of *AvrPto* or *Pto* that disrupted the interaction in yeast also abolished disease resistance in plants. The physical interaction of *AvrPto* and *Pto* provides an explanation of gene-for-gene specificity in bacterial speck disease resistance (Tang et al. 1996).

Single dominant genes usually mediate plant resistance response. These genes, however, are often members of multigene families, frequently organized in clusters (Graham et al. 2000; Michelmore and Meyers 1998). A large EST (expressed sequence tags) database is available consisting of 261,609 ESTs resulting from the Brazilian Sugarcane EST Sequencing project (SUCEST: http://www.sucest.lad.ic.unicamp.br/en). Eighty eight resistance gene analogs (RGAs) have been identified in this database (Rossi et al. 2003), 55 of which were mapped on the R 570 AFLP map (Hoarau et al. 2001). One of these clusters of two leucine-rich repeat (LLR)-like loci mapped close to the common rust major gene. Therefore, another explanation of the presence of four markers in the same genomic region that is significantly associated with yellow spot resistance could be due to the presence of such a cluster on LG 87. However, a comparison of the M 134/75 genetic map with that of R 570 and based on marker CIR12284 being linked to the yellow spot QTL (our map) and the corresponding marker mSSCIR12 on the R 570 map indicates that the yellow spot gene corresponds to HG VIII on the R 570 map and that this HG contains several mapped RGAs (Rossi et al. 2003). In order to confirm this hypothesis, RGAs need to be mapped on the M 134/75 map in order to understand the inheritance and genetic control of yellow spot resistance in this genomic region. The challenge is to identify the functional gene within this cluster.

The highly significant correlations between phenotypic data measured in both trials (locations) on plant cane and first-ratoon crops suggest that the trait value is stable across environmental and/or physiological factors. The significant correlation value is a possible indication that the trait value was not significantly different between environments, despite the contrasting environmental conditions, therefore suggesting a possible genetic control of the trait. G × E is another important component affecting trait development, especially quantitative traits. QTLs detected in one environment but not in another environment may indicate significant QTL × environment interaction (Veldboom and Lee 1996). If G × E interaction effects do exist for quantitative traits, an analysis of data from a single environment is not appropriate for an unbiased estimation of genetic parameters. Furthermore, in the absence of QTL × environment interaction, a QTL could be detected in one environment but not in another environment because the chance of simultaneous QTL detection in both environments is small (Jansen et al. 1995). Despite the non-significant effect of the G × E interaction and trait value between replications in each trial, which can justify using mean values for QTL analysis – as presented in Table 2 – QTL detection was performed on each replication in each trial and on the four different environments to determine whether this QTL behaves consistently across different environments. In this analysis, the detection of QTL across environments was consistent between environments indicating the possibility of dominant genetic control of the trait. Although in many instances G × E interaction has been detected by classical quantitative genetic analysis, other experimental results have suggested very little environment-by-QTL interaction (Tanksley 1993). In maize, Stuber et al. (1992) determined the chromosomal location of QTLs contributing to grain yield by testing a segregating population in six diverse environments, and found little or no G × E interaction. Similarly, QTL mapping for three agronomic traits in maize was consistent over four environments (Schon et al. 1994).

Consistent heavy disease pressure is required to accurately assess the potential of plant genotypes to resist the progress of infection and to determine the magnitude of the genetic factor that contributes to the resistance. Because the development of yellow spot disease is sensitive to prevailing environmental conditions, artificial inoculation cannot induce consistent disease reactions. The field plots used in this study had all of the elements favored by the pathogen, including high relative humidity, heavy morning dews and the availability of highly susceptible varieties within and around the trials. The heavy

and uniform disease pressure maintained in the field plots, combined with replicated disease evaluation experiments, made it possible to accurately assess the level of yellow spot resistance in the segregating population. The segregation ratio of progeny for the trait in this study is in agreement with the segregation data of Ramdoyal et al. (1996) who estimated that 72% of the progeny produced from crossing resistant and susceptible sugarcane varieties were yellow spot-resistant. Their conclusion was based on the types of segregation that occurred in crosses involving resistant-by-resistant and susceptible-by-susceptible plants of nine different families.

In the study we used Roman numbers from I to XI to denote the HGs. This is not the same as the nomenclature used by Hoarau et al. (2001) for HGs because in the present study we did not use any of the RFLP probes used in other sugarcane maps, thus hindering comparison. In addition, the additional SSR markers recently added to the R 570 map (Rossi et al. 2003) were those developed at CIRAD, the majority of which were not mapped on cultivar M 134/75. Consequently, it was not possible to relate HGs to the R 570 map.

Most modern commercial cultivars (i.e. R 570) are interspecific hybrids with $2n = 100–160$ and are frequently aneuploid (Burner and Legendre 1993). In such interspecific hybrids, both chromosome pairing and assortment may occur, as we found in this study. Both chromosome pairing and segregation are important to QTL detection methods. The segregating progeny of M 134/75 × R 570 may frequently display regular pairing and disomic segregation for some LGs, while other LGs may display irregular pairing and polysomic segregation. In addition, such progeny may segregate for some of the most important agronomic traits of cultivated sugarcane, such as disease resistance, sucrose and fiber content as well as for traits that differentiate *S. officinarum* and *S. spontaneum*. In this study analysis of linkages in repulsion revealed 13 cases of preferential pairing of LGs at meiosis. The high LOD scores of 5.0 applied to detect repulsion phase linkages indicate strong linkages and at the same time eliminate the possibility of artifacts. These results demonstrate incomplete polysomy in cultivar M 134/75, a typical behavior of allopolyploids. Polysomic inheritance in this cultivar could not be excluded for 82 LGs for which no repulsion phase linkages were detected between any pairwise markers, even with a low LOD of 3.0.

Knowledge of the number and the likely position of loci can provide the information required to select optimal combinations of alleles by the use of marker-assisted selection (MAS). This may be of particular relevance where linkage exists between an undesirable and desirable trait in coupling. However, for any trait, there is usually a requirement to confirm the position of the QTL before MAS becomes a viable proposition. In many cases it may be preferable to identify the likely underlying candidate gene(s) in order to establish the extent of allelic variation within the crop contributing to the trait variation. The identified major QTL in this study will be very useful for further analysis of the genetic bases of partial resistance to yellow spot in sugarcane because would appear that this QTL is controlled by a major genetic factor. Future inclusion of ESTs representing RGAs in our map would be extremely useful for the precise mapping of gene(s) controlling yellow spot resistance and in applying EST-RGA in MAS.

The results of this study show the possibility of identifying markers linked to QTLs of important disease resistance traits in sugarcane in only one generation. This will facilitate the application of MAS in sugarcane improvement early in the breeding program, and it may have an impact on the efficiency of the program, especially in reducing the number of selection cycles by directing clones to zones where yellow spot has a low prevalence.

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