Euphytica (2006) 149: 227–235
DOI: 10.1007/s10681-005-9070-7 © Springer 2006

Mapping QTLs for Witches' Broom (*Crinipellis perniciosa*) Resistance in cacao (*Theobroma cacao* L.)

Fábio Gelape Faleiro[1,2], Vagner Tibaldi Queiroz[3], Uilson Vanderlei Lopes[1,*], Cláudia Teixeira Guimarães[3], José Luis Pires[1], Milton Macoto Yamada[1], Ioná Santos Araújo[4], Messias Gonzaga Pereira[4], Raymond Schnell[5], Gonçalo Apolinário de Souza Filho[4], Cláudia Fortes Ferreira[1], Everaldo Gonçalves Barros[3] & Maurílio Alves Moreira[3]

[1]*Centro de Pesquisas do Cacau, CEPLAC, Itabuna, BA, Brazil;* [2]*Currently Centro de Pesquisa Agropecuária dos Cerrados, Embrapa, Planaltina, DF, Brazil;* [3]*Instituto de Biotecnologia Aplicada à Agropecuária, Universidade Federal de Viçosa, Viçosa, MG, Brazil;* [4]*Departamento de Ciências Agrárias, Universidade Estadual Norte Fluminense, Campos, RJ;* [5]*USDA/ARS, Miami, USA *(author for correspondence: e-mail: uilson@cepec.gov.br)*

Received 8 April 2004; accepted 6 December 2005

Key words: molecular markers, linkage map, disease resistance

Summary

Molecular markers (RAPD, AFLP and microsatellites) were used to generate a linkage map and to identify QTLs associated to witches' broom (*Crinipellis perniciosa*) resistance in cacao (*Theobroma cacao*), using 82 individuals of an F_2 population derived from the clones ICS-1 (susceptible) and Scavina-6 (resistant). Fifteen evaluations of the number of brooms have been carried out in six years (1997–2002). In order to increase the precision and accuracy in the measures of resistance, each F_2 plant was cloned in three replications in a randomized block design with single-tree plots and evaluated over 2 years. Three hundred and forty-two markers were obtained, being 33 microsatellites, 77 AFLPs and 232 RAPDs. The distribution of the number of brooms in the F_2 population was skewed to resistance, suggesting the involvement of major genes controlling resistance and the repeatability estimated for resistance was 44%. A strong putative QTL was detected as being related to witches' broom resistance. Associated to this QTL, the microsatellite mTcCIR35 explained 35.5% of the phenotypic variation in resistance. This marker is being used for marker-assisted selection in Scavina-6 progenies, including those selected in private plantations, as an auxiliary tool to the phenotypic selection.

Introduction

Cacao, *Theobroma cacao* L., is a perennial crop cultivated mainly in holdings of small farms in the tropics, under native shade trees, and is the major ingredient in the chocolate manufacture. Cacao cultivation in Brazil is of significant importance to the socio-economic standing of more than 3 million people depending directly or indirectly on this crop, generating an economy of 1.5 billion US dollars per year (ABC et al., 2001).

Since its introduction in Bahia, in 1989 (Pereira et al., 1989), witches' broom disease (causal agent *Crinipellis perniciosa* (Stahel) Singer) devastated this region economically, ecologically and socially. Losses were as high as 100% and due to the continued presence of the disease many farms were abandoned. Eradication was attempted but multi-foci and rapid spread of witches' broom deemed this measure ineffective. Within a disease management strategy many methods of control were implemented, including fungicides, biological agents (*Trichoderma stromaticum*), sanitation pruning and the use of resistant plants. Natural resistance has been preferred by most farmers because, compared to other methods, it does not involve recurrent use of costly inputs. A similar story of ruined cocoa

industries was registered in all cacao producer countries where witches' broom was introduced, including Ecuador and Trinidad, then important world cocoa producers.

The perennial nature of cacao and the time frame in which accurate evaluation of resistance can be undertaken, limits the pace in which generation of new resistant varieties can be made accessible. Further, preventive breeding to benefit countries where the disease does not occur is limited due to the difficulties in transferring germplasms and the absence of screening methods not involving germplasms transfer. Molecular marker can be an important tool in overcoming these problems.

The construction of linkage maps allows the association of markers to agronomically important traits and then marker assisted selection (Tanksley et al., 1989) speeding up the process of breeding. In the specific case of disease resistance, this would allow for the pyramiding of different genes, even when the addition of new genes cannot be phenotypically perceived. The pyramiding of genes tends to increase the durability of resistance, essential in perennial crops like cacao, which can be commercially exploited for more than 30 years (sometimes 100 years), subject to many cycles of disease infection and selection.

Many linkage maps have been developed for cacao aiming to associate molecular markers to agronomic traits (Lanaud et al., 1995, 1997; Crouzillat et al., 1996, 2000a, 2000b; Risterucci et al., 2000; Clement et al., 2001; Motilal et al., 2001; Queiroz et al., 2003). However, except that by Queiroz et al. (2003), none of them involved resistance to witches' broom. Queiroz et al. (2003), for the same population used in this paper, published a preliminary linkage map involving 193 markers (124 RAPD and 69 AFLP) and only two years of disease data. They identified two marker loci associated to the QTL of resistance to witches' broom (AV14.940 and acgcat.78). These two marker loci explained 34.7% and 25.5% of the variation in resistance, respectively. That population continued being phenotypically and molecularly evaluated and the individuals were cloned to get more accuracy in the phenotypic measurements.

This study aimed to add more markers to the preliminary map published by Queiroz et al. (2003) and to map new (or to confirm old) QTLs for witches' broom in cacao, now using much better phenotypic and molecular data than those previously used.

Materials and methods

Plant material

The mapping population consisted of 82 F_2 cacao trees, obtained by selfing the F_1 clone TSH-516, originated from the cross between the clones ICS-1 and Scavina-6. ICS-1 was selected in Trinidad and belongs to the Trinitario group, has large seeds, red pods and susceptibility to witches' broom and other diseases. Scavina-6 was selected in Peru and belongs to the Upper-Amazon group, has small seeds, green pods and is the most widely used source of resistance to witches' broom in the world. Because the F_1 (TSH-516) is self-incompatible, before selfing it, its pollen was mixed with *Herrania* spp pollen to overcome the self-incompatibility. The pollination was made protecting the female flowers 24 hours before the pollination and unprotecting 24 hours after, in order to avoid pollen contamination. The F_2 seedlings were transferred to the field in 1992.

Evaluation for witches' broom resistance

From 1998 to 2002, the number of vegetative brooms (NVV) was counted in the canopy of each of the F_2 trees, summing 15 evaluations. After counting, the brooms were removed avoiding repeated recording. In order to increase the precision and accuracy in the measures of resistance, each F_2 plant was cloned in three replicates in a randomized block design with single-tree plots and evaluated over 2 years.

Besides the number of brooms, the trunk diameter at 50 cm height was also evaluated aiming to correct NVV by the number of potential infection points, which is associated to the vigor of the tree. In many populations we have observed that the number of brooms is positively correlated with the trunk diameter, even within clones. In order to overcome the effect of the canopy size (or potential number of infection points) on the number of brooms, this number was adjusted considering the slope of the regression of the number of brooms on the trunk diameter as:

$$\mathrm{NVVC} = \mathrm{NVV} + \mathrm{b}(\overline{\mathrm{DT}} - \mathrm{DT})$$

Where, NVVC is the number of vegetative brooms corrected by the diameter of the tree; NVV is the uncorrected number of brooms; b is the angular coefficient of the regression of NVV on DT; $\overline{DT}$ is the average diameter in the F_2 and DT is the trunk diameter of tree.

The repeatability was estimated aiming to evaluate the confidence on the phenotypic data, using the yearly average of brooms per tree after correction for the diameter, as:

$$R = \sigma_G^2/(\sigma_G^2 + \sigma_w^2)$$

Where, R is the repeatability, σ_G^2 is the variance component between F_2 trees and σ_w^2 is the variance component among measurements, within trees.

DNA extraction

Leaves of each F_2 plant, the parents (ICS-1 and Scavina-6) and the F_1 (clone TSH-516) were collected and stored at −80°C until the DNA extraction. Genomic DNA was extracted using the CTAB method (Doyle & Doyle, 1990) with some modifications (Faleiro et al., 2002). After the extraction, the concentration of DNA was estimated by spectrophotometry at 260 nm (Sambrook et al., 1989). Bands of total genomic DNA separated by electrophoresis on agarose gel at 0.8% were used as an indication of the integrity and purity of the DNA extracted. After quantification, those samples with good quality DNA were diluted for 10 ng/μL.

RAPD markers

Besides the 148 RAPD markers generated by Queiroz et al. (2003), new RAPD markers were obtained using amplification reactions in a total volume of 25 μl, having Tris-HCl 10 mM (pH 8.3), KCl 50 mM, $MgCl_2$ 2 mM, 100 μM of each of the dNTPs, 0.4 μM of a primer (Operon Technologies Inc., Alameda, CA, EUA), one unit of *Taq* polymerase and approximately 30 ng of DNA. The amplifications were made in a thermocycler programmed for 40 cycles, each with the following sequence: 15 seconds at 94°C, 30 seconds at 35°C and 90 seconds at 72°C. After the 40 cycles, a stage of extension for 7 minutes to 72°C was done and, finally, the temperature was reduced to 4°C. After the amplification, 3 μl of a mixture of bromophenol blue (0.25%), glycerol (60%) and water (39.75%) were added to each sample. The samples were then applied in agarose gel (1.2%), which was submerged in TBE buffer (Tris-borate 90 mM, EDTA 1 mM). The electrophoresis lasted approximately four hours, at 90 volts. At the end of the running, the gels were stained with ethidium bromide and photographed under ultraviolet light.

Microsatellite markers

Two methods were used to obtain microsatellite markers. In method 1, used in CEPEC's laboratory (Brazil), the fragments were separated in 3% agarose gel and were stained with ethidium bromide. While in method 2, used in USDA/Miami (USA), the fragments were separated in 6% polyacrylamide gel and the markers were detected by fluorescence.

Method 1: The amplification reaction was done in a total volume of 15 μL, having Tris-HCl 10 mM (pH 8.3), KCl 50 mM, $MgCl_2$ 2.4 mM, 150 μM of each of the dNTPs, 3 pM of each of the forward and backward primers, one unit of *Taq* polymerase and, approximately, 30 ng of DNA.

The amplifications were done in a thermocycler programmed with the following sequence: 4 minutes at 94°C +10 cycles (30 seconds at 94°C + 60 seconds at 60°C −1°C at each cycle +90 seconds at 72°C) +30 cycles (30 seconds at 94°C + 60 seconds at 48°C +90 seconds at 72°C) +6 minutes at 72°C. After the amplification, the temperature of the samples was reduced to 4°C, and 3 μl of a mixture of bromophenol blue (0.25%), glycerol (60%) and water (39.75%) were added to each sample. The samples were then applied to a 3% agarose gel, submerged in TBE buffer (Tris-borate 90 mM, EDTA 1 mM). The electrophoresis lasted approximately four hours, at 90 volts. At the end of the running, the gels were stained with ethidium bromide and photographed under ultraviolet light. Standards were used to estimate the weight of the markers.

Method 2: The amplification reactions were done in a final volume of 15 μL, using the same components, concentrations and amplification program as for method 1. However, after the amplification, 2 μL of the reactions were used for the electrophoresis in 6% polyacrylamide gel, with the marker detection based on primers marked with fluorescent dye. For the detection of the markers a DNA ABI 377 automatic DNA sequencer was used with the software GeneScan. Using the GENESCAN 500 marker, the weight of each marker, in base pairs, was estimated.

AFLP markers

The same 77 markers generated by Queiroz et al. (2003) were used in this study. The methods used are detailed by those authors.

Linkage map

The linkage map was constructed with markers that segregated according to a monogenic inheritance in F_2, i.e., 3:1 for dominant markers (RAPD and AFLP) and 1:2:1 for codominant markers (microsatellites). Chi-square tests were used to confirm the monogenic inheritance of the markers, considering a significance level of 5%.

The recombination frequencies, the distance between markers and the position of them in the linkage group were determined using Joinmap (Van Ooijen & Voorrips 2001). A LOD score of 4.0 and a maximum of 40% of recombination were used. The recombination frequency was converted to distance by Kosambi's mapping function (Kosambi, 1944).

QTL identification and mapping

The identification of marker loci associated to QTLs of resistance to witches' broom was based on an analysis of variance for the effect of individual markers on the number of brooms (NVVC). The proportion of the phenotypic variance explained by individual and sets of markers was estimated by simple and multiple regressions (stepwise, with $p \leq 0.01$), using SAS and *Jump* 3.1.6.2 (SAS Institute Inc., Cary, NC). This was in part done to allow comparisons with previous studies (Queiroz et al., 2003).

The position of the QTLs in the linkage groups was determined based on the map information, using the composite interval method (Jansen, 1993; Zeng, 1993, 1994) via QTL Cartographer (Basten et al., 1994, 1999). The threshold value for mapping the QTLs was determined by the expression $\mathrm{LOD} = \chi 2_{(n)\alpha}/2\ln(10)$, with $n = 2$ degrees of freedom and $\alpha = 0.01$, resulting a $\mathrm{LOD} = 2.0$. Two markers were used as cofactors in composite interval mapping.

Results and discussion

Witches' broom resistance

Most F_2 individuals presented less than one broom/tree/evaluation (Figure 1), being similar in resistance to the resistant parent, Scavina-6. Only four individuals presented more than 10 brooms, as the susceptible parent, ICS-1. This suggests dominance for resistance, as pointed by early studies using Scavina-6 as one of the parents (Bartley 1981). Scavina-6 and its sibling (Scavina-12) are widely used as a source of resistance to witches' broom in several countries including Trinidad, Ecuador (Bartley 1983) and Brazil.

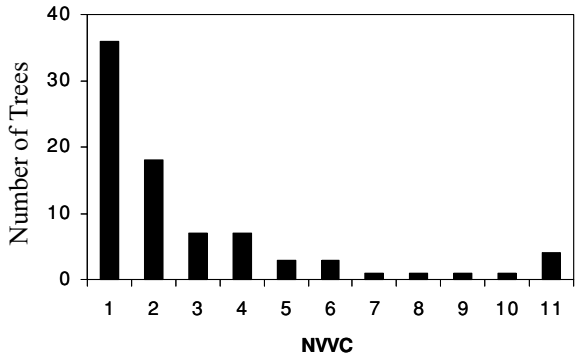

Figure 1. Number of F_2 cacao plants in different classes of number of vegetative brooms (NVVC). The class 11 represents more than 10 brooms per plant.

The repeatability for the number of brooms per tree per year, after correction for the trunk diameter, was 44%; which is quite high considering the variations in climate in the experimental period (1998–2002).

Linkage map

Three hundred and forty-two markers were used to construct the linkage map, being 33 microsatellites, 77 AFLPs and 232 RAPDs. Among these, 196 were published by Queiroz et al. (2003). Approximately 13% of the markers deviated from the expected monogenic segregation, by the χ^2 test ($P < 0.05$). Similar frequency of markers deviating from Mendelian ratios has been reported in other similar studies (Crouzillat et al., 1996; Risterucci et al., 2000, 2003; Queiroz et al., 2003).

Two hundred and forty-two, out of the 342 markers obtained, were mapped in 16 linkage groups accumulating 670 cM (Figure 2), using $\mathrm{LOD} = 4.0$, $r = 0.40$ and Kosambi's function. Among the 242 markers, 24 are microsatellites, 70 are AFLP and 148 are RAPD. Using the same mapping criteria, Queiroz et al. (2003) mapped 193 markers in 25 groups covering 1713 cM. The addition of 149 more markers in this study reduced the number of linkage groups from 25 to 16 and, thus, the map size to 670 cM.

The difference between the number of linkage groups found here (16 groups) and the haploid number of chromosomes of *Theobroma cacao* ($n = 10$) can be due to the nonsaturation of the map yet. However, around 92% of the markers are in the 10 main linkage

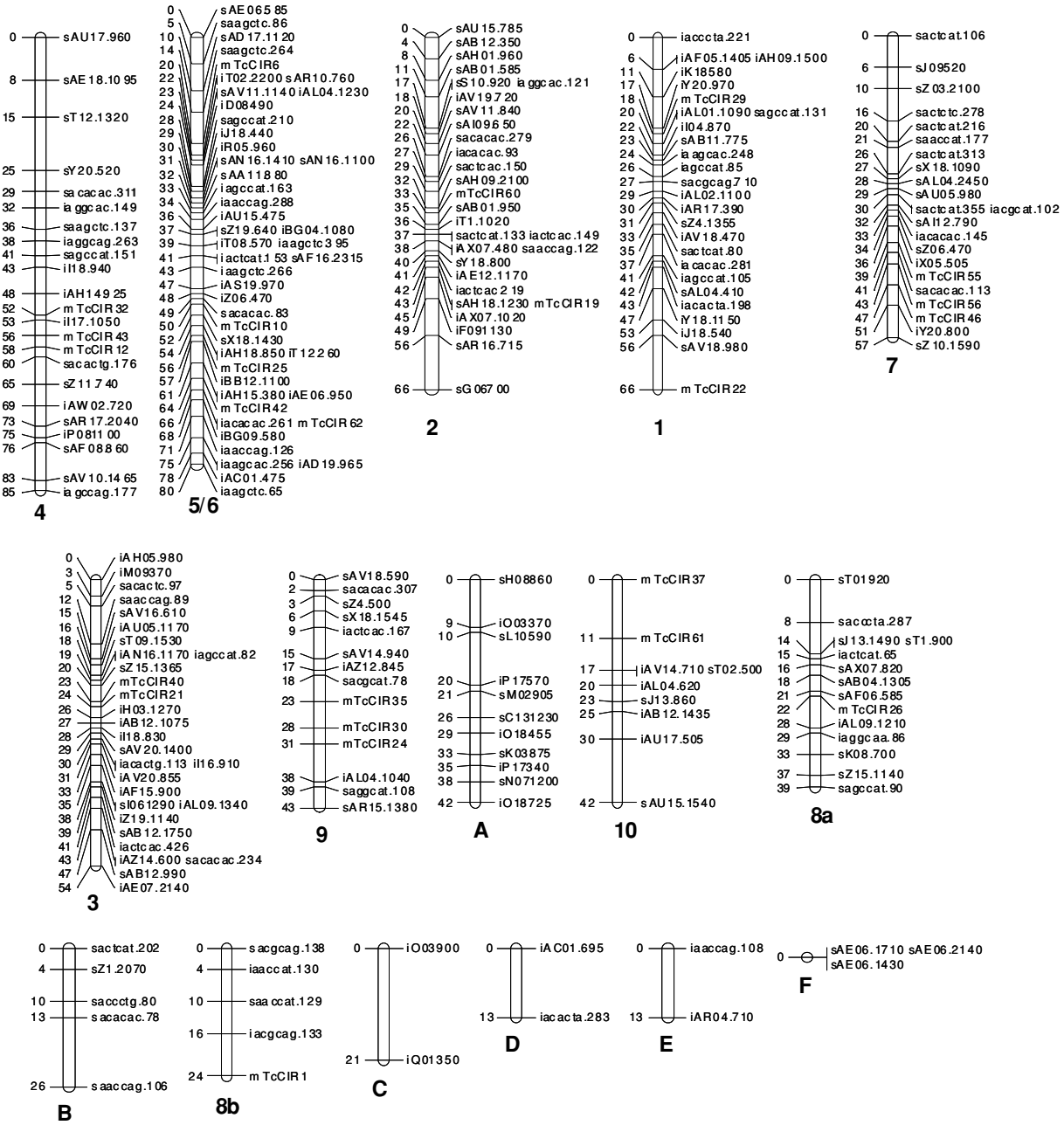

Figure 2. Linkage map of cacao (*Theobroma cacao* L.) based on segregation analysis of 342 markers, with LOD score 4.0 and maximum frequency of recombination 40%. The linkage groups were sorted by size and numbered according to the reference map by Risterucci et al. (2000) and Pugh et al. (2004). Capital letters (A-F) were used to identify groups with no match in the reference map.

groups. This was 63% in the preliminary map published by Queiroz et al. (2003).

The size of the map in Figure 2 is close to the size of other cacao maps published. Lanaud et al. (1995), using an F_1 of UPA 402 × UF 676, developed a map with 10 linkage groups and 193 loci, covering 759 cM. After saturation of this map by Risterucci et al. (2000), 885.4 cM were covered. Crouzillat et al. (2000a,b), using another F_1 progeny, Catongo × Pound 12, generated a map with 162 markers, covering 772 cM.

The 16 linkage groups ranged from 0.0 to 85.5 cM of size and the distance between markers from 0.0 to 21.2 cM, with an average intermarker distance of 2.77 cM. The intermarker distance was 8.84 cM in the map constructed by Queiroz et al. (2003), with 193 markers; 8.3 cM in that constructed by Crouzillat et al. (1996), with 138 markers; and 2.1 cM in that constructed by Risterucci et al. (2000), using 424 markers. Therefore, the distance between markers found here is comparable to that of highly saturated maps as that presented by Risterucci et al. (2000).

Also, here only 3% of the distances between markers were larger than 10 cM and only 0.4% larger than 20 cM. In the map by Queiroz et al. (2003), 7% of the distances were larger than 20 cM. This reduction in distance between markers is very important in QTL mapping (Paterson, 1996).

Identification of QTLs Associated to Witches' Broom Resistance

The microsatellite locus mTcCIR35 explained 35.5% of the phenotypic variation in resistance to witches' broom in cacao. Four other marker loci (2 SSR, 1 RAPD, 1 AFLP) explained individually more than 25% of the phenotypic variation in resistance, by simple regression analysis of the number of brooms, corrected for the trunk diameter (NVVC), on the 342 markers (Table 1). The five loci are located in the linkage group number 9, associated to a single QTL. The alleles of Scavina-6, the main source of resistance to witches' boom, at the five marker loci, had a negative indirect effect on NVVC.

The marker loci AV14.940 and acgcat.78, which in Queiroz et al. (2003), explained 34.7 and 25.5% of the variation, respectively, after the addition of two more years of phenotypic data, passed to explain 32.0 and 25.1%, not showing a clear change.

Table 1. Effect of markers (B) on the number of vegetative brooms in cacao, estimated by simple linear regression and the determination coefficient and p-value

Marker Loci	Linkage Group	$B \pm \sigma$	R^2 (%)	p-value
mTcCIR35	9	-2.42 ± 0.38	35.5	0.0001
mTcCIR24	9	-2.15 ± 0.37	32.1	0.0001
AV14.940*	9	-2.14 ± 0.35	32.0	0.0001
mTcCIR30	9	-2.11 ± 0.43	26.1	0.0001
acgcat.78*	9	-1.95 ± 0.38	25.1	0.0001

*Obtained by Queiroz et al. (2003).

Table 2. Average number of vegetative brooms (NVVC) in F_2 cacao trees with different genotypes in five marker loci (mTcCIR35, mTcCIR24, AV14.940, acgcat.78 and mTcCIR30).

Loci	Genotype[a]	Number of plants	Average NVVC
	r_1r_1	21	5.056
MTcCIR35	R_1r_1	42	0.950
	R_1R_1	12	0.841
	r_2r_2	22	4.854
MTcCIR24	R_2r_2	37	0.902
	R_2R_2	15	0.938
AV14.940	r_3r_3	22	5.470
	R_3_	59	1.177
mTcCIR30	r_4r_4	19	4.841
	R_4r_4	39	1.162
	R_4R_4	12	1.080
acgcat.78	r_3r_3	20	5.295
	R_3_	62	1.378

R_i is the allele of the resistant parent (Scavina-6) and r_i of the susceptible parent (ICS-1).

The indirect effect of the presence of Scavina-6 alleles at the five marker loci close to the QTL (mTcCIR35, mTcCIR24, AV14.940, mTcCIR30 and acgcat.78), on the witches' broom resistance, can be better visualized from the average number of brooms of the F_2 plants with and without those alleles, as shown in Table 2. The average phenotypic values of plants with Scavina-6 alleles at the microsatellite loci are lower than those with Scavina-6 alleles at the RAPD loci, evidencing the advantages of separating the heterozygous/homozygous in marker assisted selection. Among the microsatellite loci, the mTcCIR35 presented the largest indirect effect on the resistance level. The homozygous plants for the Scavina-6 allele on that locus averaged 0.841 brooms per plant, the heterozygous averaged 0.950 brooms and the homozygous for the ICS-1 allele averaged 5.046 brooms; evidencing the complete dominance of Scavina-6 alleles for resistance. The same trend of dominance for resistance was observed for the other microsatellites and the dominant markers, as can be seen by the lower average of brooms in plants having Scavina-6 alleles, either in homozygosis or heterozygosis, on these marker loci compared with plants having only ICS-1 alleles (Table 2).

The number of Scavina-6 alleles in the five marker loci, for the 10 most susceptible and the 10 most resistant plants among the 82 F_2, is presented in Table 3. The Scavina-6 alleles in mTcCIR35 and mTcCIR24 marker loci are present in all 10 most resistant and

Table 3. Genotypes of the 10 most susceptible and the 10 most resistant F_2 plants to witches' broom for five loci and average number of brooms per plant (NVVC)

		Loci [a]				
Plant	NVVC	mTcCIR35	mTcCIR24	AV14.940	mTcCIR30	acgcat.78
26	19.35	.	.	0	.	2
78	13.04	0	0	0	0	0
69	11.75	.	.	0	.	0
90	10.01	0	0	2	0	0
87	9.80	0	0	2	0	2
66	8.47	0	0	0	0	0
15	7.99	0	0	0	0	0
53	6.32	0	0	0	0	0
1	5.81	0	0	0	0	0
7	5.49	0	0	0	1	0
54	0.06	1	1	2	1	2
96	−0.01	1	1	2	1	2
35	−0.03	1	1	2	1	2
80	−0.05	2	2	2	2	2
49	−0.19	1	1	2	1	2
31	−0.31	1	1	2	1	2
23	−0.31	.	.	2	.	2
32	−0.52	1	1	2	1	2
64	−0.66	1	1	2	1	2
92	−0.68	1	2	2	1	2

[a]Codes: . = not measured; 0 = two alleles of the susceptible parent (ICS-1); 1 = heterozygous; 2 = two alleles of the resistant parent (Scavina-6) for the mTcCIR24, mTcCIR30 or mTcCIR35 loci, or the heterozygous for the AV14.940 and acgcat.78 loci.

Table 4. Effect of sets of markers (B) on the number of vegetative brooms in cacao, estimated by multiple linear regression, and partial and total determination coefficients and p-value.

Sets of Markers	Marker	$B \pm \sigma$	R^2 Partial (%)	R^2 Total (%)	P
	mTcCIR35	-2.11 ± 0.33	35.5		0.0001
1	aagcac.256	-1.10 ± 0.28	10.6	53.2	0.0003
	G06.700	0.79 ± 0.24	7.1		0.0016
	mTcCIR24	-1.90 ± 0.32	32.1		0.0001
2	aagcac.256	-0.99 ± 0.28	10.5	54.1	0.0006
	K08.700	-0.68 ± 0.25	6.6		0.0034
	G02.380	0.67 ± 0.25	4.9		0.0087
	AV14.940	-2.28 ± 0.28	32.0		0.0001
	agccat.131	-1.66 ± 0.33	12.6		0.0001
3	acacac.113	-0.93 ± 0.28	7.7	61.5	0.0007
	P08.470	-0.92 ± 0.29	4.4		0.0066
	AH01.960	0.83 ± 0.27	4.7		0.0034
	mTcCIR30	-1.55 ± 0.36	26.1		0.0001
	agccat.90	-1.06 ± 0.24	10.9		0.0011
4	actcat.216	-1.44 ± 0.30	8.7	58.9	0.0018
	AR15.1380	-0.76 ± 0.22	5.4		0.0030
	actcat.106	1.08 ± 0.31	7.8		0.0054
	acgcat.78	-1.64 ± 0.31	25.1		0.0001
	agccat.131	-1.39 ± 0.32	11.5		0.0003
	acacac.113	-1.08 ± 0.27	6.8		0.0030
5	F09.1130	-0.89 ± 0.27	6.6	63.9	0.0021
	F09.310	-1.25 ± 0.32	6.0		0.0019
	acactc.97	0.98 ± 0.29	4.0		0.0074
	mTcCIR8	1.17 ± 0.42	3.8		0.0064

absent in all 10 most susceptible, which would result in an efficiency of 100% if selection was made to choose the best individuals or to discard the worst ones in the mapping population. Also, Scavina-6 alleles for all five marker loci are present in all 10 most resistant individuals, although some of them also occur among the most susceptible. For example, Scavina-6 alleles from AV14.940, acgcat.78 and mTcCIR30 loci occur in at least one of the most susceptible.

Trying to understand the impact of the addition of other marker loci, besides the five ones in the linkage group 9 (mTcCIR35 and mTcCIR24, AV14.940, acgcat.78 and mTcCIR30), a stepwise multiple regression was done using all 342 marker loci. It can be observed in Table 4 that markers other than those in the linkage group 9 add very little in the explanation of the variation in witches' broom resistance (less than 13%).

QTL mapping

In Figure 3 the QTLs associated to witches' broom are shown along of the 16 linkage groups. A strong putative QTL is in linkage group 9, around the position 28 cM (Figure 4). Some markers are associated to the QTL, mTcCIR35 being the closest to it. It is possible that the QTL region involve a family of resistance genes, as observed in other pathosystems (Faleiro et al., 2003).

It should be pointed out that in small progenies such as the one used in this study, QTL effects can be upwardly biased (Utz & Melchinger, 1994; Utz et al., 2000) and the QTL position estimated with a high variance (van Ooijen, 1992; Mangin et al., 1994; Visscher et al., 1996; Utz et al., 2000). Since these two estimates have impact on strategies of marker assisted selection (Visscher et al., 1996), the results relative to the effect and the position of the QTL associated to resistance to witches' broom found here should be looked with certain care. Aiming to overcome these problems and to do a finer mapping of the QTL, the F_2 used here is being increased to one thousand individuals. The pollinations are underway.

In conclusion, the long time-span usually involved in identifying resistant genotypes of cacao to witches' broom can be considerably reduced by using molecular markers, as those identified in this study. In our program, at the Cacao Research Center, several populations involving Scavina-6 have been produced and

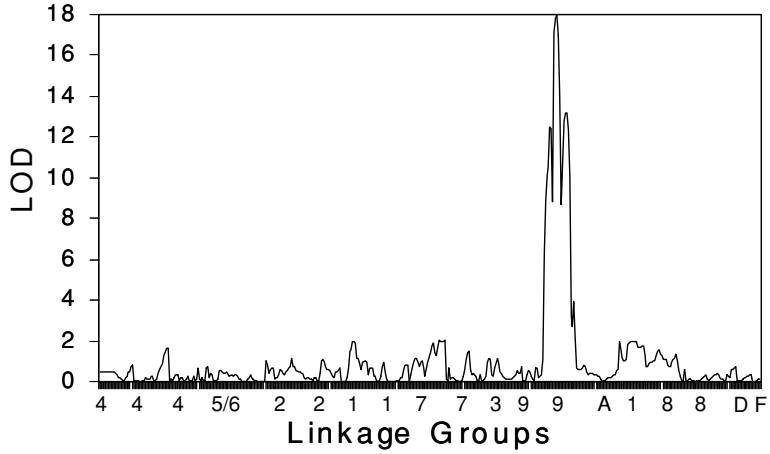

Figure 3. LOD scores along of 16 linkage groups for the number of vegetative brooms (NVVC) in cacao. A major QTL is shown in linkage group 9.

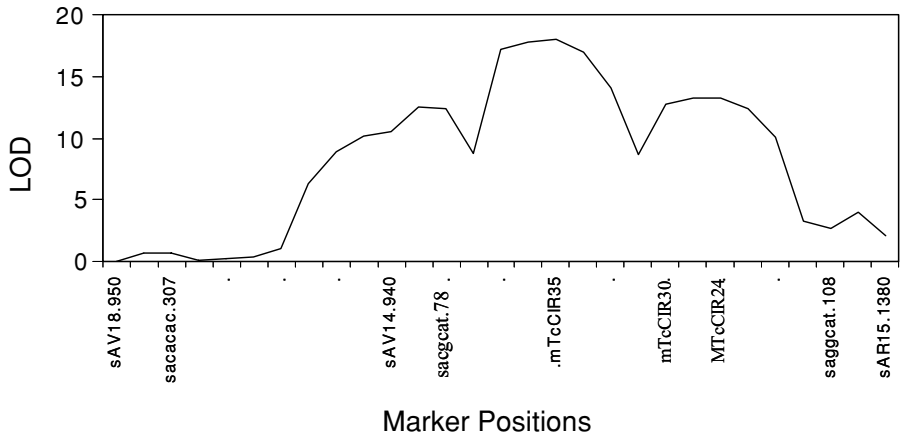

Figure 4. LOD scores along of the linkage group 9 for the number of vegetative brooms (NVVC) in cacao and position of the marker loci. A major QTL is shown.

are being screened for the presence of the QTL identified, as an auxiliary tool to the phenotypic selection currently adopted. However, the weaknesses of the estimation of QTL effect and position should not be overlooked in such programs. Certainly, saturation of the QTL region identified here, using a larger population, is desirable.

Acknowlegements

We acknowledge Dr. João Louis Pereira for the critical review and to the CFC and ICCO for the financial support to the BIOMOL project. We also acknowledge two anonymous reviewers for the suggestions for the improvement of the paper.

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