QTL evolution under natural infection using a F2 Scavina-6 x ICS1 population for witches´broom resistance in Bahia, Brazil.

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Abstract

Studies to determine the genetic bases to witches´ broom resistance were being carried out in order to identify different genetic sources of resistance and to improve the efficiency of selection using molecular markers. Scavina-6, particularly in the cocoa producing region of the Bahia state in Brazil, was initially the main resistance source. A major QTL of resistance was detected in the linkage group 9 from a F_2 Scavina 6 x ICS1 progenies (Brown et al 2005 and Faleiro et al 2006). Observations related with the number of vegetative brooms and flower cushions brooms, were carried out on143 individuals replicated three times by grafting on CEPLAC-hybrids and observed over a period between 2003 and 2008. Analyses were made with SAS, mainly to adjust phenotypic values (number of brooms) by a regression taking into account the difference of the section of each plant (vigor was made in 2008 assessing the section of the grafted portion). QTLs analyses were carried out with MapQTL.5 software. We confirm the presence of the previous QTL located in the LG9 around the mTcCIR35 marker. However, the LOD score and the percentage of variation explained by the QTL significantly decrease over time and change its position into the confidence interval. During this new period of evaluation of the disease in the field, others QTLs of resistance were also detected. The QTLs values related to these others significant QTLs of the resistance to WBD and to a QTL involved in the vigor were also presented. This result suggests a change in the relationship between the host and the pathogen.

Key words: QTL mapping, *Moniliophthora perniciosa.*

Introduction

The basidiomycete *Moniliophthora pernicosa* (Aime and Phillips-Mora. 2005), causal agent of the witches' broom disease (WBD) is the main cocoa disease in Brazil. Introduced in 1989 in two distant regions of the cocoa producing country of the south of Bahia state (Pereira et al. 1996), WBD caused significant damage to this region in, economic, social and ecological areas (Luz et al. 2005). Studies to determine the genetic bases to WBD resistance were being carried out in order to identify different genetic sources of resistance and to improve the efficiency of selection using molecular markers (Pires

2003; Albuquerque 2006). Scavina-6 source (Pound 1943) was initially the main resistance source used in Brazil and others cocoa producing countries. In CEPEC (Centro de pesquisa do cacau), research center of the CEPLAC (Comissão Executiva do Plano da Lavoura Cacauiera), QTL analyzes of resistance to WBD were made from a F_2 Scavina-6 x ICS1 progeny and a main QTL of resistance, located on the linkage group 9, has been identified from average number of vegetative brooms collected between 1996 and 2002 (Brown et al. 2005; Faleiro et al. 2006). We report here new results obtained from QTL analyzes carried out on this same progeny, with observations from two fields, on vegetative and flower cushion brooms and during a period from 2003 to 2008.

Materials and methods

Plant Material.

The F_2 Scavina 6 x ICS1 progeny was produced in CEPEC using o pollen mentor of Herrania mixed with the auto-pollen of TSH516 (incompatible) clone resulting from the cross between : Scavina-6 (resistant to WBD), Amazonian Forastero from Peru and ICS1, a Trinitario clone considered as susceptible to WBD . Initially only 82 plants were analyzed (Brown et al.2005). QTL analyzes were carried out thereafter, between 2003 and 2008 on this same field but with only 61 trees of the 82 initially observed. In the text we named this field: F2_1M. In 2001 and 2002, from the F2 population initially produced, 143 trees (genotypes) were duplicated three times by grafting and we named in the text this other field F2_1C.

Disease assement in natural disease infection

Individuals were observed during 6 years (2003-2008) in both fields F2_1M and F2_1C, for the number of vegetative brooms (VB) and the number of flower cushion brooms (FCB). Both type of brooms were collected and counted generally two time per year. We had observations only until 2008 from the F2_1M and there were observations until 2010 on F2_1C but with missing data in 2009. So, QTL analyzes were made with observations obtained between 2003 and 2008. The vigor of the tree was assessed in 2008 from the measurement section of the trunk from tree (from seedling) of the F2_1M and the average section of the grafted portion of the individuals of the F2_1C . This trait was noted Sec-08.

Molecular marker genotyping and linkage map construction

We used the genetic map published in Brown et al. (2005). Otherwise SSR markers from ESTs were recently mapped as those obtained from ESTs which have been detected on the interaction between Cocoa-*Moniliophthora. P* and Cocoa-*Ceratocystis cocoa funesta*, studies respectively mentioned in Lima et al (2010) and Santos et al (2012). The genetic map used to these QTL analyzes had 188 co-dominant markers.

Statistical analyses and QTL analyses

Descriptive statistical parameters mean, variances, normality of phenotypic traits, and others specific programs, were obtained using SAS (Statistical Analysis System, version 9.1.3, SAS Institute Inc., Cary, North Carolina, USA) or XLSTAT version 2010 (Addinsoft). A SAS statistical program was carried out to adjust phenotypic values by a regression, to take into account a number of brooms (VB and FCB) proportional to the difference of the section of each individual mainly in the period between 2006 and 2008 where the number of brooms showed a strong increase (Figure 1) and where the regression of VB and FCB with vigor was significant. The corrected value of the number of brooms for a given individual is the value observed increased by a number of brooms proportional to the difference of the section area of this individual for the average section area.. The mean value of VB and FCB corrected were respectively noted mVBcor and mFCBcor. QTL Analyses were carried out with MapQTL software, version 5.0 (Van Ooijen, JW., 2004) using the usual procedures proposed by MapQTL. QTL analysis, in simple and composite interval mapping were made using a estimation of the LOD threshold obtained for the whole genome using a program of permutation test proposed by MapQTL 5. This LOD threshold value to validate a significant QTL was found about 3.5. Otherwise, using a nonparametric procedure of Krushkal and Wallis, we considered a QTL significant with a K* (with a minimum of four stars) value which corresponds at significance P-value of 0.005.

RESULTS and DISCUSSION

The curves of evolution of the average number of brooms per tree and the histograms were presented in the Figure 1. These curves showed two periods, one between 2003 and 2005 with a weak level of average number of brooms from VB and FCB, contrasted with a second period between 2006 and 2008, where average number of both type of brooms increased and were more important. The descriptive statistics (mean = m and standar deviation=SD) with the cumulative data obtained between 2003 and 2008 were as follows: mVB=107.7 (SD=78.2) and mFCB=165.9 (SD=185) for the field F2_1M and mVB= 60.7 (SD= 42.5) and mFCB=43.8 (SD=54.7), for the field F2_1C. On the period of the highest disease pressure (2006 to 2008) and from observations of the field F2_1M, the average number of FCB was higher than the average number of VB. It was a reversed situation from observations obtained with the field F2_1C. The observations to the vigor were as follows: mSec-08 =108.3 (SD=62.3) for the F2_1M and mSec-08=56.4 (SD= 28.6) for F2_1C.

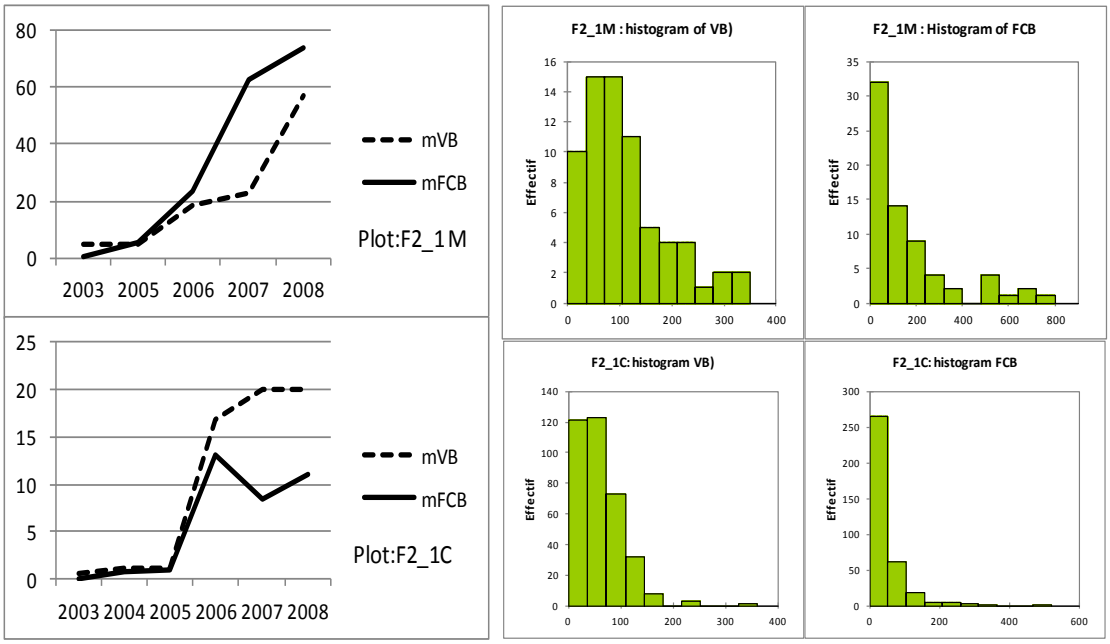

Figure 1 : Evolution from the two fields : F2-1M and F2-1C of the average number of the brooms : mVB and mFCB and the histograms.

Table 1. QTLs results obtained in both F2_1 fields :F2_1M and F1_2C, using different mapping methods

F2_1 Field	Trait	Linkage Group	Left marker	Position Peak	Right marker	Peak Marker	Peak Value	Mapping Method	% Variance Explained	Additive effect	Dominance effect
F2_1M	mVBcor_0305	9	-	7.73	-	mTcCIR266	13.371****	KW	-	-	-
F2_1M	mVBcor_0305	9	-	38.64	-	mTcCIR35	15.854******	KW	-	-	-
F2_1M	mVBcor_0305	9	0	7.73	13.52	mTcCIR266	2.89	IM	19.3	4.85439	-5.30591
F2_1M	mVBcor_0305	9	30.44	38.64	46.22	mTcCIR35	2.54	IM	17.1	5.95122	-3.34346
F2_1M	mVBcor_0305	3	69.75	81.333	85.11	mTcCir140	2.98	ResMQM	16	9.04102	-7.53594
F2_1M	mVBcor_0305	9	0	7.73	13.52	mTcCIR266	2.97	ResMQM	15.9	4.61681	-4.48855
F2_1M	mVBcor_0305	9	30.44	38.64	46.22	mTcCIR35	2.16	ResMQM	11.9	4.84745	-3.26917
F2_1M	Sec-08	9	44.22	53.16	55.98	mTcCIR178	3.39	MQM	16.3	3.603	52.3353
F2_1C	mVBcor_04	2	29.99	38.66	45.32	msEST12	2.98	MQM	11.5	-0.7489	-0.324728
F2_1C	mVBcor_06	2	29.99	37.66	45.32	msEST12	3.11	MQM	11.1	-6.5932	-4.24525
F2_1C	mVBcor_08	2	29.99	34.66	45.32	msEst12	3.45	MQM	10.6	-7.361	-2.52364
F2_1C	mVBcor_0608	2	29.99	38.66	45.32	msEST12	3.49	MQM	11.7	-16.4888	-4.35722
F2_1C	mVBcor_0308	2	29.99	38.66	45.32	msEST12	4.91	MQM	15.6	-18.4312	-3.98271
F2_1C	mVBcor_07	2	54.21	58.591	60.29	mTcCIR60	3.11	MQM	8.1	-0.854724	6.19262
F2_1C	mVBcor_03	9	30.44	35.44	41.44	mTcCIR35	9.03	MQM	32.6	0.765	-0.614
F2_1C	mVBcor_04	9	30.44	38.64	41.44	mTcCIR35	4.64	MQM	14.4	0.749834	-0.650356
F2_1C	mVBcor_07	9	35.44	41.44	44.22	mTcCIR157	3.7	MQM	9.7	1.40533	-7.00513
F2_1C	mVBcor_0305	9	29.99	38.64	41.47	mTcCIR35	5.52	MQM	16.9	1.63092	-1.4044
F2_1C	mVBcor_0608	9	38.64	41.47	44.22	mTcCIR157	3.06	MQM	8.7	5.54603	-18.4358
F2_1C	mVBcor_0308	9	38.64	41.47	44.22	mTcCIR157	4.15	MQM	11.3	8.92056	-18.6354
F2_1C	mFCBcor_0608	9	55.98	68.542	80.19	RGH2	3.28	IM	10.6	19.6193	-4.22896
F2_1C	Sec-08	9	53.16	55.166	67.54	mTcCIR8	5.44	MQM	17.4	4.72524	15.22

QTLs analysis results obtained in both fields were presented in the Table 1. From observations obtained with the field F2_1M, QTL analysis results from cumulative data between 2003 and 2005 and using a KW procedure, showed tow significant QTLs of resistance to WBD on the LG9. One QTL was centered on mTcCIR266 on the beginning of the LG9 (7.7 cM) and the other QTL, centered on the mTcCIR35 (38.64 cM) was detected is the same region of the main QTL of resistance mentioned in Brown et al. (2005) and Faleiro et al. (2006). Analysis carried out with IM or ResMQM not revealed significant QTL. However, with ResMQM, the LOD values were not far to the LOD threshold considering the QTLs detected on the LG3, centered on the mTcCIR140 and on LG9, centered on mTcCIR266, with respectively the LOD values of 2.98 and 2.97. The LOD value of the QTL centered on mTcCIR35 was lower with 2.16. QTL analyzes made during the period between 2003 to 2006 and 2003 to 2008, did not

reveal any significant or putative QTL. QTLs analysis from observations collected from the field F2_1C were carried out on the three periods, 2003-2005, 2006-2008 and 2003-2008. QTLs were detected mainly in the LG 2 and LG 9. From analyzes made in the first period 2003-2005, we had also detected the same QTL detected on the LG 9 by Brown et al (2005) centered on the mTcCIR3. The LOD values and the percentage of variance explained by the QTL, were respectively: 5.52 and 16.1 (Table 1). QTL analyzes from observation data between 2006 and 2008 and for all the period (2003-2008), showed a QTL detected in this same region of the GL9 but centered on mTcCIR157 (41.44 cM) either to 4.8 CM of the mTcCIR35. LOD value obtained from both period 2006-2008 and 2003-2008 were respectively 3.06 and 4.15 and the percentage of the variance explained by the QTL detected on the period 2003-2008 was 11.3. In the genotype matrix with 143 individuals and 188 markers, we assigned the allele "A" to ICS1 and a allele " B" to Scavina-6. In this configuration, using MapQTL 5.0, a positive number indicates a lower average number of brooms for the Scavina-6 allele in additive effect. It is important mentioned here that in Brown et al (2005) the assignment was reversed (allele "A" to Scavina-6 and allele "B" to ICS1) so in the table of QTL results presented in this paper the negative number indicated a lower of brooms for the Scavina-6 allele for additive effect. From the period between 2003 and 2008, analyzing VB, the estimated additive effect was 8.92 (Table 1). The average number of the VB of individuals include in the heterozygous class of mTcCIR157 allele was 42.18 brooms and represented the most resistant plants while the average number of VB of the individuals included in the class of homozygous Scavina-6 allele and in the class of homozygous ICS1 allele, were respectively, 55.07 and 66.17 brooms. When we compared these results those obtained with the QTL analyzes from cumulative data between 2003 and 2005 (QTL centered on mTcCIR35) on both fields F2_1M (KW analysis) and F2_1C (MQM analysis) the averages number of VB for the individuals of the three classes of, homozygous Scavina-6 allele, homozygous ICS1 allele and heterozygous, were respectively: 2,7 and 2; 5.4 and 2.2; 14.7 and 5.3. The individuals more resistant to WBD are on the homozygous Scavina-6 class.

The significant QTL detected on the LG2 from cumulative data between 2006 and 2008 and between 2003 and 2008, obtained respectively a LOD value of 3.49 and 4.91 and 15.9% of the variance was explained by the QTL analyzed in the total period (2003-2008). The msEsT12 is nearest marker of the LOD peak. This marker was recently mapped and comes from an EST sequence outcome of an expression library interaction between cocoa and *Moniliophthora p*. (Lima et al.2010). The estimated additive effect obtained from the period between 2003 and 2008 was -18.43 (Table 1). Therefore this QTL was in opposite direction to the QTLs detected on the LG9. The averages number of VB of individuals included on the classes of, homozygous Scavina-6 allele, homozygous ICS1 allele and heterozygous allele, were respectively here 86.41 49.55, and 64 brooms. So the more resistant individuals came from the homozygous status of one of the two alleles of the progenitor ICS1, which may be from Forastero ou Criollo orign (Motamayor et al. 2003). From new SSRs recently mapped on the reference map of cocoa (Allègre et al. 2011) as mTcCIR509 close to msEST12, we compared, for this marker the allele (homozygous) of a pure Criollo as B97/61/B2 with both alleles of TSH516 (heterozygous) we found a same allele suggesting that the allele associated with the allele of Scavina-6 (heterozygous of msEST12) com from Criollo origin. This hypothesis require to be strengthened.

In this study, we separated QTL analysis from the average number of vegetative broom VB those from floral cushion FCB. QTL analysis made from the field F2_1M, mainly in the period between 2006 and 2008, where the average number of FCB was in strong increase and superior to VB, did not allow to reveal significant QTLs with FCB. From QTL analyzes carried out with observation obtained on the field F2_1C between 2006 and 2008, we found one putative QTL (value just below the threshold) also on the LG9 but centered on RGH2. This marker come from of a resistance gene homologue mentioned in Khun et al.(2003). This QTL had a LOD=3.28 and 10.6 % variance explained and the estimated additive effect was of 19.6. Individuals with the least average number of FCB, belonged to the class of homozygous Scavina-6 allele with 9.3 brooms while the classes of, homozygous ICS1 allele and heterozygous allele, were respectively 48.54 and 24.69 brooms. So in this region on the LG9, the resistance to WBD came from also to Scavina-6 source.

QTL results from the estimation of the area of the section of the trunk in both fields (F2_1M and F2_1C), showed a QTL detected in the same region of the LG9. This QTL of vigor detected on F2_1M and F2_1C were centered respectively on mTcCIR8 (55.16 cM) and mTcCIR178 (53.16), so it is a most likely the same QTL (Table 1.). From the QTL analyzes made on the field F2_1C, with a LOD= 5.4 and 17.4% of variance explained, the three classes, homozygous Scavina-6 allele, homozygous ICS allele and heterozygous, were respectively, 41.48, 50.93 and 64.43 and cm^2. So from both QTL analyzes (F2_1M and F2_1C) we found a significant heterosis effect with an average vigor higher for individuals of the heterozygous class than the two other classes from the two progenitors. In the field F2_1M , vigor observations came from to the original trees (seedling) and in the field F2_1C, they came from to the vigor from average grafted portion of the repetition. These results showed that the rootstock used for grafting the F2 material, had no effect on the genetic effects of both F^2 progenitors.

QTL results presented were obtained from observations of the disease collected on F2_1M and F2_1C and for two types of brooms VB and FCB. Otherwise, QTL analysis carried out from the field F2_1M with only 61 trees observed over the 82 tree initially analyzed during the first analysis (1996-2002) by Brown et al (2005) was less powerful than the QTL analysis made from the field F2_1C with three repetitions of 143 F^2 genotypes therefore we must give more meaning to the results obtained from this second field. The major QTL of resistance to WBD detected on the GL9 and centered on the mTcCIR35 (QTL_GL9), with cumulative data on VB to 1996 until 2002, had an important effect with a LOD value of 10.5 and the percentage of variance explained by the QTL of 51.1 (Brown et al. 2005). Our results showed from analyzes on both fields a decrease of the effect of this QTL mainly on F2_1M where using the ResMQM it is no longer significant. On this same period and by comparing with the previous result , the QTL_GL9 with a LOD of 5.52 and 16.9 % of the variance explained has lost 50 % of this power and 75% of this effect. From this same field (F2_1C), QTL analyzes on VB between 2006 and 2008 and 2003 and 2008, showed that the LOD peak of the QTL_GL9 moved to the mTcCIR157 marker. This QTL was also mentioned by Brown et al. (2005). In our study, taking up the values presented earlier on average number of VB according to the three classes of alleles (homozygous Scavina-6, homozygous ICS1 and heterozygous), we found that the individuals more resistant are present in the class of heterozygous allele This would mean that in the period where the pressure of the pathogen increased, the Scavina-6 resistance

was maintained but now associated with another source here one of the two alleles of ICS that there also be from Criollo . According to the latest information obtained by Allègre et al. (2011) on the genetic map of cacao, these both QTL are included in the region of the LG9 ranging from the mTcCIR24 and mTcCIR160 markers which corresponds to a distance of 11 cM and these two QTLs (mTcCIR35 and mTcCIR157) have the same confidence interval. Otherwise, always from F2_1C, the QTL detected in the LG2 with VB and centered on the msEST12 marker, showed that a resistance to WBD coming from probably the Criollo allele of ICS1 and at this region the effect of the Scavina-6 allele alone brought a susceptibility.

In Pires et al (2009) a study carried out from average number of total brooms (VB and FCB) collected between 2001 and 2008 on various families from progenitors, susceptible or resistant (including Scavina-6), showed that the effects of the Scavina-6 resistance genes were maintained when involved with resistance genes from other sources. In an environmental context characterized by two distinct periods for the pressure of the disease, this new QTL analysis, carried out from two observation context (F2_1M and F2_1C) showed, an evolution of the expression of the resistance for the GL9 genome region between mTcCIR24 and mTcCIR160 with a displacement of mTcCIR35 to mTCIR157 when pressure of the disease increase and showing that the resistance results was due by the association of the allele of either progenitors. New QTLs of the resistance to WBD were also detected, one from another source as the case for the QTL detected on the LG2, another obtained from FCB observations like the QTL detected on the LG9 and centered on RGH2

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