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QTL analysis of field resistance to *Xanthomonas axonopodis* pv. *manihotis* in cassava

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Abstract We evaluated cassava bacterial blight (CBB) infection in an pair-cross population of 150 individuals derived from an intra-specific cross between two non-inbred cassava (*Manihot esculenta* Crantz) lines. The replicated trials were carried out in the field under high disease pressure over two consecutive crop cycles. Evaluations were conducted at 4 and 7 months after planting for the two cycles. Simple regression analysis and the nonparametric Kruskal-Wallis rank-sum test revealed that eight quantitative trait loci (QTLs) were involved in resistance. We detected changes in QTLs from crop cycle to crop cycle. The pathogen population (*Xanthomonas axonopodis* pv. *manihotis*) was also monitored over the period, using a restriction fragment length polymorphism probe and pathogenic tests. Changes in QTL detection over the 2 years could be correlated with changes in pathogen population structure. One QTL, located in linkage group D, was conserved over the two crop cycles, and in field to greenhouse evaluations. This study thus identified molecular markers useful for marker assisted-selection, a technique that can accelerate the long, multiple-season process of breeding for CBB resistance.

Keywords Cassava · Field resistance · Quantitative trait loci · Bacterial blight · Marker-assisted selection

Introduction

Cassava (*Manihot esculenta* Crantz) is a diploid outbreeder species (x=18) cultivated under a broad range of ecological conditions: between 30°N and 30°S, from 0 to 2000 m of altitude and at temperatures between 8°C and 33°C (Lozano et al. 1980). In breeding programs, cassava varieties are selected for their adaptation to specific edaphoclimatic zones (ecozones), which are described according to climatic, biotic and abiotic constraints (Hershey 1983). Cassava is a long-cycle crop: 8–12 months are required before storage roots of commercial size can be harvested. Therefore, the breeding of new varieties will benefit from marker-assisted selection schemes.

Xanthomonas axonopodis pv. *manihotis* (*Xam*) causes cassava bacterial blight (CBB), a major biotic constraint for cassava production in all cassava-cultivating regions. Molecular markers, such as restriction fragment length polymorphisms (RFLPs), reveal that a high genetic diversity exists among *Xam* strains in Latin America, the center of origin for cassava (Verdier et al. 1993; Restrepo and Verdier 1997; Verdier et al. 1998). These studies also reveal geographical differentiation of pathogen populations according to ecozone. Recent studies have shown the high potential of *Xam* populations to continually evolve, thereby, permitting an assessment of the role of host selection in pathogen population structure (Restrepo 1999). In Africa, *Xam* populations are highly homogenous (Verdier et al. 1993), although, recently, some genetic variation has been detected (Assigbétsé et al. 1998; Wydra et al. 1998). In Colombia, the analysis of pathogenic characteristics of *Xam* strains collected from three major ecozones has led to the definition of different pathotypes (i.e. according to behavior on a set of differential varieties) specific to each ecozone (Restrepo 1999). These results highlight the importance of developing cassava varieties with adequate resistance to the *Xam* strains specific to each ecozone.

Symptoms of cassava bacterial blight occur during the rainy season. In the first phase of plant colonization, the bacteria are epiphytic, being found on leaf surfaces. The bacteria then penetrate the leaf mesophyll and invade the vascular system. Host-plant resistance is mostly expressed during this second phase. Resistance and susceptibility have been evaluated under field conditions,

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using visual observations (symptoms), and in the laboratory using biochemical and histological techniques with *in vitro* cultures artificially inoculated with *Xam*. Kpémoua (1995) showed that resistance is associated with the production of phenolic compounds and the reinforcement of cell walls in the vascular system at early stages of infection.

Cassava takes to 2 years to breed from sexual seed. Thus, developing improved cultivars is delayed, and breeders are limited in their responses to changes in host-pathogen interaction (Lozano and Bellotti 1979; Lozano et al. 1980). Disease resistance is commonly identified by selecting healthy genotypes under disease pressure over several crop seasons. In cassava, disease resistance to systemic pathogens is identified by evaluating several succeeding cycles of cropping (Lozano 1975; Takatsu and Lozano 1975). Lozano and Laberry (1982) evaluated resistance in cassava cultivars in the greenhouse and in the field for four cycles. Woody stem cuttings, chosen as planting materials for subsequent cycles, were produced from the evaluation plot of the previous cycle. Susceptible varieties were easily identified as they did not survive a second cycle of disease infection. These researchers showed that resistance screening in the greenhouse and field are highly correlated. Recently, Restrepo (1999) confirmed this result by analyzing the reaction of a set of differential cultivars in their respective ecozones and in the greenhouse.

The mapping of CBB resistance, based on the evaluation of an pair-cross population's reaction to *Xam* in the greenhouse, has been reported (Jorge et al. 2000). Resistance to five different *Xam* strains was found to be controlled by nine quantitative trait loci (QTLs) located in seven different linkage groups in this particular cross. Some of these QTLs are specific to one strain, showing the complexity of host plant/pathogen interaction for CBB.

The work reported here aimed to evaluate CBB resistance under field conditions, based on a knowledge of the pathogen population structure in the field. Through marker-aided genetic analysis, it also aimed to identify the most significant QTLs for marker-assisted selection of resistance genes. We evaluated 150 individuals of an pair-cross mapping population for CBB resistance in the field during two consecutive cycles. At the same time, we monitored the *Xam* field population by sampling, isolating and characterizing the strains using both genetic and pathogenic analyses. We also compared the QTLs detected under greenhouse conditions with those detected under field conditions to define the best strategy for CBB resistance improvement.

Material and methods

Planting materials and field design

Planting materials used in this study comprised woody stem cuttings from 150 individuals from a pair-cross between two plants from elite varieties, TMS 30572 (female parent) and CM 2177–2 (male parent). Both varieties had previously provided the parents of a progeny family used to generate a genetic map of cassava (Fregene et al. 1997). Plants were first grown at the CIAT station in Palmira (Colombia, ecozone 4: low-altitude tropics, moderate temperature, dry season), a CBB-free area. Cuttings were then planted at the CIAT station in Villavicencio (Colombia, ecozone 2: lowland tropics, 26.1°C, mean precipitation of 400 mm per month) during the 1997 rainy season. Six cuttings of each parent and each individual of the pair-cross population were planted every 50 cm in rows spaced 90 cm apart. A row of a highly susceptible variety (M Cub 74) was planted every 10th row to serve as a spreader row. Each parental and progeny row was randomly replicated three times in the field. Total area planted was 0.62 ha. For the second cycle (1998), cuttings from plants (including the susceptible control M Cub 74) of the previous cycle (1997) were used.

Evaluation of disease resistance

Disease evaluations were conducted during 1997 and 1998. Each plant was evaluated at 4 and 7 months after planting, using the following scale: 1=no symptoms; 2=angular leaf spots; 3=wilting of leaves; 4=dieback of one or several apices; 5=dieback of whole plant. For each genotype, 18 plants were evaluated. Disease incidence (DI) was calculated for the susceptible control (M Cub 74) and for each progeny using the formula:

DI (%)=(number of plants showing a reaction of >3)/(total number of plants in a sample) ×100 (Bansal et al. 1994).

Disease severity (DS) was calculated, using the formula:

DS (%)=Σ (number of plants in a disease scale category× disease-scale category)/(total no. of plants×maximum disease-scale category) ×100 (Bansal et al. 1994). This DS varies from 20% to 100%.

Percentage of resistant plants (100–DI), DS and mean disease rating were used for QTL analysis.

Sampling, isolating and characterizing of *Xam* strains

In 1997 and 1998, plants presenting CBB symptoms were randomly collected 7 months after planting. The leaves were stored at room temperature for 10 days before the pathogen was isolated. Infected tissues were removed and macerated in sterilized distilled water. The resulting suspension was spread onto YPG medium (5 g l^{-1} yeast extract, 5 g l^{-1} peptone, 5 g l^{-1} glucose, 15 g l^{-1} nutrient agar, pH=7.2) and left to incubate at 30°C for 48 h. One colony per sample was selected for future characterization, and isolates were maintained on the same medium at 4°C (for routine use) or stored in 60% glycerol at –80°C.

Pathological and molecular analysis of *Xam* strains

All isolates were evaluated for aggressiveness on the susceptible cassava cultivar M Col 1522 as previously described by Restrepo and Verdier (1997). Disease severity was rated on a 0–5 scale, where 0=healthy plant and 5=complete wilting. According to the plant's disease reaction, the aggressiveness of isolates was classified into three categories: 0.0–2.4 (nonaggressive), 2.5–3.4 (weakly aggressive) and 3.5–5.0 (very aggressive). RFLP analysis, using the *pth*B probe, and statistical analysis were conducted as described by Restrepo and Verdier (1997). Each unique banding pattern generated by the probe was regarded as a haplotype. Isolates collected in 1997 had been previously characterized (Restrepo 1999). The diversity of *Xam* populations was calculated by the equation:

$$H=[n/(n\text{-}1)](1-\Sigma X_i^2),$$

where X_i is the proportion of the ith distinct *pth*B haplotype within a population, and n is the number of strains in each population (Nei and Tajima 1981).

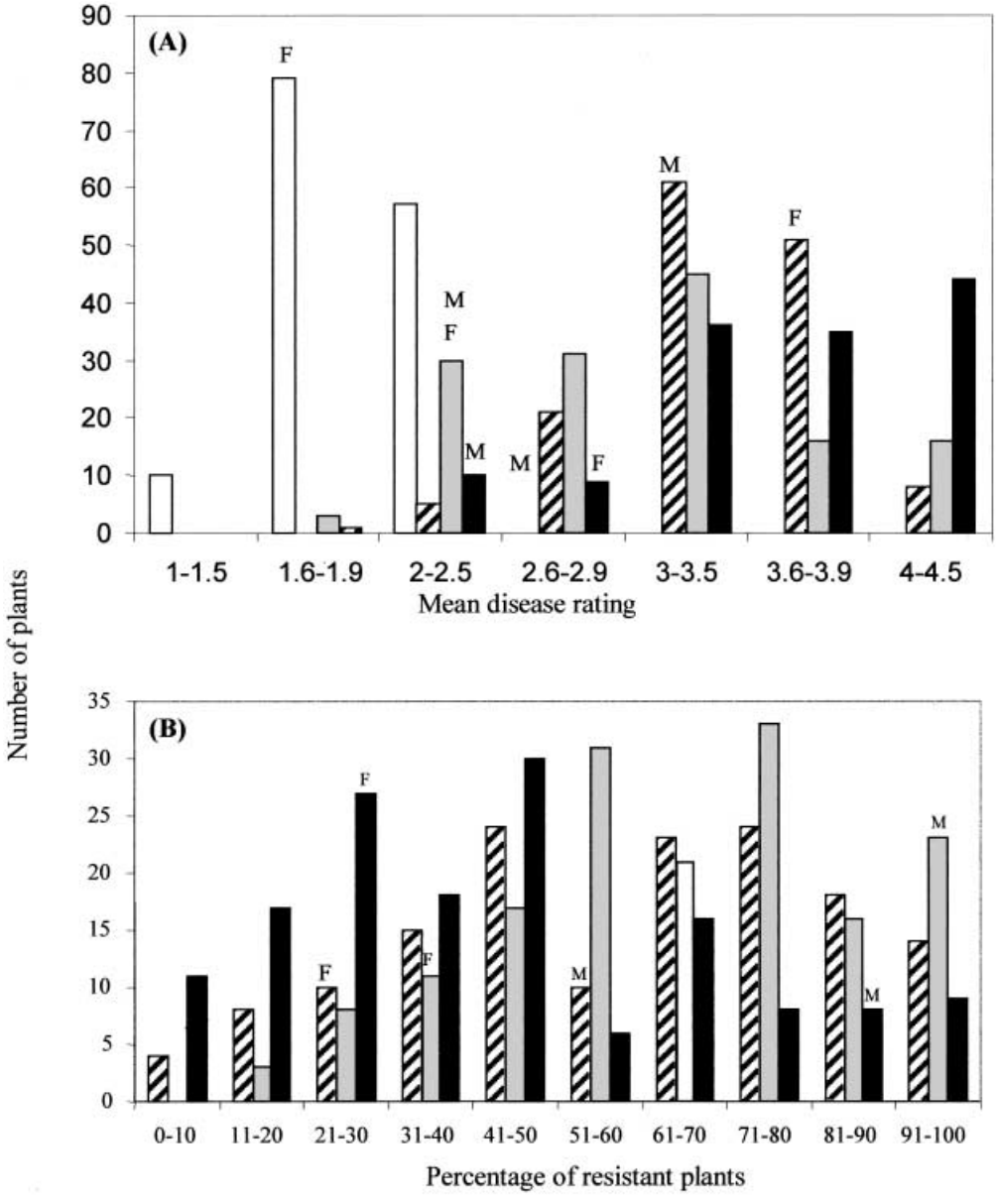

Fig. 1 Distribution of mean disease rating (**A**) and 100-DI (**B**) for the parents and 150 individuals of the cassava cross TMS 30572 (*F*) ×CM 2177–2 (*M*) and for evaluations in 1997 and 1998. *White bars* 1997, 4 months, *striped bars* 1997, 7 months, *grey bars* 1998, 4 months, *black bars* 1998, 7 months, *F*: female parent, *M*: male parent

We used the following formula to evaluate the power of the sampling strategy:

$N=\log(1-P)/\log(1-F)$,

where N is the number of samples required to detect a given haplotype present at a frequency (F) at least once with a probability of *P* (Leung et al. 1993).

Statistical analysis

The test for normal distribution of resistance, scored as 100-DI, DS and mean disease rating was performed by the Shapiro-Wilks W-statistic test using the SAS's UNIVAR procedure (SAS Institute 1989a, b). Where distributions were significantly different from normality for 100-DI, plant values were used after arcsine transformation.

Resistance data were then analyzed using the standard analysis of variance (ANOVA), performed with the SAS's GLM procedure (SAS Institute 1989a, b) for the randomized complete-block design experiment. Variance was partitioned according to experimental factors, including year, replication within year, genotype and genotype-by-year interaction. Broad-sense heritability (H^2) was calculated from the ANOVA with the formula:

$H^2=\sigma_g^2/[\sigma_g^2+\sigma_{gy}^2/y+\sigma_e^2/ny)]$,

where σ_g^2=the genetic variance, σ_{gy}^2=the genotype-by-year variance, σ_e^2=the environmental variance, n=the number of independent replications and y=the number of years.

QTL analysis

Two framework maps were used for QTL analysis, both based on the segregation of molecular markers in an population from a cross between two heterozygous parents, TMS 30572 (female) and CM 2177–2 (male). The first framework map (female-derived map) was based on the segregation of female alleles, corresponding to 142 molecular markers (Fregene et al. 1997, Jorge et al. 2000) that comprised 95 RFLP, 36 random amplified polymorphic DNA (RAPD), 3 isoenzymes, 3 microsatellites, 3 expressed sequence tags (ESTs) and 2 known genes. The second framework map

(male-derived map) was based on the segregation of male alleles, corresponding to 135 markers (Fregene et al. 1997; Jorge et al. 2000) that comprised 89 RFLP, 41 RAPD, 4 known genes and 1 EST.

Associations between molecular markers and disease rating, based on an ordinal scale, was tested using the non-parametric Kruskal-Wallis rank-sum test (Lehmann 1975). An association was considered significant if the probability of the null hypothesis (no association) was less than 0.0003 (α/number of tests, here 142, thus 0.05/142=0.0003). The arcsine transformation was applied to 100-DI before comparing the phenotypic means of the two marker classes, using a single-locus analysis of variance (SAS GLM procedure, SAS Institute, 1989a, b). A significant association between a DNA marker and CBB resistance was declared if the probability was ≤0.005 to minimize the detection of false positives. The amount of phenotypic variance explained by each marker was obtained from the regression coefficient (r^2 squared value). Total r^2 values from each QTL were computed as: (sum of squares for each markers)/(total sum of squares). Interval mapping procedures were not attempted because the linkage phase of markers in an pair-cross population is not known.

Results

Evaluation of disease resistance

In 1997, disease symptoms observed at 4 months were primarily angular leaf spots whereas, at 7 months after planting, vascular contamination had led to the more severe symptoms of exudates and wilting. In 1998, a different evolution of symptoms was observed: plants died by wilting early in the growth cycle because bacteria were present in stakes infected during the previous cycle. A disease rating of 5 was given to these plants. Plants that survived developed angular leaf spots at 4 months after planting and showed wilting of leaves and dieback at 7 months. Almost all plants of the susceptible control M Cub 74 showed symptoms, suggesting that the inoculum had been homogeneously distributed in the field. Disease incidence (DI) in 1997 on the susceptible control was 0% and 100% at 4 and 7 months after planting respectively; in 1998, DI scores were 2.2% and 94.4%, respectively. Disease severity (DS) varied from 52.6% to 54.8% at 4 months after evaluation, and 90% to 95% at 7 months, depending on the year.

Figure 1 presents the distribution of mean disease rating (Fig. 1A) and percentage of resistant plants (Fig. 1B) at 7 months in 1997 and at 4 and 7 months after planting in 1998. In 1997, at 4 months after planting, the mean disease index did not show a normal distribution, the reaction being skewed to low values of disease rating. At 7 months after planting in 1997 and in 1998 at both dates, it presented a normal distribution. Similarly, the percentage of resistant plants varied between 91% and 100% in the entire pair-cross population 4 months after planting in 1997 (data not shown). In the same year, at 7 months, values varied between 5.5% and 100%. In 1998, the percentage of resistant plants (100-DI) varied between 11.1% and 100% at 4 months and between 0% and 100% at 7 months. TMS 30572 and CM 2177–2, the female and male parents of the pair-cross population, respectively, showed different 100-DI scores in the field.

Table 1 Correlation coefficients between percentage values of resistance to cassava bacterial blight in 1997 and 1998

Year		1997 4 months	1997 7 months	1998 4 months
1997	7 months	–0.04		
1998	4 months	–0.03	0.21[a]	
1998	7 months	–0.04	0.35[b]	0.69[c]

[a, b, c] Significance at 5%, 0.1%, 0.01% levels, respectively

Table 2 Mean squares from an analysis of variance of bacterial blight resistance of an pair-cross cassava population in the field (*df*=degrees of freedom, *MS*=mean squares)

Source	*df*	MS	Variance explained (% of sum of squares)	*P>F*
Model	303	0.408	100	0.0001
Year	1	9.047	0.073	0.0001
Replication within year	4	0.357	0.011	0.0056
Genotype	149	0.575	69.4	0.0001
Year*genotype	149	0.184	22.2	0.0001
Error	596	0.097	–	–

At 4 months, 100-DI was 100% and 38.9% in 1997 and 1998, respectively, for TMS 30572, whereas it was 100% for both years for CM 2177–2. At 7 months, 100-DI was 27.8% (female) and 55.6% (male) in 1997, and 27.8% (female) and 88.8% (male) in 1998, showing that the male parent is more resistant than the female.

Correlation coefficients between the three different resistance parameters were high for all dates, except in 1997 at 4 months after planting. Correlation coefficients between both evaluations based on 100-DI at 4 and 7 months and between 1997 and 1998 are shown in Table 1. In 1997, no high correlation was found between the evaluations at 4 and 7 months, whereas in 1998, a significant positive correlation was observed. If we consider the evaluations at 7 months, a low but significant (P<0.05) positive correlation detectable between the 2 years. The results obtained by ANOVA (Table 2) show that a significant (P=0.0001) difference exists between the 2 years. Only data of the 7-month evaluation were included in this analysis of variance. A significant difference exists between replications within the year, demonstrating the need for several replications in field trials. Most of the variation (69.4%) could be explained by the factor "genotype." Based on this analysis of variance, CBB field-resistance broad-sense heritability was 0.65. Similar results were obtained with DS and the mean disease rating (data not shown).

Characterization of *Xam* strains

All strains collected in 1997 and 1998 were classified as being very aggressive (disease rating ≥3.5) after inoculation on a susceptible variety (M Col 1522). Among the

Table 3 Distribution of strains collected in 1997 and 1998, showing different haplotypes and diversity (*ND* not determined)

	Haplotypes													Total	Diversity[a]
	C4	C12	C17	C19	C26	C27	C28	C29	C34	C45	C46	C47	ND		
1997[b]	16	3	1	0	1	3	2	3	1	0	0	0	0	30	0.70
1998	1	26	1	7	0	5	0	2	0	2	1	2	3	49	0.67
Total	17	29	2	7	1	7	2	5	1	2	1	2	3	79	0.79

[a] Nei's index (Nei and Tajima 1981)
[b] Data from C.M. Velez et al. (unpublished data)

Table 4 QTLs explaining the highest values of variance for resistance in cassava based on the mean disease rating and percentage of resistant plants (100-DI) in 1997 and 1998, at 4 and 7 months after planting. Values significant at P=0.005 are in bold

100-DI											
Linkage group (map)	Markers (position in cM)[a]	1997 7 months			1998 4 months			7 months			Name of QTL
		F[b]	%V[c]	P[d]	F	%V	P	F	%V	P	
D (female)	GY219 (0)	–[e]	–	–	6.2	4.3	0.0142	**9.6**	**6.5**	**0.0024**	XMF1
	O19 (36)	–	–	–	**8.1**	**9.3**	**0.0050**	7.8	9.1	0.0066	XM8[f]
E (male)	rI4a (0)	–	–	–	8.1	8.7	0.0055	**9.3**	**9.9**	**0.0030**	XMF2
	CDY123a (21.7)	–	–	–	**11.6**	**13.1**	**0.0010**	–	–	–	
F (female)	CDY107 (0)	4.8	6.1	0.0310	6.5	8.0	0.0132	**16.5**	**18.2**	**0.0001**	
	GY37 (3.9)	5.2	3.6	0.0238	–	–	–	**8.8**	**6.0**	**0.0037**	XMF3
	GY186 (14.1)	7.5	5.2	0.0072	–	–	–	**10.7**	**7.2**	**0.0013**	
I (female)	rAC1	–	–	–	**9.2**	**10.8**	**0.0033**	–	–	–	XMF4
M (male)	nrGY67 (0)	–	–	–	**9.0**	**10.2**	**0.0036**	**11.7**	**12.9**	**0.0010**	
	rGY87 (18.7)	–	–	–	**10.4**	**11.6**	**0.0019**	**10.5**	**11.7**	**0.0017**	XMF5
	W19b (42.8)	6.4	7.7	0.0133	7.5	8.9	0.0076	**8.4**	**9.8**	**0.0049**	
	GY105 (51.3)	7.3	8.1	0.0085	**8.7**	**9.6**	**0.0041**	**8.7**	**9.6**	**0.0042**	
Mean disease rating											
B (male)	rGY72	–	–	–	**9.6**	**10.8**	**0.0027**	**9.3**	**10.6**	**0.0030**	XMF6
D (female)	GY219	6.9	4.8	0.0096	4.4	3.2	0.0376	6.5	4.6	0.0117	
E (male)	rI4a (0)	–	–	–	**9.0**	**9.9**	**0.0036**	**10.2**	**11.1**	**0.0020**	
	GY118 (10.1)	–	–	–	6.0	7.2	0.0165	**9.2**	**10.6**	**0.0033**	XMF2
	CDY123a (21.7)	–	–	–	**11.5**	**13.3**	**0.0011**	7.9	9.5	0.0064	
E (female)	rF19b (0)	**8.6**	**11.2**	**0.0045**	–	–	–	–	–	–	XMF7
	GY205 (54.5)	–	–	–	7.5	5.3	0.0071	**9.2**	**6.4**	**0.0029**	
F (female)	CDY107 (0)	5.6	7.1	0.021	**8.8**	**10.7**	**0.0042**	**14.3**	**16.4**	**0.0003**	
	GY37 (3.9)	**9.5**	**6.5**	**0.0025**	6.0	4.3	0.0156	**12.2**	**8.3**	**0.0007**	XMF3
	GY194 (9.1)	**8.2**	**5.6**	**0.0049**	7.7	5.4	0.0063	**9.2**	**6.4**	**0.0029**	
	GY186 (14.1)	**10.1**	**6.9**	**0.0019**	6.8	4.8	0.0104	**12.2**	**8.4**	**0.0006**	
M (male)	nrGY67 (0)	–	–	–	**10.7**	**12.0**	**0.0016**	**9.7**	**11.1**	**0.0020**	
	rGY87 (18.7)	–	–	–	**8.1**	**9.5**	**0.0050**	**9.1**	**10.6**	**0.0034**	XMF5
	W19b (42.8)	4.6	5.8	0.0345	5.4	6.7	0.0229	**8.7**	**10.4**	**0.0043**	
	GY105 (51.3)	5.1	6.1	0.0259	**8.0**	**9.1**	**0.0050**	**8.7**	**9.8**	**0.0042**	

[a] Distance from the first marker noted (0)
[b] F statistic from analysis of variance
[c] Percentage of variance explained (from r^2 coefficient of regression)
[d] Probability of F statistic
[e] Not significant at 5% level.
[f] QTL detected in greenhouse (Jorge et al. 2000)

30 strains collected in 1997, eight different haplotypes (Table 3) were characterized, haplotype C4 being the most abundant. In 1998, among 49 strains studied, nine haplotypes were detected, haplotype C12 being the most abundant. The diversity of *Xam* populations (Nei and Tajima 1981) was greater in 1997 than in 1998. According to the equation cited in the Materials and methods (Leung et al. 1993), the number of samples (n=79) in the present study gave us a 95% probability of detecting at least once a given haplotype present at a frequency of 3.7%. Three new haplotypes, absent in 1997, were detected in 1998 (haplotypes C19, C45 and C46), and three haplotypes present in 1997 were not detected again in 1998 (C26, C28 and C34).

QTL mapping

Table 4 shows the results of the single-marker regression analysis of percentage of resistant plants in the field (100-DI). No QTLs were found associated with 100-DI in 1997 at the $P<0.005$ level. Twelve markers spread over five different linkage groups of both the female and male-derived framework maps were found (at a significance $P<0.005$) to be involved in resistance in the 1998 trials. These markers defined six QTLs located on linkage groups D, E, F, I and M (Table 4). The QTLs explain between 7.2% and 18.2% of the variance, the most significant QTL being XMF5 located in linkage group M of the male-derived framework map. Two QTLs on linkage groups F (XMF 3) and M (XMF 5; Table 4) detected in 1998 were also significant at the $P=0.05$ level in 1997. These results are comparable with those from the nonparametric Kruskal-Wallis rank-sum test for disease resistance score based on a qualitative ranking of resistance (data not shown).

Using the mean disease rating, we found 14 markers significantly involved in resistance, spread over four different linkage groups. They define five QTLs located on linkage groups B, E, F and M (Table 4). Three of these QTLs (XMF2, XMF3 and XMF5) were also detected with the 100-DI parameter but with generally lower *F* values. According to the mean disease rating, two QTLs, not significant with 100-DI variable, are located in linkage groups E and F and appear to be significantly involved in resistance in 1997 at 7 months after planting. The QTLs in group D are no longer significant at the 0.005 level but are still significant at the 0.05 level (Table 4). QTL analysis with DS shows results equivalent to those obtained with the mean disease rating (data not shown).

Discussion

The present study analyzed the level of resistance to CBB in a cassava mapping population over 2 years in a site of high disease pressure. The high disease severity indices observed with the susceptible control M Cub 74 showed that disease incidence was high and stable across time. This region (Colombian Eastern plains, ecozone 2) has been reported as an ecozone with the highest incidence of CBB in Colombia (CIAT 1975) and was selected because the parents and the progeny are adapted to this particular ecozone. Furthermore, *Xam* populations have shown geographical differentiation by ecozone and a pathogenic specialization to the cassava material originating from each ecozone (Restrepo and Verdier 1997; Restrepo 1999).

Two or three cycles of evaluations are required to differentiate escapes from truly resistant cultivars in cassava breeding programs. The heritability of CBB resistance calculated in this study (0.69) is similar to values obtained by Umemura and Kawano (1983) and can be affected by different climatic and edaphic factors. A prolonged dry season does not allow the expression of symptoms, and a susceptible variety could be misclassified as resistant in this case. At the trial site, rainfall was abundant during the 2 years, and temperatures, averaging 26°C, were favorable for the expression of symptoms. Plant material from the first cycle was used for the second cycle of evaluation. Bacteria survive easily in cuttings that are used for further planting (Daniel and Boher 1985). Heavily contaminated planting materials from the first cycle could not survive the second cycle. Young plants established from even mildly infected stakes died rapidly. This increased the disease pressure and resulted in the rapid detection of susceptible genotypes in the second cycle. This can explain the low level of correlation observed between disease resistance parameters in the first and second cycle.

The QTL analysis method (single-marker analysis) has some limitations as no distinction can be made between the effect of the QTL and the distance between the QTL and the markers. Nevertheless, in our case average distances between markers were sufficiently small so as, to use only single-marker analysis instead of interval mapping, the latter not providing additional information to results obtained with single-marker analysis (data not shown).

Eight QTLs controlling field resistance were detected in this study using two different parameters to measure resistance: 100-DI and mean disease rating. Our results show that resistance to CBB is polygenic in this particular cross. Monogenic resistance may occur in other crosses. In the case of 100-DI, no QTLs were detected in 1997 at significant levels, whereas with the mean disease rating, two QTLs were significant at the 0.005 level. In contrast, the two QTLs in linkage group D detected in 1998 with the 100-DI were not detected with the mean disease rating. Nevertheless, three QTLs were conserved for both parameters. These results suggest that the resistance parameters measured are different and may represent different components of resistance.

No QTLs were found in the first year at 4 months after planting because the disease was in its epiphytic phase and resistance mechanisms were not fully expressed. Disease incidence was not high at the first 4-month evaluation and, thus, various susceptible genotypes were misclassified to the resistant category. In 1998, a high level of infection in the early stage of plant development induced resistance mechanisms to be expressed more strongly. Both female (TMS 30572) and male (CM 2177–2) parents had previously been reported as intermediate in resistance (CIAT 1985). In this study, CM 2177–2 showed more resistant plants than did TMS 30572. This observation correlates with the finding of highly significant QTLs associated with molecular markers of the male framework map. Nevertheless, the occurrence of individuals more resistant than the two parents and the detection of QTLs associated also with molecular markers from the female-derived map show that resistance alleles comming from both parents contribute to resistance in the progenies (transgressive segregation).

This characteristic is well-known in heterozygous species and is useful for combining resistance genetic factors in the same cultivar.

The variation in the *Xam* population structure across time (cycle or season) in the field can be explained by (1) the sampling method, which could affect the detection of rare haplotypes (i.e. random effect), or (2) plasmidic recombination, which could have occurred and thus induced changes in the haplotypes detected, because diversity detected by the *pth*B probe is principally at the plasmidic level. Kousik and Ritchie (1996) observed that new races of *Xanthomonas campestris* pv. *vesicatoria* and race changes were correlated with a loss of plasmids. In the case of *Xam*, no correlation between haplotype and pathotype (behavior of a strain on a differential set of varieties) was clearly established (Restrepo 1999). Nevertheless, recent studies have revealed a correlation between the occurrence of new haplotypes and new pathotypes (C.M. Velez unpublished data).

QTLs detected in 1997 differed from those detected in 1998, except for one which had remained constant. One hypothesis to explain this finding is that the *Xam* population structure changes rapidly across years. We detected changes in the genetic structure of the *Xam* population present in the field across time. The pathotypic structure of the *Xam* strains needs to be characterized in order to draw soundly based conclusions on the influence of pathogen population on QTL detection. In a previous study and using the same mapping population, we showed that 12 different QTLs control resistance to 5 distinct strains (Jorge et al. 2000). Specificity of resistance to different *Xam* strains was also demonstrated (Jorge et al. 2000). Other studies on *Xam* population structure have shown rapid changes in haplotypic and pathotypic structure across years, as well as strong strain×cultivar interactions (Restrepo 1999). Another hypothesis is that changes in QTLs detection can be explained by environmental factors. No significant difference between monthly average temperatures and rainfalls between the 2 years was detected.

Breeding strategies to improve CBB resistance in cassava include selecting cassava cultivars for specific ecozones. Although disease severity under field conditions is the ultimate measure of resistance, the most rapid and reliable method for measuring disease resistance is the test developed for greenhouse conditions. However, we have shown in our study that some discrepancies exist between QTLs detected in the field and those detected in the greenhouse. Both methods (field and greenhouse) have different purposes: evaluation in the greenhouse with specific strains is useful for studying specific cassava-*Xam* interactions; field evaluation of resistance is better adapted for identifying markers to select horizontal resistance in a marker-assisted selection scheme.

Characterizing the pathotypic structure of the *Xam* population in the field is important. We recommend using strains that represent the different pathotypes for screening cassava mapping populations for resistance in greenhouse.

Special emphasis must be given to linkage group D, which has many markers and low recombination frequencies. The group was found to be associated with resistance to two strains in the greenhouse (Jorge et al. 2000) and with resistance as observed in the field. We think that the QTLs in group D are involved with horizontal resistance, introgressed from *M. glaziovii*, in lines leading up to TMS 30572, the female parent of the mapping population. Seven years of strong selection against *M. glaziovii* traits has maintained the region of the linkage group D as demonstrated by the large number of markers in the linkage group and the reduced recombination frequencies (Jennings 1977). Breeders have observed that CBB resistance usually co-segregates with ACMV resistance (Hahn et al. 1980). Group D is also involved in other agronomic characteristics like dry matter content (Fregene et al. 1999). Selection for particular alleles (female alleles) from this linkage group in a marker-assisted breeding program may improve resistance to CBB and other characteristics at the same time.

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