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Genetic mapping of resistance to bacterial blight disease in cassava (*Manihot esculenta* Crantz)

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Abstract Cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), is a major disease of cassava (*Manihot esculenta* Crantz) in Africa and South America. Planting resistant varieties is the preferred method of disease control. Recent genetic mapping of an F_1 cross (TMS 30572 × CM 2177–2) led to the construction of the first molecular genetic map of cassava. To better understand the genetics of resistance to CBB, we evaluated individuals of the F_1 cross for CBB resistance by controlled greenhouse inoculations and visually assessed symptoms on days 7, 15, and 30 days after inoculation, using a scale where 0 = no disease and 5 = maximum susceptibility. Five *Xam* strains were used: CIO-84, CIO-1, CIO-136, CIO-295, and ORST X-27. Area under the disease progress curve (AUDPC) was used as a quantitative measure of resistance in QTL analysis by single-marker regression. Based on the AUDPC values, eight QTLs (quantitative trait loci), located on linkage groups B, D, L, N, and X of the female-derived framework map, were found to explain 9–20% of the phenotypic variance of the crop's response to the five *Xam* strains. With the male-derived framework map, four QTLs on linkage groups G and C explained 10.7–27.1% of the variance. A scheme to confirm the usefulness of these markers in evaluating segregating populations for resistance to CBB is proposed.

Key words Bacterial blight · Cassava · Disease resistance · Mapping QTLs

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Introduction

Cassava (*Manihot esculenta* Crantz), native to South America, is a major food crop in tropical lowlands where it grows successfully in poor soils and under drought conditions. The most common biotic constraint to production worldwide is the cassava bacterial blight (CBB), caused by a vascular pathogen, *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). Yield losses in different areas of Africa and South America may be anywhere between 12% and 90% (Persley 1977; Lozano and Laberry 1982). The variability of *Xam*, based on pathogenicity and on physiological, biochemical, and (recently) molecular characterization, reveals greater genetic diversity in Latin America than in Africa or Asia (Lozano and Sequeira 1974; Grousson et al. 1990; Verdier et al. 1993; Restrepo and Verdier 1997; Verdier et al. 1998). Genetic studies reveal that CBB was recently introduced to Africa from Latin America and that in Latin America *Xam* populations from distinct edaphoclimatic zones (or ecozones) are differentiated (Verdier et al. 1993; Restrepo and Verdier 1997; Verdier et al. 1998).

Host-plant infection occurs through stomata and epidermal wounds, causing symptoms of angular leaf spot. The pathogen then penetrates vascular tissues in stems as the disease progresses, from where it invades the whole plant (Lozano 1986). Pathogen multiplication, and the attendant production of bacterial slime, blocks vascular tissues, leading to leaf wilt, production of exudates, and dieback. Bacterial exudates spread to other plants through splashing during heavy rain and, to a much lesser extent, insects. Because cassava is vegetatively propagated, planting materials form another significant vehicle for disease dissemination (Verdier et al. 1997). The use of disease-free planting materials and adoption of appropriate cultural practices have been suggested as ways of reducing disease incidence and crop loss (Lozano 1986). However, these disease control measures have not been fully adopted in traditional cassava-producing regions, making host-plant resistance the preferred method of control (Verdier et al. 1997).

M. glaziovii and *M. esculenta* have been identified as sources of resistance to CBB (Hahn et al. 1979; CIAT 1981). Whether the two types of resistance to CBB act in the same way or are controlled by the same alleles is still unknown. Resistance from both sources is thought to be polygenic and additively inherited, with narrow-sense heritability ranging from 25% to 65% (Hahn et al. 1979; CIAT 1980).

The difference between resistant and susceptible varieties is expressed as a variation in the rate of *Xam* colonization and penetration of vascular tissues. For this reason, resistance is usually considered to be quantitative (Kpémoua 1995). Recent studies revealed that CBB resistance is associated with general defense mechanisms that limit bacterial colonization of the xylem. They include the reinforcement of constitutive barriers (lignification, callose deposition, and suberization), accumulation of phenolic compounds, and vessel occlusion (tylosis) (Kpémoua et al. 1996). No clear-cut gene relationship has been demonstrated, and hypersensitive responses have not been observed in cultivated varieties. Neither have studies been conducted on the genetics or number of disease-resistance loci involved in host-plant resistance to CBB.

Two different types of resistance to xanthomonads have been characterized and mapped in other crops. Molecular genetic mapping of host-plant resistance to bacterial blight in rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), revealed that a single locus confers resistance to a specific race or races of the pathogen. At least 18 major genes have been described, and one, *Xa*21, a broad-spectrum gene for bacterial-blight resistance in rice, has recently been cloned (Kinoshita 1991; McCouch et al. 1991; Ronald et al. 1992; Song et al. 1996; Yoshimura et al. 1996). However, ten quantitative trait loci have also been detected against three *Xoo* strains (Li et al 1999). In the common bean, 18 markers located in different genomic regions and explaining 14–29% of the phenotypic variance, account for resistance to *Xanthomonas campestris* pv. *phaseoli* (Jung et al. 1996). As the quantitative nature of disease resistance makes these genes less amenable to classical genetic analysis, more powerful methods of analysis, such as marker-assisted analysis, are required. Molecular genetic mapping is a powerful tool for dissecting complex traits and identifying quantitative trait loci (QTLs) (Tanksley et al. 1993). Methods of mapping quantitative traits in F_1 crosses between non-inbred parents have been described (Ritter et al. 1991; Bonierbale et al. 1994; Van Eck et al. 1994). The confounding effect of multiple alleles segregating from both parents requires that QTL analysis be performed with markers that detect unique polymorphisms in both parents, or that such markers be employed to buttress initial findings, using markers that detect unique polymorphisms in only one parent.

Genetic mapping with over 200 restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs), expressed sequence tags (SSTs) as markers and known genes in an intraspecific F_1 cross, between non-inbred parents, of 90 individuals has led to the construction of the first molecular genetic map of cassava (Fregene et al. 1997). The female-derived map is based on the segregation of 142 molecular markers in the gametes of the female parent, while the male-derived framework map is based on the segregation of 135 markers in the male gametes. Thirty-six markers with unique alleles from both the female and male parents, also known as allelic bridges, have been employed to identify analogous male- and female-derived linkage groups.

We describe here the QTL mapping of resistance in cassava to *Xam* by controlled inoculation of the 90 individuals of the F_1 mapping population, and a supplementary 60 progeny. The framework maps were extended to the supplementary individuals for this purpose. We also assess the phenotypic effects of the different alleles at disease resistance QTLs. The two framework maps, the male-, and female-derived maps, were employed in QTL analysis.

Materials and methods

Plant materials

Plant materials used in this study were the 90 individuals of the F_1 progeny employed in generating a molecular genetic map of cassava (Fregene et al. 1997) and 60 supplementary individuals, giving a total of 150 individuals. The F_1 progeny was multiplied in CBB-free fields at CIAT (Palmira, Colombia) in 1997 by planting 30 stakes, 20-cm long, from each individual.

Xam strains

Five *Xam* strains (CIO-1, CIO-84, CIO-136, CIO-295, and ORST X-27), collected from different ecozones (ECZs), were used for controlled inoculations in the greenhouse. These strains belong to different haplotypes; they have been described by Verdier et al. (1994) and Restrepo and Verdier (1997). Strain CIO-295 was isolated in ECZ 2/5 in Colombia in 1996 (altitude tropics and acid soils) and belongs to haplotype C30. For long-term storage, *Xam* strains were conserved in glycerol at –80°C. Strains were streaked 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 the plates incubated at 30°C for 18 h before inoculation.

Greenhouse evaluation of disease resistance

Controlled greenhouse evaluation of disease resistance was carried out on 1-month-old cassava potted plants grown from mature stem cuttings planted in sterilized soil. A standardized method for CBB resistance screening in cassava germplasm (Maraite et al. 1981; Verdier et al. 1993; Restrepo and Verdier 1997) was employed. Stems were inoculated as described by Verdier et al. (1994). Three plants per genotype were inoculated with each strain in the greenhouse under 26°/19°C (day/night temperatures), a 12-h daylight photoperiod, and 93/70% (day/night) relative humidity. Disease progress was monitored 7, 14, and 30 days after inoculation using a symptom severity scale of: 0 = no disease symptoms; 1 = necrosis around the inoculation point; 2 = gum exudation on stem; 3 = wilting of one or two leaves; 4 = wilting of more than two leaves; 5 = complete wilting and dieback. The AUDPC was calculated (Shaner and Finney 1977) and used for statistical analysis and QTL mapping.

Statistical analysis of disease resistance data

Tests for normal distribution of frequency of the AUDPC data were performed by the Shapiro-Wilks W-statistic test, using the program UNIVAR of SAS (SAS Institute, 1989a, b). Correlation between AUDPC data for the different strains was calculated using the statistical computer package "Prophet".

Framework linkage map

A subset of 68 RFLP markers from the female-derived framework map, and 15 from the male-derived map was assayed in the 60 supplementary F_1 individuals. Hybridization protocols were according to Fregene et al. (1997). The computer package MAPMAKER 2.0, running on a Macintosh Centris 650, was used for linkage test (Lander et al. 1987). A LOD score of 4.0 and recombination fraction of 0.40 served as thresholds for declaring linkage.

QTL mapping

Association between molecular markers and plant response to *Xam* was determined by a simple linear regression of AUDPC data, or by a natural log transformation of AUDPC, on marker genotype class means using the computer package Q-GENE 2.30B (Nelson 1997) (single-point analysis) running on a G3 Power Macintosh. The amount of phenotypic variance explained by each marker was obtained from the regression coefficient (r^2 value). Association between a molecular marker and CBB response was considered significant if the probability of observing an r^2 value, based on linear regression, was less than 0.002 (Lander and Botstein 1989). A search for significant inter-loci interaction was done by multiple regression, using Q-GENE's "one- and two- locus tests" command. To confirm the results of the regression analysis on AUDPC data, we employed resistance data, based on an ordinal scale, in the non-parametric Kruskal-Wallis rank-sum test (Lehmann 1975). Interval mapping procedures were not attempted because the linkage phase of markers in an F_1 population is not known. Cassava is highly heterozygous and therefore multiple alleles are also expected in genomic regions controlling the *Xam* response. Allelic bridges, i.e., markers with unique alleles segregating from both parents in the F_1, were employed to evaluate intralocus interactions and to assess effects of different alleles.

Results

Greenhouse evaluation of disease resistance in cassava

TMS 30572 and CM 2177–2, the female and male parents, respectively, of the F_1 progeny, showed different reactions to the *Xam* strains CIO-84, CIO-136, and ORST X-27: the female parent was more resistant to all three strains (Fig 1). Both parents were tolerant to strain CIO-1, while the male parent expressed higher levels of resistance to strain CIO-295. Resistance shown by the parents to these *Xam* strains is average relative to other sources (Restrepo and Verdier 1997). The distribution of response phenotypes, as measured by the area under disease curve (AUDPC), to the five *Xam* strains of the 150 F_1 individuals and their parents is shown as a set of frequency histograms in Fig. 1. AUDPC values vary from 0 to 90 (in arbitrary units). The Shapiro-Wilks test for normality revealed normal response distributions for the F_1 progeny for two of the five strains (Table 1). The three strains, CIO-84, CIO-1, and ORST X-27, with non-normal response distributions, were transformed by natural logs before simple regression analysis. Table 2 shows the

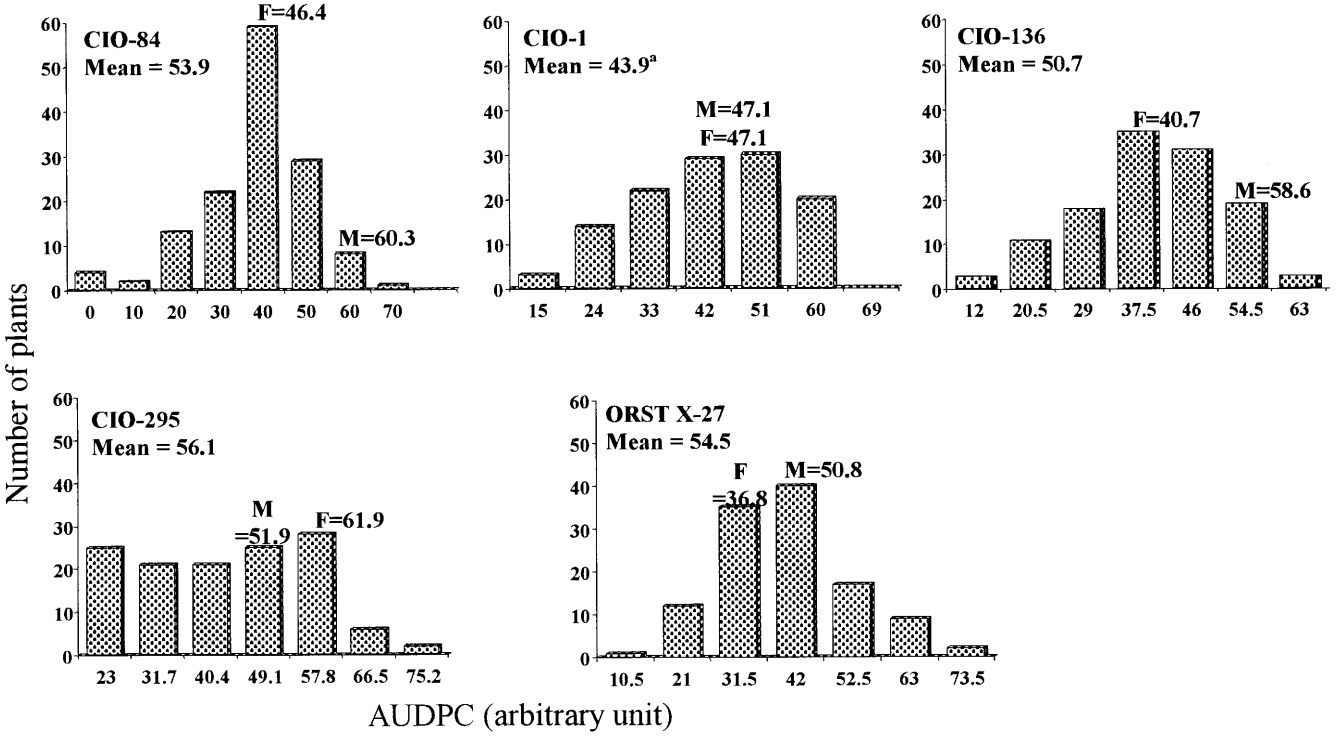

Fig. 1 Distribution of AUDPC values for the parents and 150 F_1 individuals of the cassava cross TMS 30572 (*F*) × CM 2177–2 (*M*) and for each of five strains of *Xanthomonas axonopodis* pv. *manihotis*. *F* Female parent; *M* male parent. [a] The mean is significantly different from those of all other strains

Table 1 W-statistics from the Shapiro-Wilks test for normality of AUDPC values of resistance to five strains of *Xanthomonas axonopodis* pv. *manihotis*

Strain	W statistic
CIO-84	0.96
CIO-1	0.96
CIO-136	0.97[a]
CIO-295	0.97[a]
ORST X-27	0.95

[a] Distribution is not significantly different from normal

Table 2 Correlation coefficients (*r*) with *P* value <0.05 between AUDPCs calculated for different strains of *Xanthomonas axonopodis* pv. *manihotis*

	CIO-84	CIO-1	CIO-136	CIO-295
CIO-1	0.47			
CIO-136	0.28	0.38		
CIO-295	ns[a]	0.29	0.31	
ORST X-27	0.28	0.31	0.19	0.40

[a] Not significantly different from zero

Fig. 2 Location of QTLs (identified by *XM* and *number*) for partial resistance to five strains of *Xanthomonas axonopodis* pv. *manihotis* on the female (♀) and male (♂) derived framework map. The map is based on 150 F_1 individuals from the cassava cross TMS 30572 × CM 2177–2. These results are based on AUDPC data. Distance in centiMorgans (cM) and significance levels are indicated on the *right*. The most significant markers and the corresponding QTLs are indicated in *bold* (see also Table 3)

correlation coefficients calculated among the AUDPC values for each strain.

Framework map

For genetic analysis, we used two framework linkage maps that were derived from the segregation of gametes in the female parent of 150 individuals and in the male parent of 150 individuals in order to obtain a sub-set of molecular markers. The female- and male-derived maps consist of 24 linkage groups each that spanned 1208.3 cM and 1475 cM, respectively, with an average distance of 8.5 cM and 10.9 cM between markers.

Association between molecular markers and resistance

Results of single-marker regression analysis of AUDPC values for each strain are shown in Fig. 2. Twenty-three markers on 5 linkage groups of the female-derived framework were significantly (P <0.002) linked to AUDPC resistance values with the five strains of *Xam*. Markers that accounted for the highest amount of resistance variance, based on single and multiple regression, are shown in large bold letters. Regions of chromosome D and chromosome L were found to be associated with response to *Xam* strains CIO-84 and ORST X-27, explaining more than 60% and 21%, respectively, of disease phenotype variance. Two regions of chromosome N accounted for

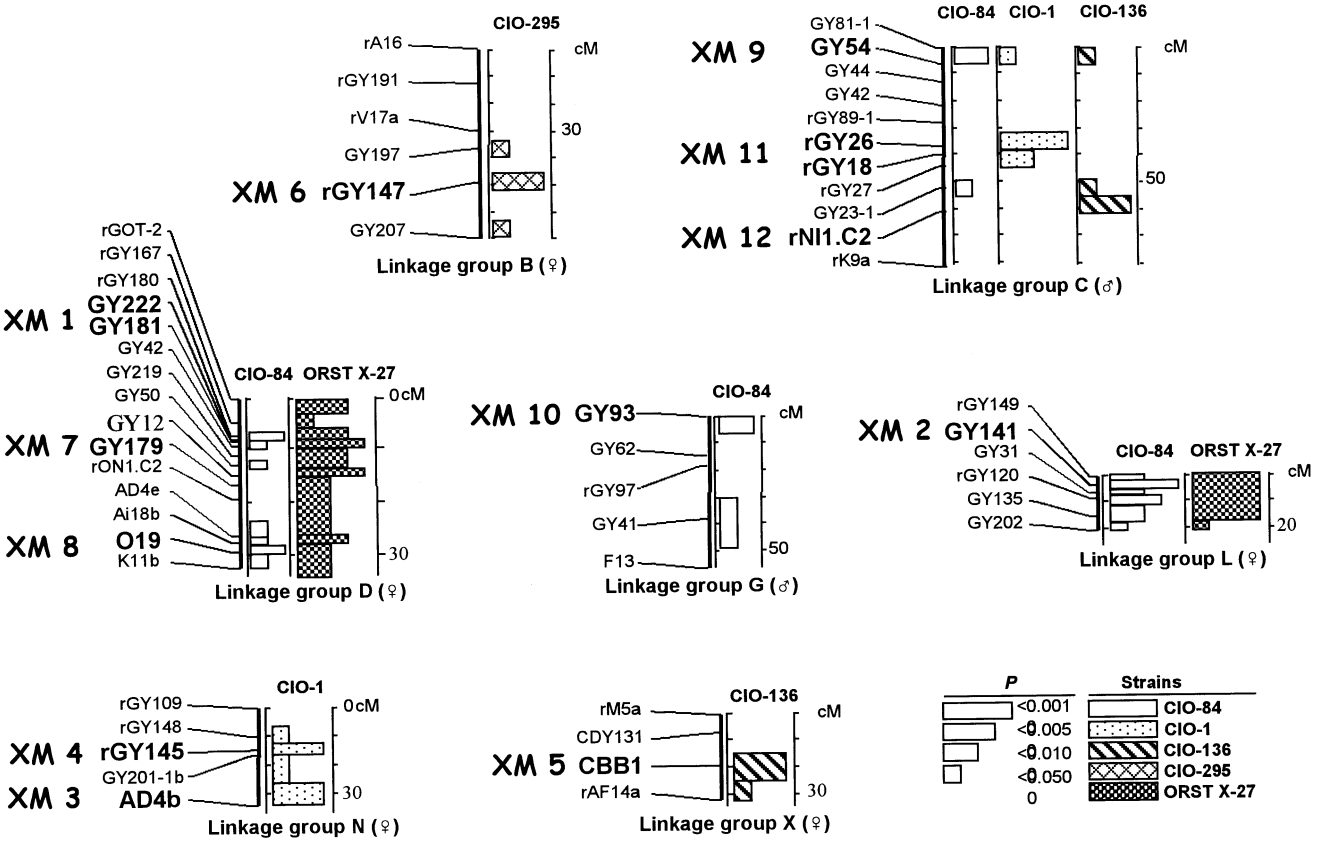

Table 3 QTLs explaining the highest values of variance for resistance in cassava based on AUDPC values for each inoculated strain of *Xanthomonas axonopodis* pv. *manihotis* (see also Fig. 2)

Strain	Map	Linkage group	Marker	Variance explained (%)	Probability	QTL name
CIO-84	Female	D	GY222	11[a]	0.008	XM 1
	Female	L	GY141	10	0.0006	XM 2
	Male	C	GY54	10.7	0.0069	XM 9
	Male	G	GY93	11.2	0.006	XM 10
CIO-1	Female	N	AD4b	13	0.004	XM 3
			rGY145	9	0.001	XM 4
	Male	C	rGY18	11.6	0.0062	XM 11
			rGY26	15.5	0.0007	
CIO-136	Female	X	CBB1	13	0.004	XM 5
	Male	C	rNI1.C2	12.4	0.0032	XM 12
CIO-295	Female	B	rGY147	10	0.003	XM 6
ORST X-27	Female	D	GY181	20	0.0002	XM 1
			GY179	12	0.0002	XM 7
			O19	12	0.006	XM 8
	Female	L	GY141	19	0.0004	XM 2

[a] Trait value for each genotype

22% of the variance to strain CIO-1, while one region each on chromosomes B (10%) and X (13%) were identified as putative QTLs for strains CIO-295 and CIO-136, respectively. Based on the segregation of alleles from the male, five markers were found linked to resistance to three *Xam* strains (CIO-84, CIO-1, and CIO-136). No significant association with resistance to the strains CIO-295 or ORSTX-27 were found. Three regions of the linkage group C explained 10.7%, 27.1%, and 12.4% of the phenotypic variance associated with the response of the CIO-84, CIO-1, and CIO-136 strains, respectively. One region of linkage group G explained 11.2% of the phenotypic variance to CIO-84. The results of the regression analysis are summarized in Table 3. These results are comparable with those from the non-parametric Kruskal-Wallis rank-sum test for disease resistance score based on a qualitative ranking of resistance (data not shown). The F_1 progeny has also been evaluated for resistance to CBB in the field (Villavicencio, Eastern Colombia), in replicated trials, over two growing seasons. *Xam* strains endemic in this area include CIO-1 and CIO-136. The results from QTL mapping of disease resistance are comparable to those obtained with the field evaluation (V. Jorge, unpublished).

Intra-locus and inter-loci interactions of QTLs

The gene-tagging population is an F_1 cross between non-inbred parents, and the genetic maps employed here are drawn from the segregation of unique alleles in the gametes of the female and male parents. An obvious drawback is that F_1 progeny are grouped together according to maternal marker alleles with the paternal alleles being ignored. Important effects segregating from the male parent can be overlooked by considering only maternal segregation data. Markers with unique alleles from both parents, also known as allelic bridges and segregating in a ratio of 1:1:1:1, can be used to resolve this problem. However, only 36 of such markers, distributed on 10 linkage groups, are known for cassava. Two, GY50 and GY120, were found in regions of the QTLs for response to strains CIO-84, and ORST X-27, with significant (P <0.01) linkage to disease response. Disease response data were grouped according to four genotypic classes of the allelic bridges, and the association with *Xam* disease resistance re-analyzed (Table 4). Two measurements of disease resistance were used in this analysis: average disease rating and AUDPC values. In both cases, the results revealed no significant differences in single-marker regression, using the four genotypic classes, compared with only two genotypic classes based on maternal alleles for the same marker. As Table 4 shows, two favorable alleles segregating from the female parent explain why no differences were found, whether the di-allelic or multi-allelic approach was used. Two-loci interaction tests, by multiple regression, revealed a significant (P <0.002) interaction between GY141, on linkage group L, and GY170, on linkage group H, for response to strain CIO-84.

Discussion

Quantitative resistance to *Xam* was studied in a F_1 progeny derived from an interspecific cross between two cassava varieties, TMS 30572 and CM 2177–2. This cross was selected originally to map resistance to the African cassava mosaic disease (ACMD); TMS 30572, the female parent, is highly resistant to ACMD, whereas CM 2177–2 is susceptible. The F_1 mapping population was found to segregate for resistance to different strains of *Xam* and thus was an excellent population for mapping QTLs that control disease resistance. Controlled greenhouse evaluation of disease resistance has previously shown good reproducibility, and correlation with field screening has been demonstrated earlier (V. Verdier, *unpublished*); we therefore used this method for resistance

Table 4 Effect of allele substitution on average disease rating and area under disease progress curve (AUDPC) values in response to two strains of *Xanthomonas axonopodis* pv. *manihotis* in the cassava F_1 mapping population

Marker	Trait	Strain	Multi-allelic analysis[a]						Di-allelic analysis	
			aa	ab	ac	bc	$\frac{aa+ab}{2}$	$\frac{ac+bc}{2}$	a_	c_
GY50	Average disease rating	CIO-84	4.390	3.74	3.18	3.26	4.06	3.22	4.04	3.29
		ORST X-27	4.178	4.04	3.12	2.88	4.11	3.00	4.00	3.01
	AUDPC	CIO-84	65.780	57.66	54.07	52.44	61.72	53.25	61.3	53.70
		ORST X-27	61.120	62.63	53.22	47.35	61.87	50.29	61.6	50.90
			aa	ab	ac	bc	$\frac{aa+ac}{2}$	$\frac{ab+bc}{2}$	a_	b_
GY120	Average disease rating	CIO-84	3.080	3.75	3.40	3.24	3.24	4.070	3.25	4.12
		ORST X-27	3.043	3.48	3.52	3.28	3.28	3.765	3.24	3.78
	AUDPC	CIO-84	50.43	58.49	54.55	52.49	52.49	63.550	52.4	62.60
		ORST X-27	48.67	58.02	54.63	51.65	51.65	59.460	51.3	61.50

[a] a, b, and c are different alleles at RFLP marker loci segregating from female and male parent

evaluation. The five *Xam* strains employed were selected on the basis of their (1) their ecozone of origin; (2) haplotypes based on the RFLP pattern observed with the *pth*B probe (Restrepo and Verdier 1997), which corresponds to a pathogenicity gene related to the *avrBs*3 gene family (Verdier et al. 1996); and (3) capacity to induce differential responses on a set of cassava cultivars that included the parents of the F_1 progenies (Restrepo 1999).

Using the five *Xam* strains, we characterized 12 QTLs that explained 9% to 27.1% of the phenotypic variance, suggesting that several genes are probably required for resistance to CBB. This finding agrees with what is known about cassava's response mechanism to *Xam*, which has been found to involve a series of different events, including several biochemical and enzymatic reactions (Kpémoua et al. 1996) that inhibit cell to cell movement of the bacteria. Several studies in other crops have also shown a quantitative nature of resistance to phytopathogenic bacteria (Camargo et al. 1995; Sandbrink et al. 1995; Zhikang et al. 1995; Jung et al. 1996; Miklas et al. 1996; Thoquet et al. 1996; El Attari et al. 1998). The number of markers found to be involved in resistance in these studies, based on greenhouse tests, varies between 5 and 32 markers. QTLs XM 3 to XM 6 are specific to strains CIO-1, CIO-136, and CIO-295, whereas QTL XM 1 and XM 2 are common to CIO-84 and ORST X-27. Similar results were observed in a study of resistance to *Phytophthora infestans* in potato, using two strains of the pathogen. It revealed 5 QTLs involved in non-specific resistance and 14 QTLs involved in specific resistance to race 0 or race 1 of *P. infestans* (Leonards-Schippers et al. 1994). Regions of the cassava genome involved in resistance to *Xam* differ according to the strain inoculated, indicating that specific resistance factors are probably involved with respect to the different *Xam* strains. This hypothesis would be reinforced by testing multiple strains per haplotype. The main difference between each strain used in this study is based on their RFLP pattern, using a pathogenicity gene (*pth*B, Restrepo and Verdier 1997) and their pathotypes (Restrepo 1999). These pathogenicity factors may interact with resistance factors in a quantitative mode.

Several F_1 progenies show a higher level of resistance than the parents for all *Xam* strains used, a phenomenon known as transgressive segregation. Blocks of dominant genes from both parents is to date the most convincing explanation of transgressive segregation (Bingham 1998). This suggests that some QTLs for CBB resistance do show dominance gene action. Evidence for this can be found in the intra-locus interaction analysis of QTLs controlling resistance to strains CIO-84 and ORST X-27. Two alleles from the female parent account for a certain level of resistance, irrespective of the other allele from the male parent. Furthermore, QTLs with a positive effect on CBB resistance have been detected from both female and male parents.

This is the first report of a QTL analysis in cassava, and also the first to identify that genes control CBB resistance in cassava. Identifying markers for CBB resistance can be used to increase the efficiency of selecting resistant genotypes for use as parents in cassava breeding. Cassava is a long-cycle crop (8 to 12 months), and resistance to CBB is routinely screened over three to four crop cycles. In addition cassava breeders currently have to contend with the inconvenience of escapes from inefficient natural infection or mass inoculation methods. Evaluation of the F_1 progeny has been conducted under natural field infection conditions in Colombia to confirm results obtained in the greenhouse, the results of which reveal the robustness of some of the QTLs detected (V. Jorge, *unpublished*).

Several genes conferring resistance to pathogens have been characterized at the molecular level, and most of these genes are probably involved in signal transduction mechanisms (Baker et al. 1997). One gene (*Xa*21), conferring resistance to six races of the xanthomonad *X. oryzyae* in rice, was employed to clone a homologue in

cassava, CBB1 (Bonierbale et al. 1997). The cassava homologue was mapped to the existing cassava map, on linkage group X, where it showed a significant association with resistance to the CIO-136 *Xam* strain (Fig 2). The *Xa*21 gene in rice confers resistance by pathogen recognition. Our results therefore suggest that pathogen recognition, through a specific interaction between pathogen strain and plant resistance factors, may also be involved in *Xam* response in cassava. This finding is of interest and could further clarify the evolution of genes governing plant response to a family of related pathogens.

QTLs for resistance against two strains, CIO-84 and ORST X-27, appear to be introgressions from a wild *Manihot* species. These QTLs are located on linkage group D of the female-derived map, which has a large number of polymorphic markers and shows much lower recombination frequency than the rest of the genome. TMS 30572, the female parent, is a third backcross derivative of an inter-specific cross involving cassava and a wild relative, *M. glaziovii*. The nature of linkage group D suggests that it is a vestige of the *M. glaziovii* genome, implying that resistance to at least two strains of *Xam* may have introgressed from this wild relative.

Future activities include adding more allelic bridges to the genetic map in order to study, on a larger scale, intra-locus interactions. Markers such as simple sequence repeats will be useful because of their higher levels of polymorphism. In addition, a second population of 244 individuals has been developed at CIAT by backcrossing 5 F_1 individuals to the female parent TMS 30372. The BC_1 progeny has been evaluated by greenhouse inoculation for CBB resistance and is currently being genotyped with molecular markers from the framework map. Further information with respect to meiosis will lead to a more precise mapping of CBB resistance, confirm the genetic model of resistance, and will provide individuals that are homozygous for some QTLs, thus permitting identification of resistance loci with recessive effects. Fine mapping of regions involved in resistance will allow contiguous mapping with candidate clones from a cassava BAC library already available (M. Fregene *unpublished*), then to the eventual cloning of resistance genes. This will facilitate, through genetic transformation, a more efficient movement of resistance genes in adapted germplasm to susceptible cassava varieties.

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