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Kerstin Wydra, Valerien Zinsou, Véronique V. Jorge, Valérie Verdier

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K. Wydra, V. Zinsou, V. Jorge, and V. Verdier

First and second authors: Institute of Plant Diseases and Plant Protection, University of Hannover, Herrenhäuser Str. 2, 30419, Germany; second author: Faculté des Sciences Agronomique, Université Nationale du Bénin, Cotonou, Benin; and third and fourth authors: Centro Internacional de Agricultura Tropical (CIAT), Institut de Recherche pour le Développement (IRD), Apartado Areo 6713, Cali, Colombia.

Current address of V. Jorge: INRA—Unité Amélioration, Génétique et Physiologie Forestières, Avenue de la Pomme de Pin, BP 20619 Ardon, 45166 Olivet Cedex, France.

Current address of V. Verdier: Laboratoire Génome et Développement des Plantes, UMR CNRS 5096, Université de Perpignan, 52, Avenue de Villeneuve, 66680 Perpignan, France.

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ABSTRACT

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Cassava suffers from bacterial blight attack in all growing regions. Control by resistance is unstable due to high genotype–environment interactions. Identifying genes for resistance to African strains of *Xanthomonas axonopodis* pv. *manihotis* can support breeding efforts. Five F_1 cassava genotypes deriving from the male parent 'CM2177-2' and the female parent 'TMS30572' were used to produce 111 individuals by backcrossing to the female parent. In all, 16 genotypes among the mapping population were resistant to stem inoculation by four strains of *X. axonopodis* pv. *manihotis* from different locations in Africa, and 19 groups with differential reactions to the four strains were identified, suggesting that the strains represent different pathotypes. Four genotypes were resistant to leaf inoculation, and three were resistant to both stem and leaf inoculations. Genotypes with susceptible, moderately resistant, and resistant reactions after leaf and stem inoculation partly differed in their reactions on leaves and stems. Based on the genetic map of cassava, single-marker regression analysis of disease severity after stem-puncture inoculation was performed. Eleven markers were identified, explaining between 16 and 33.3% of phenotypic variance of area under disease progress curve. Five markers on three and one linkage groups from the female- and male-derived framework of family CM8820, respectively, seem to be weakly associated with resistance to four strains of *X. axonopodis* pv. *manihotis*. Based on the segregation of alleles from the female of family CM8873, one marker was significantly associated with resistance to two *X. axonopodis* pv. *manihotis* strains, GSPB2506 and GSPB2511, whereas five markers were not linked to any linkage group. The quantitative trait loci (QTL) mapping results also suggest that the four African strains belong to four different pathotypes. The identified pathotypes should be useful for screening for resistance, and the QTL and markers will support breeding for resistance.

Additional keyword: molecular markers.

Cassava is a basic component of the farming system in most areas of Sub-Saharan Africa (36). It provides more than 60% of the daily calorie intake of some 500 million people in the sub-Saharan region of Africa (14). Bacterial blight, caused by *Xanthomonas axonopodis* pv. *manihotis* Vauterin et al. (46), previously *X. campestris* pv. *manihotis* Bondar (7), is one of the most important cassava diseases in Africa (3,52,54). Symptoms include angular leaf spots, blight, wilting, and stem dieback after systemic infection. Among the proposed control measures (50,53), host-plant resistance is the most suitable for farmers. Resistance of African cassava cultivars to bacterial blight originated from interspecific-cross breeding with the wild species *Manihot glaziovii* and is assumed to be polygenic (19). Resistance in cassava is quantitative (23), because a hypersensitive reaction has not been reported in cassava cultivars in the interaction with *X. axonopodis* pv. *manihotis* strains. Race- and cultivar-specific reactions never were observed between cassava cultivars and *X. axonopodis* pv. *manihotis* strains, although pathotypes and haplotypes were evident among Latin American strains (38–40). The variability of *X. axonopodis* pv. *manihotis*, based on pathogenicity and on physiological, biochemical, and molecular characterization, revealed greater diversity in Latin America than in Africa (2,6,15,18,38–40,47,51).

Quantitative trait loci (QTL) mapping can reveal the regions of specific resistance loci and may identify race-specificity of partial resistance genes (57). For instance, molecular genetic mapping of resistance to bacterial blight in rice revealed that a single locus confers resistance to a specific race of the pathogen (30,56). In potato, 5 of the 11 genomic regions showed no specificity against the two races of *Phytophthora infestans* tested. However, the six others were effective against just one race (29). Several QTL are known against bacterial wilt of tomato, with loci on chromosome six playing a predominant role in resistance to *Ralstonia solanacearum* (11,37,44,48). Separate QTL may be responsible for different resistance mechanisms. In soybean, QTL on two linkage groups conferred separate components of resistance to *Fusarium solani* f. sp. *glycine*, causing the sudden death syndrome (33,34).

Resistance in the form of preformed mechanisms, such as deposition of lignin and other phenolic compounds (16,26,37), as well as latex and leaf pectins (50) were suggested to be involved in resistance of cassava against bacterial blight. Genetics of resistance to bacterial blight in cassava and genes governing resistance

Corresponding author: K. Wydra; E-mail address: wydra@ipp.uni-hannover.de

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were investigated by inoculating a mapping population of 244 genotypes with four Colombian and one African strain of *X. axonopodis* pv. *manihotis* (21–23). Six markers were involved in resistance to these strains.

The objectives of the present studies were to (i) test 111 genotypes of the mapping population derived from an intraspecific cross of 'TMS30572' and 'CM2177-2' for their reaction to bacterial blight using leaf and stem inoculation methods, and identify (ii) possible *X. axonopodis* pv. *manihotis* pathotypes and (iii) bacterial blight resistance-related genetic markers.

MATERIALS AND METHODS

Cassava genotypes. The five F_1 genotypes used in this study were chosen for their fertility from the cassava mapping population comprising 90 F_1 plants from an intraspecific cross. TMS30572 was the female parent, an elite cassava cultivar developed at the International Institute of Tropical Agriculture (IITA), Nigeria, and CM2177-2, a successful cassava cultivar resulting from breeding at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, was the male parent. Genotype TMS30572 is partly resistant (1,13,55) and genotype CM2177-2 is tolerant to cassava bacterial blight. The overall crossing scheme was designed to combine the resistance of TMS30572 with the tolerance and quality of CM2177-2. Genotype TMS30572 derives from an interspecific cross of *M. esculenta* genotype F279 (Java) and *M. glaziovii*, of which the hybrid has been backcrossed and the progeny selected for several characters and pollinated with several landraces, resulting in genotype TMS30572. In our previous studies, genotype TMS was the overall most resistant genotype across ecozones; nevertheless, it showed susceptible reactions in some environments (3,13,61). Cassava genotypes used in this study were the two largest families of backcross (BC_1) populations, CM8820+B (= CM8820 × CM8820B, the reciprocal cross) and CM8873+B (= CM8873 × CM8873B, the reciprocal cross) derived from the backcross of two F_1 genotypes (CM7857-4 and CM7857-77) with TMS30572, from which the genetic map was built (17,21). In all, 111 genotypes derived from the backcross of five F_1 individuals (CM7857-4, CM7857-10, CM7857-51, CM7857-77, and CM7857-115) to the female recurrent parent TMS30572 were used. These genotypes were grouped in seven populations (CM8820 and CM8820B [reciprocal cross], CM8870, CM8872, CM8873, and CM8873B [reciprocal cross], CM8877) (Table 1). Genotype 'Ben86052' was used as a susceptible reference genotype (13).

All experiments were conducted in the greenhouse. Stem cuttings 20 cm in length from apparently healthy plants were received from IITA, Nigeria. They were planted in plastic pots 16 cm in diameter containing sandy soil at the IITA station in Cotonou, Benin, in six replicates per inoculated bacterial strain. After 6 weeks, plants were transferred to the glasshouse (26°C, 70% relative humidity) for inoculation and the subsequent studies. Six plants per bacterial strain and per genotype were used. Plants were kept under quarantine conditions and no diseases other than bacterial blight occurred. IITA Ibadan, Nigeria, could not provide sufficient planting material for all the trials (e.g., the leaf infiltration) because only a few individual plants were kept from the mapping population. Due to these obstacles and the space-consuming trial set up under greenhouse conditions, repetitions of trials were not possible, though six replicates were used under controlled conditions.

Bacterial strains and their origin. The three highly virulent strains of *X. axonopodis* pv. *manihotis*, GSPB2506, GSPB2507, and GSPB2511 (Göttinger Sammlung Phytopathogener Bakterien, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Germany), were isolated by K. Wydra, IITA, in Cotonou, Benin, and Ibadan and Onne, Nigeria, respectively. The strain Uganda 12 was isolated by B. Boher, IRD France, in Uganda. The four strains were used for stem inoculation. In addition, strains GSPB2506, GSPB2511, and Uganda 12 also were used for leaf infiltration inoculations.

Stem inoculation and symptom evaluation. Four-week-old plants of 111 genotypes (six plants per genotype) were inoculated in the stem at the third leaf axil from the top by inserting a sharp toothpick, contaminated with $\approx 10^7$ CFU/ml by passing through a 48-h-old culture of *X. axonopodis* pv. *manihotis* (G. Sanchez, S. Restrepo, and V. Verdier, *unpublished data*) grown on nutrient glucose agar (NGA: nutrient broth, 8 g/liter; glucose, 11 g/liter; yeast extract, 3 g/liter; agar, 15 g/liter; pH 7.2). Plants were evaluated 5, 10, 15, 20, 25, and 30 days after inoculation. Disease scores were 0 = no symptom, 1 = 1 leaf wilted, 2 = 2 to 4 leaves wilted, 3 = more than 4 leaves wilted, 4 = dieback of plant or shoot. The area under the disease progress curve (AUDPC) was calculated for each inoculated plant from the disease reaction scores 5, 10, 15, 20, 25, and 30 days after inoculation by using the following formula $\text{AUDPC} = \Sigma_i[(D_i + D_{i-1}) \times (t_i - t_{i-1})]/2$, where D_i = disease score at time t_i using the 0-to-4 scores and t_i = time measured in days after inoculation (41). AUDPC was calculated to compare the resistance of each genotype to the four strains and the aggressiveness of strains toward the cassava genotypes after inoculation of stems. On the basis of the percentage of AUDPC of each strain used in stem inoculations (the reaction on the highly susceptible genotype CM8820-47 being set as 100%), resistant (0 to 33.2%), moderately resistant (33.3 to 49.9%), and susceptible genotypes (50 to 100%) were identified for each strain inoculated. Calculating the mean AUDPC values of the four strains per genotype, groups of resistant, moderately resistant, and susceptible genotypes were defined using the same percentage ranges as above. The strains were ranked for their virulence considering their reaction on all the genotypes (GSPB2507 = GSPB2511 > Uganda 12 > GSPB2506). To identify differential reactions, AUDPC values of the four strains were compared for each genotype, and genotypes with values differing by ≥33% in the reverse direction of GSPB2507 = GSPB2511 > Uganda 12 > GSPB2506, in order of decreasing virulence, were selected as differential genotypes. Additionally, genotypes for which strains differed by ≥43% were defined as differential genotypes. Evaluations following these criteria allowed the most detailed description of the disease reactions. Incremental principal component analysis (IPCA) based on AUDPC values after stem inoculation of 43 genotypes was established to demonstrate the position of differential genotypes compared with the other genotypes.

TABLE 1. Cassava genotypes derived from the backcross of five F_1 individuals (CM7857-4, CM7857-10, CM7857-51, CM7858-77, and CM7857-115) to the female recurrent parent TMS30572

Population	Genotype code
CM8820	1, 2, 4, 5, 9, 11, 12, 13, 16, 19, 23, 27, 30, 31, 32, 33, 34, 38, 40, 43, 46, 47, 48, 50, 53, 56, 58, 61, 64, 67, 70, 72, 75
CM8820B	3, 6, 7, 8, 10, 12, 13, 14, 17, 20, 21, 23, 25
CM8870	1, 3, 6, 13, 14, 15, 17
CM8872	3, 13, 14, 15, 16
CM8873	1, 7, 11, 12, 13, 14, 18, 25, 28, 30, 31, 35, 38, 39, 40, 42, 44, 48, 54, 58, 63, 64, 67, 69, 71, 74, 77, 78, 80, 81, 84, 86
CM8873B	1, 3, 5, 7, 9, 14, 15, 16, 17, 20, 25, 31, 35, 36
CM8877	6, 8, 9, 11, 13, 15, 52
F_1 individuals	CM7857-4, -10, -51, -77, -115

Leaf infiltration and symptom evaluation. Eight genotypes identified as resistant and moderately resistant in the previous stem inoculation test (CM8873-64, CM8873-69, CM8820-34, CM8820-40, CM8877-11, CM8820B-23, CM8870-1, and CM8820-30) were used. The abaxial surfaces of the first two nearly fully expanded leaves were infiltrated by means of a glass atomizer (20) with a bacterial suspension of *X. axonopodis* pv. *manihotis* (10^6 cells/ml) in 0.01 M $MgSO_4$ obtained from 48-h-old strains on NGA agar. Infiltration was carried out until a temporary water-soaking appeared without leaf damage. Two leaves per plant and five plants per genotype and bacterial strain were used. Control plants were treated similarly with sterile 0.01 M $MgSO_4$ solution. The area of water-soaked and necrotic lesions on leaves was determined each third day starting 14 days postinoculation (dpi) using a transparent plastic sheet on which the areas were reproduced and calculated. The two-dimensional biplot of the principal component analysis (PCA) on 10 leaf-infiltrated genotypes based on the AUDPC (leaf-AUDPC) was calculated from the lesion area measures 14, 17, 20, 23, 26, and 29 dpi by using the formula above, with D lesion area on leaves, t days after inoculation, and i evaluation days. The lesion area of dropped leaves was added to the measured area at each evaluation date. Genotypes were identified as resistant (AUDPC 0 to 16.6%), moderately resistant (16.7 to 33.2%), and susceptible (33.3 to 100%) compared with the reaction of the most susceptible reference genotype Ben86052, which was set as 100%. The leaf-AUDPC of three strains was established to compare genotypes and demonstrate their relatedness in reaction.

Restriction fragment length polymorphism analysis. A set of 58 restriction fragment length polymorphism (RFLP) markers was selected from markers of the cassava genetic map. They were distributed on 17 of the 20 linkage groups of the published linkage map (17). The other three linkage groups did not carry RFLP markers or the RFLP was not polymorphic in the population. Hybridization protocols were identical to those published (17).

Microsatellites. Screening for simple sequence repeats (SSRs) and design of primers has been performed previously (17,32). A set of 63 microsatellites were chosen to evaluate the BC_1 population and construct the genetic map. DNA amplification was performed in 25 µl of the following reaction: 25 µg of DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM each primer, and 2.5 units of Ampli-*Taq* (Perkin-Elmer-Cetus, Emeryville, CA). Amplification was performed according to the following steps: one denaturation cycle was performed at 94°C for 5 min, prior to 34 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min; and a final extension at 72°C for 5 min. Polymerase chain reaction-amplified products were separated on 6% polyacrylamide gels for ≈2 h. Gels were silver stained and dried before reading. Scoring was repeated twice, by two different readers.

Construction of linkage map and QTL analysis of cassava bacterial blight resistance. Linkage maps were constructed using the pseudotestcross strategy and the Mapmaker program (28) as previously explained (17). In this strategy, only markers segregating 1:1 were considered and linkage analysis was performed separately for markers coming from female and male parents, considering each case as a backcross-type population. We built two linkage maps, one for each parent, using the 58 RFLP and 63 SSR markers from 17 of the 20 linkage groups from the original map (17,32), while three linkage groups did not carry any RFLP markers or the RFLP markers were not polymorphic in our population. A log of the likelihood ratio (LOD) score, representing the likelihood ratio between the two best maps, of 3.0 and recombination fraction of 0.30 served as the threshold for declaring linkage. Orders of markers were verified by the "ripple" command (LOD threshold at 2.0). The 67 and 56 markers of female and male maps, respectively, of family CM8820+B were associated in 18 linkage groups. Also, 50 and 45 markers identified for the female and male map, respectively, of family CM8873+B were associated in 18 linkage groups.

A simple linear regression between molecular markers and AUDPC of *X. axonopodis* pv. *manihotis* strains was performed per marker with the QTL cartographer program (QTL Cartographer version 1.13; Department of Statistics, Raleigh, NC) (5). We considered also, in this case, two linkage maps, one derived from each parent. For each marker, two classes were used: presence (A_1A_0) and absence (A_0A_0) of the marker; and regression analysis using the linear model $y_i = b_0 + b_1x_i + e$, where y_i is the phenotype of the ith individual and x_i is an indicator variable for the marker phenotype (for backcrosses $0 = A_0A_0$ or $1 = A_0A_1$), and b_0 and b_1 are the basic parameters of the linear regression. A significant association between DNA marker and cassava bacterial blight resistance was declared, when the probability obtained from regression was equal to or less than 0.05 and the likelihood ratio (LR) statistic was more or equal to 6 in order to minimize the detection of false positives. The phenotypic variance explained by each marker was computed as sum of squares associated with the marker per total sum of squares.

RESULTS

Stem inoculation. In stem inoculations of 111 genotypes with four highly virulent *X. axonopodis* pv. *manihotis* strains from diverse African origin, 16 genotypes showed a resistant reaction, 26 showed a moderately resistant reaction, and 69 a susceptible reaction (data not shown) (58). Strains GSPB2507 and GSPB2511 were the most virulent, followed by Uganda 12 and GSPB2506, with mean values per strain between 28.3 and 37.0 considering the 111 genotypes (Table 2). Among the 111 genotypes, 29 genotypes expressed a differential reaction to the four strains. Additionally, values of all the resistant genotypes and representatives of the moderately resistant and susceptible groups are given, demonstrating the high variability in these groups. The reference genotypes BEN86052 and TMS30572 were susceptible and resistant, respectively.

From the stem inoculation data, a set of 19 differential genotype groups from 29 genotypes were identified after analyzing strain–genotype interactions (Table 3). The susceptible (S), moderately resistant (MR), and resistant (R) reactions of genotypes to the four strains suggested that the strains were of four different pathotypes. Using a more stringent differentiation by combining MR and S reactions resulted in the formation of eight differential groups of genotypes (data not shown). The two-dimensional biplot of the PCA demonstrates the positions and relatedness of genotypes in terms of their reaction to the four strains (Fig. 1). In all, 12 genotypes showing low means of stem-AUDPC, with lowest values for genotypes CM8877-11 (G42) and CM8873B-25 (G43), belonged to the resistant group; 9 genotypes (G23 to G30 and G41) were moderately resistant; and 22 genotypes with high means of stem-AUDPC, with highest values for genotypes CM8820-47 (G1), CM8873-44 (G2), and CM8873-74 (G3), belonged to the susceptible group. Eleven genotypes with high differential reactions (IPCA 2 > 0.5) and nine genotypes with low differential reactions (IPCA 2 < –0.5) were identified. An additional 9 genotypes, identified as differential genotypes (Table 3), and 14 nondifferential genotypes were located closer to the horizontal zero line.

Leaf inoculation. Considerable differences in lesion areas and day of first leaf drop were observed when eight stem-resistant genotypes, the susceptible genotype BEN86052, and the resistant TMS30572 were leaf infiltrated with three highly virulent strains of *X. axonopodis* pv. *manihotis* (GSPB2506, GSPB2511, and Uganda 12) (Table 4). Total lesion areas of inoculated leaves continued to increase in some genotypes after the first leaf drop (data not shown). Thus, the resistant genotypes TMS30572, CM8820-40, CM8820-30, and CM8873-69 developed limited lesion areas,

with late (23 to 26 dpi), early (17 dpi), late, or no leaves shed, respectively. The susceptible genotypes developed large lesion areas and shed leaves early or late. Genotypes CM8873-69, CM8820-30, and CM8820-40 were identified as resistant; CM8870-1 and CM8870-11 as susceptible due to their reaction to strain GSPB2506; and the susceptibility of BEN86052 and general resistance of TMS30572 were confirmed. Strain GSPB2506 was most virulent, followed by Uganda 12 and GSPB2511. Differential reactions (S and R) suggest occurrence of two pathotypes, strains Uganda 12 and GSPB2506, differentiated by their resistant and susceptible reactions on genotypes CM8877-11 and CM8870-1 (Table 5). Genotypes (i) Ben86052, (ii) CM8877-11 and CM8870-1, and (iii) the genotypes showing moderately resistant and resistant reactions after leaf infiltration are suitable to differentiate the two pathotypes.

The two-dimensional biplot of the PCA of genotype reactions after leaf infiltration, showing the relatedness of genotypes and comparing their reaction to three inoculated strains, revealed—additionally to the differential genotypes CM8873-69 and TMS30572 with high and CM8873-64 with low IPCA 2 scores—as genotypes with diverse reactions to the inoculated strains (data not shown). The individuals of the mapping population selected for resistance to stem inoculation are located far from the highly susceptible reference genotype BEN86052 with high IPCA 1 score.

TABLE 2. Reaction of a set of 29 differential cassava genotypes, 4 genotypes with representative susceptible and moderately resistant reactions, and 8 resistant genotypes selected from 111 BC_1 mapping population genotypes after stem puncture inoculation with four highly virulent strains of *Xanthomonas axonopodis* pv. *manihotis*

	Stem-AUDPC for bacterial strain[s]					
Cassava genotype	GSPB2507	GSPB2511	Uganda 12	GSPB2506	Mean[t]	Reaction[u]
G1 CM8820-47	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0	60.8 ± 2.7	60.2	S[v]
G2 CM8873-44	36.6 ± 5.9	56.0 ± 2.2	60.0 ± 0.0	57.5 ± 0.7	52.5	S[w]
G3 CM8873-74	50.8 ± 9.4	52.9 ± 1.3	37.5 ± 1.4	60.0 ± 0.0	50.3	S[w]
G4 CM8873-48	36.5 ± 3.0	58.3 ± 1.9	50.0 ± 8.8	22.5 ± 3.3	41.8	S[w]
G5 CM8873-81	30.0 ± 0.0	60.0 ± 3.8	42.5 ± 2.2	30.0 ± 0.0	40.6	S[w]
G6 CM8820-61	48.3 ± 0.9	34.5 ± 1.8	20.0 ± 0.0	52.5 ± 0.0	38.8	S[w]
G8 BEN86052[x,y]	37.5 ± 5.0	40.8 ± 2.2	42.9 ± 2.7	33.7 ± 5.4	38.7	S
G7 CM8820B-17	28.7 ± 3.6	49.0 ± 3.3	35.0 ± 0.0	40.0 ± 2.2	38.2	S[w]
G9 CM8820-16	50.0 ± 0.0	26.2 ± 6.3	25.0 ± 0.0	47.5 ± 2.4	37.2	S[w]
G10 CM8877-9	34.0 ± 7.1	52.5 ± 2.2	40.0 ± 0.0	13.2 ± 1.8	34.9	S[w]
G11 CM8873-39	29.1 ± 4.7	48.7 ± 2.0	30.8 ± 2.1	30.4 ± 3.7	34.8	S[w]
G12 CM8820-46	23.3 ± 5.4	41.2 ± 1.9	42.0 ± 1.7	30.6 ± 8.6	34.3	S[w]
G13 CM8873-7	35.8 ± 1.2	27.9 ± 4.1	48.7 ± 4.5	24.1 ± 4.9	34.1	S[w]
G14 CM8873B-1	27.9 ± 4.3	39.1 ± 6.7	51.2 ± 4.6	16.2 ± 4.0	33.6	S[w]
G15 CM8873B-7	45.4 ± 4.8	32.5 ± 9.1	42.5 ± 3.8	13.7 ± 4.2	33.5	S[w]
G16 CM8820-56	34.1 ± 9.7	54.5 ± 9.4	32.0 ± 1.2	12.5 ± 0.0	33.3	S[w]
G17 CM8820B-25	44.5 ± 2.2	44.5 ± 0.0	0.0	42.0 ± 1.9	33.0	S[w]
G18 CM8873-35	19.1 ± 2.5	32.0 ± 1.7	40.0 ± 0.0	35.8 ± 5.0	31.7	S[w]
G19 CM8820-13	22.5 ± 5.2	23.3 ± 4.8	52.5 ± 0.0	28.3 ± 9.5	31.7	S[w]
G20 CM8873B-31	50.0 ± 0.0	20.8 ± 7.0	33.7 ± 4.4	20.8 ± 7.7	31.3	S[w]
G21 CM8820-9	36.2 ± 6.8	32.9 ± 6.3	32.9 ± 6.2	22.0 ± 3.2	31.0	S[v]
G22 CM8877-52	30.0 ± 3.6	46.0 ± 7.9	24.1 ± 0.9	37.0 ± 5.5	30.7	S[v]
G23 CM8820-38	24.0 ± 3.3	30.0 ± 0.0	46.6 ± 1.2	18.5 ± 2.7	29.8	MR[w]
G24 CM8820B-7	25.8 ± 4.3	37.5 ± 1.4	50.4 ± 2.7	0.0	28.4	MR[w]
G25 CM8873B-20	22.0 ± 5.9	44.1 ± 10.2	35.4 ± 7.6	9.5 ± 3.1	27.8	MR[w]
G26 CM8873B-36	31.6 ± 6.4	0.0	35.8 ± 2.0	27.5 ± 3.9	23.7	MR[w]
G27 CM8820-4	37.5 ± 0.0	27.5 ± 0.0	26.2 ± 0.6	0.0	22.8	MR[w]
G28 CM8873-25	32.9 ± 2.2	27.5 ± 0.7	2.5 ± 0.0	27.9 ± 3.0	22.7	MR[w]
G29 CM8873B-16	18.7 ± 1.6	31.6 ± 4.2	17.9 ± 2.7	16.5 ± 2.6	22.2	MR[v]
G30 CM8820-64	25.0 ± 3.0	45.0 ± 5.2	11.8 ± 3.1	0.0	20.5	MR[w]
G31 CM8820-30[y]	30.4 ± 0.4	21.5 ± 4.4	18.6 ± 5.9	11.2 ± 1.7	20.4	MR[v]
G32 CM8873-86	15.4 ± 0.4	37.9 ± 5.6	15.8 ± 0.6	9.5 ± 2.2	19.7	R[w]
G33 CM8873-69[y]	25.0 ± 4.1	21.5 ± 4.4	17.5 ± 4.3	12.5 ± 0.0	19.1	R
G34 CM8873-78	17.5 ± 1.8	45.0 ± 2.0	0.0	13.3 ± 2.9	19.0	R[w]
G35 CM8870-1[y]	16.2 ± 0.6	16.2 ± 2.0	16.6 ± 2.2	15.0 ± 1.9	16.0	R
G36 CM8873-64[y]	34.1 ± 7.5	22.9 ± 7.7	6.8 ± 1.3	0.0	16.0	R[w]
G37 CM8870-17	40.0 ± 8.6	0.0	5.0 ± 0.0	17.0 ± 3.8	15.5	R[w]
G38 TMS30572[x,y]	13.0 ± 1.3	18.3 ± 2.4	16.6 ± 1.2	10.0 ± 1.4	14.5	R
G39 CM8820B-23[y]	14.5 ± 0.4	21.8 ± 8.2	15.0 ± 0.0	3.1 ± 0.4	13.6	R
G40 CM8820-34[y]	25.0 ± 2.9	15.6 ± 2.1	11.6 ± 2.1	0.0	13.1	R
G41 CM8820-40[y]	19.0 ± 2.2	12.5 ± 0.0	11.6 ± 0.4	6.8 ± 0.4	12.4	R
G42 CM8877-11[y]	10.0 ± 0.0	6.8 ± 1.3	0.0	0.0	14.2	R
G43 CM8873B-25	0.0	0.0	0.0	0.0	0.0	R
Means of 41 genotypes[z]	28.7	31.6	22.6	20.1	…	…
Means of 111 genotypes[z]	37.0	36.8	32.9	28.3	…	…

[s] Stem-AUDPC = area under disease progress curve after stem inoculation. *X. axonopodis* pv. *manihotis* strains from Ibadan (GSPB2507) and Onne (GSPB2511), Nigeria, Uganda (Uganda 12), and Cotonou (GSPB2506), Benin.

[t] Mean of highest stem-AUDPC values of four strains used as 100% to determine the reaction group.

[u] S = susceptible, means of AUDPC of four strains correspond to 50 to 100% of highest mean AUDPC with values 30.1 to 60.2; MR = moderately resistant, 33.3 to 49.9% with values 20.1 to 30.1; and R = resistant, 0 to 33.2%, with values 0 to 20.0.

[v] Genotypes selected among the 111 tested genotypes, which show highest or lowest reactions in the susceptible and moderately resistant groups; all genotypes of the resistant group are included in the table.

[w] Genotype belongs to the groups of differential genotypes.

[x] BEN86052 and TMS30572 are susceptible and resistant controls, respectively.

[y] Genotype selected for leaf inoculation.

[z] Mean of 41 and 111 (data not shown; 65) individuals of the mapping population without consideration of the control genotypes.

Comparison of reaction of genotypes to stem and leaf inoculation. Among the resistant and moderately resistant genotypes selected after stem inoculation, two were grouped as susceptible due to their reaction to strain GSPB2506 and three as moderately resistant after leaf inoculation (Table 4). Genotypes TMS30572, CM8820-40, and CM8873-69 were resistant to the four strains after both leaf and stem inoculations, and genotype CM8820-30, moderately resistant after stem inoculation, was resistant after leaf inoculation.

Comparison of virulence of *X. axonopodis* pv. *manihotis* strains after stem and leaf inoculations. Strains Uganda 12 and GSPB2506 belong to different pathotypes according to their reactions on stems and leaves (Tables 2 and 5), whereas strain GSPB2511 showed no differential reaction with the genotypes after leaf inoculation. Strain GSPB2507 was not tested on leaves (sufficient cuttings not available). Strains GSPB2507 and GSPB2511 were most virulent after stem inoculation, followed by Uganda 12 and GSPB2506 (Table 2). The converse was observed

TABLE 3. Pathotypes of *Xanthomonas axonopodis* pv. *manihotis* identified according to their reaction after stem puncture inoculation of 111 cassava genotypes, and a set of 19 differential cassava genotype groups, clustered according to the percentage of the area under disease progress curve into resistant (R), moderately resistant (MR), and susceptible (S) genotypes

	Reaction with strain[y]				
Genotypes	GSPB2507	GSPB2511	Uganda12	GSPB2506	Differential genotypes[z]
G2 CM8873-44					
G3 CM8873-74	S	S	S	S	1
G4 CM8873-48	S	S	S	MR	2
G7 CM8820B-17					
G11 CM8873-39					
G12 CM8820-46	MR	S	S	S	3
G13 CM8873-7					
G20 CM8873B-31	S	MR	S	MR	4
G9 CM8820-16	S	MR	MR	S	5
G5 CM8873-81	MR	S	S	MR	6
G10 CM8877-9					
G15 CM8873B-7					
G16 CM8820-56	S	S	S	R	7
G17 CM8820B-25					
G6 CM8820-61	S	S	R	S	8
G18 CM8873-35	R	S	S	S	9
G19 CM8820-13	MR	MR	S	MR	10
G14 CM8873B-1					
G24 CM8820B-7					
G25 CM8873B-20	MR	S	S	R	11
G26 CM8873B-36	S	R	S	MR	12
G27 CM8820-4	S	MR	MR	R	13
G28 CM8873-25	S	MR	R	MR	14
G23 CM8820-38	MR	MR	S	R	15
G36 CM8873-64	S	MR	R	R	16
G30 CM8820-64	MR	S	R	R	17
G37 CM8870-17	S	R	R	R	18
G32 CM8873-86					
G34 CM8873-78	R	S	R	R	19

[y] S = area under disease progress curve after stem inoculation (stem-AUDPC): 30.4 to 60.8; MR = stem-AUDPC: 20.3 to 30.3; R = stem-AUDPC: 0 to 20.2.
[z] Groups of differential genotypes. Genotypes 2 and 3 showed susceptible reactions with all strains, but differed in reaction to strain GSPB2507 and Uganda12, respectively, by more than 33% from the highest symptom value caused by these strains and, therefore, are grouped under differential genotypes (differential group 1).

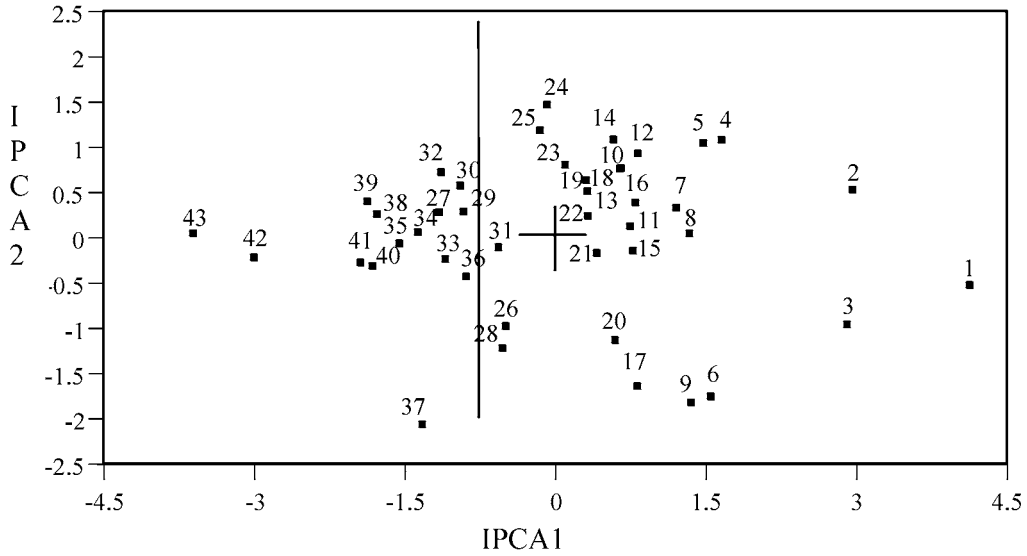

Fig. 1. Principal component analysis (PCA) of area under disease progress curves after stem inoculation of 43 cassava genotypes with four *Xanthomonas axonopodis* pv. *manihotis* strains, with cassava genotypes G1-G22 (susceptible), G23-G31 (moderately resistant), and G32-G43 (resistant).

after leaf inoculation with three of the strains, with GSPB2506 being the most virulent (Table 4). Genotype CM8873-64 was moderately resistant after stem and leaf inoculation with strain GSPB2511 (Table 3), but resistant to strains Uganda 12 and GSPB2507 using both inoculation methods.

Detection of QTL for cassava bacterial blight resistance. Single-marker regression analysis of AUDPC values from stem inoculation of each strain, based on the genetic linkage map of cassava, revealed 11 markers associated with resistance to the four strains of *X. axonopodis* pv. *manihotis* (Table 6). These markers can be classified in two types, based on the significance level of the simple linear regression. The first type corresponds to six markers that were significant at P value of <0.005, and the second type to five markers significant only at P values between 0.005 and 0.05. The latter type should be considered with precaution and confirmed by further studies.

Among the 11 markers associated with resistance to the four strains, 6 markers were identified as related to five (O, N, C, A, and ND) linkage groups, whereas 5 markers were not linked to any group. Five markers on three and one linkage groups of the female- and male-derived framework of family CM8820, respectively, were linked to disease reactions caused by the four strains of *X. axonopodis* pv. *manihotis* (Table 6). Based on the segregation of alleles from family CM8873, one marker (SSRY83) in the female-derived framework map was found to be associated with resistance to *X. axonopodis* pv. *manihotis* strains GSPB2506 and GSPB2511. The position of the six markers associated with resistance is shown on linkage groups of genetic maps of families CM8820+B and CM8873+B, developed by Jorge (21) (Fig. 2).

Two regions within group O (QTL 1 and 2) were found to be associated with strains GSPB2507 and GSPB2511, groups N and C (QTL 3 and 4) with strain Uganda 12, one region of group A (QTL 5) with strains Uganda 12 and GSPB2507, and group ND (QTL 6) was linked to strains GSPB2511 and GBPB2506. These results underlined that the four African strains belong to four different pathotypes. Among the 10 genotypes inoculated by leaf infiltration, 7 (e.g., CM8820B-23, CM8820-30, CM8820-34, CM8820-40, CM8873-69, CM8873-64, and TMS30572, which also showed a resistant or medium resistant reaction against three strains) have marker data. This small number of genotypes does not allow analysis of linkage between leaf resistance and markers.

DISCUSSION

Host–pathogen interactions among 111 genotypes of a cassava mapping population and four strains of *X. axonopodis* pv. *manihotis* from different geographic origins in Africa were studied using stem puncture and leaf infiltration methods. Results from

TABLE 4. Lesion areas on leaves expressed as area under disease progress curve (AUDPC) and day of first leaf drop (Drop) for eight genotypes of the cassava mapping population and for reference genotypes after leaf infiltration with three highly virulent strains of *Xanthomonas axonopodis* pv. *manihotis*[w]

	GSPB2511		Uganda 12		GSPB2506			
Genotypes	AUDPC (mm^2)	Drop (dpi)	AUDPC (mm^2)	Drop (dpi)	AUDPC (mm^2)	Drop (dpi)	Means of strains[x]	Reaction[y]
G8 BEN86052[z]	10,756.0 ± 1,534.5	23	36,377.7 ± 2,834.8	17	51,382.7 ± 7,721.1	20	32,939.1 ± 17,835.3 a	S
G42 CM8877-11	9,322.0 ± 3,248.7	23	5,975.8 ± 474.4	17	27,873.5 ± 14,277.3	17	13,757.1 ± 12,828.3 b	S
G35 CM8870-1	7,582.7 ± 4,116.0	23	6,288.5 ± 1,621.1	17	22,267.7 ± 5,203.9	17	12,046.3 ± 8,324.8 bc	S
G36 CM8873-64	16,726.2 ± 5,867.6	23	6,756.2 ± 473.3	17	8,211.0 ± 3,419.1	17	10,574.5 ± 5,829.9 bc	MR
G39 CM8820B-23	7,057.3 ± 1,668.9	17	12,034.6 ± 3,586.2	17	11,413.6 ± 1,117.7	20	10,168.0 ± 6,942.2 cd	MR
G40 CM8820-34	5,050.6 ± 602.4	20	12,616.2 ± 473.3	20	9,902.1 ± 3,548.2	20	9,189.6 ± 2,212.9 cd	MR
G38 TMS30572[z]	9,459.6 ± 602.4	23	3,176.1 ± 682.3	26	3,240.9 ± 451.7	23	5,302.2 ± 3,206.9 d	R
G41 CM8820-40	4,560.4 ± 623.2	17	3,913.1 ± 1078.5	17	3,457.7 ± 764.1	17	4,157.1 ± 941.4 e	R
G31 CM8820-30	2,185.7 ± 267.9	23	4,028.8 ± 1351.2	23	4,358.0 ± 1,162.5	23	3,184.0 ± 1,756.3 e	R
G33 CM8873-69	0.0 ± 0	…	397.5 ± 120.0	…	342.9 ± 61.2	…	246.9 ± 195.9 f	R
Means	7,270.0 ± 473.3 c	…	9,156.4 ± 911.0 b	…	14,245.0 ± 1570.9 a	…	…	…

[w] AUDPC calculated from lesion areas measured in square millimeters after infiltration of two leaves per plant with 10^6 CFU/ml.
[x] Mean of reaction (AUDPC of lesion area development) of three strains. Highest mean value used as 100%.
[y] S = susceptible, 33.3 to 100% (mean of strains-AUDPC = 10,968.7 to 32,939.1); MR = moderately resistant, 16.7 to 33.2% (mean of strains-AUDPC = 5,500.8 to 10,935.8); R = resistant, 0 to 16.6% (mean of strains-AUDPC = 0 to 5,467.9).
[z] BEN86052 and TMS30572 are the susceptible and resistant reference genotypes, respectively.

TABLE 5. Reaction of seven genotypes selected as resistant, one as moderately resistant after stem inoculation, and two reference genotypes to stem and leaf inoculations[u]

	GSPB2507	GSPB2511		Uganda 12		GSPB2506	
Genotypes	Stem[v]	Stem	Leaf[w]	Stem	Leaf	Stem	Leaf
G8 BEN86052[x]	S	S	MR	S	S	S	S
G42 CM8877-11[y]	R	R	MR	R	R	R	S
G35 CM8870-1[y]	R	R	R	R	R	R	S
G36 CM8873-64[y]	S	MR	MR	R	R	R	R
G39 CM8820B-23	R	MR	R	R	MR	R	MR
G40 CM8820-34	MR	R	R	R	MR	R	MR
G31 CM8820-30[z]	S	MR	R	R	R	R	R
G33 CM8873-69	MR	MR	R	R	R	R	R
G38 TMS30572	R	R	MR	R	R	R	R
G41 CM8820-40	R	R	R	R	R	R	R

[u] Strain GSPB2507 not used for leaf inoculation due to insufficient number of cuttings.
[v] Stem: S = susceptible, 50 to 100% (stem-AUDPC 30.4 to 60.8); MR = moderately resistant, 33.3 to 49.9% (stem-AUDPC 20.3 to 30.3); and R = resistant 0 to 33.2% (stem-AUDPC 0 to 20.2).
[w] Leaf: S = susceptible, 33.3 to 100% (leaf-AUDPC 17,110.5 to 51,382.7); MR = moderately resistant, 16.7 to 33.2% (leaf-AUDPC 8581.0 to 17,070.7); and R = resistant, 0 to 16.6% (leaf-AUDPC 0 to 8,529.5); reactions defined for each genotype–strain reaction.
[x] Susceptible control.
[y] Set of differential genotypes identified by leaf inoculation (genotypes with R and S).
[z] Moderately resistant genotype after stem inoculation.

stem inoculation with *X. axonopodis* pv. *manihotis* suggest the existence of 19 differential genotype groups and 3 differential genotype groups by leaf inoculation, which allow differentiation of pathotypes. Four *X. axonopodis* pv. *manihotis* pathotypes were described after stem inoculation and two pathotypes after leaf inoculation. Also Banito (3) identified high strain–genotype interactions and identified the same strains as pathotypes after stem inoculation by six groups of differential genotypes, using improved and local Togolese cassava cultivars. Although not all of the 111 genotypes of the mapping population were compared by both stem and leaf infiltration, the results showed obvious differences between genotypes and strains after inoculation with each of the two methods. Sample size was limited due to limited availability of cuttings and of greenhouse space for experiments.

Both stem inoculation and leaf infiltration were revealed to be necessary to screen for resistance to bacterial blight in order to identify genotypes with a combined resistance. The leaf infiltration method was also recommended by other authors (15,20, 25,42) to determine the resistance of genotypes against cassava and cowpea bacterial blight and bacterial pustule of soybean, whereas this method was not recommended by Restrepo et al. (38), who did not find resistant reactions in cassava leaves. These contradictory observations may be due to the different leaf inoculation method used by the latter authors (e.g., inoculation by placing 10 µl of a bacterial suspension in a small hole in the leaf without detailed symptom observation). The inoculum concentration of 10^6 cells/ml was identified as the most effective concentration to differentiate between the reactions of genotypes in previous trials with different inoculum levels (58).

After leaf infiltration, the resistant genotypes developed small symptom areas with no, early, or very late leaf abscission. The resistant genotype CM8820-40, which dropped inoculated leaves early, also was resistant after stem inoculation. Thus, an early leaf drop may indicate a resistance mechanism. Indications for resistance mechanisms in leaves and stems were also found in our former studies (58,60), when the bacterial multiplication in leaves and stems was reduced in the resistant genotype TMS30572 compared with a moderately resistant genotype and the susceptible genotype Ben86052. A similar observation was reported only for the stem by Restrepo et al. (38). Kpémoua et al. (26) suggested that suberin and tyloses in vessels may limit disease extension in stems of a resistant genotype. Only recently, possible resistance mechanisms on leaf level were suggested, such as cassava cell wall pectins (50) and latex production and, in cell culture studies, PR-proteins (9,24). Our results suggest that resistance mechanisms in leaves and stems are not associated and depend on different resistance mechanisms. Similar observations are reported from resistance of potato cv. Pimpernel to *P. infestans*, where resistance of the foliage and quantitative resistance of the tubers were not associated (45).

The four *X. axonopodis* pv. *manihotis* strains were identified as different pathotypes according to their reactions on leaves and stems. Strain–genotype interactions on the leaf level might have been more discriminative after inoculation of more host genotypes. Among the same strains used in this study, tested with different host genotypes (3), and also among Latin American strains, strain–genotype interactions in stems were detected and related to the possible presence of pathotypes (38–40,44,45). The ranking of genotypes based on the percentages of the mean of AUDPC over strains was similar to the ranking according to the PCA and confirmed the effectiveness of the method. Nevertheless, the identification of differential genotypes by our resistance class grouping after stem inoculation was more discriminative than the PCA, though the PCA better describes the relatedness between reactions of genotypes.

Six QTL and five unlinked markers that explained between 16 and 33.3% of the phenotypic variance were characterized using quantitative data of symptom development after stem inoculation by the four African strains. Nevertheless, some of these QTL and markers have to be confirmed by further studies because the population size was small, but they give some evidence that, with a larger sample size, we could be able to detect more QTL, especially in the CM8820 family. Our results suggest that several genes are involved in resistance to cassava bacterial blight. Microscopic observations of resistance reactions within infected vessels support the concept of a polygenic base of resistance to bacterial blight (26). Quantitative resistance based on strain-specific QTL has been reported from various crop–pathogen systems (8,27,29,43). Complex resistance traits are measured quantitatively and the identified QTL were defined by Young (57) as synonymous to partial resistance loci.

In the F_1 population, Jorge et al. (22) identified 12 QTL for resistance to five different strains. For the African strain ORST X-27 and one Colombian strain, resistance QTL appeared to be introgressions from a wild *Manihot* sp. and are located on one linkage group 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 (22). Using the same BC_1 population as in the present study, Jorge (21) identified five QTL linked to bacterial blight resistance against four Colombian

TABLE 6. Markers showing most significant likelihood ratio (LR) values (threshold = 6) after simple linear regression (quantitative trait loci [QTL] cartographer) for each female- and male-derived map and for each strain inoculated, and identified QTL

Family, map	Strain	Linkage group[y]	Marker	LR	P^z	Variance explained (%)	QTL
CM8820							
Female	GSPB2507	O	SSRY6	7.9	0.010	16	QTL1
	GSPB2511	O	SSRY6	6.7	0.011	18	QTL1
	…	…	SSRY170	6.5	0.012	19.7	QTL2
	Uganda 12	N	SSRY55	7.4	0.027	18.1	QTL3
	…	C	SSRY93	6.7	0.011	23.2	QTL4
Male	GSPB2507	A	GY12	6.1	0.015	21.8	QTL5
	Uganda 12	A	GY12	6.0	0.015	33.3	QTL5
	…	NL	SSRY17	6.7	0.011	17.4	…
CM8873							
Female	GSPB2507	NL	SSRY104	6.1	0.015	12.8	...
	GSPB2511	ND	SSRY83	15.4	0.000	19.6	QTL6
	Uganda 12	NL	SSRY104	7.6	0.007	19.3	…
	GSPB2506	ND	SSRY83	9.5	0.002	18.2	QTL6
	…	NL	SSRY104	8.0	0.005	23.4	…
Male	GSPB2507	NL	SSRY7	8.2	0.005	18.4	…
	GSPB2511	NL	SSRY84	8.6	0.004	21.8	…
	GSPB2506	NL	SSRY157	7.9	0.005	22.6	…

[y] NL = not linked and ND = no name assigned.
[z] Significance.

and one African strain. Among these QTL, two were located on linkage groups N and O, where we also found markers linked to resistance in the present study.

The low number of QTL detected in the case of the BC_1 population compared with the F_1 population (21) could be due to the number of markers selected for the BC_1 mapping (121 markers) compared with the number selected for the F_1 population (142 markers). Although the limited data did not allow the analysis of linkage between leaf resistance and markers, it may be speculated that different loci may be significant after leaf and stem inoculation. Effects of QTL on phenotype varying according to inoculation method were identified in the pathosystem *R. solanacearum* and tomato, indicating, that different QTL may act in different plant organs (10).

In barley, three QTL, accounting for nearly 30% of the phenotypic variation detected on chromosomes 3 and 7, are involved in partial resistance to bacterial leaf streak (*X. axonopodis* pv. *hordei*) (12). In tomato, resistance to *R. solanacearum* is controlled by QTL on several chromosomes, and the number of detected QTL, which partly are strain-specific, is increasing with each study (10,44). In bean, seven QTL are involved in resistance to common bean bacterial blight (*X. axonopodis* pv. *phaseoli*) (35); whereas, in rice, 10 QTL were detected correlated to resistance against rice bacterial blight (30). Thus, resistance based on strain-specific resistance can be improved by introducing the QTL underlying the resistance into a desirable genetic background or using them in gene pyramiding. Knowledge about the race- or pathotype-specific nature of partial resistance loci is the basis for this process (57).

Strain-specific resistance loci may contribute to explain the high genotype–environment interaction observed in selection of cassava genotypes for resistance to bacterial blight (3,49,55,59–61). Therefore, to identify genotypes with more widespread resistance, inoculation with different pathotypes is necessary. We recommend that breeders select resistant genotypes after stem and leaf inoculation with a representative set of *X. axonopodis* pv. *manihotis* pathotypes under glasshouse conditions to identify genotype–pathogen interactions.

The newly identified markers for cassava bacterial blight resistance can be used to increase the efficiency of identifying resistant genotypes for Africa. Incorporation of resistance loci in new lines by gene pyramiding and identification of additional resistance loci will contribute to selection of cassava genotypes with more effective and possibly durable resistance to *X. axonopodis* pv. *manihotis*.

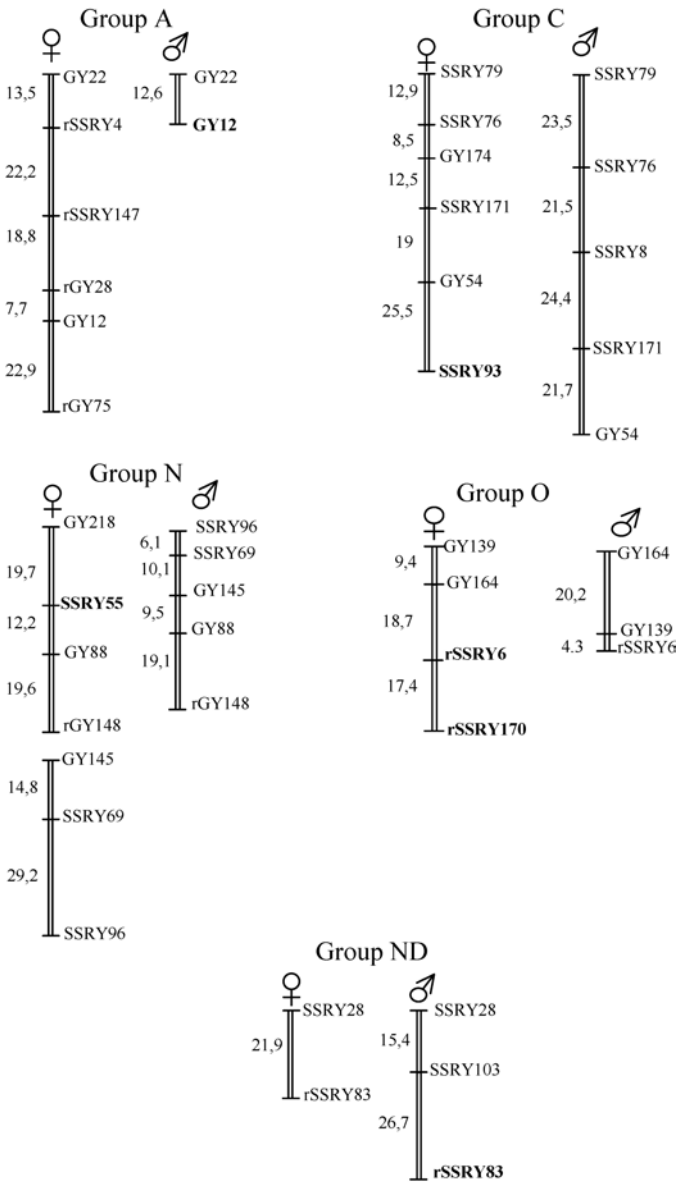

Fig. 2. Markers on five linkage groups of the genetic map developed by Jorge (24), based on marker segregation restriction fragment length polymorphism (GY) and microsatellites (SSRY) in parent gametes TMS30572 (♀) and CM7857-4 (♂) of backcross (BC_1) populations CM8820 + CM8820B (groups A, C, N, an O), and in parent gametes TMS30572 (♀) and CM7857-77 (♂) of BC_1 populations CM8873 + CM8873B (group ND). Markers with "r" are linked in repulsion. Distances at left are in centimorgans. Newly identified markers to African strains are in bold.

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LITERATURE CITED

1. Akparobi, S. O., Togoun, A. O., and Ekanayake, I. J. 1998. Assessment of cassava genotypes for resistance to cassava mosaic disease, cassava bacterial blight and cassava green mite at a lowland and mid-altitude site in Nigeria. Afr. Crop Sci. J. 6:385-396.
2. Assigbétsé, K., Verdier, V., Wydra, K., Rudolph, K., and Geiger, J. P. 1998. Genetic variation of the cassava bacterial blight pathogen, *Xanthomonas campestris* pv. *manihotis*, originating from different ecoregions in Africa. Pages 223-229 in: Plant Pathogenic Bacteria. A. Mahadevan, ed. Centre for Advanced Study in Botany, University of Madras, India.
3. Banito, A. 2003. Integrated control of cassava bacterial blight in West Africa in relation to ecozones, host plant resistance and cultural practices. Ph.D. thesis. University of Hannover, Germany.
4. Banito, A., Kpémoua, K., and Wydra, K. 2002. Ecozonal variation in reaction of cassava genotypes from Togo to bacterial blight. New Aspects of Resistance Research on Cultivated Plants. Bacterial Diseases. Beit. Züchtungsforschung BAZ 9:67-70.
5. Basten, C. J., Weir, B. S., and Zeng, Z.-B. 1994. Zmap—a QTL cartographer. Pages 65-66 in: 5th Congr. Genet. Appl. Livestock Production: Computing Strategies and Software. C. Smith, J. S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J. P. Gibson, and E. B. Burnside, eds. Guelph, Canada.
6. Boher, B., and Agbobli, C. A. 1992. La bactériose vasculaire du manioc au Togo: Caracterisation du parasite, répartition géographique et sensibilité variétale. Agron. Trop. 46:131-136.
7. Bondar, G. 1915. Molestia bacteriana da mandioca. Bol. Agric. Sao Paulo 16:153-524.
8. Concibido, V., Young, N. D., Lange, D. A. Denny, R. L., Danesh, D., and Orf, J. H. 1996. Targeted comparative genome analysis and quantitative mapping of a major partial-resistance gene to the soybean cyst nematode. Theor. Appl. Genet. 93:234-241.
9. Cooper, R. M., Kemp, B., Day, R., Gomez-Vasquez, R., and Beeching, J. R. 2001. Pathogenicity and resistance in *Xanthomonas* blight of cassava. Pages 319-323 in: Plant Pathogenic Bacteria. S. DeBoer, ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.
10. Danesh, D., Aarons, S., McGill, G. E., and Young, N. D. 1994. Genetic dissection of oligogenic resistance to bacterial wilt in tomato. Mol. Plant-Microbe Interact. 7:464-471.

11. Danesh, D., and Young, N. D. 1994. Partial resistance loci for tomato bacterial wilt show differential race specificity. Tomato Genet. Coop. Rep. 44:12-13.
12. El Attari, H., Rebai, A., Hayes, P. M., Barrault, G., Dechamp-Guillaume, G., and Sarrafi, A. 1998. Potential of doubled-haploid lines and localization of quantitative trait loci (QTL) for partial resistance to bacterial leaf streak (*Xanthomonas campestris* pv. *hordei*) in barley. Theor. Appl. Genet. 96:95-100.
13. Fanou, A. 1999. Epidemiological and ecological investigations on cassava bacterial blight and development of integrated methods for its control in Africa. Ph.D. thesis. University of Göttingen, Germany.
14. FAO. 1997. Draft working notes on selected chapters of The World Cassava Economy: Recent trends and medium-term outlook. Global Cassava Development Strategy: Progress Review Workshop. International Fund for Agricultural Development, Rome, Italy.
15. Fessehaie, A. 1997. Biochemical/physiological characterization and detection methods of *Xanthomonas campestris* pv. *manihotis* (Berthet-Bondar) Dye, the causal organism of cassava bacterial blight. Ph.D. thesis. University of Göttingen, Germany.
16. Flood, J., Cooper, R. M., Deshappriya, N., and Day, R. C. 1995. Resistance of cassava (*Manihot esculenta*) to *Xanthomonas* blight in vitro and in planta. Asp. Appl. Biol. 42:277-284.
17. Fregene, M., Angel, F., Gomez, R., Rodriguez, F., Chavarriaga, P., Roca, W., Tohme, J., and Bonierbale, M. 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). Theor. Appl. Genet. 95:431-441.
18. Grousson, F., Pages, J., and Boher, B. 1990. Etude de la variabilité d'un agent pathogène, *Xanthomonas campestris* pv. *manihotis*, par l'analyse factorielle multiple. Agronomie 4:627-640.
19. Hahn, S. K., Terry, E. R., Leuschner, K., Akobundu, I. O., Okali, C., and Lal, R. 1979. Cassava improvement in Africa. Field Crops Res. 2:193-226.
20. Hokawat, S., and Rudolph, K. 1991. Variation in pathogenicity and virulence of strains of *Xanthomonas campestris* pv. *glycines*, the incitant of bacterial pustule of soybean. J. Phytopathol. 131:73-83.
21. Jorge, V. 2000. Cartographie de la résistance du manioc à la bactériose vasculaire du manioc causée par *Xanthomonas axonopodis* pv. *manihotis*. Ph.D. thesis. Université Paris XI, France.
22. Jorge, V., Fregene, M., Duque, M. C., Bonierbale, M. W., Tohme, J., and Verdier, V. 2000. Genetic mapping of resistance to bacterial blight disease in cassava (*Manihot esculenta* Crantz). Theor. Appl. Genet. 101:865-872.
23. Jorge, V., Fregene, M., Velez, C. M., Duque, M. C., Tohme, J., and Verdier, V. 2001. QTL analysis of field resistance to *Xanthomonas axonopodis* pv. *manihotis* in cassava. Theor. Appl. Genet. 102:564-571.
24. Kemp, B. P., Beeching, J. R., and Cooper, R. M. 2001. Pathogenicity and resistance in *Xanthomonas* blight of cassava. (Abstr.). In: 10th Int. Conf. MPMI, ISPNI, Madison, WI.
25. Khatri-Chhetri, G. 1999. Detection and characterization of *Xanthomonas campestris* pv. *vignicola* strains, incitant of cowpea bacterial blight and pustule, and studies on genotype/strain-interactions. Ph.D. thesis. University of Göttingen, Germany.
26. Kpémoua, K., Boher, B., Nicole, M., Calatayud, P., and Geiger, J. P. 1996. Cytochemistry of defence responses in cassava infected by *Xanthomonas campestris* pv. *manihotis*. Can. J. Microbiol. 42:1131-1143.
27. Kreike, C. M., de Koning, J. R. A., Vinke, J. H., van Ooijen, J. W., and Stiekema, W. J. 1994. Quantitatively-inherited resistance to *Globodera pallida* is dominated by one major locus on *Solanum spegazzinii*. Theor. Appl. Genet. 88:764-769.
28. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.
29. Leonards-Schippers, C., Gieffers, W., Schaferpregl, R., Ritter, E., Knapp, S. J., Salamini, F., and Gebhardt, C. 1994. Quantitative resistance to *Phytophthora infestans* in potato—a case study for QTL mapping in an allogamous plant species. Genetics 137:67-77.
30. Li, Z.-K., Luo, L. J., Mei, H. W., Paterson, A. H., Zhao, X. H., Zhong, D. B., Wang, Y. P., Yu, X. Q., Zhu, L., Tabien, R., Stansel, J. W., and Ying, C. S. 1999. A 'defeated' rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Mol. Gen. Genet. 261:58-63.
31. Mangin, B., Thouquet, P., Olivier, J., and Grimsley, N. 1999. Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci. Genetics 151:1165-1172.
32. Mba, R. E. C., Stephenson, P., Edwards, K., Melzer, S., Nkumbira, J., Gullberg, U., Apel, K., Gale, M., Tohme, J., and Fregène, M. 2001. Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: Towards an SSR-based molecular genetic map of cassava. Theor. Appl. Genet. 102:21-31.
33. Njiti, V. N., Doubler, T. W., Suttner, R. J., Gray, L. E., Gibson, P. T., and Lightfoot, D. A. 1998. Resistance to soybean sudden death syndrome and root colonization by *Fusarium solani* f. sp. *glycine* in near-isogenic lines. Crop Sci. 38:472-477.
34. Njiti, V. N., Shenaut, M. A., Suttner, R. J., Schmidt, M. E., and Gibson, P. T. 1996. Soybean response to sudden death syndrome: Inheritance influenced by cyst nematode resistance in Pyramid × Douglas progenies. Crop Sci. 36:1165-1170.
35. Nodari, R. O., Tsai, S. M., Gilbertson, R. L., and Gepts, P. 1993. Toward an integrated linkage map of common bean. 3. Mapping genetic factors controlling host–bacteria interactions. Genetics 134:341-350.
36. Nweke, F. I., Dixon, A. G. O., Asiedu, R., and Folayan, S. A. 1994. Attributes of cassava varieties desired by farmers in sub-Saharan Africa. Pages 65-92 in: 5th Symp. Int. Soc. Trop. Root Crops—Africa Branch. M. O. Akoroda, ed. Uganda.
37. Pereira, L. F., Goodwin, P. H., and Erickson, L. 2000. Peroxidase activity during susceptible and resistant interactions between cassava (*Manihot esculenta*) and *Xanthomonas axonopodis* pv. *manihotis* and *Xanthomonas cassavae*. J. Phytopathol. 148:575-577.
38. Restrepo, S., Duque, M. C., and Verdier, V. 2000. Characterization of pathotypes among isolates of *Xanthomonas axonopodis* pv. *manihotis* in Colombia. Plant Pathol. 49:680-687.
39. Restrepo, S., Valle, T., Duque, M. C., and Verdier, V. 1999. Assessing genetic variability among Brazilian isolates of *Xanthomonas axonopodis* pv. *manihotis* in Colombia. Appl. Environ. Microbiol. 63:4427-4434.
40. Restrepo, S., and Verdier, V. 1997. Geographical differentiation of the population of *Xanthomonas axonopodis* pv. *manihotis* in Colombia. Appl. Environ. Microbiol. 63:4427-4434.
41. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.
42. Sikirou, R. 1999. Epidemiological investigations and development of integrated control methods of bacterial blight of cowpea caused by *Xanthomonas campestris* pv. *vignicola* (Burkholder) Dye. Ph.D. thesis. University of Göttingen, Germany.
43. Song, W., Wang, G., Chen, L., Kim, H., Pi, L., Holsten, T., Gardner, J., Wang, B., Zhai, W., Zhu, L., Fauquet, C., and Roland, P. 1996. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270:1804-1806.
44. Thoquet, P., Olivier, J., Sperisen, C., Rogowsky, P., Laterrot, H., and Grimsley, N. 1996. Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii 7996. Mol. Plant-Microbe Interact. 9:826-836.
45. Turkensteen, L. J. 1993. Durable resistance of potatoes against *Phytophthora infestans*. Pages 115-124 in: Durability of Disease Resistance. T. Jacobs and J. E. Parleviet, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
46. Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472-489.
47. Verdier, V., Restrepo, S., Mosquera, G., Duque, M. C., Gerstl, A., and Laberry, R. 1998. Genetic and pathogenic variation of *Xanthomonas axonopodis* pv. *manihotis* in Venezuela. Plant Pathol. 47:601-608.
48. Wang, J.-F., Olivier, J., Thoquet, P., Mangin, B., Sauviac, L., and Grimsley, N. H. 2000. Resistance of tomato line Hawaii 7996 to *Ralstonia solanacearum* Pss4 in Taiwan is controlled mainly by a major strain-specific locus. Mol. Plant-Microbe Interact. 13:6-13.
49. Wydra, K. 2002. The concept of resistance, tolerance and latency in bacterial diseases: Examples from cassava and cowpea. New Aspects of Resistance Research on Cultivated Plants. Bacterial Diseases. Beitr. Züchtungsforschung BAZ 9:36-43.
50. Wydra, K., Agbicodo, E., Ahohuendo, B., Banito, A., Cooper, R. M. C., Dixon, A. G. O., Jorge, V., Kemp, B., Kpémoua, K., Rudolph, K., Verdier, V., Witt, F., Zandjanakou, M., and Zinsou, V. Integrated control of cassava bacterial blight by (1) combined cultural control measures and (2) host plant resistance adapted to agro-ecological conditions, and (3) improved pathogen detection. 9th Trienn. Symp. Int. Soc. Trop. Root Crops—Africa Branch (ISTRC-AB). (In press.)
51. Wydra, K., Fessehaie, A., Fanou, A., Sikirou, R., Janse, J., and Rudolph, K. 1999. Variability of strains of *Xanthomonas campestris* pv. *manihotis*, incitant of cassava bacterial blight, from different geographic origins in pathological, physiological, biochemical and serological characteristics. Pages 317-323 in: Plant Pathogenic Bacteria. A. Mahadevan, ed. Centre for Advanced Study in Botany, University of Madras, India.
52. Wydra, K., and Msikita, W. 1998. Overview of the present situation of cassava diseases in West Africa. Pages 198-206 in: Root Crops Poverty Alleviation, 6th Trienn. Symp. Int. Soc. Trop. Root Crops—Africa Branch (ISTRC-AB), Lilongwe, Malawi. M. O. Akoroda and I. J. Ekanayake, eds. ISTRC, IITA and Government of Malawi.
53. Wydra, K., and Rudolph, K. 1999. Development and implementation of integrated control methods for major diseases of cassava and cowpea in West-Africa. Göttinger Beitr. Land–u. Forstwirtschaft i.d. Tropen u. Subtropen. 133:174-180.

54. Wydra, K., and Verdier, V. 2002. Occurrence of cassava diseases in relation to environmental, agronomic and plant characteristics. Agric. Ecosyst. Environ. 93:211-226.
55. Wydra, K., Zinsou, V., and Fanou A. 1999. The expression of resistance against *Xanthomonas campestris* pv. *manihotis*, incitant of cassava bacterial blight, in a resistant cassava variety compared to a susceptible variety. Pages 583-592 in: Plant Pathogenic Bacteria. A. Mahadevan, ed. Centre for Advanced Study in Botany, University of Madras, India.
56. Yoshimura, S., Umehara, Y., Kurata, N., Nagamura, Y., Sasaki, T., Minobe, Y., and Iwata, N. 1996. Identification of a YAC clone carrying the *Xa-1* allele a bacterial blight resistance gene in rice. Theor. Appl. Genet. 93:117-122.
57. Young, N. D. 1996. QTL mapping and quantitative disease resistance in plants. Annu. Rev. Phytopathol. 34:479-501.
58. Zinsou, V. 2001. Studies on the expression of resistance of cassava genotypes including individuals of the mapping population to cassava bacterial blight. M.Sc. thesis, University of Göttingen, Germany.
59. Zinsou, V. 2003. Studies on host plant resistance to cassava bacterial blight in combination with cultural control measures adapted to ecozones of West Africa. Ph.D. thesis. University of Hannover, Germany.
60. Zinsou, V., Wydra, K., Agbicodo, E., Ahohuendo, B., and Rudolph, K. 2001. Studies on the resistance of cassava genotypes to bacterial blight. Pages 301-305 in: Plant Pathogenic Bacteria. S. DeBoer, ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.
61. Zinsou, V., Wydra, K., Ahohuendo, B., and Hau, B. Genotype × environment interactions in symptom development and yield of cassava genotypes with artificial and natural cassava bacterial blight infections. Eur. J. Plant Pathol. (In press.)