Genetic mapping of resistance factors to *Phytophthora palmivora* in cocoa

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Abstract: *Phytophthora palmivora* causes pod rot, a serious disease on cocoa widespread throughout the producing regions. In order to ascertain the genetic determination of cocoa resistance to *P. palmivora*, a study was carried out on two progenies derived from crosses between a heterozygous, moderately resistant Forastero clone, T60/887, and two closely related and highly susceptible Forastero clones, one completely homozygous, IFC2, and one partially heterozygous, IFC5. The cumulative size of both progenies was 112 individuals. Plants were subjected to natural and artificial inoculation of *P. palmivora* in Côte d'Ivoire. The genetic maps of T60/887 and of IFC5 were constructed using amplified fragment length polymorphism (AFLP) markers and microsatellites. The map of T60/887 comprised 198 markers assembled in 11 linkage groups and representing a total length of 793 cM. The map of IFC5 comprised 55 AFLP markers that were assembled into six linkage groups for a total length of 244 cM. Ratio of rotten over total number of fruit under natural infection was measured for each tree over two harvests. Artificial inoculations were performed on leaves and pods. These tests were weakly correlated with the pod rot rate in the field. Five quantitative trait loci (QTLs) of resistance were detected for T60/887 but none were common between the three traits measured. Stability and reliability of the experimental procedures are discussed and revealed the difficult use of these artificial tests on adult trees for a good prediction of field resistance.

Key words: *Theobroma cacao*, *Phytophthora palmivora*, cocoa black pod disease, genetic map, quantitative trait locus (QTL).

Résumé : Le *Phytophthora palmivora* provoque la pourriture des cabosses, une maladie sévère du cacaoyer, répandue dans toutes les régions de production. Pour comprendre le déterminisme génétique de la résistance à *P. palmivora* une étude a été menée sur deux descendances dérivées de croisements entre un clone Forastero hétérozygote modérément résistant, T60/887, et deux clones Forastero génétiquement proches et très sensibles, l'un complètement homozygote, IFC2, l'autre partiellement hétérozygote, IFC5. La taille cumulée des deux descendances est de 112 individus. Les plants sont soumis à des inoculations naturelles et artificielles de *P. palmivora* en Côte d'Ivoire. Les cartes génétiques de T60/887 et de IFC5 ont été construites à l'aide de marqueurs AFLPs et microsatellites. La carte de T60/887 comprend 198 marqueurs réunis en 11 groupes de liaison et représente une taille de 793 cM. La carte de IFC5 comprend 55 marqueurs AFLP (polymorphisme de longueur des fragments amplifiés) rassemblés en six groupes de liaison représentant une longueur totale de 244 cM. Le pourcentage de fruits pourris sous infection naturelle a été mesuré pour chaque arbre pendant deux récoltes. Les inoculations artificielles ont été conduites sur feuilles et cabosses. Celles-ci sont faiblement corrélées aux taux de pourriture au champ. Cinq loci de caractère quantitatifs (QTLs) ont été détectés pour T60/887 mais aucun n'est commun entre les trois caractères mesurés. La stabilité et la fiabilité des procédures expérimentales sont discutées et révèlent la difficulté d'utiliser les tests artificiels sur arbres adultes pour prédire le taux de pourriture au champ des arbres.

Mots clés : *Theobroma cacao*, *Phytophthora palmivora*, pourriture brune du cacaoyer, carte génétique, locus de caractère quantitatifs.

Introduction

Black pod on cocoa is caused by different *Phytophthora* species. *Phytophthora palmivora* (Butler 1919) was initially considered the only species causing *Phytophthora* pod rot on cocoa, but in the 1970s, several authors revised the classification of *Phytophthora* isolates found on cocoa, based on the size and the number of chromosomes (Sansomé et al.

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1975). This permitted the discrimination of several other species, namely *P. megakarya* (Brasier and Griffin 1979), *P. capsici* (Tsao and Alizadeh 1988), and *P. citrophthora* (Babacauh 1980).

Phytophthora palmivora has a worldwide distribution, and losses can be as high as 30% when climatic conditions are favourable to the parasite. The first symptom of the disease is a brown to black spot on the fruit, which increases in size. Several days after infection, a white mycelium, with sporangia, develops on the surface of the diseased pod, and fruits become mummified. Humid conditions are favourable for the development of *P. palmivora* in the fruit (Thorold 1975). The inoculum develops from the soil (Muller 1974) or different parts of the tree such as infected roots (Ward and Griffin 1981), stems, leaves (Blaha 1974), trunk bark, and floral bases (Babacauh 1980). It can be spread by rainwater splashes and runoff, by pod contact, by insects (ants), or by vertebrates (rodents). To reduce black pod disease, infected fruits are harvested and destroyed. Chemicals can be used for control but they are a costly and polluting method. Genetic resistance could be an alternative effective way of reducing yield losses due to *Phytophthora*.

Different artificial inoculation tests were developed in order to enable an early prediction of cocoa resistance to *P. palmivora*. Some tests used roots (Partiot 1975) or stems (Blaha 1974) but they were destructive to the tree. Recently a new method was developed on leaves by Nyassé et al. (1995). This method was first evaluated based on a design including five clones, each being represented by 20 adult trees (Nyassé 1997). A significant, positive correlation between the mean leaf test data and the mean pod rot rate in the field was observed. This suggested that the leaf test could be used as an early predictor of resistance in the field.

Among all the germplasm analysed for resistance, no tree has ever been found completely resistant in the field. Nevertheless, variability for resistance exists, and a polygenic determination of the various tests (yield loss in the field, leaf test, pod test) has been suggested (Blaha and Lotodé 1976; Enriquez and Salazar 1980; Tan and Tan 1990; Warren 1994; Enriquez and Soria 1999; Cilas et al. 1999). Moreover, Cilas et al. (1999) showed that genetic factors involved in the resistance in the field are additive. Mapping of a quantitative trait locus (QTL) for resistance to *P. palmivora* has already been performed. Lanaud et al. (1999*a*) studying a progeny obtained from a cross between a Forastero and a Trinitario identified a QTL for field rot rate, located on chromosome 1 of the Trinitario clone, explaining 15% of the variation of the trait. Other putative QTLs for the same trait were identified on chromosome 9 in both parents of the progeny. Analyses of QTLs of resistance evaluated by leaf test revealed putative QTLs located in the same regions of chromosome 1 and 9 but only in the Forastero parent. Another region of chromosome 10 was identified on Forastero parent for this trait, but the leaf test appeared to be poorly reproducible in this experiment conducted on leaves from adult trees. In another experiment, Risterucci et al. (2000*a*) analysed in another progeny QTLs of resistance towards several species of *Phytophthora* (*P. palmivora*, *P. megakarya*, *P. capsici*) evaluated by tests on leaves from young plants in a greenhouse. Several QTLs were identified on one parent of the progeny, a hybrid with SCA6 (a highly resistant Forastero clone). Species-specific as well as common QTLs of resistance towards the several species of *Phytophthora* were revealed on chromosomes 1, 3, 5, and 6 showing a good repeatability of results obtained by these tests. QTLs of resistance to *P. palmivora*, evaluated by artificial pod test, were also identified by Crouzillat et al. (2000) on chromosomes 2, 4, 5, and 9 of Forastero clones.

In this study, QTL analyses were developed to study resistance to *P. palmivora* in another Forastero progenitor selected for yield. The use of different methods of resistance evaluation, field rot rate, leaf and pod tests, on the same progeny will allow comparison at the genome level.

Materials and methods

Plant materials and *Phytophthora* isolate

Two full-sib families planted in 1988 at Zagné, Côte d'Ivoire, were studied. They are part of a completely randomized design where each genotype is represented by a single tree. This design contains a total of 10 full-sib families. The two families studied have a common male parent. The first family was derived from a cross between T60/887, an upper-Amazon Forastero clone (derived from a cross between Na34 and IMC60), and IFC2, an Amelonado, which is a Forastero clone from Lower Amazonia. This family contains 59 individuals. The second family was derived from a cross between T60/887 and IFC5, another Amelonado highly related to IFC2 as revealed by restriction fragment length polymorphism (RFLP) markers (Laurent et al. 1994). This family contains 56 individuals. RFLPs showed that IFC2 and IFC5 clones were more homozygous than T60/887. IFC2 showed 100% homozygosity based on 27 RFLP probes, IFC5 showed 77% homozygosity based on 30 probes, and T60/887 showed 60% homozygosity based on 20 probes. T60/887 has a moderate level of resistance to *P. palmivora* in the field when analysed in crosses in a factorial design (Cilas et al. 1999). IFC2 and IFC5 are described as susceptible to *P. palmivora* in the field (Paulin 1990).

Based on previous leaf test experiments (I. Kebe, unpublished results), we selected an isolate of *P. palmivora*, Div4, which was considered moderately aggressive compared with other isolates from Côte d'Ivoire. This isolate was harvested in Divo, Côte d'Ivoire. It was used for all artificial inoculations performed on pods and leaves.

Resistance evaluation

Field resistance evaluation data from two important harvest years, the 5th and the 6th, were available. The pod rot rate of each tree (PRR) was measured as the number of infected pods over the total number of pods for the 112 progeny individuals.

Two artificial inoculation methods were developed, one performed on the leaves and the other performed on the fruits. The leaf resistance test was carried out according to Nyasse et al. (1995). Leaf discs with a diameter of 15 mm were inoculated on the underside with 10 μL of an inoculum suspension containing 3×10^5 zoospores/mL. Experiments were conducted at 25°C under near 100% humidity. Scores were attributed according to the following scale: 0 for no symptoms, 1 for presence of penetration points, 2 for clusters of penetration points, 3 for clusters of patches, 4 for marbled patches, 5 for solid patches. Three successive sets of leaf tests were performed. The first set, S0, was performed in November 1997 and the second and third sets, S1 and S2, were performed in may 1998 at 3-day intervals. For S0, 10 leaf discs were used to evaluate each of the 112 individuals. This was repeated four times in four distinct batches. This trial was repeated twice giving a total number of 80 discs per individual. Symptoms were observed the 7th day after inoculation (LT7-S0). For S1 and

S2, five leaf discs were used to evaluate each of the 112 individuals. This was repeated four times in four distinct batches totalling 20 leaf discs per individual. Symptoms were scored the 7th day after inoculation, giving traits LT7-S1 and LT7-S2, for experiments S1 and S2, respectively.

Pod resistance evaluation was carried out on fruits still attached to the tree. Ten fruits were inoculated per individual. Each fruit was given a single 0.8-cm-deep wound with a pin, and 30 µL of a suspension of zoospores at a concentration of 3×10^5 zoospores/mL was injected into the wound. The length and width of the rot spot were measured the 1st, the 5th, and the 6th day after inoculation. The rot areas, FT, were calculated (mm^2) and were then used for QTL detection.

Molecular genotyping

DNA extraction was performed according to Risterucci et al. (2000*b*). DNA was isolated from fresh adult leaves. One gram of tissue was crushed in liquid nitrogen, and the powder was mixed with 5 mL of extraction buffer [1.4 M NaCl, 100 mM Tris–HCl pH 8.0, 20 mM EDTA, 10 mM Na_2SO_3, 1% PEG 6000, 2% MATAB (mixed alkyltrimethylammonium bromide)] preheated to 75°C. After homogenization, the extract was incubated for 30 min at 75°C. After cooling to room temperature, an equal volume of chloroform – isoamyl alcohol (24:1, v/v) was added, followed by emulsification; then the tube was centrifuged at 7000 × *g* for 30 min. The supernatant was precipitated at –20°C overnight, after adding an equal volume of isopropanol. The DNA was removed and resuspended in 1 mL of 0.7 M NaCl, 50 mM Tris–HCl, 10 mM EDTA, pH 7.0 buffer. After total resuspension, DNA was cleaned by using a Qiagen genomic-tip as recommended by the supplier.

Microsatellite and amplified fragment length polymorphism (AFLP) markers were used to establish the map. For microsatellites, primers for 28 loci were available (Lanaud et al. 1999*b*). DNA was amplified using a final concentration of 0.4 ng/µL. The reaction mixture (1× buffer, 200 mM dNTP, 1.5 mM $MgCl_2$, primers 0.2 mM each; 0.04 U/µL Taq) up to a final volume of 20 µL with water. The 5′ extremity of one primer was labelled with ^{33}P by a kinase. The following PCR conditions were used: 1 cycle at 94°C for 5 min; 35 cycles at 94°C for 30 s, 46°C or 51°C for 1 min, and 72°C for 1 min; 1 cycle at 72°C for 8 min. The temperature was kept at 4°C after cycling. The products were separated by polyacrylamide gel electrophoresis at 55 W for 2 h in 0.5× TBE buffer. For AFLPs, analyses were run as described by Vos et al. (1995) using kits developed by Gibco BRL (Paisley, U.K.). The results were then revealed by autoradiography.

Statistical analyses

Field data were adjusted by the Papadakis method, which takes into account measures of neighbouring trees to adjust data and correct localized effects (Papadakis 1937). This method was performed using the SAS system (SAS 1997). For leaf test traits, genotype and replication effects were tested. Replication in each trial effect and genotype × trial interaction were also tested for LT7-S0. This was performed with the procedure GLM of the SAS system (SAS 1997). Normality of trait distribution was tested with the Wilk and Shapiro test (Shapiro and Wilk 1965). Spearman (1904) phenotypic rank correlation was calculated between characters.

A genetic map was established for T60/887 and IFC5. No locus was segregating for IFC2. The mapping data were analysed according to a double pseudo-test-cross strategy (Grattapaglia and Sederoff 1994) using the JOINMAP 1.4 program (Stam 1993). Genetic distances between markers were estimated using the Kosambi mapping function (Kosambi 1944).

QTLs were detected by using the interval mapping procedure (Lander and Botstein 1989) implemented in MAPQTL 3.0 (Van Ooijen and Maliepaard 1996) software. LOD thresholds were estimated for each chromosome according to Rebaï et al. (1994) with the help of a Fortran computer program kindly provided by A. Rebai, Institut national de la recherche agronomique (INRA), Toulouse, France. The nonparametric marker by marker test of Kruskal and Wallis (1952) was also performed to take into account possible bias of the interval mapping test when traits are not normally distributed. The QTL analysis procedure was performed in three steps. First, the LOD score threshold corresponding with a global type I error of $P = 0.05$ was computed for each chromosome (Rebaï et al. 1994). For each trait, a QTL was declared as present when the LOD score curve exceeded this threshold. Second, the LOD score corresponding with a global type I error of $P = 0.20$ was computed for each chromosome. For a given trait, a QTL was declared as present if a QTL was also detected at the same map position for another trait at this step or at the previous step. Third, a Kruskal and Wallis test was computed for all QTLs as a control to take into account possible effect of the non-normality of distribution on the LOD score test.

Table 1. Spearman correlation (*r*) between the different traits of resistance estimated on fruit by the rot area (mm^2), and on leaf, by the score as defined in Materials and methods, after inoculation.

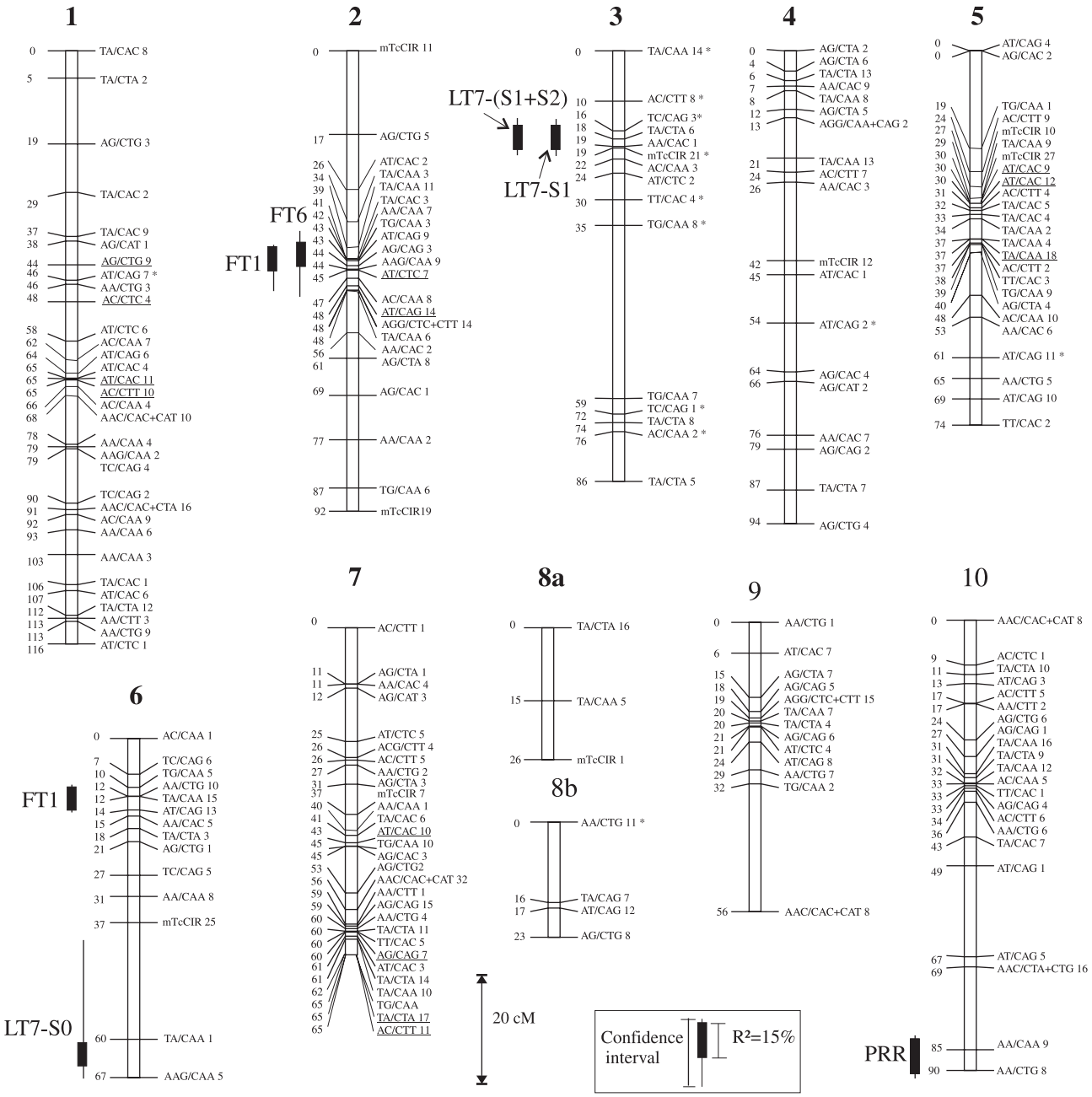
	Traits related to the fruit test	
	5 days	6 days
Traits related to the leaf test	*r*	*r*
November	–0.164	–0.086
May (1)	0.177	0.213*
May (2)	0.193*	0.226*
May (1 and 2)	0.249*	0.181

Note: Asterisks indicate a significant correlation at $P = 0.05$.

Results

Differences between the means of the two populations, T60/887 × IFC5 and T60/887 × IFC2, were tested for all traits. A difference appeared as significant ($P < 0.05$) for only one trait, LT7-S1. This permitted combining data of the two families for QTL analyses, except for LT7-S1 for which an adjustment to the mean of the family was performed. Frequency distributions have been analysed. Distributions were normal for leaf test traits ($P = 0.05$) but were not for the pod rot rate in the field or for fruit test traits FT5 and FT6. ANOVAs performed for the leaf test traits permitted to test genotype and replication effects for S1 and S2, and genotype, trial, replication in trial, and genotype × trial interaction effects for S0. All effects were highly significant ($P < 0.001$). Significant trial, genotype × trial, replication in trial, and replication effects indicate that the leaf test is highly influenced by environment and that its repeatability is low. Nevertheless, the high genotype effect also indicates that the search for QTLs is worthwhile. A significant rank correlation ($P < 0.05$) was found between LT7-S1 and LT7-S2, but none was found between those two traits and LT7-S0. Measures of the rot area on pods at different dates were all significantly correlated ($P < 0.001$; $r > 0.6$), as expected, since those measures were not independent. Rank correlation between artificial inoculation tests on leaves and fruits are shown in Table 1. Some were found significant ($P < 0.05$) but values were low. No significant correlation between leaf and fruit tests and the PRR were detected.

Fig. 1. Genetic map of T60/887 and location of QTLs for resistance to *P. palmivora* constructed with JOINMAP using the distance of Kosambi. Segregation distortion at a threshold of 5% are represented by (*). Each chromosome is designated by a number by comparison with the reference map (see text). AFLP markers are indicated by the pair of primers *Eco*RI and *Mse*I who served to amplify the DNA. For some AFLP markers, three different primers were used simultaneously, one primer *Eco*RI and two primers *Mse*I, and are indicated by (+). The number refers to the polymorphic band on the gel. Underlined AFLP markers correspond to markers present in T60/887 and in IFC5 (see text). Each QTL is indicated by a rectangle. The confidence interval of each QTL is represented by (–). For precise information on each QTL refer to Table 2.



Among the 28 microsatellites tested for polymorphism, 9 appeared as segregating among the gametes of T60/887. A single one showed a segregation distortion at $P < 0.05$. No microsatellite was segregating among the gametes of IFC5. For AFLP, 26 pairs of primers were used, revealing 252 segregating markers. Among them, 184 markers were present only in parent T60/887, 55 markers were present only in parent IFC5, and 13 were present in both T60/887 and IFC5. Eleven AFLP markers, present in T60/887 only, showed a segregation distortion to the expected 1:1 ratio at $P < 0.05$.

The genetic map of T60/887 was established by using (*i*) the AFLP markers heterozygous in T60/887 and absent in either IFC2 and IFC5, which showed a theoretical 1:1 segregation ratio, and (*ii*) the markers heterozygous in both T60/887 and IFC5, and showing a 3:1 segregation ratio (Fig. 1). The linkage map of T60/887 comprised 198 mark-

ers assembled in 11 linkage groups. Eight AFLP markers, present in T60/887 only, remained unlinked. The total length of the map was 793 cM. The average distance between two markers was 3.7 cM. The length of individual linkage groups varied from 23 to 118 cM. Among the markers presenting a segregation distortion, eight AFLP markers were localized on chromosome 3, one AFLP was present on each chromosome 1, 4, 5, and on linkage group 10, and one microsatellite was localized on chromosome 3. For T60/887, identity of chromosomes was determined by comparison of marker position with the reference map derived from the cross UPA402 × UF676 (Lanaud et al. 1995). AFLP and SSR markers have recently been added to this map (Risterucci et al. 2000*b*) which now comprises 424 markers, covering 885 cM. The identity of a given chromosome was assessed on the basis of at least one SSR marker in common or at least two AFLP markers. Indeed, chromosomes 2–8 were identified based on one or two SSRs, and chromosome 1 was identified based on three AFLPs. The three other linkage groups were assigned to chromosomes 8, 9, and 10, thanks to microsatellites mapped on a distinct population derived from the same cross (unpublished data).

The map of IFC5 was also performed using both markers heterozygous in IFC5 only and markers heterozygous in T60/887 and IFC5 (Fig. 2). The genetic map of IFC5 comprised 55 markers mapped onto six linkage groups. Thirteen markers, present in IFC5 only, remained unlinked. The length of individual groups varied from 19 to 84 cM, and the total map length covered 244 cM. No markers were common with the reference UPA402 × UF676 map. Identity of chromosomes was determined using markers present in both parents and by comparison of linkage groups identified in T60/887 map. Homology was assessed based on at least two AFLPs. Indeed identity of chromosomes 1, 7, 5, and 2 was assessed based on four, four, three, and two AFLP markers, respectively. Two linkage groups had no common marker with the map of T60/887 and remained unidentified.

Results of QTL analyses on the T60/887 map are summarized in Table 2 and in Fig. 1. Three QTLs were detected in the first step of the procedure. One QTL was detected at the end of linkage group 10 for PRR at a LOD score of 4.2. It accounted for 17% of the total phenotypic variation. One QTL was detected for LT7-S0 and another one was detected for FT1, both on chromosome 6 but at different map positions. Both of them accounted for 12% of total variation. In the second step of the procedure, four additional QTLs were detected. Two, for LT7-S1 and LT7-(S1+S2), were colocated on chromosome 3, and two, for FT1 and FT6, were detected on chromosome 2 (Table 2). For PRR and FT6, which are not normally distributed, the nonparametric test of Kruskal and Wallis was also significant at $P < 0.001$ and $P < 0.005$, respectively (Table 2).

The QTL detection performed on the IFC5 map gave no significant hits. This result is not surprising considering the low portion of the genome covered by the map and the small size of the progeny having IFC5 as a parent (59 individuals).

Discussion

The linkage map constructed for T60/887 included 200 markers that were assembled into 11 linkage groups. This

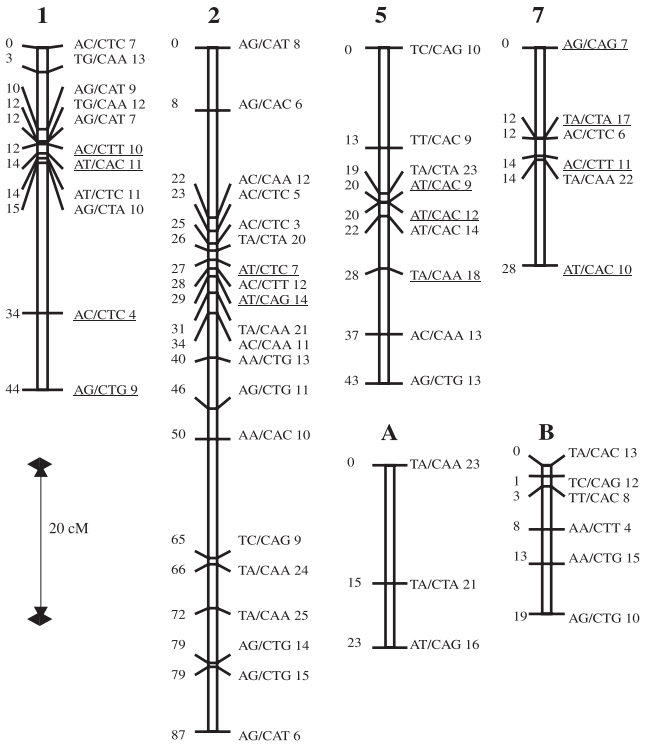


Fig. 2. Genetic map of IFC5 constructed with JOINMAP using the distance of Kosambi. Each chromosome is designated by a number by comparison with the T60/887 map (see text). The two unidentified groups are indicated by letters A and B. AFLP markers are indicated by the pair of primers *Eco*RI and *Mse*I, which served to amplify the DNA. The number refers to the polymorphic band on the gel. Underlined AFLP markers correspond to markers present in T60/887 and in IFC5 (see text).

indicates that the map was not completely saturated, since the basic chromosome number of cocoa is $x = 10$. Moreover, 8 markers remained unlinked. Nevertheless, by comparison with the length of the saturated reference map performed on the cross UPA402 × UF676 (Risterucci et al. 2000*b*), the map of T60/887 may cover up to 90% of the cocoa genome. The map of IFC5 included 55 markers, assembled into 6 linkage groups, and 13 markers remained unlinked. The total length of the map covered only 27% of the cocoa genome based on the length of the reference map. Based on what we know about the story of cocoa introductions in Africa at the beginning of the century (Toxopeus 1985), we can hypothesize that the markers segregating in the gametes of IFC5 were inherited from the Criollo genetic group. Regarding the genome proportion, which is close to a quarter, and the distribution of markers in a few linkage blocks, it is possible that IFC5 may be derived from a first back-cross generation on the Amelonado genetic background after a first hybridization between an Amelonado and a Criollo.

Traits used to account for resistance to *P. palmivora* were of three types, (*i*) an evaluation of yield losses in the field, (*ii*) a symptom evaluation after artificial inoculation of the fungus on leaves, and (*iii*) a symptom evaluation after artificial inoculation of the fungus on pods. Yield loss in the field is the most important trait accounting for resistance since it is directly related to the impact of the disease on plant pro-

Table 2. QTLs detected for different traits measuring resistance to *Phytophthora palmivora* in the map of T60/887.

Traits	Interval position	Linkage group	LOD_5[a]	LOD_{20}[b]	LOD[c]	R2 (%)[d]	P[e]
Pod rot rate (%)	AA/CAA9–AA/CTG8	10	2.7	2.0	**4.2**	17	0.001
Leaf test, November	TA/CAA1–AAG/CAA5	6	2.5	1.9	**2.7**	12	0.0005
Leaf test, May (1)	TA/CTA6–mTcCIR21	3	2.6	2.0	2.2	9	0.005
Leaf test, May (1 and 2)	TA/CTA6–mTcCIR21	3	2.6	2.0	2.2	9	0.001
Fruit test, 1 day	TG/CAA5–AT/CAG13	6	2.5	1.9	**2.8**	12	0.001
Fruit test, 1 day	TA/CAA1–TG/CAA3	2	2.7	2.1	2.4	11	0.001
Fruit test, 6 days	TA/CAA11–TG/CAA3	2	2.7	2.1	2.4	11	0.005

[a]LOD score threshold associated with a global type I error of 5%.
[b]LOD score threshold associated with a global type I error of 20%.
[c]LOD scores in bold characters indicate that a QTL was detected with a global type error of 5%.
[d]Percentage of the phenotypic variation explained by a QTL.
[e]Type I error associated with the Kruskal and Wallis individual test.

duction. Nevertheless its measure is highly time and space consuming. Its heritability at the family level has been estimated to be $h^2 = 0.6$ (Cilas et al. 1999). No estimation was made at the single plant level, but it is probably very low as for most traits related to yield. Leaf and pod tests have been developed as early screening procedures for resistance to *P. palmivora* (Nyassé et al. 1995; Blaha and Lotode 1976). A clonal trial involving five genotypes represented by 20 trees each established that the mean PRR was significantly correlated with the mean value of the leaf test (Nyassé 1997). Nevertheless, the present data show that leaf tests are highly susceptible to environmental conditions and, like pod tests, are poorly correlated with yield loss.

Our experiment permitted detection of five QTLs for several traits accounting for resistance to *P. palmivora*, one for PRR, two for leaf test traits, and two for fruit test traits. No QTL was found to be common between characters representing the three types of evaluation methods. Similar results were obtained by Lanaud et al. (1999*a*) who observed no common QTL for PRR and leaf test traits in another population. The global size of the population derived from the parent T60/887 was 112 individuals. Although this number is high regarding field trials in trees, it is not high regarding the statistical power of QTL detection. Moreover, the repeatability of the different traits measured to characterize the resistance was low. Simulation experiments (Beavis et al. 1994) show that a combination of a small population size and a low heritability are bad conditions for an accurate QTL detection. This permits detection of only a sample of QTL segregating in the progeny, and their effect and position cannot be precisely estimated. This could explain the absence of coincidence between QTLs for the different traits. Improving the power of QTL detection will necessitate an increase of the repeatability of tests and (or) the size of progenies. The repeatability of tests may be improved by the clonal multiplication of individual progeny trees and an optimization of single tree test procedures. The increase of population sizes may be achieved by setting up new experiments specifically designed for QTL detection. Multifamily approaches (Muranty 1997) may also be a key issue by (*i*) validating existing designs, (*ii*) treating several combinations of parents at a time, and (*iii*) increasing the representation of each parent at the whole-design scale.

The lack of coincidence between QTLs revealed by the different traits could also account for different resistance mechanisms, and thus could have a different genetic determination, but our results do not permit a clear conclusion on this aspect, at this stage. Cocoa resistance to *P. palmivora* is probably complex, and interaction with other biological traits such as fruit morphology, pod ripening period, or length of the harvesting period have to be considered. Such factors would be interesting to study in relation with the expression of field resistance of cocoa.

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