Genetic mapping of quantitative trait loci for black pod resistance in cocoa

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Black pod on cocoa is caused by different *Phytophthora* species. *P. palmivora* (Butler, 1919) has a worldwide distribution and losses can be as high as 30%. Several other species have been described, namely *P. megakarya* (Brasier and Griffin, 1979), responsible for large losses in central Africa, *P. capsici* (Tsao and Alizadeh, 1988) and *P. citrophthora* (Babacauh, 1980). Like many other agronomic traits, resistance to *Phytophthora* exhibits a continuum of phenotypic variations in the species *T. cacao,* suggesting the implication of several genes. A polygenic control of resistance has already been suggested by several authors (Blaha and Lotodé, 1976; Enriquez and Salazar, 1980; Rodriguez *et al.*, 1985; Tan and Tan, 1990; Warren, 1994; Enriquez and Soria, 1996; Cilas *et al.*, 1996). Cilas *et al.* (1999) also showed that genetic factors involved in resistance in the field were additive.

Genome mapping studies were developed under the CAOBISCO project in order to specify the genetic bases of cocoa resistance to *Phytophthora*. Using this approach, it is possible to identify the several genetic components, or QTL, that

affect a complex quantitative trait. The QTL approach allows the calculation of the number of genetic factors involved in the resistance trait and the localization of them in the cocoa genome. It is also possible to estimate, for each one, the particular phenotypic variation it demonstrates, and to determine the parental origin of favourable alleles. The plant-pathogen interaction could be analysed in detail, along with the possible identification of genes involved successively in various stages of interaction, with respect to different strains or species of *Phytophthora*. Such an approach was widely used to study the resistance of plant species to various types of pathogens—virus, fungus, bacteria (Young, 1996).

QTL analyses allow the variability of resistance genes among several cocoa clones known for their good level of resistance to be determined, and the possibility of increasing the resistance level in cocoa trees by cumulating different resistance genes to be evaluated. Several types of resistance evaluation were carried out (artificial tests on leaves or pods, rot observations in the field), and the approach based on genome mapping made it possible to observe whether the same genes were involved in the different observations of resistance. In addition, other traits linked to production or to the biological cycle of the trees, were analysed in order to study their possible interactions with resistance traits.

To try to clarify the functional role of QTL, markers corresponding to genes with a known biological function were mapped and a co-segregation between these genes and the QTL was looked for. Such a "candidate genes" approach, based on known resistance or defence genes already isolated from other species, was developed in this project to try to understand resistance mechanisms and find selection markers located in the genes directly involved in resistance. This information is likely to facilitate the combining of the most useful QTL for cocoa resistance improvement using tightly linked markers for efficient marker-assisted selection. It could also be a starting point for map-based cloning of alleles at QTL of interest.

Progenies analysed and *Phytophthora* species involved

Several progenies located in Ivory Coast, Cameroon, Trinidad and France (Montpellier) were analysed to identify, in several clones, the regions of the genome (QTL) involved in quantitative resistance to black pod due to various species of *Phytophthora* (table 1). The progeny studied in Montpellier was used for a simultaneous study of resistance to three different *Phytophthora* species: *P. palmivora, P. megakarya, P. capsici.*

Resistance was evaluated either by the rot rate in the field or by artificial inoculation tests on pods or leaves taken from adult or nursery trees. The progenies in Ivory Coast and Cameroon were mainly used to study resistance in the field, plus

resistance evaluated by artificial inoculation tests (on pods or leaves from adult trees), whereas the progenies located in Trinidad and Montpellier were only used to study resistance evaluated by leaf tests on young nursery plants (table 1).

Table 1. Progenies analysed during the project, nature of resistance traits observed and species of *Phytophthora* involved in black pod disease.

Progenies	Country	Resistance traits observed			Species of *Phytophthora* involved in black pod
		Field rot rate	Leaf test	Pod test	
UPA 402 x UF 676	Ivory Coast	x	x		*P. palmivora*
T60/887 x Amelonado		x	x	x	
IMC78 x Catongo		x			
DR1 x Catongo		x			
S52 x Catongo		x			
IMC 57 x Catongo	Trinidad		x		*P. palmivora*
TSH1077 x Catongo					
ICS 84 x UPA 134	Cameroon	x	x	x	*P. megakarya*
SNK 10 x UPA 134		x	x		
IMC 67 x SNK 413		x	x		
(Sca 6 x H) x IFC 1	France				*P. palmivora* (2 strains)
			x		*P. megakarya* (2 strains)
					P. capsici (2 strains)

Markers used for mapping

A high-density reference map had already been established for cocoa by Risterucci *et al.* (2000), using the progeny UPA402 x UF676 located in Ivory Coast. UPA402 is an Upper Amazon Forastero and UF676 is a Trinitario. The map is mostly composed of RFLP and AFLP, along with a few RAPD and microsatellite markers.

Efficient molecular markers are needed to establish the maps and compare QTL locations easily among them. AFLP were used for rapid saturation of the maps. They require only a small amount of DNA, but they do not allow a comparison to be made between different maps when used alone. Microsatellite markers were developed to make these comparisons possible. Microsatellites are particularly useful for these purposes: they are PCR markers that require only a small amount of DNA, they are co-dominant, locus-specific, highly polymorphic, and offer repeatable results or analyses. A first set of microsatellites was produced by Lanaud *et al.* (1999*b*). The production of new microsatellites markers was carried out at CIRAD in collaboration with the French Centre National de Séquençage (CNS) and CAOBISCO.

Thirty-five new polymorphic microsatellites were isolated using two methods of enrichment: a protocol adapted from that by Edwards *et al.* (1996), and a modified protocol of the Streptavidin MagneSphere Paramagnetic Particles kit, Promega (Billotte *et al.*, 1999). Twenty-five newly produced microsatellites were mapped on the UPA402 x UF676 reference map. A total of 46 microsatellites produced by CIRAD and called mTcCIRx have been mapped. Nineteen microsatellites, named Cx or Cax, identified by D. Crouzillat of Nestlé, were also mapped. In all, 64 microsatellites have now been mapped on the reference map (Lanaud *et al.*, in press).

These markers were distributed on all the chromosomes. The map length remained stable at 887.3 cM. The new reference map now includes 468 markers: 64 microsatellites, 191 AFLP, 178 RFLP, 30 RAPD and 5 isozymes. Microsatellite markers will be easier to use for a marker-assisted selection made in tropical countries.

Mapping QTL for resistance to *Phytophthora palmivora* on progenies from Ivory Coast

In Ivory Coast, only *P. palmivora* was detected during our experiments, even though *P. megakarya* existed at the border between Ghana and Ivory Coast. *P. palmivora* causes between 10 and 25% pod losses (Kébé, 1994; Cilas *et al.*, 1999). Five progenies located in Ivory Coast were studied for their resistance to *P. palmivora* (Lanaud *et al.*, 1999*a*; Flament *et al.*, 2002; Clément *et al.*, 2003*a,b*). These were located in four different places: Bingerville, Zagné, Divo and Abengourou. Disease pressure is higher in Bingerville and Zagné than in Divo and Abengourou. One progeny was studied in both Zagné and Divo. QTL analyses for resistance traits were carried out on clones belonging to different genetic groups: Trinitario (UF676, DR1, S52) and Upper Amazon Forastero (UPA402, T60/887, IMC78). Traits for resistance, yield and morphology were studied in most of the progenies to test possible relationships among traits.

Several significant QTL of resistance evaluated by the percentage of rotten pods in the field were identified in the different parents: one QTL was located on chromosome 1 of UF676, another was located on chromosome 10 of T60/887 and another was located on chromosome 4 of IMC78. Other putative QTL with lower LOD score values were also identified and need to be confirmed—like those identified on chromosome 8 of T60/887, on chromosomes 1 and 9 of UPA402, on chromosome 4 of DR1, and on chromosome 10 of S52. However the putative QTL identified on chromosome 4 of DR1 was located in the same region as significant QTL related to that trait identified in IMC78, and the putative QTL identified on chromosome 1 of UPA402 was located in the same region as the significant QTL related to resistance identified in UF676. An improved trial design, adapted to QTL

analyses (larger number of individuals, reduced environmental effects) is required to increase the power of QTL detection.

In both progenies UPA402 x UF676 and T60/887 x Amelonado from Zagné, QTL of resistance evaluated by the leaf test on adult trees were identified on several chromosomes, but low repeatability was found between the different sets of experiments. Some QTL were nevertheless identified on the mean of the sets of experiments. These tests are very sensitive to environmental factors, which may prevent detection of significant effects, and which may also explain the lack of correlation observed between the different resistance evaluation methods used on these old trees: only one co-localization with a putative QTL related to resistance evaluated in field was observed in UPA402.

The identification of different regions involved in resistance to *P. palmivora* suggests that a pyramiding strategy of different resistance genes from various parents is possible to improve their level of resistance to *P. palmivora*. Marker-Assisted Selection would facilitate this accumulation of resistance genes.

The possible interaction with other morphological or agronomic traits of the trees was also studied in the five progenies from Ivory Coast. In IMC78 and DR1, a co-localization was found between the QTL related to field resistance and the QTL related to the mean pod weight; this QTL was also co-localized with a QTL related to vigour (trunk circumference) and yield in IMC78. In T60/887, studied in a progeny located in Zagné, for which vigour and yield traits were also observed, the bigger QTL for field resistance located on chromosome 10 of T60/887 was not co-localized with other QTL related to yield or vigour. However a putative QTL explaining 10% of the variation of the field resistance trait was located in the same region of 30 cM of chromosome 8 as a QTL involved in pod number and trunk circumference. In this same region, a QTL for mean pod weight was identified in T60/887, studying a progeny located in Divo. These co-localizations could correspond to direct interactions between the vigour and mean pod weight of the tree and its resistance, or could also correspond to a close linkage between different genes involved in these traits. A larger population is needed to confirm these results.

Progenies from Cameroon

Phytophthora megakarya, which is found only in Africa, is the most destructive *Phytophthora* species on cocoa. When no treatment is used, losses can be as high as 80% in Cameroon (Despréaux *et al.*, 1989; Berry and Cilas, 1994). Using an artificial inoculation test on fruits still attached to the tree, 100 clones were evaluated for resistance and classified (Blaha and Lotodé, 1976). Among these clones, six were chosen based on their different levels of susceptibility. Then a diallel trial was composed with the six clones used as parents. Despréaux *et al.* (1989) studied this trial and showed that genetic factors involved in the resistance

to *P. megakarya* were additive and could imply polygenic resistance. Three progenies of the diallel trial, involving five different cocoa clones, were studied to specify the genetic control of cocoa resistance to *P. megakarya* (Flament, 1998).

The diallel trial was put in place in 1979. After nearly 20 years in the plantation, these old trees were not always in good, general state, which could explain the large heterogeneity observed for all observations made on these progenies and the difficulty in having clear, significative, statistical results. For this reason, an adjustment with the Papadakis method (Papadakis, 1937) was chosen to analyse field data.

The results show that methods of resistance evaluation in the field, and fruit and leaf tests, are strongly affected by environmental conditions. Resistance expression in the field, as indicated by a variance analysis, showed the existence of a plot-and-year effect. Moreover the heritability of this trait was low (0.2). Also, no correlation was detected between artificial inoculation tests and the pod rot rate estimated in the field. However Nyassé (1995, 1997) showed that when the test was carried out on five clones, there was a correlation between the leaf resistance trait and the pod rot rate in the field. It is possible that a strong environmental effect might mask a real correlation that could not be detected as it involved a small number of individuals. It can also be imagined that different genes were involved in the resistance mechanisms evaluated by the three different methods and that each method could measure a different type of resistance trait.

In spite of these strong environmental effects, five QTL were detected for all traits and for different parental clones. As resistance trait distributions were not normal, QTL detected by interval mapping were also analysed by a non-parametric approach of the Kruskal and Wallis type. Three of the five QTL detected were detected by the two methods, but two were detected at a higher probability than the other. Indeed a QTL was detected for leaf resistance traits in UPA134 on chromosome 9 by interval mapping at a LOD of 4 and confirmed by Kruskal and Wallis at $P = 0.0005$. Moreover this QTL was also detected by variance analysis when the number of individuals was increased to 104. Another QTL was detected for fruit resistance traits in UPA134 on chromosome 2. This QTL was detected for two different sets of experiments, confirming its existence. The threshold was intentionally chosen low, since our number of individuals was small, to increase QTL detection, but increasing the risk of detecting a false QTL.

SNK413 is a very resistant clone in Cameroon. The lack of QTL identification in this clone could be due to strong environmental heterogeneity, but also to the possible homozygous status of resistance alleles in this clone, which could explain the absence of segregation of resistance alleles in the progeny and the high resistance of SNK413. No QTL was common to the three methods of resistance evaluation. Different genes could therefore be involved in the genetic control of resistance to *Phytophthora*, as suggested by the absence of correlation. However, other common QTL could exist without being detected because of the low trial power (few individuals) and the strong environmental impact.

Due to many off-types detected in the progenies, the low number of individuals per progeny (58 to 78) limited the power of QTL detection. Only a common parent (UPA134) present in two progenies allowed, after adjustment, the cumulation of data for both progenies and the confirmation of QTL detected by leaf test on chromosome 9. This fact revealed the difficulty in working on trees already planted. They were planted in 1974 with a different objective than QTL detection. Moreover, the trial was not really adapted, since the absence of tree replication is a disadvantage in QTL detection. These results are preliminary and need to be confirmed. Other factors could interact with resistance evaluation, such as the ripening period, which was not taken into account during this study. Indeed this factor is assumed to act as resistance by escape (Berry and Cilas, 1994), whereby trees produce their fruit before the zoospore production period.

Progeny from Trinidad

Among diseases affecting cocoa in Trinidad, black pod rot caused by *Phytophthora* is currently the main cause of production losses. The two species of *Phytophthora* existing in Trinidad are *P. palmivora* and *P. capsici*, the former being the more widespread and more aggressive in Trinidad (Iwaro *et al.*, 1998).

A progeny composed of 155 plants derived from the cross between IMC 57 (female parent) and Catongo (male parent) was studied (Motilal *et al.*, 2002). IMC57 is an Upper Amazon Forastero and was chosen for its resistance to *P. palmivora* (assessed by an artificial inoculation test on leaves) and its high heterozygosity rate. Catongo is a Lower Amazon Forastero and was chosen for its low level of resistance to *P. palmivora* (assessed by the leaf test). This study enabled us to detect QTL for resistance to *P. palmivora* in IMC57.

Resistance to *P. palmivora* was assessed in the nursery, using the leaf inoculation test developed by Thévenin and Motilal (1998). Each plant was subjected to four series of inoculations. This led to the identification of numerous significative QTL located on chromosomes 1, 2, 4, 6, 9, and 3 or 8. Each of the QTL accounts for between 6.5 and 10% of the total variation. In most cases, the QTL were identified on the means obtained from five series of inoculations, and they account for a very large part (almost 80%) of the variation for this trait.

The studied progeny was planted out in the field in July 2000. Its level of resistance to *P. palmivora* will be also tested by artificial inoculations on pods, in accordance with the protocol described in Iwaro *et al.* (2000), and then by estimating the percentage of rotten pods under natural infection conditions. The QTL obtained from these analyses will be compared to those obtained by the leaf inoculation test and presented in this work. This QTL analysis confirms that many genes are involved in cocoa resistance to *P. palmivora*.

Thus, a cumulation of several favorable alleles using a marker-assisted selection could permit the level of resistance of cocoa clones to be greatly improved.

Progeny studied in France

This study aimed to compare the genetic control of cocoa resistance to three different species of *Phytophthora*: *P. palmivora*, *P. megakarya* and *P. capsici* (Risterucci *et al.*, in press). It is important to know whether selection for resistance to one of the *Phytophthora* species could increase the level of resistance to the other species, and whether common genes of resistance usable as a priority for marker-assisted selection can be located on the genome. The study was conducted on 151 hybrid progenies from the cross (Sca6 x H) x IFC1, created in Ivory Coast and grown in a greenhouse in Montpellier. Using microsatellite markers, the clone H, initially unknown, was identified as a Trinitario clone close to the clone GS36. As not all *Phytophthora* species exist in a single growing zone, leaf tests were carried out in the greenhouse in Montpellier, where a large collection of different *Phytophthora* strains and species exists. *Phytophthora* resistance was screened by leaf test inoculation with two different strains per species.

Selection of the best individuals for resistance to *P. palmivora* at a 10% selection rate would lead to a genetic gain of 47% for *P. palmivora,* 21% for *P. megakarya* and 42% for *P. capsici*. A comparison of the 30 most resistant individuals selected for each species revealed that six trees on average were common, i.e. 20% of the resistant trees. This result confirms that there is little interaction between species and genotype and that selection for resistance to a single species (e.g. *P. palmivora*) would provide genetic gains for improving resistance to the other species. These results are in agreement with the QTL analysis. Indeed, some QTL for resistance to three and two species were detected in common regions. QTL were identified using composite interval mapping and located in six genomic regions. One of these QTL was detected on chromosome 5 with five strains from the three *Phytophthora* species. Another was detected on chromosome 6 with three strains of two species and one other QTL was detected on chromosome 1 for two strains of two different species. Three additional QTL were detected for only one strain of *Phytophthora* species. Each QTL accounted for between 8 and 12% of the phenotypic variation. For each strain, between 11.5 % and 29.6 % of the total phenotypic variation could be accounted for by the QTL identified.

For some QTL, the Sca6 or H (Trinitario) origin of favourable resistance alleles was determined by very close markers. Not all the favourable QTL were provided by Sca6 alone, but the Trinitario H involved also provided some favourable alleles. This was particularly the case for those of chromosomes 1 and 5 provided by the Trinitario; the favourable resistance alleles located on chromosomes 3 and 6 were provided by Sca6. Observation of the molecular banding patterns for Sca6 also showed that these favourable alleles were in the homozygous state in Sca6.

These results show that both specific and non-specific QTL were identified in both the Trinitario and the Forastero clones. This is particularly interesting in the present situation of progression of *P. megakarya* in Africa. Indeed, *P. megakarya* is responsible for the largest yield losses, and even though only *P. palmivora* is currently present in Ivory Coast, when selecting for resistance to *P. palmivora*, it should be possible to increase the resistance of clones to *P. megakarya*. It could be particularly worthwhile transferring these common QTL into an elite clone through a marker-assisted selection scheme.

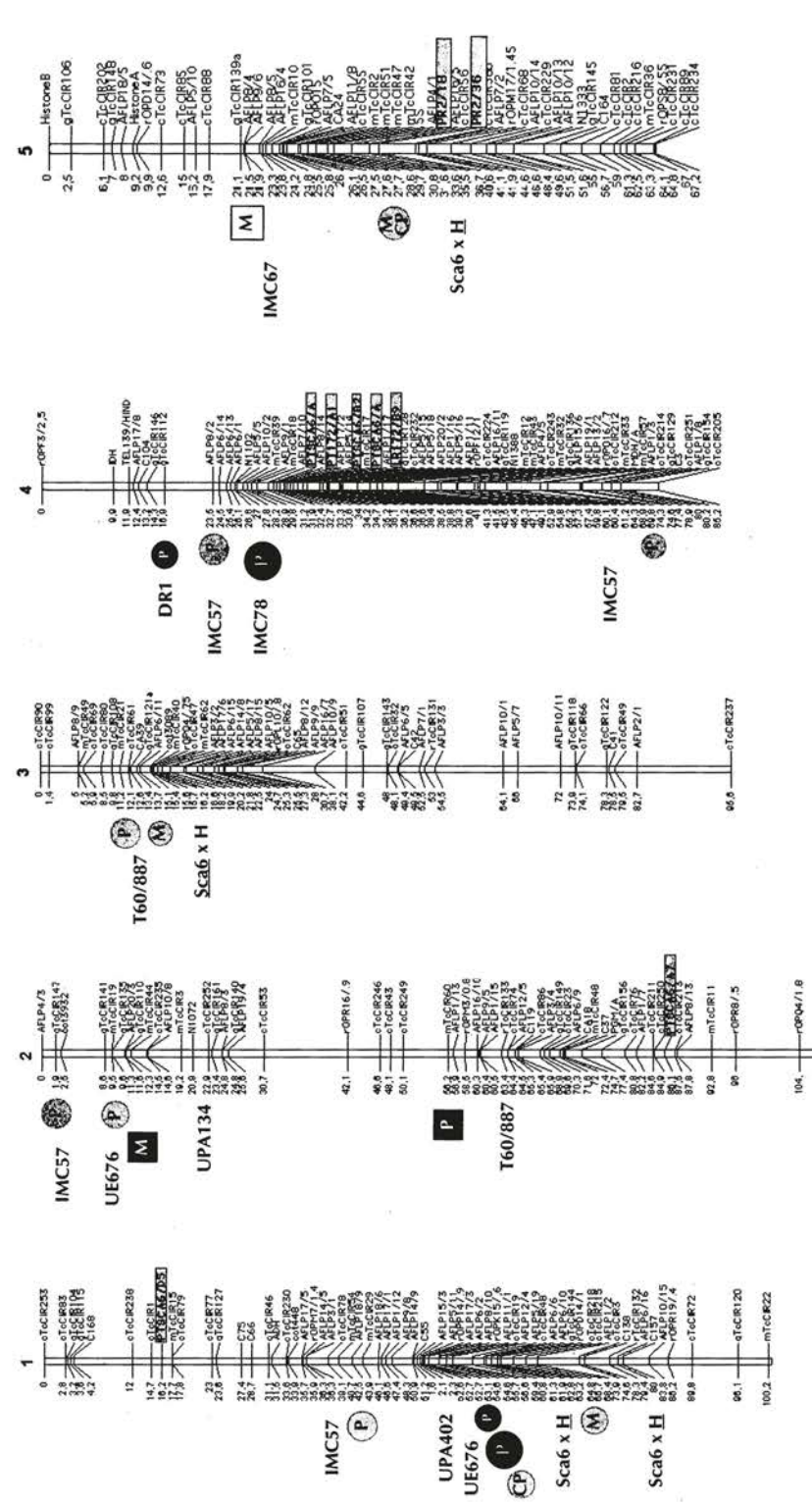
Characterization and genetic mapping of resistance and defence gene analogues in cocoa

Disease resistance and defence gene analogue (RGA/DGA) sequences were isolated in cocoa (Lanaud *et al.*, in press) using a polymerase chain reaction (PCR) approach with degenerate primers designed from conserved domains of plant resistance and defence genes: the NBS (nucleotide binding site) motif present in a number of resistance genes such as the tobacco *N*, sub-domains of plant serine/threonine kinases such as the *Pto* tomato gene and conserved domains of two defence gene families (pathogenesis-related proteins (PR) of classes 2 and 5). Nucleotide identity between 36 sequences isolated from cocoa and known resistance or defence genes varied from 58 to 80%. Amino acid sequences translated from corresponding coding sequences produced sequences without stop codons, except for one NBS-like sequence.

Most of the RGA could be mapped on the cocoa genome and three clusters of genes could be observed: NBS-like sequences clustered in two regions located on chromosomes 7 and 10; *Pto*-like sequences mapped in five genome regions of which one, located on chromosome 4, corresponded to a cluster of five different sequences; PR2-like sequences mapped in two regions located on chromosome 5 and 9 respectively.

Co-localizations observed between QTL related to *Phytophthora* resistance and candidate resistance and defence genes

Results of QTL and RGA/DGA mapping are reported in figure 1.


1
2
3
4
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IMC57
UE676
UPA134
T60/887
Sca6 x H
DR1
IMC78
UPA402
IMC67

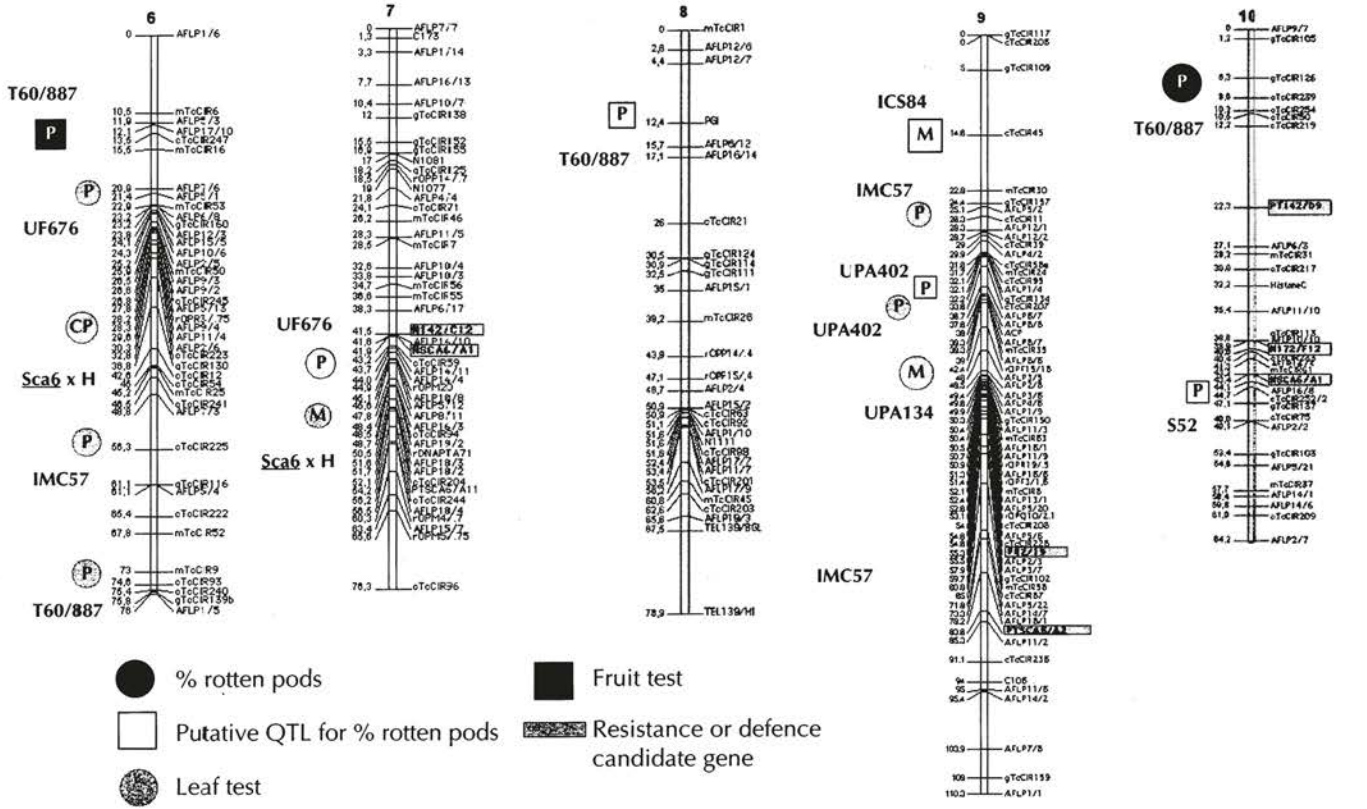

Figure 1. Mapping of QTL for resistance to P. palmivora *(P),* P. megakarya *(M) or* P. capsici *(C). The name of the clones where the QTL has been identified is mentioned near the QTL. In the case of the hybrid Sca6 x H, the parental origin of the favourable allele (Sca6 or H) is underlined.*

Co-localizations between QTL related to *Phytophthora* resistance

Several QTL related to resistance evaluated by the percentage of rotten pods were identified on chromosomes 1, 4, 5, 8, 9, and 10. Co-localizations between these QTL were found on chromosome 1 for UF676 and UPA402 (putative QTL), chromosome 4 for IMC78 and DR1 (putative QTL), and chromosome 9 for ICS84 and UPA402 (both putative QTL). These co-localizations were observed each time on clones belonging to different genetic groups and were not specific to one particular genetic group.

Co-localizations among QTL identified by the leaf test were also noted on chromosome 2 for IMC57 and UF676 (putative QTL), chromosome 3 for Sca6 and T60/887, chromosome 4 for IMC57 and UPA402 (putative QTL), chromosome 6 for Sca6 and IMC57 and chromosome 9 for IMC57, UPA402 and UPA134.

Some co-localizations were also observed between QTL related to resistance evaluated on different clones by the leaf test, pod test or by the percentage of rotten pods in the field, on chromosome 1, chromosome 2, chromosome 4 and chromosome 9.

Co-localizations between RGA, DGA and QTL regions for resistance to *Phytophthora*

Using common markers (particularly microsatellites) located in the several maps established, several co-localizations between RGA and DGA identified in this project and QTL for resistance could be observed.

This is the case for the cluster of RGA located in chromosome 4. In the same region of about 10 cM, four QTL for resistance to *P. palmivora* have been identified: one QTL explaining 13.2% of the variability of resistance to *P. palmivora* evaluated by a fruit test was identified in a Forastero clone (Pound 12) at LOD 3.4 by Crouzillat *et al.* (2000) while studying a progeny located in Costa Rica. Two other QTL of resistance to *P. palmivora* were identified in a Forastero clone (IMC78) and in a Trinitario clone (DR1) at LOD 7.4 and 2.5 respectively by Clément *et al.* (2003*b*) while studying progenies located in Ivory Coast. These QTL accounted for 22.6% and 10.1% respectively of the variability of the percentage of rotten pods observed in the field on cumulated data over a six-year harvesting period. Another QTL, located in this same chromosome region, and accounting for 8.1% of the variability of resistance evaluated by leaf test, was identified at LOD 2.2 by Motilal *et al.* (2002) in a Forastero clone (IMC57) by studying the progeny located at Trinidad.

Another region of 15 cM located in chromosome 5 gathers two DGA (PR2 analogues) and QTL for resistance evaluated by leaf test by Risterucci *et al.* (in press) toward three different species of *Phytophthora* (*P. palmivora*,

P. megakarya, P. capsici). These QTL were identified at LOD 2.9 to 3.9 and accounted for between 7.5 and 12.4% of the variability of the trait according to strains and *Phytophthora* species studied.

Another case of co-localization could be observed in chromosome 7 where 2 RGA containing NBS and two QTL for resistance evaluated by leaf test are co-localized, identified by Risterucci *et al.* (in press) in a Trinitario clone (H), and by Lanaud *et al.*, (1999a) in another Trinitario clone (UF676). These QTL were identified at LOD 3.1 and LOD 2.5 respectively, and accounted for 8% and 10% respectively of the variability of this resistance trait.

Discussion and general conclusion

Resistance to *Phytophthora,* which is already known to be partial, is a complex trait depending on several genes. The mapping approach was developed to acquire more precise knowledge of its genetic bases and to localize in the genome the regions (QTL) involved in resistance expression. This approach could also make it possible to compare the various sources of resistance, depending on the clones.

Several progenies involving clones belonging to different genetic groups were studied. Most of the cocoa clones recognized for their resistance have various levels of heterozygosity and QTL mapping studies have mainly been carried out using direct crosses already existing between those clones. Hence, only the resistance genes present with a heterozygous status could segregate in the progenies and be revealed from these direct crosses. The more resistant clones could have their resistant alleles in a homozygous status, and in that case direct crosses involving those clones are not appropriate for revealing their resistant alleles. This might explain why no QTL was identified in a very resistant clone, such as SNK413 in Cameroon, which was studied on a direct cross. The study of such resistant clones would require an examination of backcrosses or test cross models involving these clones. Scavina 6 is a very resistant clone with a high level of homozygosity. A test cross was produced with Scavina 6 during the project, and the study of the progeny (Sca6 x H) x IFC1 led to the identification in the genome of several QTL of resistance whose favourable alleles, originated from Sca6, are in a homozygous state in the Scavina 6 parent.

A large number of QTL related to resistance to *Phytophthora*, evaluated in the field or by artificial inoculations, were identified in the various progenies. Some chromosome regions appeared to be particularly involved, as on chromosomes 1, 4 and 9, where several co-localizations were observed. Other strong genetic effects were identified on chromosomes 5 and 10 for some clones studied. QTL for resistance to *P. palmivora* were also identified in the same regions of chro-

mosomes 1 and 4 by Crouzillat *et al.* (2000), studying by pod tests a progeny located in Costa Rica and resulting from a cross involving Pound12 and Catongo.

As a first step, existing trials, for which observations had been carried out for several years, were used for these analyses. However, the designs of these trials were not optimized for QTL analyses that require a large number of individuals analysed per progeny and plants observed under uniform conditions. While several significant QTL related to field resistance were identified, many other QTL appeared as putative QTL. New trials, with appropriate designs involving larger progenies, need to be planted for further QTL analyses.

Early artificial tests on leaves have been developed as an alternative method to evaluate the resistance of clones without needing to observe several years of harvests. The repeatability of leaf tests on leaves from adult trees in the field or on young trees in the nursery appeared to be very different. In the first case, low repeatability was observed between several sets of experiments carried out on the same plants at different periods, and the QTL were identified with low LOD score values. It was not the case for leaf tests on young nursery plants, for which high repeatability of genetic effects was observed between experiments associated with higher LOD score values. These differences could be due to several reasons: the larger number of plants studied in young progenies and also the stronger environmental effects on adult trees in the field, which might induce variations in leaf reactions after artificial inoculations.

This fact probably explains the lack of co-localisation between QTL related to field resistance and artificial inoculation tests on adult trees. For these reasons, the QTL studies based on data from leaf tests carried out on adult trees and reported here did not really permit the establishment of links between resistance evaluated by the percentage of rotten pods and the leaf tests; and they could not be used to test the predictive value of the leaf test to select resistant plants. In view of their greater stability, the leaf tests applied to young nursery plants seemed to be more promising and experiments were set up during the project in order to establish, on a genome level, the relationship between leaf tests applied to young plants and the percentage of rotten pods on adult trees.

Both specific and non-specific QTL for resistance to two or three different species of *Phytophthora* (*P. palmivora*, *P. megakarya* and *P. capsici*), were identified by leaf tests in the two genotypes belonging to two different genetic groups: Scavina 6, a Forastero clone and H, a Trinitario clone. These results are particularly interesting and, when selecting for resistance to *P. palmivora*, they should make it possible to increase the resistance of clones to *P. megakarya*, which is responsible for the largest yield losses in Africa, even though only *P. palmivora* is currently present in most of producing countries.

The search for candidate genes has been another approach developed under this project, to try to identify genes involved in cocoa resistance and provide markers for early screening of resistant plants among all the germplasm. Several cocoa DNA fragments homologous to resistance or defence genes have been isolated

and mapped, and some co-localizations between these loci and QTL for resistance have been observed. Due to the large number of QTL and candidate genes identified, some co-localizations could also be artefacts. However, three chromosome regions gathering RGA or DGA, with several QTL of resistance from different clones, could be of particular interest: one is located on chromosome 4, and gathers cocoa sequences homologous to the Pto R gene from tomato, to the rust resistance gene LRK10 from wheat, and to four QTL related to resistance evaluated in field or by leaf test. Another region of chromosome 7 gathers wo RGA and two QTL for resistance evaluated by leaf tests, and another region gathers defence PR2-related genes and QTL related to resistance to several species of *Phytophthora*.

However, other factors depending on the biological traits of the trees could also be important in resistance expression. Biological traits related to vigour and yield were studied on five progenies from Ivory Coast. Co-localizations were observed on chromosome 4 of IMC78 between QTL related to vigour and mean pod weight and a QTL of field resistance. Another co-localization was found on chromosome 8 of T60/887 between a QTL for pod number and a QTL for field resistance. Larger progenies would be necessary to determine whether it is a unique gene involved in several traits or different, closely linked genes.

The 10 progenies studied revealed the existence of various resistance genes. This situation is favourable for improving resistance in parents by possible pyramiding of different resistance genes using a marker-assisted selection strategy. Several QTL of resistance evaluated in the field or by the leaf test in some parents may be good candidates for marker-assisted selection. That is the case for the QTL of resistance to several species of *Phytophthora* identified in Sca6 and in the Trinitario clone H. Other QTL identified with strong genetic effects observed after leaf tests, or observations over several years of harvests, could also be good candidates for initial testing of marker-assisted selection. Such is the case, for example, for the QTL identified by leaf tests in IMC57 or for the QTL for field resistance identified in UF676, T60/887 or IMC78.

Improving clones for resistance cannot be carried out independently from the improvement of other important traits, such as yield or quality. The usefulness of a mapping approach involving the main traits of interest also includes the ability to identify the linkage between favourable and unfavourable alleles of each trait of interest, and to more effectively estimate and manage the number of plants needed to select worthwhile combinations. On chromosome 4 of IMC78, an association was found between favourable alleles for field resistance, vigour and yield, and selection of all that area of the genome will be interesting to carry out with Marker-Assisted Selection. In other areas, recombinations would be needed to recombine favourable alleles of several traits.

Marker-assisted selection could make it possible to optimize selection for resistance traits and to construct favourable associations in a controlled way using markers close to the QTL. Locus-specific markers, like microsatellites, are highly

polymorphic and easy to reveal by PCR, and will be particularly useful in this strategy.

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