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# A meta–QTL analysis of disease resistance traits of *Theobroma cacao* L.

**C. Lanaud · O. Fouet · D. Clément · M. Boccara · A. M. Risterucci · S. Surujdeo-Maharaj · T. Legavre · X. Argout**

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**Abstract** *Theobroma cacao*, is a tropical understorey tree that is a major economic resource to several tropical countries. However, the crop is under increased threat from several diseases that are responsible for 30% loss of harvest globally. Although QTL data related to the genetic determinism of disease resistance exist in cocoa, QTL mapping experiments are heterogeneous, thus making comparative QTL mapping essential for marker assisted selection (MAS). Sixteen QTL experiments were analysed, and the 76 QTLs detected were projected on a progressively established consensus map. Several hot spots, with QTLs related to different *Phytophthora* species and other diseases, were observed. The likely number of "real" QTLs was estimated by using a meta-analysis implemented in BioMercator software. There was a twofold reduction in average confidence interval observed when compared to the confidence interval of individual QTLs. This alternative approach confirms the existence of several sources of resistance to different diseases of cocoa which could be cumulated in new varieties to increase the sustainability of cocoa resistance using MAS strategies.

**Keywords** Meta-analysis · QTL · Genetic-map · *Theobroma cacao* · Disease resistance · *Phytophthora* resistance

C. Lanaud (✉) · O. Fouet · D. Clément · M. Boccara · A. M. Risterucci · S. Surujdeo-Maharaj · T. Legavre · X. Argout
Centre de Coopération Internationale en Recherche Agronomique pour le Développment (CIRAD), CIRAD-BIOS TA A96/03, UMR DAP, Avenue Agropolis, 34398 Montpellier Cedex 5, France
e-mail: claire.lanaud@cirad.fr

## Introduction

*Theobroma cacao*, is a tropical tree from which cacao beans—'food of the gods' are derived. Cacao is a highly prized commodity with its beans being a major export commodity for several countries in West Africa (68% of world production), providing major economic resources to Ivory Coast, Cameroon, Nigeria and Ghana (Guiltinan et al. 2008). Currently, the worth of worldwide cacao industry is estimated at $73 billion dollars (Ploetz 2007) and provides 60,000 jobs (Morais 2005). Traditionally grown under thinned, forest shade, cacao affords sustainable benefits not only to the farmer but also to the environment through maintaining biological diversity and preventing land erosion by retention of soil moisture (Evans 2002; Belsky and Seibert 2003). However, the sustainability of cocoa is under increasing threat from both coevolved and newly-encountered diseases, which now constitute the most serious constraint to production in the Neotropics (Evans 2007).

Cacao diseases reduce the crops' potential globally by almost 30% annually, with some farms experiencing 100% losses (Keane 1992; Bowers et al. 2001). The three most crippling diseases of *T. cacao* since 1989 have been identified as black pod, frosty pod and witches' broom (Fulton 1989). Black pod is found in all cocoa growing areas, and is due to several *Phytophthora* species which vary in both their aggressiveness and the level of crop loss (Cilas and Despreaux 2004). One species, *P. megakarya*, spreading throughout West Africa, is particularly aggressive and can cause between 60 and 100% crop loss (Djiekpor et al. 1981). *P. palmivora* is less aggressive (responsible for 25% of crop losses), but widespread in all cocoa areas. *P. citrophthora* and *P. capsici* are present only in America. Frosty pod, caused by *Moniliophthora roreri*, and witches' broom, attributable to *Moniliophthora perniciosa* are only present in the Americas.

These diseases impact on cacao production, and socioeconomic consequences have been reviewed by Fulton (1989) and more recently by Evans (2007). Efforts to overcome these biotic stresses through eradication (phytosanitation) or by chemical means have not been practical to the resource poor farmer. A more promising approach is to identify varieties having higher levels of tolerance to diseases among germplasm collections and utilise them in well designed breeding programs. Consequently, disease resistance is the primary trait targeted by cacao breeders and sources of resistance have been identified for black pod (Iwaro et al. 2006), witches' broom (Paim et al. 2006; Surujdeo-Maharaj 2008) and frosty pod (Phillips-Mora and Wilkinson 2007.

QTL studies, aiming to understand the genetic bases of resistance to these diseases, have been carried out in recent years in order to identify and manage more efficiently, through marker assisted selection, the accumulation of resistance factors in new varieties. Most of these QTL studies, conducted in the frame of an international project funded by CAOBISCO (Lanaud et al. 2004a), were related to resistance to several *Phytophthora* species, while a more limited number of them aimed to analyse the genetic bases of witches' broom and frosty pod resistance. Besides their use in marker assisted selection, QTL studies are crucial for identifying genes that underlie trait variation, after cloning them and characterising their allelic diversity and their effects in the genetic resources.

QTL studies were generally developed from controlled crosses (Crouzillat et al. 1996, 2000a, b, 2003; Lanaud et al. 1999, 2003, 2004a, b; Motilal et al. 2000; Clément et al. 2003a, b; N'Goran et al. 2000; Flament 1998; Flament et al. 2000; Risterucci et al. 2003; Queiroz et al. 2003; Albuquerque and Figueira 2004; Brown et al. 2005, 2007; Faleiro et al. 2006; Figueira et al. 2006), but more recently, they are developed by association mapping strategies (Pugh 2005; Schnell et al. 2005; Marcano et al. 2007, 2009). In total, 76 QTLs were detected for various resistance traits.

Several statistical methods have been described to detect QTLs (Jansen 1996). Most of the QTLs were generally detected from controlled crosses and were characterised by their map position, their contribution in trait variation ($R2$), their LODscore value and their confidence interval (CI). However, each studied population reveals only a limited number of QTLs that are restricted to the allelic composition of the progenies from the two parents at the origin of the progeny. For each individual experiment, estimates of numbers of QTLs per trait are generally low (<8) and around four. Additionally, the confidence interval for each QTL location is not very precise (generally between 10 and 30 cM) (Kearsey and Farquhar 1998; Chardon et al. 2004).

QTL mapping experiments are generally totally heterogeneous, because they could involve different types of populations (F1, F2, Back crosses, etc.), different sample sizes, parents of different genetic origins, and under different environmental conditions, different methods of trait evaluation, and even different markers to establish genetic maps. Under such heterogeneous conditions, the question arises: do the QTLs detected in one particular progeny or population for a given trait correspond to QTLs identified in another population for a same or a related trait? Recently, marker assisted selection programs have been initiated for cocoa in several research institutions. For these reasons, the comparative QTL mapping is a difficult but necessary challenge to synthesise all this QTL information that could be fully valorised for cocoa improvement using marker assisted selection (MAS) strategies.

Until now, bioinformatics tools, integrated in the database COCOAGENDB (Ruiz et al. 2003; Argout

et al. 2006) allowed users to compare a part of QTLs detected in different maps, using CMAP, a web-based tool that allows users to view comparisons of genetic and physical maps (http://gmod.sourceforge.net/cmap/). This comparison was based on the common markers between maps which allow alignment of homologous groups and their corresponding QTL.

Several approaches have been more recently developed for the comparison and integration of multiple QTL mapping experiments, after establishment of a consensus map. Software like JoinMap (Stam 1993) allows constructing consensus map from raw segregating data of several populations. Such a composite map was recently established by Brown et al. (2007) by combining data from the Brazilian F2 population, data from an F1 population grown at CATIE (Brown et al. 2005, 2007) and data from the original reference map from CIRAD (Pugh et al. 2004).

However, raw data are generally not accessible for all populations. In alternative strategies, (Arcade et al. 2004) only loci positions, always available, can be used to merge genetic maps. As such, common markers are used to establish bridges between maps and to project the remaining loci and QTLs on a single map. This strategy offers an improved view of the genetic control for traits of interest compared to single population analyses. For cases where multiple QTLs for a given trait (at least 10), detected in independent experiments, are located in a common region, Goffinet and Gerber (2000) proposed a meta-analysis approach. This approach allows the use of existing published QTL information (location, $R2$, confidence interval) to determine the most probable real number of QTLs, their position and new confidence interval. Such a process can be applied to merge a large number of genetic maps and QTLs by iterative projection, and to refine their position on the genome.

The advantages of this method is not only to localise all markers and QTLs in a single figure representing the linkage groups, but also to synthetize all the information related to a cluster of QTL by identifying consensus QTLs.

Meta-analyses, first used in medical and social sciences, were recently applied in human genetics and evolution (Allison and Heo 1998; Lohmueller et al. 2003; Etzel and Guerra 2003) and in plant genetics to study the genetic architecture of flowering time in Maize (Chardon et al. 2004), resistance to soybean cyst nematode (Guo et al. 2006) and fiber development in cotton (Rong et al. 2007).

The present work attempts to synthesise most of the results of QTL or association studies related to cocoa resistance that are available. For this purpose, numerous QTL results were collected from publications, congress communications and internal programmes. Using our high density reference genetic map (Pugh et al. 2004), we projected on this single map 15 other genetic maps with their 76 corresponding QTLs related to disease traits after supplementary SSR mapping on some of them. This approach carried out with the "biomercator" software (Arcade et al. 2004) allowed highlighting the hot spots of QTL for each trait. In order to estimate the real number of QTLs included in these "hot spot", their consensus position and more probable confidence interval, we used the meta-analysis approach developed by Goffinet and Gerber (2000) and implemented in the Biomercator software.

## Materials and methods

### Collection of genetic maps and QTL results

With the objective of establishing a consensus map where all QTLs will be projected, we collected data from 16 QTL or association studies (1) by making a bibliographic review, (2) using data stored in Cocoagen DB and (3) using internal unpublished data. All information related to each population used for these analyses is reported in Table 1.

Information pertaining to all genetic maps was first collected or estimated according to the published maps. A first step of standardisation of linkage groups, names and orientation, and of marker names was carried out.

Information was also collected for each QTL or marker/trait association identified: original map position, $R2$ (% phenotypic variance explained by the given trait), confidence interval (CI) and LOD value (when known). The confidence interval was generally estimated by LOD value-1. When the CI was not given, it was estimated according to the method of Darvasi and Soller (1997) by the formula: $CI = 530/N \times R2$, where $N$ corresponds to the number of individuals of the studied population and $R2$ is the percent of phenotypic variance explained by the trait.

**Table 1** QTL studies considered for projection on a consensus map

| Popid | Reference | Progeny parents | Type of population | Population size (ind.) | Traits | Methods used |
|---|---|---|---|---|---|---|
| B1 | Brown et al. (2005) | (SCA6 × ICS1)AF | F2 | 79 | WBN | IM, MQM, Res. MQM |
| B2 | Brown et al. (2006) | P7 × UF273 | F1 | 256 | FRP BPP | IM, MQM |
| C1 | Clément et al. (2003a) | IMC78 × Catongo | F1 | 125 | BPF | IM, CIM |
| C2 | Clément et al. (2003a) | DR1 × Catongo | F1 | 96 | BPF | IM, CIM |
| Z1 | Crouzillat et al. (2000a) Crouzillat et al. (2000b) | P12 × Catongo | F1 | 55 | BPP | IM |
| Z2 | Crouzillat et al. (1996) Crouzillat et al. (2000b) | (Catongo × P12) × Catongo | BC | 131 | BPP | ANOVA, IM |
| FA | Faleiro et al. (2006) | (SCA6 × ICS1)AF | F2 | 82 | WBN | CIM |
| G1 | Figueira et al. (2006) | ICS39 × CAB208 | F1 | 168 | WBN | IM |
| G2 | Figueira et al. (2006) | ICS39 × CAB214 | F1 | 116 | WBN | IM |
| F1 | Flament et al. (2000) | T60/887 × amelonado | F1 | 112 | BPF BPL BPP | IM, KW test |
| F2 | Flament (PhD thesis) | ICS84 × UPA134 | F1 | 62 | BPL | IM, KW |
| | | SNK10 × UPA134 | | 78 | | |
| L1 | Lanaud et al. (1999) | UPA402 × UF676 | F1 | 114 | BPF BPL | |
| L3 | Lanaud et al. (2004) | 17-3/1 × 36-3/1 | F1 | 345 | BPL | IM, KW |
| PU | Pugh (2005) | Trinitario type | Collection | 150 | BPP | Ass. study Anova |
| RI | Risterucci et al. (2003) | (SCA6 × H) × IFC1 | F1 | 151 | BPL | MQM |
| MO | Motilal et al. (2000) | IMC57 × Catongo | F1 | 155 | BPL | IM |

The following method of QTL analyses were specified for each study: single interval mapping (IM), composite interval mapping (CIM), multiple QTL mapping (MQM), restricted MQM (Res. MQM), Single factor analysis (SFA), non parametric Kruskal and Wallis test (KW)

The several traits observed are reported in the text of material and methods

Candidate gene mapping was also integrated to the consensus map for a further comparison with QTL mapping. Several types of candidate genes were included: genes related to disease resistance or defence mechanisms (Lanaud et al. 2004c; Kuhn et al. 2003) and WRKY transcription factors potentially involved in resistance gene regulation (Borrone et al. 2004; Brown et al. 2005).

## Complementary microsatellite mapping and QTL analyses

Most of the maps established before microsatellite development, were constituted mainly of RFLP and AFLP markers. To carry out comparative mapping with the more recent QTL studies, based on maps constructed mainly with microsatellites, it was first necessary to locate additional microsatellites on the first maps established for QTL studies when it was possible. For this purpose, about 50 microsatellites were added on the maps established for three progenies: DR1 × Catongo, IMC78 × Catongo (Clement et al. 2003a, b) and (SCA6 × H) × IFC1 (Risterucci et al. 2003).

These new maps were then established with JoinMAP V.4 (Van Ooijen 2006) and new QTL analyses were subsequently carried out using interval mapping implemented in MapQTL software V.5 (Van Ooijen 2004).

## Traits observed

Several classes of traits were observed and are reported in Table 1 for each studied population.

Disease resistance to:

- Black pod caused by *P. palmivora, P. megakarya and P. capcisi*.

Resistance was evaluated in the field by the percentage of rotten pods (BPF) and under laboratory conditions, by leaf tests (BPL) or pod test (BPP). The letter, p, m, or c was added to the name of the QTL to differentiate the experiments made with *P. palmivora, P. megakarya* and *P. capcisi*, respectively.

- Witches' broom disease caused by *Moniliophthora perniciosa*.

Resistance was evaluated by the percentage of infected shoots (WBN) observed in field.

- Frosty pod caused by *Moniliophthora roreri*.

Resistance was evaluated by pod observations made in field, scoring for both internal (FRI) and external (FRX) infection of pods.

## Genetic maps and QTL projection on a unique consensus map

The map established by Pugh et al. (2004) was chosen as the reference to align QTLs detected in the 16 QTL studies. Indeed, it is the map which gathers the higher number of co dominant markers, with a majority of SSR and RFLP, linking together the first maps established mainly with RFLP markers and the more recent maps established mainly with SSR markers.

It was not possible to integrate in our analyses, maps established only with AFLP or RAPD markers, like those of Queiroz et al. (2003) due to the lack of common markers that allow the establishment of bridges between maps.

A consensus map integrating markers and QTLs, was constructed using BioMERCATOR software version 2.1 (Arcade et al. 2004), by iterative projection of each individual map and QTL on the unique consensus map progressively established from the reference map: this was done by projecting a first map (with its specific QTLs) on the reference map (initially chosen as a frame for the projection). This results in the first consensus map C1 between these first two maps. Next, a third map, with its QTLs, is projected onto the consensus map C1, thus giving rise to a second consensus map C2. Then a fourth map and its corresponding QTLs are then projected on the C2 map to give the new consensus map C3 etc., until all maps with their respective QTLs could be projected.

Only linkage groups from individual maps having QTL or localised candidate gene were projected on the consensus map. To avoid a too higher density of molecular markers on the consensus map, AFLP and RAPD markers were not projected, except in cases where they were the unique markers present at the side of the QTL and useful for the QTL projection.

In the few cases where the order of some common markers showed a discrepancy compared to the reference map, they were excluded from the projection process and only the next flanking markers were then used.

Each QTL projected was renamed: the first two letters corresponding to the first publication author, followed by letters identifying the trait as mentioned above. QTL for which the CI covered the whole length of the chromosome was excluded from the analysis.

The reference map was chosen as the main frame to integrate all the other maps. In some cases, linkage groups from other maps could be longer than LGs of the reference map (due to additional AFLP or RAPD markers not projected on the consensus map, for example). If, in these particular LGs, the CI of a QTL was extended until the end of the LG, its projection on the LG of the consensus map exceeded the end of the corresponding consensus LG. In that case, its length was reduced accordingly.

## Development of a website giving access to all QTL information and their projection on the consensus map

CocoaGEN DB (http://cocoagendb.cirad.fr) was chosen as the support to visualise the consensus map established with the iterative projection of all individual maps. This database was able to allow both the storing of genetic mapping data for all QTLs projected on the consensus map and their display through CMAP.

## Meta-analyses of QTLs

When the number of QTLs detected in a same chromosome for a given or related trait was close or higher than 10, we used the approach developed by

Goffinet and Gerber (2000), implemented in the BioMercator software (Arcade et al. 2004), to estimate the likely number of "real" QTLs, their probable location and CI. This approach compares different models for the "real" position of the n QTLs using an Akaike information criterion (Akaike 1973, 1992). It groups the several QTLs in different classes, according to their position and CI, and provides for the best model a number of consensus QTLs with their estimated position and CI.

## Results

### Marker and QTL projection on a consensus map

The reference map (UPA402 × UF676) is the most complete map having the largest part of RFLP and SSR markers produced. For this reason, this map was chosen to project the other maps successively.

The number of bridge markers, used to integrate linkage groups and corresponding QTL, varied according the maps projected; the linkage groups were integrated with at least 2 common markers with the consensus map, but generally more:

- the maps, C1, C2, F1, F2, L1, L2, L3, R1, Mo, were constructed with AFLP, and RFLP and SSR markers produced by CIRAD. All RFLP and SSR used to establish the maps were also mapped in the reference map. Microsatellites newly mapped in some progenies, provided between 2 and 9 markers common with the reference map for each linkage groups (with an average of four common markers/linkage group). This commonality allowed for the establishment of bridges with the reference map.
- maps from Crouzillat et al. (1996, 2000a, b) were mainly established with RFLP markers produced by Nestlé. However, some RFLP probes mapped reciprocally on CIRAD and NESTLE maps allowed bridging with 2–4 common markers per linkage groups having QTLs.
- the maps (B1, B2) constructed by Brown et al. (2005, 2007) were mainly established with SSRs produced by CIRAD and all mapped in the reference map. In this case, the number of bridge markers varied from 4 to 23 according to the linkage groups, (with an average of 10 common markers/linkage group). Similarly, the maps G1 and G2 established by Figueira et al. (2006), had 4–8 SSR markers/linkage group, allowing bridges to be established with the linkage map.

**Fig. 1** Consensus map established from the iterative projection of 16 different maps on the reference map established by Pugh et al. (2004) and projection of 76 QTLs detected using the BioMERCATOR software. QTLs were identified for resistance to several diseases caused by *Phytophthora palmivora, P. megakarya, P. capcisi, Moniliophthora perniciosa* and *Moniliophthora roreri*. **a** CH1, CH2, CH3, **b** CH4, CH5, CH6, **c** CH7, CH8, CH9, CH10. QTL names are explained in "material and methods" ▶

Following successive projections of each individual map on the reference map, a consensus map was established with 676 markers including 299 SSR, 264 RFLP, 28 candidate genes, and 85 other types of markers (mainly AFLP) needed for the QTL and CI projection.

This consensus map is represented in Fig. 1a, b and c.

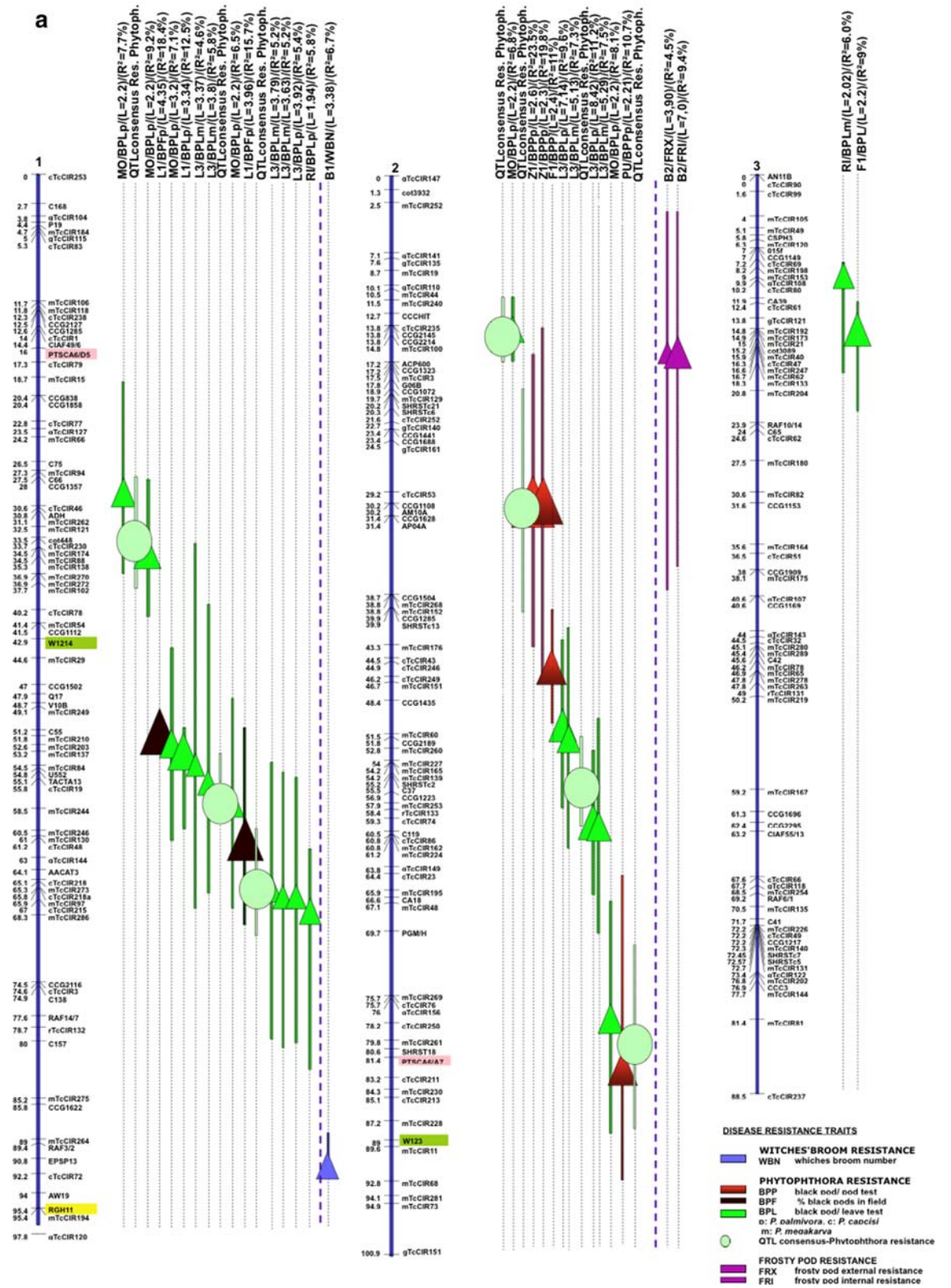
A total of 76 QTLs related to disease resistance were identified from 16 QTLs or association studies and projected on the consensus map as represented in Fig. 1a, b and c and summarised in Table 2.

### *Phytophthora* resistance

Sixty-five QTLs for *Phytophthora* resistance were detected in all chromosomes, with some hot spots observed in CH1, CH2, CH4 and CH5, where at least 10 QTLs from the different QTL or association studies could be detected. Generally, these hot spots gather QTLs related to *Phytophthora* resistance detected by different type of observations (rotten fruits in field or artificial inoculation tests) and different methods (pods infected in field or artificially) and different methods (pod or leaf inoculation tests).

A meta-analysis study was then carried out on CH1, CH2, CH4 and CH5, which satisfy the conditions of the Goffinet and Gerber (2000) approach. Both individual and consensus QTLs are reported on the consensus map (Fig. 1a, b, c). Forty-eight QTLs from the 65 detected QTLs were reduced to 13 consensus QTLs.

Thus, for example, in CH5, 12 QTLs were reduced to two consensus QTLs. The first consensus QTL was established from seven individual QTLs with effects varying from 5.6 to 11.4% while the second


a
1
2
3
MO/BPLp/(L=2.2)/(R²=7.7%)
QTLconsensus Res. Phytoph.
MO/BPLp/(L=2.2)/(R²=9.2%)
L1/BPFp/(L=4.35)/(R²=18.4%)
MO/BPLp/(L=3.2)/(R²=7.1%)
L1/BPLp/(L=3.34)/(R²=12.5%)
L3/BPLm/(L=3.37)/(R²=4.6%)
L3/BPLm/(L=3.8)/(R²=5.8%)
QTLconsensus Res. Phytoph.
MO/BPLp/(L=2.2)/(R²=6.5%)
L1/BPFp/(L=3.96)/(R²=15.7%)
QTLconsensus Res. Phytoph.
L3/BPLm/(L=3.79)/(R²=5.2%)
L3/BPLm/(L=3.63)/(R²=5.2%)
L3/BPLp/(L=3.92)/(R²=5.4%)
RI/BPLp/(L=1.94)/(R²=5.8%)
B1/WBN/(L=3.38)/(R²=6.7%)
QTLconsensus Res. Phytoph.
MO/BPLp/(L=2.2)/(R²=6.8%)
QTLconsensus Res. Phytoph.
Z1/BPPp/(L=2.6)/(R²=23.5%)
Z1/BPPp/(L=2.3)/(R²=19.8%)
F1/BPPp/(L=2.4)/(R²=11%)
L3/BPLp/(L=7.14)/(R²=9.6%)
L3/BPLm/(L=5.13)/(R²=7.3%)
QTLconsensus Res. Phytoph.
L3/BPLp/(L=5.26)/(R²=7.5%)
MO/BPLp/(L=2.2)/(R²=8.1%)
PU/BPPp/(L=2.21)/(R²=10.7%)
QTLconsensus Res. Phytoph.
B2/FRX/(L=3.90)/(R²=4.5%)
B2/FRI/(L=7.0)/(R²=9.4%)
RI/BPLm/(L=2.02)/(R²=6.0%)
F1/BPLL/(L=2.2)/(R²=9%)
DISEASE RESISTANCE TRAITS
WITCHES'BROOM RESISTANCE
WBN whiches broom number
PHYTOPHTHORA RESISTANCE
BPP black pod/ pod test
BPF % black pods in field
BPL black pod/ leave test
p: P. palmivora. c: P. capcisi
m: P. meoakarva
QTL consensus-Phytoophthora resistance
FROSTY POD RESISTANCE
FRX frosty pod external resistance
FRI frosty pod internal resistance

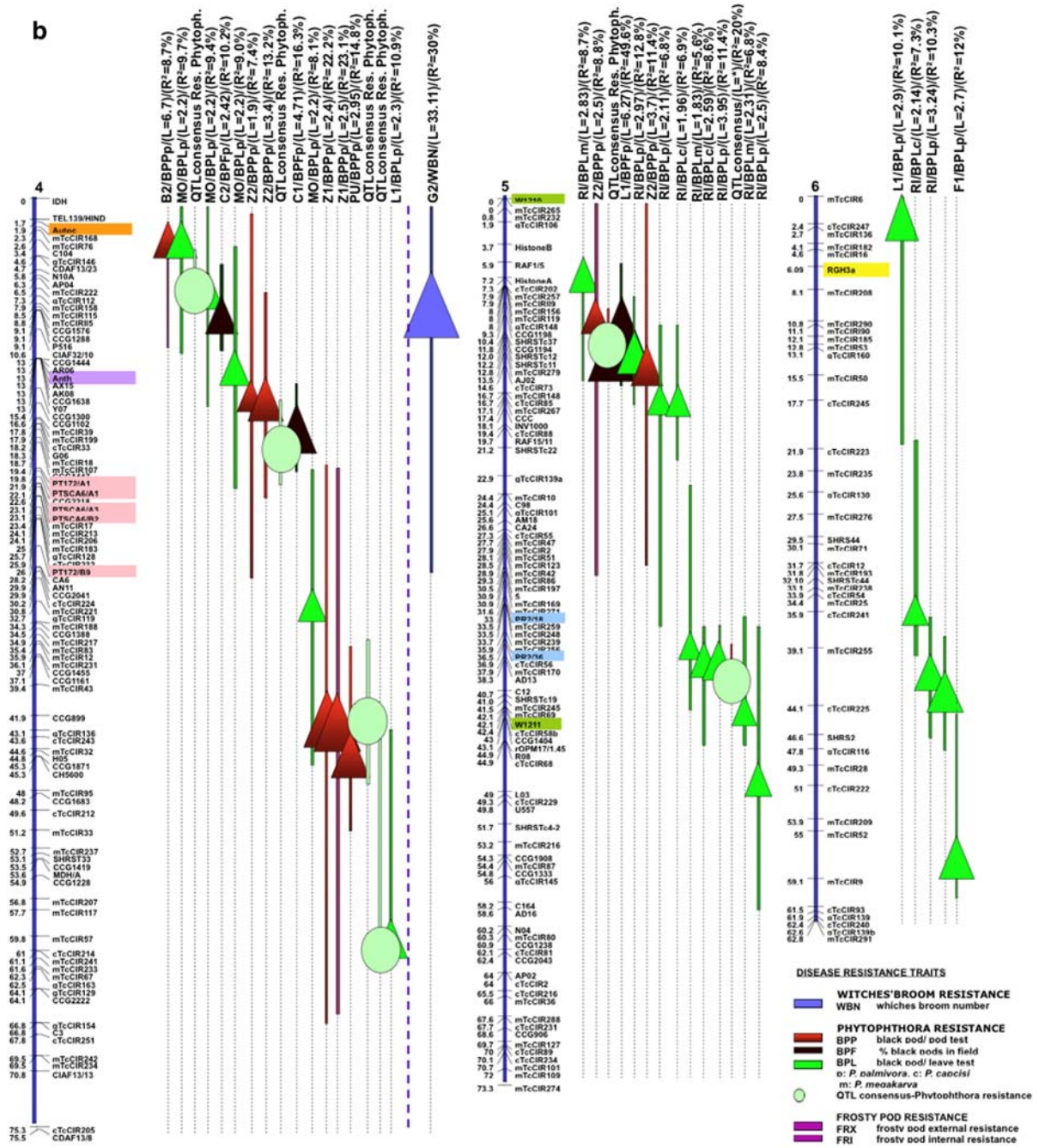



**Fig. 1** continued

consensus QTL corresponded to five individual QTLs with effects varying from 6.9 to 49.6%.

Microsatellite markers closely linked to these QTLs and corresponding consensus QTL could be easily identified for use in marker assisted selection, owing to the high density of markers in these regions. For example, mTcCIR156 and mTcCIR119 are located 0.7 cM from the upper consensus QTL, and mTcCIR170 is only 0.9 cM from the second consensus QTL. These two consensus QTLs combine QTLs

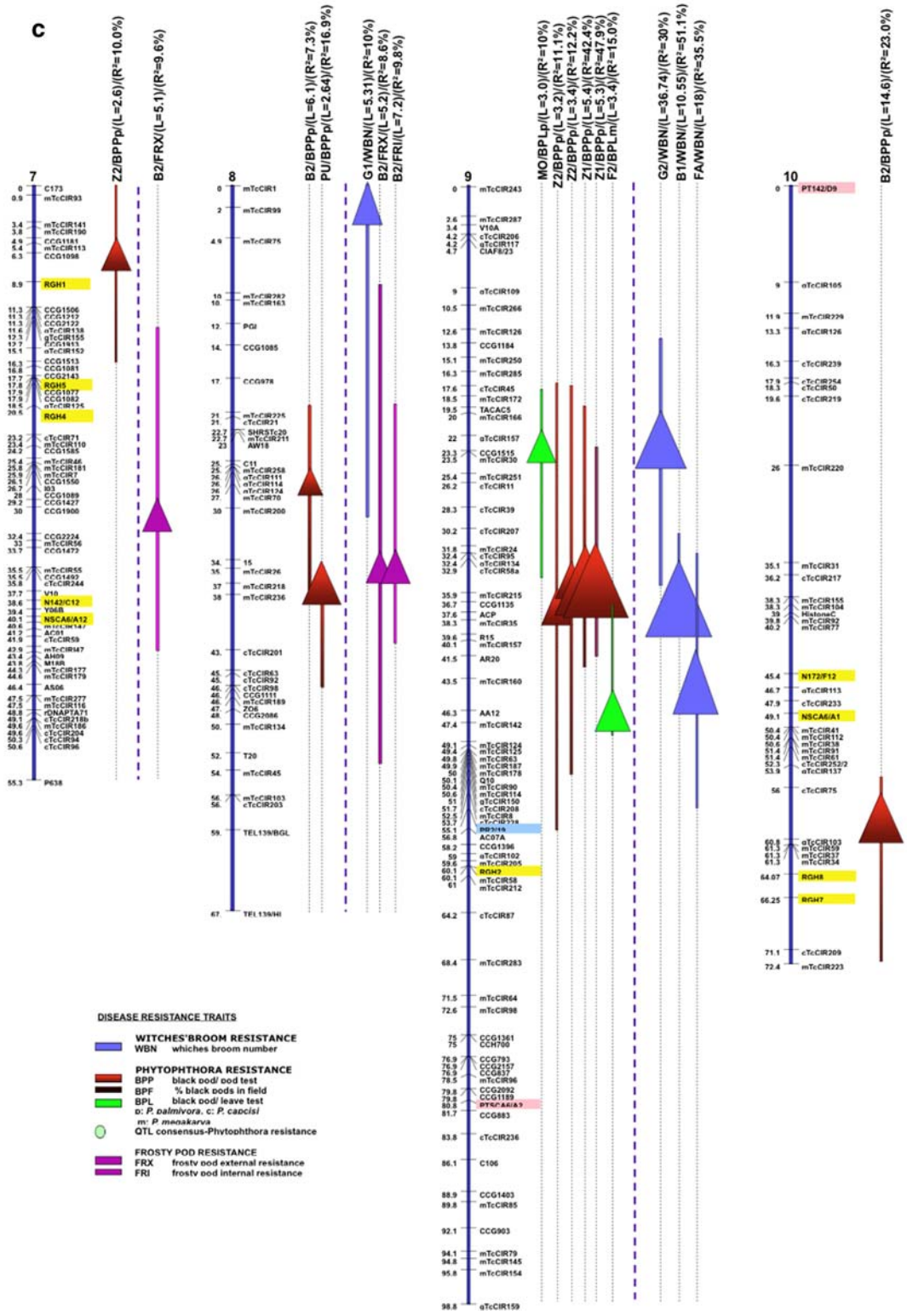



**Fig. 1** continued

**Table 2** Repartition of the 76 individual QTLs detected in the several studies and of consensus QTLs projected in the consensus map, according to chromosomes and to traits considered

| Chromosome number of QTLs for: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Phytophthora* resistance | 13 | 10 | 2 | 13 | 12 | 5 | 1 | 2 | 6 | 1 | 65 |
| Number of consensus QTLs for *Phytoph.* resist. | 3 | 4 | | 4 | 2 | | | | | | 13 |
| Number of individual QTLs/consensus QTL | 2/7/5 | 1/3/5/2 | | 5/4/4/1 | 7/5 | | | | | | |
| Witches' broom resistance | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 0 | 6 |
| Frosty pod resistance | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 5 |

The consensus QTLs were computed using the Biomercator software as indicated in material and methods

related to resistance for three different species of *Phytophthora*: *P. palmivora, P. megakarya* and *P. capcisi* and will be of particular interest to be considered for marker assisted selection.

In considering the average CI of the 13 consensus QTLs (8.98 cM) compared to those of the 48 individual QTLs (19.7 cM), the meta-analysis has allowed a twofold reduction in the QTL CI.

Some common regions were detected for resistance to different *Phytophthora* species as is the case with CH1, CH2, CH5, CH6, and CH9. In CH1, CH2 and CH5 where meta-analysis could be made, these QTLs were included in same meta–QTLs.

### *Co-localisation between QTL for resistance to Phytophthora and candidate genes*

Some co-localisations were observed between QTL and candidate genes (Fig 1 a, b, c, and Cocoagen DB web site):

- On CH2 and CH4, Serine threonine kinase class resistance genes are localised within the CI of a consensus QTL for *Phytophthora* resistance.
- On CH5, Pathogenesis Related proteins (PR2) are found within the CI of the consensus QTL for *Phytophthora* resistance.
- On CH6, CH7 and CH10, NBS class resistance genes are localised within the CI of individual QTLs, but these QTLs need to be confirmed by other QTL studies.

### Witches' broom resistance

Six QTLs have been detected in four different studies. They are present in CH1, CH4, CH8, and CH9 where three QTLs have been detected.

### Frosty pod resistance

Five QTLs were detected on CH2, CH7 and CH8 from only one QTL study (Brown et al. 2007).

### Common regions detected for various disease resistance

QTLs for resistance to various diseases were detected in common chromosome regions such as:

- CH2 and CH8: QTLs for black pod and frosty pod resistance.
- CH4 and CH9: QTLs for black pod and witches' broom resistance.

## Discussion

A large number of QTLs related to disease resistance have been detected up to now in cocoa. Our main concerns are to compare and identify them more precisely from the different genetic sources, in order to optimise the accumulation of favourable alleles in improved varieties through a marker assisted selection strategy.

Although the number of individuals per cocoa population studied was sometimes low, the presence of QTLs (identified from independent experiments and environmental conditions) within the same chromosome region, allow confirming their identification; their alignment on a same common map offers a more complete picture of the genetic control of traits compared to results from individual maps. Comparative mapping implies having common markers to compare and align several maps to be used in the generation of a consensus map where all QTLs

can be projected. For this purpose, supplementary microstellite markers were localised in four previously mapped progenies, where together, RFLP and microsatellite markers allowed the establishment of bridges among most of the QTL mapping studies.

The iterative projection of maps onto our reference map was chosen because of the inaccessibility of raw data for other maps, and because most of existing microsatellite and RFLP markers was mapped in the reference map, allowing comparative mapping. Seventy-six QTLs already detected for several resistance traits in cocoa in a wide series of experiments and genetic backgrounds were then projected in this consensus map.

Although a large body of QTL information exists for *Phytophthora* resistance in cocoa, the merit of this QTL overview has been to highlight genome "hot spots", where QTLs detected in different studies are localised within the same genome region. At least eight genomic regions appear to be clearly involved in *Phytophthora* resistance, suggesting that it is possible to cumulate different sources of *Phytophthora* resistance to improve the sustainability of cocoa resistance.

This situation is similar to QTL studies carried out on potato for resistance to *Phytophthora infestans* (Leonards-Schippers et al. 1994) or on pepper for resistance to *Phytophthora capcisi* (Lefebvre and Palloix 1996; Thabuis et al. 2003) where several loci are also involved in quantitative resistance to *Phytophthora*.

Although, 'hot spot' regions were identified in this study, it was difficult to evaluate the real number of QTLs identified by the different studies. Thus, the question remained that some QTLs located within the same genome region could either be the same QTL or not. In that case, the meta-analysis approach, developed by Goffinet and Gerber (2000), provided a new way to resolve this complex situation allowing an estimation of the real number of QTLs and refining their position in the genome by reducing their CI. Using this approach, 48 individual QTLs related to *Phytophthora* resistance, were reduced to 13 consensus QTL with a CI two fold smaller than for individual QTLs.

The iterative projection of all QTLs in a common genetic map has also highlighted common genome regions with QTLs related to different diseases. This is the case in CH9, where the same region is involved in *Phytophthora* and witches' broom resistance. Such common localisation could reflect common genes or cluster of genes involved in these resistances.

Recently a large EST collection has been generated (Argout et al. 2008) and provides the basis for large functional genomic and gene mapping studies. High throughput genotyping tools based on SNP (single nucleotide polymorphisms) and SSR defined from this EST collection will facilitate the construction of very high density gene maps. Comparative QTL and candidate gene mapping will be the first step towards the discovery of genes underlying trait variations; an increased precision of QTL localisation provided by such a multi QTL approach will facilitate the establishment of links between genetic and genomic approaches. *Theobroma cacao* is a species classified in the same rosid II as *Arabidopsis*, the model plant (APG II 2003) for which extensive work has been done in functional genomics. A synteny approach using *Arabidopsis* could also help discover genes underlying QTLs. This approach was recently carried out to study the Maize (*Zea mays*) flowering traits; indeed, genes for flowering time, identified in rice, *Oryza sativa*, belonging to the same family, were mapped in the Maize genome and permitted identification of 19 associations between genes for flowering time and traits (Chardon et al. 2004).

The present work demonstrates the interest in carrying out such multi QTL analysis to confirm and refine the existence of already detected QTLs. However, for most traits in cocoa, QTL information remains limited. It is therefore necessary to continue QTL detection for most traits in cocoa, allowing to accumulate these results from different experiments conducted under varying environmental conditions and, subsequently, carry out meta-analyses.

The synthetic information provided by this work confirms the existence of several sources of *Phytophthora* resistance which could be cumulated in the same variety to improve the resistance sustainability in *T. cacao* to *Phytophthora* species. A marker assisted selection for enhancement of cocoa resistance to *Phytophthora* is being carried out in Cameroun, where major losses in production are due to *Phytophthora megakarya*. The meta-analysis conducted in this work will help a better choice of QTLs to be used for the MAS strategies, by focusing

mainly on those having a good reliability following multiQTL analyses from different genetic sources and environmental conditions.

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