Mapping QTLs for Black pod (*Phytophthora palmivora*) resistance in three hybrid progenies of cocoa (*Theobroma cacao L.*) using SSR markers

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Abstract- To identify QTLs for cacao resistance to *Phytopthora palmivora*., a study was conducted in three hybrid progenies of CNRA in Côte d'Ivoire obtained from crosses (SCA6 x H) x C1, (P7 x ICS100) x C1 and (P7 x ICS95 x) x C1. SCA6 (Scavina 6) and P7 (Pound 7) are resistant Forastero clones, while ICS100, ICS95 and H are moderately susceptible Trinitario clones and C1 (IFC1), a susceptible Forastero clone. Resistance to *P. palmivora* was assessed by means of pod rot rate (PRR) in field and leaf discs inoculation with zoospores (Folres) in both half-sib progenies, while only PRR was investigated in the progeny derived from (SCA6 x H) x C1. For each progeny, a SSR-based linkage map was constructed and Kruskal-Wallis test and interval mapping were applied, associating phenotypic data. Over the three progenies, 8 QTLs of PRR and 3 QTLs of Folres were detected on different chromosomes. One QTL of Folres (R^2 = 17.3 %) has been identified on chromosome 10, for the first time. The chromosome 1, carrying QTLs of PRR and Folres, is an interesting support of cacao resistance to *Phytophthora.* Chromosomes 4 and 6, each, of of two different parents carry QTLs of PRR In addition, the association mTcCIR291-PRR, on the chromosome 6 was noted in the family derived from (SCA6 x H) x C1, as well as in one of the two half-sib families. Markers linked to QTLs are valuable potential candidates for a future exploiting of these QTLs as selection tools.

Index Terms- Quantitative trait loci (QTL), Resistance to *Phytophthora palmivora*. Simple sequence repeats (SSR), *Theobroma cacao* L.,

I. Introduction

Cacao (*Theobroma cacao* L.) is an important export commodity crop in many countries in Latin America, Africa and Asia. It provides a livelihood for over 120 million people worldwide (Green America, 2014). However, cocoa production has been constrained by a number of factors, which include low productivity; pests and diseases. The *Phytophthora* Pod Rot (PPR) or black pod, caused by fungi of the genus *Phytophthora*, is one of the most devastating diseases in cacao cultivation because of its direct effects on yield losses that ranged from 10 to 100 % of the total annual production, depending on country, *Phytophthora* species and zones (Dakwa, 1987; Akrofi *et al*., 2003; Ploetz, 2007; Pokou *et al*., 2008). Despite the application of chemicals, PPR is increasingly difficult to control and in most countries chemical application is being restricted, for globally durable cultivation purpuses. Environmentally safer alternative controls are being attempted (Opuku *et al.*, 2003; Mpika *et al*., 2009a, 2009b; Adebola and Amadi, 2010; Gadji *et al*., 2015; Fister *et al*., 2015). There is thus an urgent need for durable resistant cacao cultivars. Resistance to pathogens is a complex trait controlled by many genes (Warren and Pettitt, 1994; Ndoumbé *et al*., 2001; Tahi *et al*., 2006a; Nyassé *et al*., 2007) and is still being well unknown.

Since many decades, steps towards resistance to diseases in cacao breeding programs by means of traditional methods, crosses and generations of phenotypic backcrossing or selfing, have been limited. This phenotypic-based selection is laborious and time consuming for cacao, a slow-growing plant, in addition to be expensive. Thus, despite these efforts, many clones and varieties are susceptible to *Phytophthora spp* (Eskes and Lanaud, 2001). Up to now, no genotype with complete resistance to black pod disease has been found or created. Thus, the introduction of molecular markers in the selection process, via markers-assisted selection, should improve the efficiency and should be a convenient alternative to phenotypic selection. Indeed, DNA markers are very efficient and powerful tools in plant breeding for the genetic dissection of quantitative traits and the early screening of desired genotypes in perennial crops, such as cacao, thereby offering new opportunities for genetic improvement in cacao.

In cacao, few studies have been conducted to analyze markers-trait associations and to detect QTL for resistance to *Phytophthora* (Lanaud *et al*., 1999, 2001, 2004; Crouzillat *et al*., 2000a, 2000b; Flament *et al*., 2001; Motilal *et al*., 2001; Clement *et al*., 2003a, 2003b; Risterucci *et al*., 2003; Brown *et al*., 2007). In these studies, markers were combined: either, markers other than microsatellite or a few SSR with markers of other types. In these cases where different types of markers were mixed, problems occurred in interpreting the results with complex profiles (Herran *et al*., 2000) and accordingly should be so in implementing MAS.

Microsatellite, (SSR), is an ideal PCR-based DNA marker for genetic mapping and MAS because of their multiple advantageous features (Röder *et al*., 1998; Araújo *et al*., 2007; Santos *et al*;, 2012; Ting *et al*., 2014; Stack *et al*., 2015) that

make them more suited for molecular genetic studies in developing countries like Côte d'Ivoire.

The present study aimed at identifying SSR markers-based QTLs for resistance to *Phytophthora palmivora*, in three related hybrid populations monitored over a 3-year period. QTL stability across time was also analyzed.

II. MATERIALS AND METHODS

Plant materials

Three mapping hybrid cacao populations generated from crosses (SCA6 x H) x C1, (P7 x ICS100) x C1 and (P7 x ICS95) x C1, comprised, respectively, of 179, 173 and 183 plants and set up at the Centre National de Recherche Agronomique (CNRA) research station in Bingerville, south-east of Côte d'Ivoire, were studied. C1 (or IFC1), the common male parent, a highly homozygous (Risterucci *et al*., 2003) Lower Amazon Forastero clone local type Amelonado, is susceptible to pod rot (Paulin, 1990). ICS100, ICS95 and H are Trinitario clones. ICS95 is moderately susceptible to *Phytophthora sp*. (Blaha and Lotode, 1976; Nyassé *et al*., 2007), while ICS100 is moderately susceptible to *P. megakarya* (Nyassé *et al*., 2007). P7 (or Pound 7) and SCA6 (Scavina 6) are Upper Forastero amazon clones from wild origin (Paulin, 1990 ; Nyassé *et al*., 2007). P7 is resistant to *Phytophthora palmivora* (Iwaro *et al*., 2006; Tahi *et al*., 2006b; Nyassé *et al*., 2007) and able to transmit high resistance levels to its offsprings (Tahi *et al*., 2006a). It has been one of the progenitors of commercial varieties in Côte d'Ivoire, especially in crosses with Lower Amazon Forastero clones (Amelonado). SCA6 is known for its high resistance level to black pod disease (Iwaro *et al*., 1997a, 1997b, 2005, 2006; Risterucci *et al*., 2003 ; Tahi *et al*., 2006b).

Experimental design

The trial, set up in 1997 according to a completely randomized mating design of rows with border without shade trees, spreaded on 1.25 ha. Each tree represented a unique genotype. Cacao trees were planted at 3 m x 3 m spacing. Parental trees were not included in the trial. Pollination was not controlled. Hand weedings were supplemented with chemical treatments, if needed, by spraying Kalach 360 SL (360 g/L) at the rate of 10 mL/L water. Pesticides treatments were directly applied with Thiodan 50 CE at the rate of 12.5 mL/L water. Regular pruning was performed on the exceeding plagiotropic and orthotropic branches, along with the removal of parasitic epiphytes (*Loranthus* spp). No fertilizer was applied.

Resistance to *Phytophthora palmivora* assessment

Resistance assessment on a single tree basis, was carried out following two different methods: field pods rot data-based assessment (pod rot rate due to black pod disease: PRR) and artificial leaf discs inoculation test (''resistance assessed on'flesf discs'': Folres).

During three cocoa campaigns (september to august), a sanitary harvest was carried out every two weeks and healthy ripe and rotten (or infected) pods counted. The PRR was measured as the percentage of cumulated rotten pods number in relation to the total number of pods (healthy ripe and rotten pods) over the three harvest periods, from 6th to 9th year, after planting.

The artificial inoculation method (Folres) adopted was the leaf disc test as described and performed in Akaza *et al*. (2009). Only trees of progenies derived from crosses (P7 x ICS100) x C1 and (P7 x ICS95) x C1 were evaluated by this latter method.

DNA extraction

Five grams of the lamina of a young green leaf of each tree were ground to a fine powder in liquid nitrogen. The frozen powder was suspended in 5 ml of extraction buffer [100 mM Tris-HCl (pH8), 1.4 M NaCl, 20 mM EDTA, 2 % MATAB (Mixed Alkyltrimethylammonium Bromide), 1% PEG 6000, 0.5 % Sodium Sulphite] contained in a 15 ml tube and preheated to 74 °C in water bath. The suspension was mixed and the mixture incubated in water bath for 30 min at 74 °C. Every 5 min, it was homogenized. After cooling at room temperature, 5 ml of Chloroform:Isoamylalcohol (CIAA) (24:1, v/v) were added and the tube was inverted gently till obtaining a homogeneous emulsion that was then centrifuged at 4,000 rpm for 20 min at 4 °C. The resulting upper aqueous phase was carefully transferred into a new 15 ml tube. About, 0.7 volume of cold isopropanol was added to that aqueous phase and DNA was precipitated by gentle inversions of the tube. The DNA pellet was removed, ambient air-dried and suspended in 500 µl of low-salt TE buffer [50 mM Tris-HCl (pH8), 0.7 M NaCl, 10 mM EDTA, H_2O]. DNA was kept for dissolving either at room temperature for 48 hours or at 40 °C for 24 hours. DNA solutions were then preserved at -20 °C until use.

Crude DNA solutions were, later, purified. Concentrations of purified solutions were evaluated and these solutions diluted in sterile bi-distilled water to a final concentration of 0.5 ng/µl before use for microsatellite analyses.

SSR analysis

The mixture for amplification, up to a final volume of 10 µl with sterile deionized water, contained 5 µl of 0.5 ng/µl of genomic template DNA solution, 1 x of the supplied PCR buffer (100 mM KCl, 10 mM Tris-HCl pH 8.3, 0.05 % w/v gelatin), 200 µM dNTP mix, 0.5 mM $MgCL_2$, 0.08 µM forward primer 5' labelled with the M-13 tail sequence 5'-CACGACGTTGTAAAACGAC-3', 0.1 µM reverse primer, 0.1 µM M13 fluorescent Infrared Dye (IRDye) 700 or 800 (Li-Cor) that matched the sequence of the M13 tail and 0.1 U/µl Taq DNA polymerase (Eurobio, France). To reduce the likelihood of amplifying nonspecific sequences, the touchdown PCR amplification profile used consisted of 5 min at 95 °C of initial DNA denaturation cycle, followed by first ten cycles of 30 s denaturation at 95 °C, 45 s for annealing at 55 °C or 60 °C according to the primer and 45 s extension at 72 °C, with 1 °C decrease in annealing temperature per cycle, then 25 cycles of 95 °C denaturation for 30 s with constant annealing temperature (46 °C, 51 °C, 53 °C or 55 °C, according to the primer) and a final extension at 72 °C for 8 min. PCR were performed either in a MJ Research PTC-200™ thermal cycler (Waltham, MA, USA) in 96-well microplates or in an Eppendorf MastercyclerR ep384 (Perking Elmer) thermal cycler in 384-well PCR plates.

IR700 or IR800-labeled PCR products of each marker were diluted 8-fold either with blue formamide (98 % deionized formamide, 10 mM EDTA, 0.05 % bromophenol blue and 0.05 % xylene cyanol) or with urea blue, as stop/loading buffer.

Products of at most four markers were pooled and subjected to electrophoresis for SSR alleles resolving in denaturing 6.5 % polyacrylamide precast sequencing gels, 25-cm long and 0.25-mm thick, with 7 M urea in 10 x TBE, 175 ml of 10 % fresh ammonium persulphate and 25 µl TEMED. An IRDye700-labelled 50-350 bp concentrated sizing standard (Li-Cor) was included at both right and left border lanes to facilitate semiautomatic analysis of the gel and sizing of fragments.

Electrophoresis and visualization of SSR alleles, under 41 W constant power at 50 °C for 1.5 to 2 h were carried out using either Li-Cor 4200-IR^2 or Li-Cor 4300-IR^2 automated DNA Analyzer (Li-Cor, Inc., Lincoln, NE, USA). Migration images were analyzed and alleles sized (scored) using Saga^{GT} Generation 2 Version 3.2.1 automated microsatellites analysis software (Li-Cor, 2004) and coded with Jelly 0.1 (Rami, unpublished).

Two hundred and nineteen (219) chromosome-specific microsatellites (mTcCIR) developed by CIRAD and mapped on other progenies were selected on the basis of their informations in published findings (Lanaud *et al*., 1999; Risterucci *et al*., 2000, 2003; Pugh *et al*., 2004 ; Fouet *et al*., 2011) and so that to cover the 10 chromosomes of cacao genome. After screening on the parents of each mapping population, polymorphic SSR were subsequently used to genotype individuals of that population.

III. Data analyses

Phenotypic data

Normal distribution of the data of each trait was verified applying the chi square test (χ^2) and/or the Agostino test at the threshold $\alpha = 5$ % . Variances homogeneity was also checked by the test of Bartlett and/or the test of Brown-Forsythe. Means, correlation coefficients (r) according to Pearson between PRR and Folres and analyses of variance were calculated or performed with STATISTICA 7.1 software (StatSoft Group Inc., Tulsa, Okla.), SPSS 17.0 and XLSTAT-Pro 7.5. Trees that did not produce any pod were discarded from descriptive statistical analyses. Natural logarithm transformation, square root transformation or arc sine transformation were applied, according to the type of trait analyzed, in case of evident anormality. Non parametric tests were performed when transformations did not achieve a sufficiently normal distribution.

Genetic map construction

The genetic mapping of polymorphic SSR was performed using the JoinMap 4.0 software (Van Ooijen, 2006). Owing to the high homozygosity level of the C1 clone, the unique male parent, the genotyping data were analyzed according to a pseudo-test-cross strategy. Thus, the genetic maps are obtained from the female parents, respectively: the hybrids derived from SCA6 x H, P7 x ICS100 and P7 x ICS95.

Marker loci fitting the expected 1:1 Mendelian segregation ratios at $p < 0.05$ through the Chi^2 square (χ^2) test for goodness-of-fit in relation with the codominant form as prescribed by JoinMap 4.0 were included in linkage analyses. The linkage groups (LGs) were established with the LOD score varying from 2.0 to 10.0 which gave the number of LGs closest to the 10 expected. The order of the markers within each linkage group was estimated using the least-square method, through an iterative process, at a maximum recombination rate of 0.3 and a minimum LOD of 4.0. Recombination frequencies were converted into map distances in centiMorgans (cM) with the Kosambi's mapping function (Kosambi, 1944). Chromosomes (LGs) carrying QTLs were produced visually by using MapChart 2.2 software (Voorrips, 2002) and/or Spidermap software v1.4.7b (Rami, 2009).

QTL mapping

MapQTL software version 5.0 (Van Ooijen, 2004) was used to apply two QTL detection methods: the marker by marker test or single-marker locus analysis (SMA) and the simple interval mapping (SIM). The SMA of MapQTL uses the Kruskal-Wallis (KW) test, a non parametric rank sum test, equivalent of the one-way ANOVA, that does not require any hypothesis on the phenotypic distribution of a quantitative trait, was first applied to detect associations between markers and individual traits, at a very highly significant (****) probability threshold of 0.005 (5 ‰). According to Van Ooijen (2004), the Kruskal-Wallis (KW) test produces more significant tests when using co-dominant than dominant markers..Then in the SIM analysis, the presence of a QTL was declared significant at $p < 0.05$, and a LOD value of 2,0 for which this QTL is one-hundred-fold more likely than its absence. Finally, a QTL was retained significant, in this study, when satisfied both thresholds adopted.

In addition, the percentage (%) of the phenotypic variance explained by a single QTL, that represents the coefficient of determination (R^2), was estimated as the ratio of sum of squares of the marker (QTL) to the total sum of squares by the maximum-likelihood estimation method.

IV. RESULTS

Phenotypic data

Distribution of data was normal ($p > 0.05$) for PRR in the family derived from (SCA6 x H) x C1 and for Folres in both half-sib families, but quasi normal for PRR in the family derived from (P7 x ICS100) x C1. It deviated to normal distribution for data of PRR in the family derived from (P7 x ICS95) x C1. For this trait, distributions remained non normal despite transformations performed. Non-parametric tests were, thus, applied. Homogeneity of variances was observed for both PRR and Folres.

PRR and Folres were not correlated. The analysis of variance revealed different influences of the factor genotype **(a** tree = a single genotype**)** on the variation of traits: significant ($p < 5$ %) for PRR, very highly significant ($p < 0.1$ %) for Folres.

Genetic linkage maps

The main characteristics of the three maps are presented in table 1. The sizes of the linkage groups were highly correlated with the number of markers per map unit for the parents "SCA6 x H" and "P7 x ICS95", with correlation coefficients of, respectively, r = 0.84 and r = 0.96, clearly indicating the relatively even distribution of the markers within the linkage groups. On the contrary, the markers distribution was less even in the map of the parent "P7 x ICS100" as supported by r = 0.43.

The length and the number of markers of the most densely populated, longest and shortest chromosomes in each female parent are given in table 2. In the parent "P7 x ICS95', LG 3 was

the most densely populated and also the shortest. In both parents "SCA6 x H" and "P7 x ICS100", LG 2 was the longest. It was longer in the parent "P7 x ICS100" with 131.2 cM, but contained little less (11) markers than in the parent "SCA6 x H" with 108.2 cM and 19 markers

Sixty-one, fifty-six and fifty-five markers were common to maps of parents "SCA6 x H'' and ''P7 x ICS100'', ''SCA6 x H'' and ''P7 x ICS95'', ''P7 x ICS100'' and ''P7 x ICS95'', respectively. Thirty-eight markers were common to the three maps. Common markers to homolog LGs allowed comparison of markers ordering among these three maps. For each LG, a high co-linearity was observed (Fig. 1). Thirty-five markers were mapped at the same positions in parents ''P7 x ICS100'' and ''P7 x ICS95''.

Fourteen markers (14 %) in the parent "P7 x ICS100" and 11 (13,9 %) in the parent "P7 x ICS95" were skewed, which showed a deviated segregation from the expected 1:1 ratio, at significance level $P < 0.05$. These markers are marked with asterisks (Fig. 1). No skewed marker was observed in the patent "SCA6 x H".

QTL mapping

Based on the combination of Kruskal-Wallis test and simple interval mapping approach, QTLs were obtained (Fig. 1) and summarised, with the main characteristics, in table 3. The phenotypic variation for traits explained by individual QTLs, R^2, ranged from 5.9 to 34.3 %.

QTL of resistance to *Phytophthora palmivora*

Height QTLs of resistance to *P. palmivora* assessed by means of the pod rot rate (PPR) in field were detected: one each on LGs 2 and 4 of the parent "P7 x ICS95", three each in parents "SCA6 x H" on LGs 1, 6, 8 and "P7 x ICS100" on LGs 4 and 6. Regarding resistance evaluated by means of the leaf discs assay (Folres), three QTLs were identified only in the parent "P7 x ICS100", on LGs 1, 3 and 10. Chromosome 1 carried QTLs for resistance assessed by both PPR and Folres approaches: one QTL of PPR in the parent "SCA6 x H" and one QTL for Folres in the parent "P7 x ICS100". Chromosome 4 of parents ''P7 x ICS100'' and ''P7 x ICS95'' and chromosome 6 of parents ''SCA6 x H'' and ''P7 x ICS100'' carried QTL of PPR. The highest LOD score (4.35) for PPR was obtained on chromosome 6 of the parent ''SCA6 x H'', whereas that (3.48) for Folres was obtained on chromosome 10 of the parent ''P7 x ICS100'' (Table 6). R^2 of PPR QTLs, relatively high and ranging from 13.2 to 27.6 %, were more diverse in progenies derived from crosses (SCA6 x H) x C1 and (P7 x ICS100) x C1 than that derived from cross (P7 x ICS95) x C1. In the progeny derived from (P7 x ICS100) x C1, R^2 of QTLs for PPR and Folres (from 13.8 to 17.3 %) were similar.

V. DISCUSSION

Phenotypic data

Normal distribution of values of indicates their polygenic inheritance. That is the case of Folres in the family derived from (P7 x ICS100) x C1, Folres in the family derived from (P7 x ICS95) x C1. Traits exhibiting non normal distribution of values can also be poly-genetically inherited. Besides, the polygenic inheritance is suggested by the transgressive segregation of phenotypic data in each family. That was demonstrated in wheat (Kumar *et al*., 2006).

The highly significant effects of the genotype (tree) on the variation of the resistance to *Phytophthora* predict high probabilities of detecting QTLs involved in its expression; the search for QTLs is worthwhile.

Genetic maps

The current maps are the first SSR-based genetic linkage maps used for searching QTL in *T. cacao*. Ten linkage groups (LG), corresponding to the ten haploid chromosomes of *T. cacao*, were obtained for parents (SCA6 x H) and (P7 x ICS95), while nine were obtained for the parent (P7 x ICS100). Six LGs were obtained by Flament *et al*. (2001) and higher numbers of LGs have also been obtained: 12 (Crouzillat *et al*., 2000b; Motilal *et al*., 2001), 11 (Flament *et al*., 2001), 25 (Queiroz *et al*., 2003), 16 (Faleiro *et al*., 2006). Similar results were obtained in oil palm (*Elaeis spp*,) (Ting *et al*., 2014), in pine (Cloutier *et al*., 2010). Many reasons underlign the gap to the haploïd number of chromosomes. It could be due to the small size of the population studied, that could reduce the power of linkages detection between markers, given the low number of meiotic recombinations between markers and also to the non saturation of the map (Crouzillat *et al*., 2000b; Flament *et al*., 2001; Faleiro *et al*., 2006). The successive works conducted in cacao by Queiroz *et al*. (2003), Faleiro *et al*. (2006) and Araujo *et al*. (2009) using the same mapping population and criteria corroborate the latter reason. Indeed, these authors reduced map length from 1,713 to 670 cM and LGs number from 25 to 14 by increasing markers number from 193 to 273. Cloutier *et al*. (2010) stated more precisely that markers density was not sufficient to fill up unmarked zones of the LGs. Average distances between neighboring markers, 5.96 cM, 8.33 cM and 13.79 cM in map of parents ''SCA6 x H'', ''P7 x ICS100'' and ''P7 x ICS95'', respectively, are inferior to 20 cM, that is commonly regarded as the distance below which searching for QTL can be performed efficiently (Murranty, 1996). Indeed, 85.22, 62.22, 33.82 % of the intervals of these maps are below 10 cM and, 95.65, 94.44, 66.18 % are inferior to 20 cM.

A high co-linearity, based on common SSR markers, was

Table 1: Main characteristics of the three linkage maps.

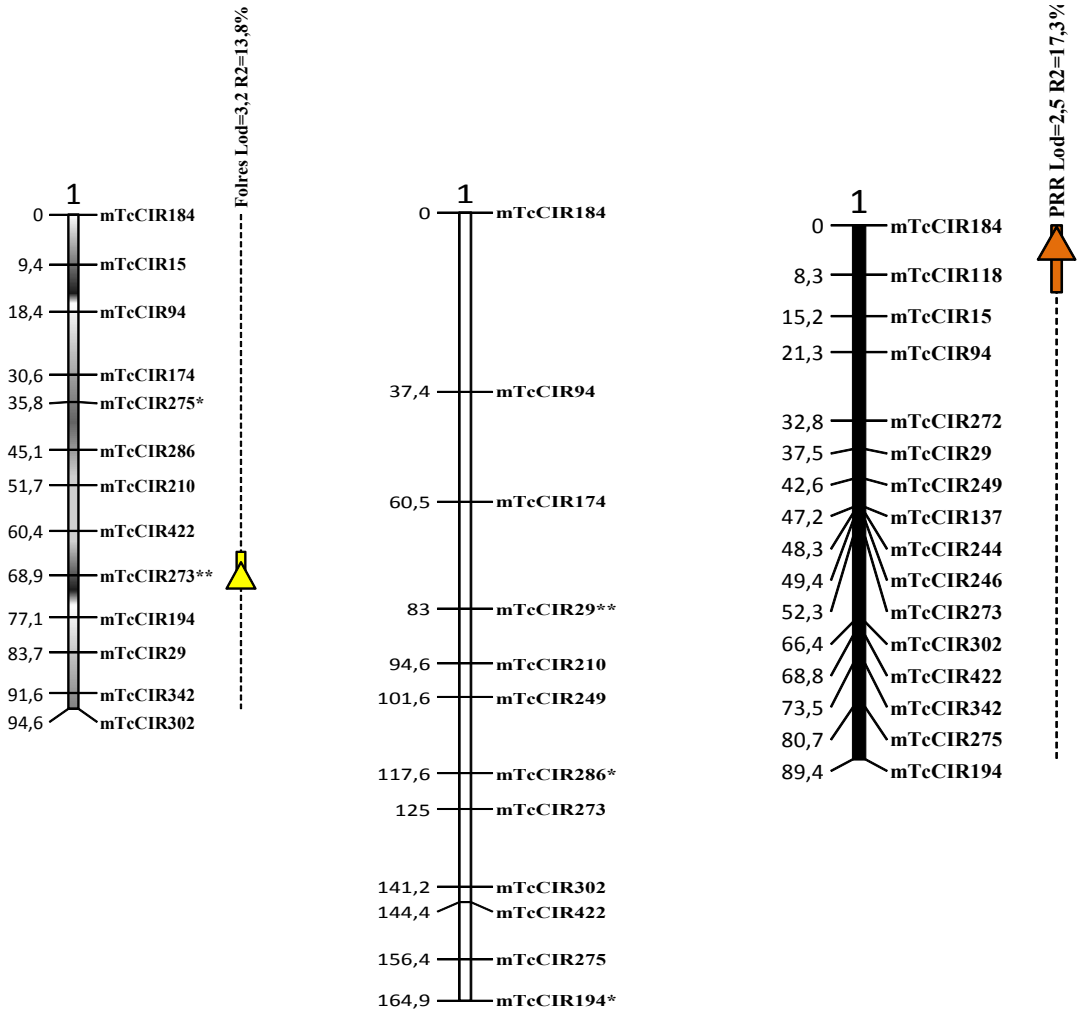
Female parent	Number of markers	Map length (cM)	Number of linkage groups	AD (cM)	% intervals inferior to 20 cM	% intervals inferior to 10 cM)	r
(SCA6 x H)	122	727.3	10	5.96	95.65	85.22	0.84
(P7 x ICS100)	98	816.1	9	8.33	94.44	62.22	0.43
(P7 x ICS95)	79	1089.2	10	13.79	66.18	33.82	0.96

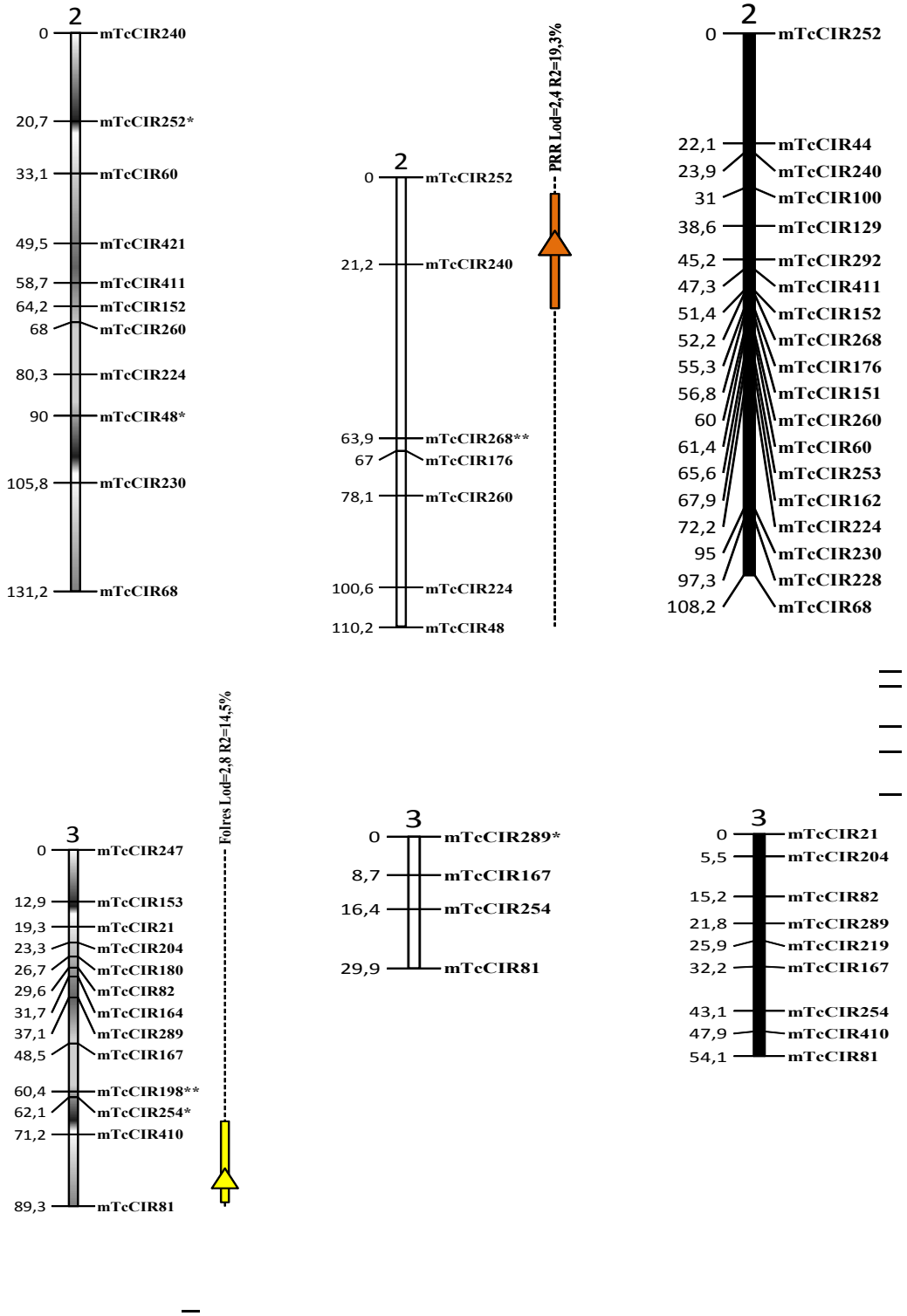
AD : Average distance between adjacent loci; r: correlation coefficient value between linkage groups size and the number of markers per map unit

Table 2: Length and number of markers of most densely populated, longest and shortest linkage groups in the female parent of each of the mapping populations.

Female parent	most densely populated LG :		Longest LG		Shortest LG	
	Number of markers	Length (cM)	Number of markers	Length (cM)	Number of markers	Length (cM)
(SCA6 x H)	LG4 12	61,5	LG 2 19	108.2	LG 6 9	52.4
(P7 x ICS100)	LG 6 13	74,6	LG 2 11	131.2	LG 8 7	52.8
(P7 x ICS95)	LG 3 4	29,9	LG 9 13	202.7	LG 3 4	29.9

LG: Linkage group;




2
0 mTcCIR240
20,7 mTcCIR252*
33,1 mTcCIR60
49,5 mTcCIR421
58,7 mTcCIR411
64,2 mTcCIR152
68 mTcCIR260
80,3 mTcCIR224
90 mTcCIR48*
105,8 mTcCIR230
131,2 mTcCIR68
2
0 mTcCIR252
21,2 mTcCIR240
63,9 mTcCIR268**
67 mTcCIR176
78,1 mTcCIR260
100,6 mTcCIR224
110,2 mTcCIR48
PRR Lod=2,4 R2=19,3%
2
0 mTcCIR252
22,1 mTcCIR44
23,9 mTcCIR240
31 mTcCIR100
38,6 mTcCIR129
45,2 mTcCIR292
47,3 mTcCIR411
51,4 mTcCIR152
52,2 mTcCIR268
55,3 mTcCIR176
56,8 mTcCIR151
60 mTcCIR260
61,4 mTcCIR60
65,6 mTcCIR253
67,9 mTcCIR162
72,2 mTcCIR224
95 mTcCIR230
97,3 mTcCIR228
108,2 mTcCIR68
3
0 mTcCIR247
12,9 mTcCIR153
19,3 mTcCIR21
23,3 mTcCIR204
26,7 mTcCIR180
29,6 mTcCIR82
31,7 mTcCIR164
37,1 mTcCIR289
48,5 mTcCIR167
60,4 mTcCIR198**
62,1 mTcCIR254*
71,2 mTcCIR410
89,3 mTcCIR81
Folres Lod=2,8 R2=14,5%
3
0 mTcCIR289*
8,7 mTcCIR167
16,4 mTcCIR254
29,9 mTcCIR81
3
0 mTcCIR21
5,5 mTcCIR204
15,2 mTcCIR82
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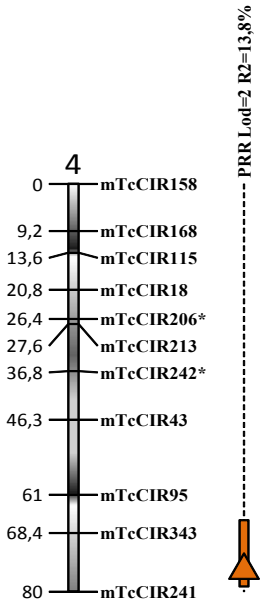
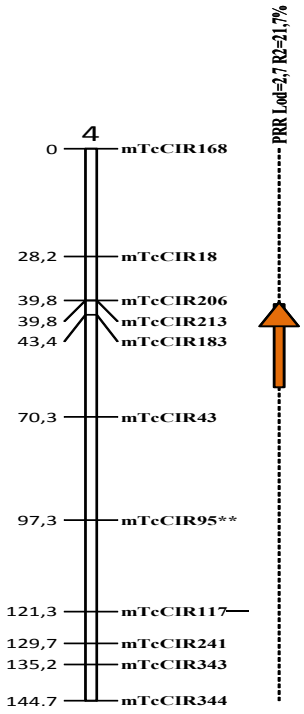
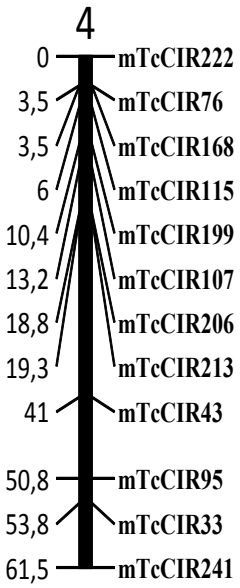
47,9 mTcCIR410
54,1 mTcCIR81


4
PRR Lod=2 R2=13,8%
0 mTcCIR158
9,2 mTcCIR168
13,6 mTcCIR115
20,8 mTcCIR18
26,4 mTcCIR206*
27,6 mTcCIR213
36,8 mTcCIR242*
46,3 mTcCIR43
61 mTcCIR95
68,4 mTcCIR343
80 mTcCIR241


4
PRR Lod=2,7 R2=21,7%
0 mTcCIR168
28,2 mTcCIR18
39,8 mTcCIR206
39,8 mTcCIR213
43,4 mTcCIR183
70,3 mTcCIR43
97,3 mTcCIR95**
121,3 mTcCIR117
129,7 mTcCIR241
135,2 mTcCIR343
144.7 mTcCIR344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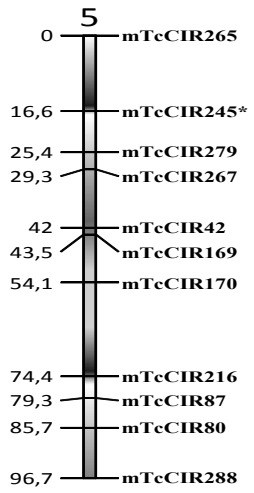
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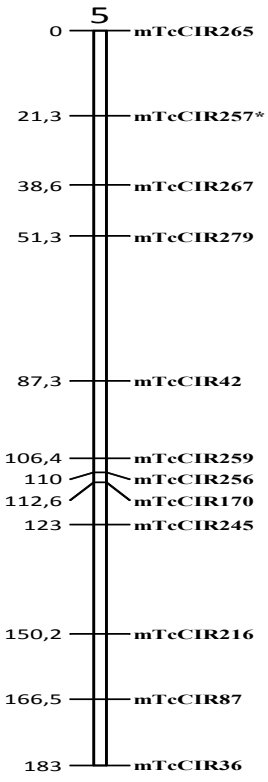
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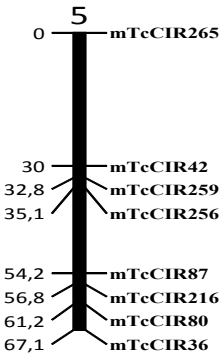
166,5 mTcCIR87
183 mTcCIR36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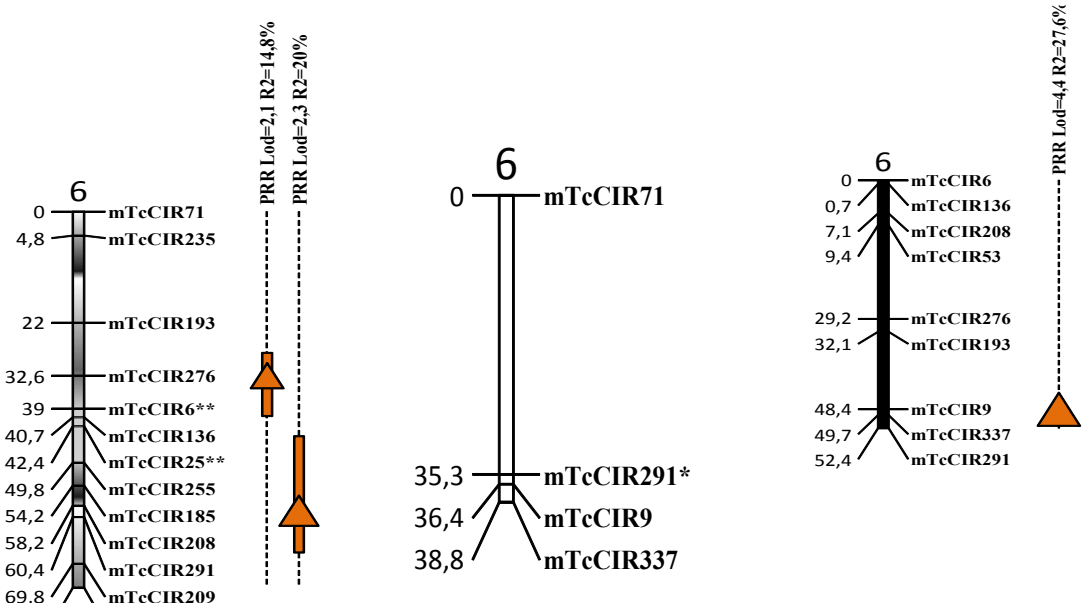
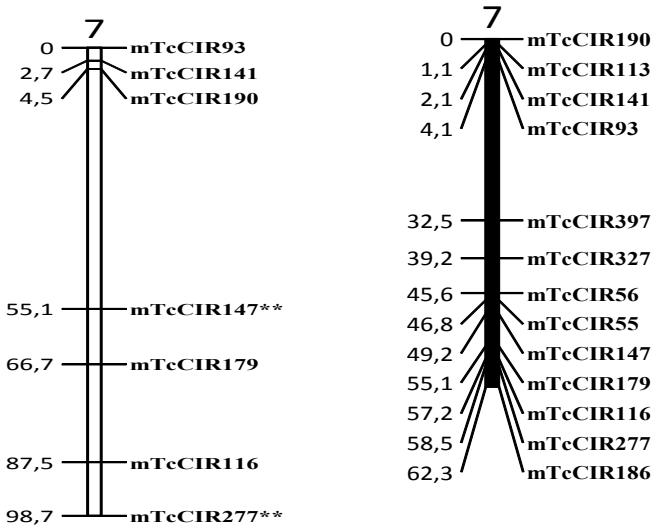
67,1 mTcCIR36


6
0 mTcCIR71
4,8 mTcCIR235
22 mTcCIR193
32,6 mTcCIR276
39 mTcCIR6**
40,7 mTcCIR136
42,4 mTcCIR25**
49,8 mTcCIR255
54,2 mTcCIR185
58,2 mTcCIR208
60,4 mTcCIR291
69.8 mTcCIR209
PRR Lod=2,1 R2=14,8%
PRR Lod=2,3 R2=20%
6
0 mTcCIR71
35,3 mTcCIR291*
36,4 mTcCIR9
38,8 mTcCIR337
6
0 mTcCIR6
0,7 mTcCIR136
7,1 mTcCIR208
9,4 mTcCIR53
29,2 mTcCIR276
32,1 mTcCIR193
48,4 mTcCIR9
49,7 mTcCIR337
52,4 mTcCIR291
PRR Lod=4,4 R2=27,6%

=


7
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4,5 mTcCIR190
55,1 mTcCIR147**
66,7 mTcCIR179
87,5 mTcCIR116
98,7 mTcCIR277**
7
0 mTcCIR190
1,1 mTcCIR113
2,1 mTcCIR141
4,1 mTcCIR93
32,5 mTcCIR397
39,2 mTcCIR327
45,6 mTcCIR56
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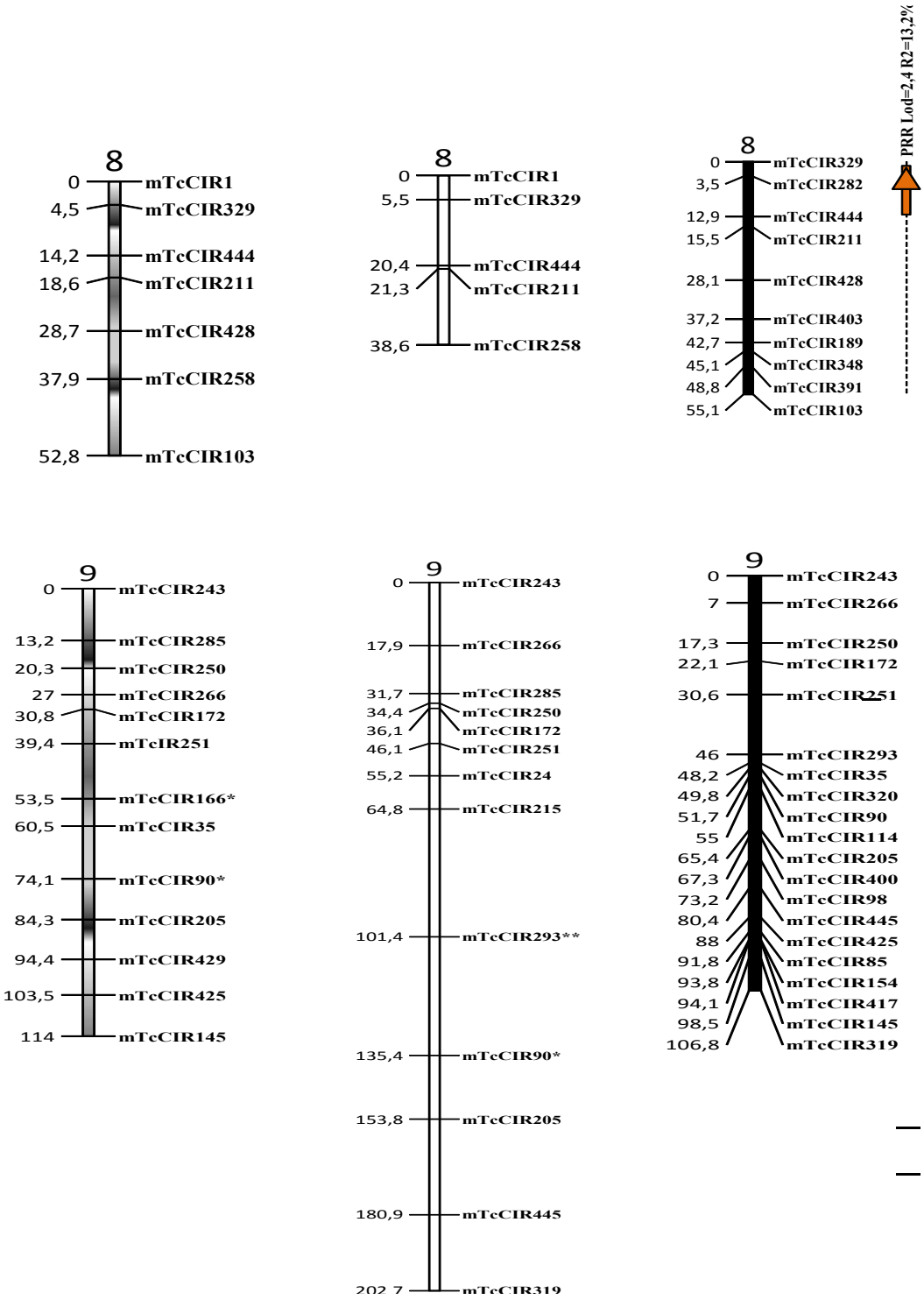
8
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14,2 mTcCIR444
18,6 mTcCIR211
28,7 mTcCIR428
37,9 mTcCIR258
52,8 mTcCIR103
8
0 mTcCIR1
5,5 mTcCIR329
20,4 mTcCIR444
21,3 mTcCIR211
38,6 mTcCIR258
8
0 mTcCIR329
3,5 mTcCIR282
12,9 mTcCIR444
15,5 mTcCIR211
28,1 mTcCIR428
37,2 mTcCIR403
42,7 mTcCIR189
45,1 mTcCIR348
48,8 mTcCIR391
55,1 mTcCIR103
PRR Lod=2,4 R2=13,2%
9
0 mTcCIR243
13,2 mTcCIR285
20,3 mTcCIR250
27 mTcCIR266
30,8 mTcCIR172
39,4 mTcIR251
53,5 mTcCIR166*
60,5 mTcCIR35
74,1 mTcCIR90*
84,3 mTcCIR205
94,4 mTcCIR429
103,5 mTcCIR425
114 mTcCIR145
9
0 mTcCIR243
17,9 mTcCIR266
31,7 mTcCIR285
34,4 mTcCIR250
36,1 mTcCIR172
46,1 mTcCIR251
55,2 mTcCIR24
64,8 mTcCIR215
101,4 mTcCIR293**
135,4 mTcCIR90*
153,8 mTcCIR205
180,9 mTcCIR445
202,7 mTcCIR319
9
0 mTcCIR243
7 mTcCIR266
17,3 mTcCIR250
22,1 mTcCIR172
30,6 mTcCIR251
46 mTcCIR293
48,2 mTcCIR35
49,8 mTcCIR320
51,7 mTcCIR90
55 mTcCIR114
65,4 mTcCIR205
67,3 mTcCIR400
73,2 mTcCIR98
80,4 mTcCIR445
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106,8 mTcCIR319

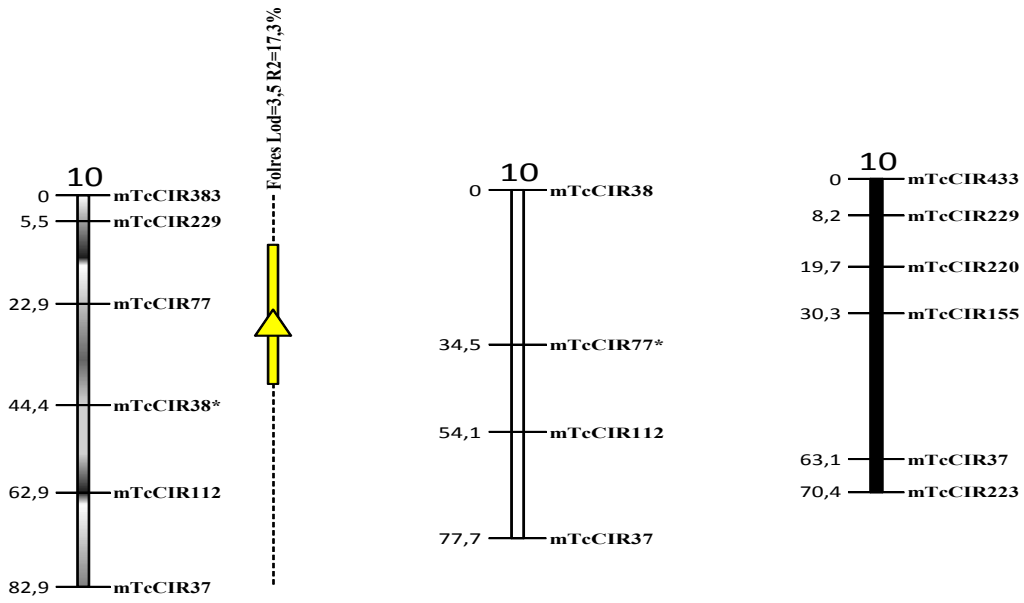

Figure 1 :**Graphical representation of the linkage groups (LGs) of the three female parents carrying their respective detected QTLs.** Vertical lanes representing LGs of the female parent "P7 x ICS100" in black-white, those of the female parent "P7 x ICS95" in white and of the female parent "SCA6 x H" in black. On the right side of the LG, the triangle is proportional to the percentage of the phenotypic variance of the trait explained by the QTL. On the left, cumulative map distances in cM and the locus names are indicated. Markers loci with asterisks deviated significantly from a 1:1 ratio.

Legend : PRR: pod rot rate; Folres: resistance to *P. palmivora* assessed on leaf discs

Table 3: Summary of data of QTLs for resistance to *Phytophthora palmivora* detected in the three progenies studied.

Traits	Progeny derived from cross	chr	KW (SMA)		SIM			
			K*	SL	Position LOD peak	Bording marlers	LOD peak	R^2
PRR	(SCA6 x H) x C1	1	10,034	4*	3,000	mTcCIR184 – mTcCIR118	2,54	17,3
		6	16,903	7*	48,400	mTcCIR337 – mTcCIR291	4,35	27,6
		8	7,956	4*	4,000	mTcCIR329 – mTcCIR282	2,43	13,2
	(P7 x ICS100) x C1	4	9,437	4*	75,035	mTcCIR343 – mTcCIR241	2,00	13,8
		6	10,251	4*	32,551	mTcCIR276 – mTcCIR6	2,08	14,8
		6	7,668	4*	59,211	mTcCIR208 – mTcCIR291	2,29	20,0
	(P7 x ICS95) x C1	2	7,960	4*	16,000	mTcCIR252 – mTcCIR240	2,40	19,3
		4	13,853	6*	43,378	mTcCIR213 – mTcCIR183	2,72	21,7
Folres	(P7 x ICS100) x C1	1	14,187	6*	68,869	mTcCIR422 – mTcCIR273	3,21	13,8
		3	11,276	5*	82,178	mTcCIR410 – mTcCIR81	2,75	14,5
	(P7 x ICS95) x C1	10	14,285	6*	26,903	mTcCIR77 – mTcCIR38	3,48	17,3
		-	-	-	-	-	-	

chr : chromosome ; KW : Kruskal-Wallis test ; K*: Kruskal-Wallis statistic value; SL: significance level; 1* = 0.1; 2* = 0.05; 3* = 0.01; 4* = 0.005; 5* = 0.001; 6* = 0.0005; 7* = 0.0001; SIM : Simple Interval Mapping ; R^2 : Phenotypic variation for trait explained by individual QTL.

Legend : PRR: pods rot rate (Phytophthora pod rot); Folres: resistance to *Phytophthora palmivora* assessed by leaf discs inoculations.

observed among the three maps obtained in this study and with other maps of Lanaud *et al*. (1995, 1999, 2009), Crouzillat *et al*. (1996, 2000a, 2000b), N'Goran *et al* . (1997), Risterucci *et al*. (2000), Flament *et al*. (2001), Clement *et al*. (2003a, 2003b), Pugh *et al*. (2004), Brown *et al*. (2005, 2007, 2008) and Fouet *et al*. (2009), particularly the cacao reference maps (Pugh *et al*., 2004, Fouet *et al*., 2011; Allegre *et al*. (2012).

The level of skewed segregation ratios, 4.92, 7.14 and 7.60 %, observed in populations derived, respectively, from (SCA6 x H) x C1, (P7 x ICS100) x C1 and (P7 x ICS95) x C1, were comparable with that has been observed in maps of other crosses : 4 % in Crouzillat *et al*. (1996), 6,57 % in Flament *et al*. (2001), 5,6 % in Risterucci *et al*. (2003), and 7,3 % in Clement *et al*. (2003a). These ratios were, however, superior to 2.0 and 1.8 % obtained by Clement *et al*. (2003a), and inferior to 9 % of Lanaud *et al*. (1995), 9,4 % of Risterucci *et al*. (2000), 8.57 % of Crouzillat *et al*. (2000b), 28.9 and 18.9 % of Queiroz *et al*. (2003), 21.1 % of Brown *et al*. (2007) 11.3 and 18.3 % of Allegre *et al*. (2012). In cotton, another Malvaceae species, higher skewed ratios were reported: 24.27 % (Zhang *et al*., 2009) and 9.8% (An *et al*., 2010). Severe one (52.49 %) was obtained by Shen *et al*. (2007). Despite the observed segregation distortion, the linkage associations between the markers mapped in this study were strongly supported.

QTL detection

We combined single marker analysis (SMA), via Kruskal-Wallis test at 0.5 % (****), with simple interval mapping (SIM) at LOD of 2.0. This LOD value was also adopted by N'Goran, (1994), N'Goran *et al*. (1997), Crouzillat *et al*. (2000a, 2000b, 2000c), Faleiro *et al*. (2006). Although slightly lower than 2.15, 2.40 and 3.20 used by Clement *et al*. (2003a, 2003b), it is higher than 1.5 to 1.9) in Crouzillat *et al*. (1996) and Flament *et al*. (2001).

QTLs of *Theobroma cacao* resistance to *Phytophthora palmivora*

Height QTLs of resistance to *Phytophthora* assessed by pod rot rate (PRR) in field were detected: one each on LGs 2 and 4 of the parent "P7 x ICS95", three each in the parents "SCA6 x H" on LGs 1, 6, 8 and "P7 x ICS100" on LGs 4 and 6. R^2 varied from 13.2 to 27.6 %. Regarding resistance evaluated by leaf discs assay (Folres), three QTLs, accounting for 13.8 to 17.3 % of the phenotypic variance of Folres, were identified only in the parent "P7 x ICS100", on LGs 1, 3 and 10.

Crouzillat *et al*. (2000b) identified, in two Forastero progenies of Costa Rica, six QTLs for resistance to *P. palmivora* evaluated by inoculations of detached pods. QTLs detected, in this study, respectively, on LG 1 (R^2 = 17.3 %), LG 2 (R^2 = 19.3 %) and LG 4 (R^2 = 21.7 %), by means of PRR, could correspond to three QTLs of these six QTLs. The QTL localized on LG 2 could also correspond to that was mapped by Flament *et al*., (2001), on this LG 2, by inoculating attached pods in two Forastero full-sib families in Côte d'Ivoire. On LG 1, we mapped one QTL of Folres with R^2 = 13.8 %. Risterucci *et al*. (2003) mapped also three QTLs of Folres (R^2 varying from 8.0 to 10.0 %) on this LG 1 in Forastero background. On LG 3, the QTL of Folres we identified (R^2 = 14.5 %), is different from that in Flament *et al*. (2001) (R^2 = 9 %) and Risterucci *et al*. (2003) (R^2 = 11.5 %). Clement *et al*. (2003b) identified, in two progenies of Trinitario/Forastero and Forastero backgrounds, two QTLs of PRR (R^2 = 10.1 and 22.6 %, respectively), in a region of LG 4, where we localized one QTL of PRR (R^2 = 21.7 %) in the family derived from (P7 x ICS95) x C1. One QTL of resistance to three species of *Phytophthora* was found in the same zone of LGs 5 and 6 (R^2 varying from 8.6 to 11.0 %) (Risterucci *et al*., 2003). One QTL (R^2 = 12.0 %) of PRR of detached pods and one QTL (R^2 = 12.0 %) of Folres were localized on LG 6 (Flament *et al*., 2001). QTLs of PRR (R^2 = 14.8 and 20.0 %) detected in the present work on LG 6 are in the same region that in Risterucci *et al*. (2003). The only one QTL of PRR in field (R^2 = 13.2 %) identified on LG 8 in the parent ''SCA6 x H'' is the third QTL of resistance to *P. palmivora* detected on this LG 8, after those detected by N'Goran *et al*. (1997) (R^2 = 31 %), and Brown *et al*. (2007) (R^2 = 7.3 %) using artificial inoculations of detached pods. In the current study and for the first time, one QTL of Folres (R^2 = 17.3 %) was identified on LG 10. Indeed, Lanaud *et al*. (2009), in a meta–QTL analysis found no QTL of Folres on LG 10. The location of this QTL could be the same that that of the QTL of PRR (R^2 = 23.0 %) identified by Brown *et al*. (2007) by inoculations of detached pods. Flament *et al*. (2001) localized also on LG 10, one QTL (R^2 = 17 %) of PRR. N'Goran (1994) detected, respectively on LGs 1 and 8, one QTL of PRR. These QTLs are different from those here on these two chromosomes.

QTL stability across years

Although no QTL of PRR and Folres was declared stable during the strict minimum of a three-year period monitoring, different associations were observed, which are the same, either in the family derived from (SCA6 x H) x C1 and in one of the two half-sib families, or in both half-sib families. In the former case, on the LG 6, the association mTcCIR291-PRR was noted.

Pleiotropic and polygenic QTLs

Different regions of LGs 1, 4 and 6 are involved, as found in this study, in the expression of *T. cacao* resistance to *P. palmivora*. Lanaud *et al*. (2001) and Clement *et al*. (2003b) reported also the implication of different chromosome regions in the expression of this trait.

The current findings show that the chromosome 1 is an interesting support of cacao resistance to *Phytophthora*, since it carries QTLs of both PRR and Folres, the main two resistance assessment methods to black pod in cacao. Chromosomes 4 and

6 are also important supports, since, in two different individuals (parents), they carry, each one, QTLs of PRR.

VI. CONCLUSION

This work has achieved to the construction of the first SSR-based genetic linkage maps used for searching QTLs in *T. cacao*. They were suitable to this purpose and, accordingly, were used to map QTLs for resistance to *P. palmivora* assessed by means of the pod rot rate (PRR) in field and the leaf discs assay (Folres). Height QTLs of PRR were mapped over the three progenies on different chromosomes among which chromosomes 4 and 6 in two of these progenies. Three QTLs of Folres were also detected on different chromosomes in only one of the two half-sib progenies investigated. The chromosome 1, carrying QTLs of both PRR and Folres, is an interesting support of cacao resistance to *Phytophthora*.

Some QTLs detected in the current investigation are equivalent to some QTLs in some previous works. Some others are new. For the first time, one QTL of Folres (R^2 = 17.3 %) was identified on chromosome 10. In addition, the association mTcCIR291-PRR, on the chromosome 6 was noted in the family derived from (SCA6 x H) x C1, as well as in one of the two half-sib families.

The findings contribute to increasing the understanding of the inheritance of this character and the prospects for marker-aided breeding. Markers linked to QTLs are valuable potential candidates for a future exploiting of these QTLs as selection tools.

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