Theor Appl Genet (2000) 100:975–984

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Identification of QTLs involved in the resistance to South American leaf blight (*Microcyclus ulei*) in the rubber tree

Received: 1 August 1999 / Accepted: 27 August 1999

Abstract South American leaf blight (SALB) is a disease of the rubber tree caused by the fungus *Microcyclus ulei*. Quantitative trait loci (QTLs) for resistance were mapped using 195 F_1 progeny individuals derived from the cross between a susceptible cultivated clone, PB260, and a resistant clone, RO38, derived from interspecific hybridization. The resistance level of the progeny individuals was evaluated in controlled conditions. The reaction type (RT) and the lesion diameter (LD) were measured on immature leaves after artificial inoculation of the fungus. Five different strains of the fungus were used, all highly sporulating on PB260. Among those, four did not sporulate and one sporulated partially on RO38. Both pseudo-testcross parental genetic maps and the consensus map were constructed. The search for QTLs was performed using the Kruskal-Wallis marker-by-marker test and the Interval-Mapping method for the three maps. Eight QTLs for resistance were identified on the RO38 map. Only one QTL was detected on the PB260 map. The analysis of the F_1 consensus map confirmed results obtained with the parental maps. A common QTL was detected for resistance to the five strains for both RT and LD. Two QTLs were common for complete resistance to four strains, for RT and LD respectively. Resistance determinism for complete and partial resistance, and perspectives for breeding for durable resistance to SALB are discussed.

Key words *Microcyclus ulei* · South American leaf blight · *Hevea* · QTL mapping

Communicated by P.M.A. Tigerstedt

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Introduction

Originating from South America, the rubber tree (*Hevea* spp.) is cultivated for its latex, mainly in South-East Asia and Africa. It is an outcrossing perennial crop, vegetatively propagated by grafting. South American leaf blight (SALB) is caused by the ascomycete fungus *Microcyclus ulei* (P. Henn.) v. Arx which was first described in 1900 (Ule 1905). It is endemic to the Amazon basin where it is the most important disease for rubber tree. This disease began to cause damage as soon as the first industrial plantations of rubber tree where set up (Drost 1910; Kuyper 1911; Labroy and Cayla 1913; Stahel 1915) and is responsible for the failure of the crop on this continent. It is currently absent in Asia and Africa. Infection results in repeated defoliation, dieback of the canopy and even the death of trees.

Source of complete resistance to SALB, characterized by the absence of spores of the fungus on the leaves, are present in some wild clones of *Hevea brasiliensis* and also in clones of other species such as *Hevea benthamiana*, *Hevea guianensis*, *Hevea pauciflora*, or *Hevea spruceana* (Holliday 1970; Dean 1987; Simmonds 1990). Nevertheless, most resistance sources appear to be non-durable since they were rapidly overcome after their use in breeding schemes (Simmonds 1989).

No genetic study was ever performed on controlled progeny, and genetic determinism of resistance to *M. ulei* remains doubtful. Complete resistance to SALB is assumed to rely on a mono- or oligo-genic determinism. This is assumed to be a mechanism that inhibits sporulation, because no strict barrier to the germination process or penetration through the leaf cuticule could be observed (Blasquez and Owen 1963; Hashim et al. 1978; Hashim and Pereira 1989). Partial resistance may be more durable because it is assumed to have a polygenic determinism (Simmonds 1982; Rivano 1997). It is characterized by a reduced rate of epidemic development despite a susceptible infection type (Parlevliet and van Ommeren 1975). Under this assumption, different studies (Chee et al. 1985; Hashim and Pereira 1989;

Junqueira et al. 1990; Rivano 1992) were targeted to characterize resistance by certain basic traits, such as the latent period, infectious period, leaf deformation, reaction type (based on the ability to produce spores), lesion diameter and lesion number. Reaction type and lesion diameter appear to be the most accurate of the traits. In the research presented here, a QTL study for the complete and the partial resistance to *M. ulei* has been conducted based on a F_1 population of 195 progeny for which a high-density molecular map has been constructed (Lespinasse et al. 1999).

Materials and methods

Plant material

A population of 195 F_1 progeny clones was obtained from a cross between two heterozygous genotypes, PB260 and RO38 (Lespinasse et al. 1999). PB260 is a *H. brasiliensis* high-yielding widely grown clone, bred in South East Asia and belonging to the so called Wickham material. As for all other Wickham clones, it is extremely susceptible to SALB. RO38 is a poor-yielding interspecific hybrid (*H. benthamiana* × *H. brasiliensis*) highly reesistant to SALB in the ecological conditions of French Guyana (Rivano 1992). The 195 progenies and the two parents were clonally propagated by grafting and were grown in polyethylene bags in a greenhouse in Kourou, French Guyana. Two to five plants were available for each individual and 20 plants were available for each parent. Three weeks before the inoculation of the fungus for resistance scoring, the plants were cut back to induce sprouting of new shoots.

Fungus strains

Five *M. ulei* strains were used in this study, G70, G77, Man1, San91 and Una2. They were collected on the leaves of susceptible clones of rubber trees in Brazil and in French Guyana. Strains G70, Man1, San91 and Una2 did not sporulate on the RO38 parent, but sporulated highly on the PB260 parent. The strain G77 sporulated moderately on the RO38 parent, and sporulated highly on the PB260 parent. Each of the five strains presented a specific pattern of virulence (Table 1) according to the host range established by Junqueira et al. (1986) and modified by Rivano (1992).

The five strains used were stored in a strain library in Kourou. Since the spore cloning method is not well-defined, the strains were purified from single sporulating lesion before storage. First, spores were collected from a single leaf lesion and inoculated on a leaf of another susceptible clone. After spore development, a second round of sampling from a single sporulating lesion followed by a second inoculation was performed. Spores were collected again in a single lesion, inoculated on a sterile culture medium M4 (Junqueira et al. 1984), and placed at 24 ± 2°C under dark conditions in a growth chamber. M4 medium permits the slow development of a mycelium stroma from conidia.

Disease evaluations

To induce spore production, stroma were subcultured on M3 medium (Junqueira et al. 1984) and transferred to an incubator (photoperiod 12 : 12, temperature 24 ± 2°C). Conidia were obtained within 2–3 weeks. They were inoculated with a paint brush on immature leaves of a susceptible genotype, called the multiplicator, in order to amplify the conidial stock. The standard inoculum was then prepared from 2 to 7 day-old conidia collected with a paintbrush from the multiplicator leaves. They were suspended in sterile water with Tween 80 (0.1%). The final concentration was ad-

Table 1 Geographical origin of the *M. ulei* strains studied, and reaction type on a rubber tree host range and on the parents of the mapping population. +: dense sporulation on leaves 11 days after inoculation; +/–: weak sporulation on leaves 11 days after inoculation: –: absence of sporulation on leaves 11 days after inoculation: *: no data. N, number of clones from the host range for which the strain is able to sporulate after inoculation

Strains	Origin		Host range											N	Parents	
	Country	State	IAN710	FX4098	FX3925	MDF180	FX985	IAN3087	FX3899	IAN717	FX25	FX2804	FX2261	F4542		RO38	PB260
G70	French G.	–	+	+	–	–	+/–	*	–	+/–	–	*	–	*	4	–	+
Una2	Brazil	Bahia	+	+	–	+	+	–	–	–	–	–	–	*	4	–	+
San91	Brazil	Mato Grosso	+	+	–	+/–	–	+/–	–	–	+/–	–	+	*	6	–	+
Man1	Brazil	Amazonas	+	+	+/–	+	–	+/–	–	+/–	+	+/–	+	–	9	–	+
G77	French G.	–	+	+	+	+/–	+	+/–	+/–	+	+/–	+/–	–	+/–	11	+/–	+

justed to 5×10^4 spores/ml. Inoculum was prepared about 1 h before each experiment.

Plants showing young brown-red leaves, were transferred from the greenhouse to an inoculation chamber with a temperature of 25 ± 2°C, relative moisture at 85–100%, luminosity at 110–180 $mE.cm^{-2}.s^{-1}$ and a photoperiod of 12 : 12. The lower surface of two the three young leaves was inoculated for each plant with the conidia suspension using a MK3 Defensor atomiser. Shoots with inoculated leaves were covered with a polyethylene bag to provide a 100% relative humidity and plants were kept in the dark for 16–20 h (Rivano 1992). Bags were then removed and the plant kept under a 12 : 12 photoperiod.

Inoculation experiments were carried out twice a week. In a given experiment, enough room was available for 25 progeny plants presenting leaves at the proper stage for inoculation, plus the two parents used as a control. For the five different strains, at least 163 of the 195 progenies have been inoculated. Some individuals have been repeated in a second experiment (see Table 2). All these data were collected between 1995 and 1998.

Two parameters were used to evaluate the level of resistance of progeny clones, the lesion diameter (LD) and the reaction type (RT). They were measured on inoculated leaves 11 days after inoculation. The LD is scored according to the comparison of the surface of lesions observed on the inoculated leaf with a set of dots of known sizes. The RT is based on the observation of the symptoms on the inoculated leaf with a binocular and is scored on a 1–5 scale modified from Junqueira (Junqueira et al. 1986). Notes 1 and 2 correspond to non sporulating lesions. Note 1 corresponds to chlorotic or necrotic lesions and note 2 to local depression of the lamina without necrosis symptoms. Notes 3, 4 and 5 correspond to sporulating lesions, with very weak, moderate, and high sporulation, respectively. The grade value of each progeny clone for LD and RT is the mean grade scored for all lesions on all inoculated leaves. After evaluating the symptoms, the plants were cut back and kept in the greenhouse to produce new shoots for an eventual new inoculation experiment.

Map construction

A saturated linkage map has been constructed with 717 RFLPs, AFLPs, microsatellites and isozymes, based on a sample of 106 progeny individuals among the 195 used here (Lespinasse et al. 1999). This map is a consensus map issued from the merging of parental maps, established according to a pseudo-testcross strategy (Grattapaglia and Sederoff 1994). PB260 and RO38 pseudo-testcross maps encompass 594 and 295 markers, respectively (Lespinasse et al. 1999). Two pseudo-testcross core maps of 158 and 233 markers each were deduced from the saturated RO38 and PB260 maps, respectively. Core markers, uniformly distributed every 10–15 cM on the parental maps, were chosen, A priority was given to the more-informative RFLP, microsatellite and isozyme markers presenting three or four segregating alleles in the progeny. The marker-data matrix was extended for core markers to the 89 progeny individuals not used to construct the saturated reference map. A F_1 consensus map of 253 loci was then constructed based on the core markers scored for the 195 progeny individuals with the software JoinMap 1.4 (Stam 1993).

Statistical analysis and QTL detection

Normality of the different traits was assessed according to the Shapiro and Wilk test calculated with the PROC UNIVARIATE of SAS (SAS Institute 1989). Pearson correlation coefficients between traits were calculated with the PROC CORR.

QTL analysis was performed using the computer software MAPQTL 3.0 (van Ooijen and Maliepaard 1996). Three different approaches were employed. First, the non-parametric rank-sum test of Kruskal-Wallis (van Ooijen et al. 1992) was applied individually to each segregating locus. A threshold value of $P = 0.005$ was used for individual tests. Second, interval mapping (Lander and Botstein 1989) was performed on the two parental maps. The markers with LOD score values higher than 2.5 were then taken as co-factors for running a multiple-QTL method (MQM) (Jansen 1993; Jansen and Stam 1994). A threshold value of 3 was retained according to the method developped by Rebaï (1994) to obtain a global type-I error of 5%. The permutation method (Doerge and Rebaï 1996) was also applied using QTL Cartographer software (Basten et al. 1995) and it confirmed this threshold value of 3 for most traits. Third, interval mapping was applied to the consensus map. A threshold value of 3 was chosen.

A QTL was retained when statistics exceeded the threshold defined above for both the Kruskal-Wallis test and the MQM interval mapping. It was considered as putative when the LOD score value comprised between 2 and 3 for MQM interval mapping and a QTL was detected in the same position for another trait (i.e. another parameter or another strain). We arbitrarily considered that two QTLs had the same map position when LOD-score peaks were less distant than 20 cM.

Results

SALB resistance data

Data for LD and RT obtained for each strain were analysed as separate traits. The distribution of RT and LD values for the five strains are presented in Fig. 1. None was normally distributed ($P < 0.0001$) according to the test of Shapiro and Wilks. The grade of progeny clones for LD and RT was between the parental values for strains G70, Man1, San91 and Una2, indicating the absence of transgressive segregation in this population. In contrast, transgressive segregation was observed for LD and RT measured for strain G77.

Strong correlations were found between the two repetitions for RT and LD scored for strains G70, Una2 and G77 (Table 2). A strong correlation was found between repetitions for RT only with strain San91. Moderate correlations were observed for RT and LD with strain Man1, and for LD only with strain San91.

Significant correlations between LD and RT ($P < 0.0001$) were found for each strain (Table 3). Significant correlations ($P < 0.0001$) were also found for LD and RT respectively measured with different strains (Table 3). In order to classify the strains according to their pathogenicity on the progeny, the progeny mean for RT was compared for the five strains with a Student *t*-test (Table 4). G77 was the most pathogenic of the five strains followed by San91. The mean RT of G70 and Man1 were not significantly different. Una2 was the least pathogenic.

RO38 map

The QTL analysis of RT for the five strains allowed for the identification of five distinct QTLs (Table 5). One major QTL, located on chromosome g13 (peak at marker EM36/14), was detected for the five strains at a LOD score higher than 8. It contributed to at least 13% of the phenotypic variance for all strains. This contribution reached 34% for strain G77. One minor QTL, located on chromosome g11, was detected for all strains except G70

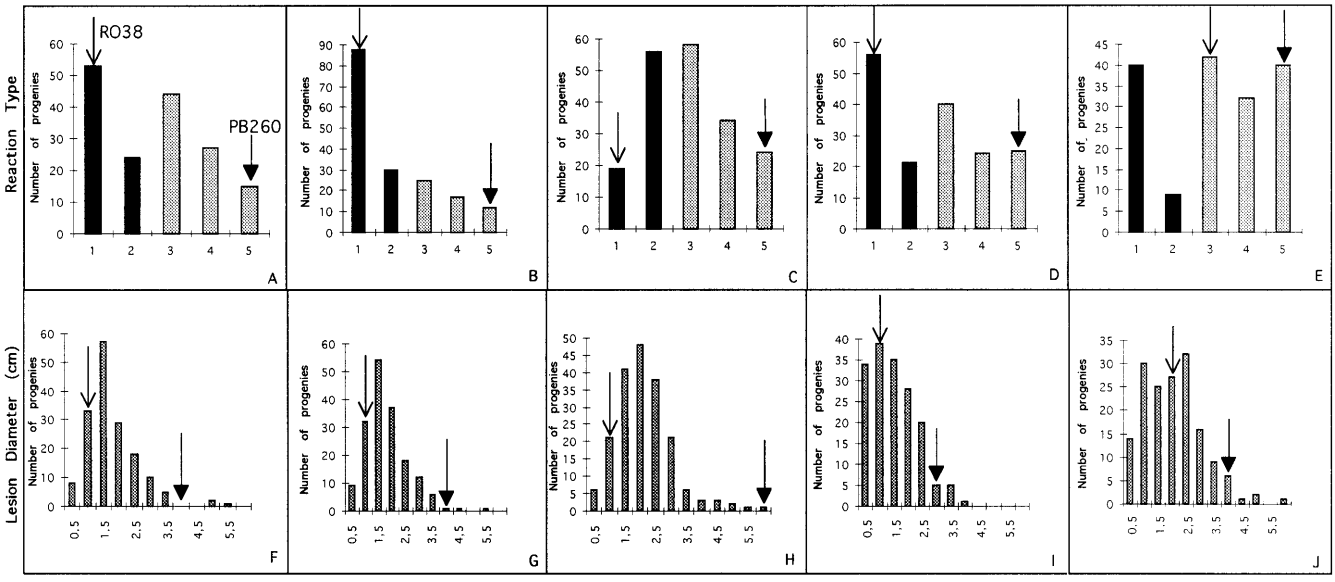

Fig. 1 Distribution of the reaction type and lesion diameter for the five *M. ulei* strains G70 (*A*, *F*), Una2 (*B*, *G*), San91 (*C*, *H*), Man1 (*D*, *I*) and G77 (*E*, *J*). Parental trait values are indicated by *arrows*. For reaction type, non-sporulating and sporulating individuals are in *black and grey*, respectively

and G77. Nevertheless, it was putative for strain G70. This QTL contributed between 4 and 10% of the phenotypic variance, depending on strains. A major QTL located on chromosome g15 was detected for strains Una2, San91 and Man1. LOD score values were higher than 8 for the three strains, and the QTL contribution to the phenotypic variance was higher than 15%. Another QTL was detected on chromosome g13 (peak at marker EM30/2) for strain San91 only. It was putative for strain Man1. It contributed to 6% of the phenotypic variance for each strain. One minor QTL, located on chromosome g10, was detected for strain G70 and contributed to 9% of the phenotypic variance. The QTL analysis of LD for the five strains allowed for the identification of three QTLs and one putative QTL. One major QTL was detected for the

Table 2 Number of individuals inoculated for each *M. ulei* strain (Ni), number of progeny inoculatd twice in two distinct experiments (Nt) and Pearson correlations, r_{RT} and r_{LD}, between repetitions for reaction type (RT) and lesion diameter (LD), respectively

Strain	Ni	Nt	r_{RT}	r_{LD}
G70	163	44	0.82**	0.80**
Una2	171	25	0.71**	0.65*
San91	191	166	0.70**	0.42**
Man1	167	90	0.51**	0.39*
G77	163	85	0.60**	0.61**

* $P < 0.001$; ** $P < 0.0001$

Table 4 Comparison of the RT progeny mean between *M. ulei* strains based on a least significant difference Student *t*-test. The least significant difference was 0.27. Means with the same letter are not significantly different at $P < 0.05$

Strain	Mean	*t* grouping
G77	3.13	A
San91	2.81	B
G70	2.35	C
Man1	2.22	C
Una2	1.89	D

Table 3 Pearson correlations between the reaction type (RT) and the lesion diameter (LD) for each *M. ulei* strain and between the strains for RT and LD respectively. All coefficients are significant at $P < 0.0001$

Strain		**G70**		**Una2**		**San91**		**Man1**		**G77**	
		RT	LD	RT	LD	RT	LD	RT	LD	RT	LD
G70	RT										
	LD	0.70									
Una2	RT	0.48	0.41								
	LD	0.48	0.54	0.62							
San91	RT	0.55	0.45	0.68	0.46						
	LD	0.45	0.50	0.46	0.50	0.53					
Man1	RT	0.50	0.35	0.59	0.41	0.72	0.39				
	LD	0.48	0.43	0.41	0.55	0.45	0.48	0.59			
G77	RT	0.51	0.47	0.38	0.43	0.40	0.37	0.37	0.41		
	LD	0.49	0.48	0.33	0.51	0.40	0.38	0.37	0.43	0.76	

Table 5 Results of the QTL analysis for reaction type (RT) and lesion diameter (LD) from the cross PB260 × RO38, according to various hypothesis. C is the chromosome name. LOD peak is the marker nearest to the highest LOD-score position. Nb is the number of progeny individuals with available data for QTL detection. Pos is the LOD score position on the chromosome from the peak marker. R^2(%) is the proportion of the explained phenotypic variance. Weight is the effect of the alleles from the F4542 grandparent of RO38. The putative QTLs (see text) description is indicated with a italic font

Analysis[a]	Trait	C	LOD peak	G70					Una2					San91					Man1					G77				
				Nb	Pos	LOD	R^2 (%)	Weight	Nb	Pos	LOD	R^2 (%)	Weight	Nb	Pos	LOD	R^2 (%)	Weight	Nb	Pos	LOD	R^2 (%)	Weight	Nb	Pos	LOD	R^2(%)	Weight
I	RT	g10	EM36/7	163	+6	3.6	9	0.77	171	–	–	–	–	191	–	–	–	–	167	–	–	–	–	163	–	–	–	–
		g11	EM3/24	*163*	*0*	*2*	*4*	*0.53*	171	–20	4.3	8	0.68	191	–10	6.3	10	0.96	167	–10	3.8	9	0.82	163	–	–	–	–
		g13	EM36/14	163	0	8.2	18	1.1	171	0	11.6	22	1.12	191	0	9.8	13	1.1	167	0	8.3	15	1.09	163	0	15.1	34	1.76
		g13	EM30/2	163	–	–	–	–	171	–	–	–	–	191	+5	4	6	0.77	*167*	*+5*	*2.8*	*6*	*0.69*	163	–	–	–	–
		g15	gHbCIR45	163	–	–	–	–	171	0	8.8	15	0.97	191	+5	15.3	23	1.5	167	–8	10.1	26	1.44	163	–	–	–	–
	LD	g10	EM36/7	*163*	*+14*	*2.2*	*6*	*0.41*	171	–	–	–	–	191	–	–	–	–	167	–	–	–	–	163	–	–	–	–
		g12	LAP	163	+6	4.8	13	0.59	171	–5	4.6	8	0.45	191	+1	3.8	7	0.5	167	+15	4.2	9	0.47	163	–	–	–	–
		g13	EM36/14	163	+5	7.1	18	0.7	171	0	18.4	36	0.95	191	0	7.9	16	0.72	167	+5	8	23	0.74	163	0	7.6	18	0.88
		g13	EM30/2	163	–	–	–	–	171	–	–	–	–	191	–	–	–	–	167	0	4.1	8	0.48	163	–	–	–	–
II	$RT_{0–1}$	g10	EM36/7	*163*	*+6*	*2.7*	*7*	*0.26*	171	–	–	–	–	191	–	–	–	–	167	–	–	–	–	163	–	–	–	–
		g13	EM36/14	163	0	7.5	17	0.42	171	0	12.9	25	0.47	191	+9	3.3	5	0.23	167	+5	5.4	14	0.38	163	0	10.6	26	0.47
		g15	gHbCIR45	163	–	–	–	–	171	+5	8.7	18	0.4	191	+5	13.1	29	0.55	167	+5	5.8	15	0.4	163	–	–	–	–
III	RT_s	g11	EM3/24	86	–	–	–	–	54	–20	5.7	44	1.54	117	–10	5.2	18	1.1	90	0	3.6	12	0.79	114	–	–	–	–
		g13	EM36/14	86	–	–	–	–	54	–	–	–	–	117	0	8.8	25	1.27	90	0	3.7	13	0.84	114	0	3.4	12	0.75
		g15	gHbCIR45	86	–	–	–	–	54	–	–	–	–	117	+10	3.6	11	0.95	90	0	4	14	0.99	114	–	–	–	–
		g2	gHbCIR393	86	–	–	–	–	54	–16	3.1	18	1.02	117	–	–	–	–	90	–	–	–	–	114	–	–	–	–
IV	LD_r	g12	LAP	77	–	–	–	–	–	–	–	–	–	74	–15	2.7	13	0.47	77	–	–	–	–	49	–	–	–	–
		g13	EM36/14	77	–	–	–	–	117	0	10.5	30	0.73	74	0	3	15	0.53	77	–	–	–	–	49	–	–	–	–
		g13	EM30/2	77	0	3.9	19	0.43	117	–	–	–	–	74	–	–	–	–	77	–	–	–	–	49	–	–	–	–
		g4	gHbCIR88	77	–	–	–	–	117	–4	3.3	7	0.32	74	–	–	–	–	77	–	–	–	–	49	–	–	–	–
	LD_s	g12	LAP	86	–5	5.5	27	0.91	54	–10	3.5	22	0.87	*117*	*+1*	*2.3*	*8*	*0.56*	90	–10	4.3	16	0.67	114	–	–	–	–
		g13	EM36/14	86	–	–	–	–	54	–	–	–	–	117	0	3.9	13	0.74	90	+5	5.3	28	0.89	114	–	–	–	–
		g4	gHbCIR88	86	–	–	–	–	54	–	–	–	–	117	–	–	–	–	90	–	–	–	–	*114*	*0*	*2.4*	*9*	*0.57*
V	RT	g15	gHbCIR45	163	0	3	9	0.75	171	–	–	–	–	191	–	–	–	–	167	–	–	–	–	163	–	–	–	–
VI	RT	g6	SKD	163	–	–	–	–	171	0	3.7	16	–	191	0	5	20	–	167	–	–	–	–	163	–	–	–	–
		g10	EM36/7	163	+5	3.1	12	–	171	–	–	–	–	191	–	–	–	–	*167*	*–4*	*2.2*	*12*	–	163	–	–	–	–
		g11	EM3/24	163	–	–	–	–	*171*	*+20*	*2.7*	*9*	–	*191*	*+15*	*2.8*	*10*	–	*167*	*+5*	*2*	*6*	–	163	–	–	–	–
		g13	EM36/14	163	0	9	25	–	171	+5	9.7	30	–	191	+5	9.2	24	–	167	+5	8.7	27	–	163	0	17.7	42	–
		g15	gHbCIR45	163	0	3.5	10	–	171	+5	7.5	21	–	191	+5	14.6	34	–	167	+5	8.7	25	–	163	–	–	–	–
	LD	g10	EM36/7	*163*	*+5*	*2*	*11*	–	171	–	–	–	–	191	–	–	–	–	167	–	–	–	–	163	–	–	–	–
		g12	LAP	163	–8	3.7	12	–	171	–13	3.5	9	–	191	–3	2.8	7	–	167	+17	4.2	12	–	163	–	–	–	–
		g13	EM36/14	163	+5	8.4	24	–	171	0	15.8	38	–	191	+5	8.3	21	–	167	+5	8.8	27	–	163	+5	10	29	–
		g16	gHbCIR161	163	0	3.9	12	–	171	–	–	–	–	191	–	–	–	–	167	–	–	–	–	163	–	–	–	–

[a] I: analysis of the RO38 pseudo-testcross map; II: analysis of the RO38 pseudo-testcross map for RT scored as a binary trait (see text); III: analysis of the RO38 pseudo-testcross map for RT based on sporulating individuals only; IV: analysis of the RO38 pseudo-testcross map for LD based on non-sporulating (LD_r) and sporulating (LD_s) progeny individuals; V: analysis of the PB260 pseudo-testcross map; VI: analysis of the consensus F_1 map

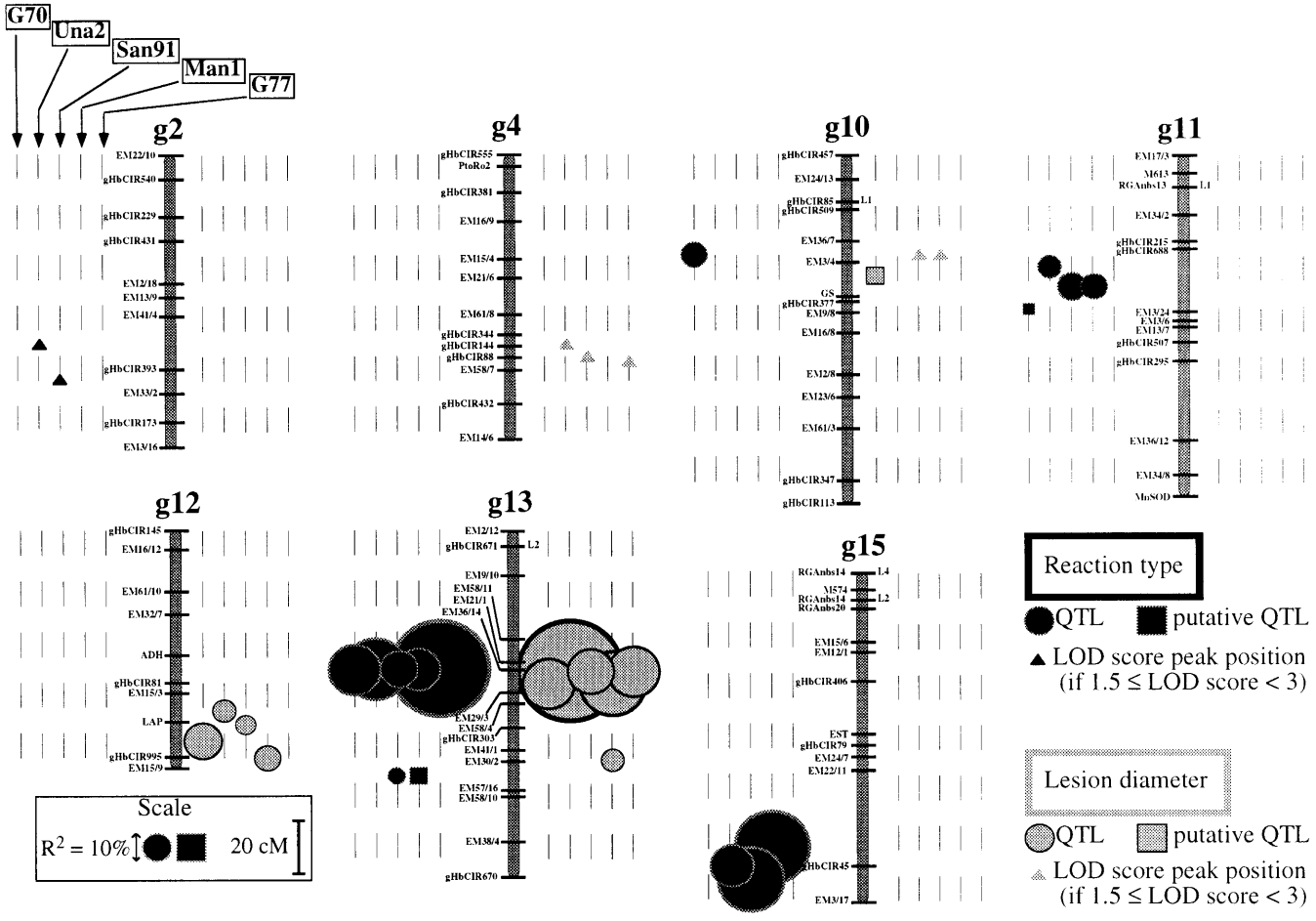

Fig. 2 Synthesis of QTL linkage map locations for reaction type and lesion diameter identified in the cross PB260 × RO38 with the RO38 pseudo-testcross map. The variance explained by each QTL is indicated by the diameter of the *circle* (QTL) or the *square* (putative QTL). The QTLs positions correspond to the LOD score peak positions. If no QTL is detected but the LOD score is higher than LOD 1.5 the score peak position is indicated by a *triangle*. QTLs and LOD score peak positions are represented with two different colours according to the parameter for which they were detected. Duplicated loci in the RO38 map are indicated by *Lx*, in accordance with the map published by Lespinasse et al. (1999)

five strains on chromosome g13 (peak at marker EM36/14) and co-localized with the major QTL detected on the same chromosome for the RT measured for the five strains. It contributed to at least 16% of the phenotypic variance, and reached 36% for strain Una2. One minor QTL was detected for strains G70, Una2, San91 and Man1 on chromosome g12. This QTL contributed to at least 7% of the phenotypic variance. Another minor QTL was detected on chromosome g13 (peak at marker EM30/2) for strain Man1, and contributed to 8% of the phenotypic variance. One putative QTL detected for strain G70 was located on chromosome g10. It co-localized with the QTL detected for RT measured for strain G70 and contributed to 6% of the phenotypic variance.

In summary, six regions were found to be associated with resistance to *M. ulei* on five among the 18 chromosomes (Fig. 2). One QTL on chromosome g13 was detected using LD and RT with the five strains. For all QTLs, the favourable allele, i.e. the allele providing resistance, was inherited from the *H. benthamiana* parent of RO38, F4542, and none were inherited from the Wickham *H. brasiliensis* parent of RO38, AVROS 363.

We then conducted a series of particular analyses on modified trait values or progeny samples for RT and LD. In a first round of analysis, we tried to better understand the biological significance of the scale used to measure RT. RT is a composite scale which mixes information on the ability of progeny to permit sporulation of the fungus (RT = 1 or 2 for non-sporulating symptoms versus RT = 3, 4 or 5 for sporulating symptoms) and the intensity of sporulation (RT = 3, 4 or 5). We first scored RT as a qualitative trait called RT_{0-1} separating non-sporulating (RT_{0-1} = 0) from sporulating (RT_{0-1} = 1) progeny individuals. We then used the RT value of sporulating progeny individuals (notes 3-4-5) only. This new trait was called RT_s. A QTL detection was conducted for RT_{0-1} and RT_s (Table 5). The main QTL on g13 and the QTL on g15 were detected in the analysis of both traits for most of the strains, suggesting that these QTLs may be involved in both the prevention and the reduction of sporulation. No QTL was detected in the RT_s analysis on g13 with strain G70, and on g13 and g15 with strain Una2. This may be due to the particularly low progeny

size observed for these strains with this trait. The QTL on g11 was only detected in the analysis of RT_s. This suggests that this QTL may only be implicated in the reduction of sporulation. The QTL on g10 for strain G70 was detected for RT_{0-1} but not for RT_s. Nevertheless, this may be due to the low detecting power of RT_s caused by a reduced progeny size. A new QTL was detected for RT_s on g2 for strain Una2.

In a second round of analysis, we tried to investigate whether the genetic basis of LD was different for non-sporulating and for sporulating lesions. We thus compared QTL analyses for LD on two distinct progeny samples, one on non-sporulating progeny individuals ($RT \leq 2$), and another on sporulating progeny individuals ($RT > 2$) (Table 5). The QTL on g12 was found for all strains except G77 on the sporulating progeny, whereas it was found only with the strain San91 on the non-sporulating progeny. The QTL on g13 (peak marker EM36/14) fluctuated according to the strain and progeny sample. We observed a new QTL for strain G70 on chromosome g13 and for strain Una2 on g4 on the non-sporulating progeny. The QTL on g4 was putative for strain G77 on the sporulating progeny.

PB260 map

One QTL for the RT of strain G70 was identified on chromosome g15. It explained 9% of the variance of the trait (Table 5). No QTL was identified for any other trait.

Consensus map

We made a supplementary QTL analysis on the F_1 consensus map because loci presenting three or four alleles inherited from both parents allow a more accurate QTL detection than loci heterozygous in a single parent (Williams 1998). Eighty three of these markers were available in the F_1 core map. The QTL analysis of RT and LD for the five strains allowed for the identification of six QTLs on chromosomes g6, g10, g12, g13, g15 and g16 (Table 5). QTLs were detected on g10, g12, g13 and g15 in similar positions to the ones detected on the RO38 map. A putative QTL was detected on g10 for RT measured with strain Man1. Two new QTLs were detected on g6 for RT measured with strains Una2 and San91, and on g16 for LD measured with strain G70. These QTLs were also detected on the analysis of the RO38 pseudo-testcross map with simple interval mapping (data not shown), but not with the MQM method. Simultaneous mapping of multiple QTLs is more efficient and more accurate than simple interval mapping (Jansen and Stam 1994; Knapp 1991). Consequently we did not retain these new genomic regions as potential regions implicated in resistance to SALB. No QTL was found on the g11 chromosome, but LOD score values were higher than 2 for RT measured with strains Man1, San91 and Una2 near marker EM3/24.

Discussion

Accuracy of traits measured

The repeatability of RT and LD was not always very high as could be assessed by the correlations observed between repetitions of measures (r = 0.39–0.82). This moderate repeatability is due to difficulties in standardizing the inoculation process of the fungus. Several factors may be responsible for this, such as spore concentration, leaf maturity and homogeneity of the inoculum. The influence of spore concentration, leaf maturity and homogeneity of the inoculum. The influence of spore concentration has been tested based on different inoculation experiments on both parents, but this factor did not seem to influence RT and LD (unpublished data). The influence of leaf maturity has been tested by conducting a series of inoculations of the two parents at different stages of leaf development. We observed that small differences in maturity could be responsible for variations of RT and LD (unpublished data). Since, for practical reasons, inoculations could only be performed twice a week, the stage of maturity could have been different from one inoculation to another and may explain at least a part of the variations observed. The heterogeneity of the inoculum may also be an important source influencing repeatability. First, cloning has not been mastered yet for *M. ulei*. Strains have been purified twice from a single lesion before their use for inoculation but this does not guarantee a perfectly pure clonal content of each strain. Moreover, the strains had to be inoculated regularly on a multiplicator plant to provide fresh inoculum, since storage was not possible for more than a few hours. These numerous multiplication processes might have selected the most aggresive strains in a mixture of genotypes. Second, differences in the germination rates of conidia were observed for a different inoculum of the same strain. This suggests non-mastered qualitative variations of conidia. All these factors may explain the variation observed in our experiment. No sensible experimental test could be applied to estimate the environmental noise, because sprouting is rather random. It is thus not possible to predict in advance the genotype composition of a given experiment. This may explain why the level of genotypic variation explained by all the QTLs detected for RT or LD never exceeded 50% in our study. This could be overcome in the future by improving the inoculation conditions or by raising the number of repetitions or the size of our working population.

Genetic interpretation of the traits measured

For the five strains, the QTL analysis performed on the RO38 map allowed for the identification of six QTLs associated with resistance, distributed on five of the 18 linkage groups of the genetic map. One QTL, localized on chromosome g13 near the marker EM36/14, had a large effect on RT and LD for the five strains. This QTL

is probably responsible for the phenotypic correlations observed between RT and LD within and between strains. Two other QTLs were comon to the four strains for which RO38 expressed complete resistance: one for RT on g11 and one for LD on g12. Finally, strain-specific QTLs were found on three other chromosomes, g15, g13 (peak at marker EM30/2) and g10.

The scale we used for scoring RT was complex because it mixed a qualitative phenomenon, i.e. the ability of the fungus to complete its life cycle (sporulation vs non sporulation), and a quantitative phenomenon, i.e. the intensity of sporulation when sporulation is possible. To better understand its biological meaning, we conducted particular QTL analysis on modified RT values and selected progeny samples. These analyses suggested that QTLs located on g13 and g15 may be involved in both qualitative and quantitative resistance and that the QTL on g11 may only concern quantitative resistance.

We also investigated separately the genetic basis of LD on the sporulating and on the non-sporulating progeny populations. A QTL on g12 was detected in only the sporulating population for strains G70, Man1 and Una2. A QTL was detected for G70 on g13, and for Una2 on g4, on the non-sporulating population but not on the sporulating population. This might suggest that the genetic determinism controlling LD for sporulating and non-sporulating individuals is slightly different. Nevertheless, the reduced population sizes may have lowered the QTL detection power in these analyses.

Partial vs complete resistance

The diversity of pathogenicity and the neutral diversity are not well known for *M. ulei*. It is, thus, difficult to estimate how the strains we studied here are representative of the fungus species. Nevertheless, based on the RT measure on host range, the RT mean of the whole progeny and the geographic origin, the five strains studied appear as quite different from each other. The RT measure of the five strains on the host range can be compared to the classification of Junqueira based on a similar host range (Junqueira et al. 1986) that allowed for the distribution of 16 strains collected from 11 states in Brazil into three different groups. Strains G70, Man1, San91 and Una2 which do not sporulate on the RO38 parent are close to the strains of group II defined by Junqueira. G77, the only strain for which RO38 is partially resistant, is probably closer to group I. None of our strains resembled those of Junqueiras' group III.

According to our results, complete resistance observed for strains G70, Man1, San91 and Una2 was quantitatively expressed in the progeny. This resistance is based on four or five loci depending on the strain. This is contrary to the previous hypothesis, which was based on field records, and suggested a monogenic determinism for complete resistance (Simmonds 1990). A polygenic determinism for complete resistance has also been reported in a potyvirus-pepper interaction (Caranta and Palloix 1996).

A single QTL was detected for strain G77 responsible of partial resistance. It contributed to 34% of the phenotypic variance for RT, and 18% of the phenotypic variance for LD. This is in contrast to other studies reporting on fungus and virus-plant interactions which showed that the genetic basis of partial resistance is governed by several QTLs (Leonards-Schippers et al. 1994; Wang et al. 1994; Caranta et al. 1997; Albar et al. 1998).

The same QTL detected for strain G77 was also detected for the four strains for which RO38 presents complete resistance. This genomic region could thus be implicated in both partial and complete resistance to SALB. The complete resistance would then be the result of the basic gene action of the g13 QTL, accompanied by other QTLs. Moreover, on the basis of symptom observations, it seems that there is no clear-cut separation between complete and partial resistance. Both types of resistance could share a common resistance mechanism and partial resistance may correspond more to a complete resistance that has partially failed, than to a biological response of a different nature. Only one strain presenting a partial resistance on RO38 was analysed so it will be necessary to confirm these results with different strains for which RO38 might also present partial resistance.

In our study, genetic resistance to *M. ulei* relies on 1–5 QTLs, among which two have a major effect. A similar determinism with one or two major QTLs and several minor QTLs has been reported for other plant-pathogen interactions (Leonards-Schippers et al. 1994; Wang et al. 1994; Lefebvre and Palloix 1996; Caranta et al. 1997). Our results are consistent with other studies showing that quantitative resistance is more oligogenic than polygenic (Young 1996; Lefèvre et al. 1998; Newcombe 1998; Pernet et al. 1999). Except for the major QTL on g13 which was found for all strains, other QTLs were strain-specific, even QTLs associated with quantitative variation of LD. Specificity of QTLs involved in quantitative resistance has been described for other species (Young 1996). Quantitative resistance, therefore, may not guarantee durability of resistance.

Breeding for durable resistance

A single QTL was detected for strain G70, and no QTL was detected for the other strains in the analysis of the PB260 map. This result is in accordance with the observation that clones belonging to the Wickham genepool are usually not resistant to *M. ulei*. Since this material is quite inbred (Besse et al. 1994), the QTL analysis of PB 260 is probably representative of most other Wickham clones. This suggests that it may not be worth looking for resistance to SALB in the Wickham material. This argument is reinforced by the origin of alleles observed for the QTL detected in the RO38 map. All the alleles conferring resistance were inherited from the wild *H. benthamiana* grandparent, F4542, and no favourable allele for resistance was inherited from AVROS363, the Wickham grandparent.

The non-specificity of the QTL detected on g13 is encouraging for the durability of resistance. Pathogen diversity must now be investigated in detail. It will allow for the assesment of the representativity of strains studied here, and to test new original strains. Even if specificity of most QTLs was observed, the QTLs on g15, g12 or g11 were detected with three or four strains. This is encouraging for the feasibility of combining different QTLs in a same genotype for resistance to several strains. QTL studies for resistance to *M. ulei* should now be enlarged to other crosses issuing from new resistance backgrounds, especially from wild *H. brasiliensis and H. pauciflora*. This will facilitate the identification of new alleles eventually more favourable for durability. Molecular markers allowed us to better understand the complex interaction between rubber and *M. ulei*. The historical failure of rubber breeding regarding resistance to *M. ulei* may be more attributable to the poor screening procedure for resistance than to the absence of resistance in the germplasm. When the genetic bases for resistance to *M. ulei* is clearer, marker-assisted selection will offer a promising way to transfer resistance into high-yielding clones.

Acknowledgments We thank K. Kaye and D. Tharreau for critical reading of the manuscript, and C. Mattos for helpful comments. The experiments comply with the current laws of France where the experiments were performed.

References

Albar L, Lorieux M, Ahmadi N, Rimbault I, Pinel A, Sy AA, Fargette D, Ghesquières A (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTL identification and relationship between resistance and plant morphology. Theor Appl Genet 97:1145–1154

Basten CJ, Weir BS, Zeng ZB (1995) QTL cartographer. North Carolina State University, USA

Besse P, Seguin M, Lebrun P, Chevallier MH, Nicolas D, Lanaud C (1994) Genetic diversity among wild and cultivated populations of *Hevea brasiliensis* assessed by nuclear RFLP analysis. Theor Appl Genet 88:199–207

Blasquez CH, Owen JH (1963) Histological studies of *Dothidella ulei* on susceptible and resistant *Hevea* clones. Phytopathology 53:58–65

Caranta C, Palloix A (1996) Both comon and specific genetic factors are involved in polygenic resistance of pepper to several potyviruses. Theor Appl Genet 92:15–20

Caranta C, Palloix A, Lefebvre V, Daubeze AM (1997) QTLs for a component of partial resistance to cucumber mosaic virus in pepper: restriction of virus installation in host-cells. Theor Appl Genet 94:431–438

Chee KH, Darmono TW, Zhang KM, Lieberei R (1985) Leaf development and spore production and germination after infection of *Hevea* leaves by *Microcyclus ulei*. J Rubber Res Inst Malaysia 33:124–137

Dean W (1987) Brazil and the struggle for rubber. Cambridge University Press, UK

Doerge RW, Reba A (1996) Significance thresholds for QTL interval-mapping tests. Heredity 76:459–464

Drost AW (1910) Verlsag over het jaar. Meded Dep Landb Suriname 21

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics 137:1121–1137

Hashim I, Pereira JCR (1989) Lesion size, latent period and sporulation on leaf discs as indicators of resistance of *Hevea* to *Microcyclus ulei*. J Nat Rubber Res 4:56–65

Hashim I, Chee KH, Duncan EJ (1978) Reaction of *Hevea* leaves to infection with *Microcyclus ulei*. J Rubber Res Inst Malaysia 26:67–75

Holliday P (1970) South american leaf blight (*Microcyclus ulei*) of *Hevea brasiliensis*. Phytopathol Papers 12:1–30

Jansen RC (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205–211

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Junqueira NTV, Chaves GM, Zambolin L, Romeiro RS, Gasparotto L (1984) Isolamento, cultivo e esporulaçao de *Microcyclus ulei*, agente etiologico do mal-das-folhas da seringueira. Rev Ceres 31:322–331

Junqueira NTV, Chaves GM, Zambolin L, Gasparotto L, Alfanas AC (1986) Variabilidade fisiologica de *Microcyclus ulei*. Fitopatol Bras 11:823–833

Junqueira NTV, Lieberei R, Kalil Filho AN, Lima MIPM (1990) Components of partial resistance in hevea clones to rubber tree leaf bight, caused by *Microcyclus ulei*. Fitopatol Bras 15: 211–214

Knapp SJ (1991) Using molecular markers to map multiple quantitative trait loci: models for backcross, recombinant inbred, and doubled-haploid progeny. Theor Appl Genet 81:333–338

Kuyper J (1911) Eine Hevea blattkrankheit in Suriname. Rec Trav Bot Neerl 8:371–379

Labroy MO, Cayla MV (1913) Culture et exploitation du caoutchouc au Brésil. Société Générale d'Impression, Paris, pp 235

Lander BS, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lefebvre V, Palloix A (1996) Both epistatic and additive effects of QTLs are involved in polygenic-induced resistance to disease: a case study, the interaction pepper – *Phytophtora capsici* Leonian. Theor Appl Genet 93:503–511

Lefèvre F, Goué-Mourier C, Faivre-Rampant P, Villar M (1998) A single gene cluster controls incompatibility and partial resistance to various *Melampsora larici-populina* races in hybrid poplars. Phytopathology 88:156–163

Leonards-Schippers C, Gieffers W, Schafer-Pregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an alogamous plant species. Genetics 137: 67–77

Lespinasse D; Rodier-Goud M, Grivet L, Leconte A, Legnate H, Seguin M (1999) A saturated linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellite, and isozyme markers. Theor Appl Genet 100:127–138

Newcombe G (1998) Association of *Mmd1*, a major gene for resistance to *Melampsora medusae* f. sp. *deltoidae*, with quantitative traits in poplar rust. Phytopathology 88:114–121

Oijen JW van, Maliepaard C (1996) MAPQTL(tm) version 3.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen

Ooijen JW van, Sandbrink H, Purimahua C, Vrielink R, Verkerk R, Zabel P, Lindhout P (1992) Mapping quantitative genes involved in a trait assessed on an ordinal scale: a case study with bacterial canker in *Lycopersicon peruvianum*. In: Yoder JI (ed) Molecular biology of tomato. Technomic, Lancaster PA, pp 59–74

Parlevliet JE, van Ommeren A (1975) Partial resistance of barley to leaf rust, *Puccinia Hordei*. II. Relashionship between field trials, micro-plot test and latent period. Euphytica 24:293–303

Pernet A, Hoisington D, Dintinger J, Jewell D, Jiang C, Khairallah M, Letourmy P, Marchand JL, Glaszmann JC, Gonzalez de Leon D (1999) Genetic mapping of maize streak virus resistance from the Mascarene source. II. Resistance in line CIRAD390 and stability across germplasm. Theor Appl Genet 99:540–553

Rebï A, Goffinet B, Mangin B (1994) Approximate thresholds of interval mapping tests for QTL detection. Genetics 138: 235–240

Rivano F (1992) La maladie sud-américaine des feuilles de l'*Hevea*. Etude, en conditions naturelles et contrôlées, des composants de la résistance partielle à *Microcyclus ulei* (P. Henn.) V.Arx. PhD thesis, University of Paris-Sud, France

Rivano F (1997) South American Leaf Blight of *Hevea*. I. Variability of *Microcyclus ulei* pathogenicity. Plantations, Recherche, Dével 4:104–114

SAS Institute (1989) SAS/STAT user's guide. Release 6.06 SAS Institute, Cary, N.C.

Simmonds NW (1982) Some ideas on botanical research on rubber. Trop Agric 59:2–8

Simmonds NW (1989) Rubber breeding. In: Webster CC, Baulkwill WJ (eds) Rubber. Longman Scientific and Technical, New York, pp 85–124

Simmonds NW (1990) Breeding horizontal resistance to south american leaf blight of rubber. J Nat Rubber Res 5:102–113

Stahel G (1915) De hevea-bladziekte van Zuid-Ammerika. Meded Dep Landb Suriname 1

Stam P (1993) Construction of integrated genetic linkage maps by means by a new computer package: JoinMap. Plant J 3:739–744

Ule E (1905) Katuschukgewinnung und Kautschukhandel am Amazonen-strome. Tropenfplanzer 6:1–71

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421–1434

Williams CG (1998) QTL mapping in outbred pedigrees. In: Paterson AH (ed) Molecular dissection of complex traits. CRC press, New York, pp 81–94

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501