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ORIGINAL PAPER

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Molecular mapping of genes conferring field resistance to South American Leaf Blight (*Microcyclus ulei*) in rubber tree

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Abstract The South American Leaf Blight (SALB), caused by the fungus *Microcyclus ulei*, is the major rubber tree disease in all Central and South America. A population of 192 progeny individuals derived from a cross between a resistant clone and a susceptible cultivated clone was planted in a field trial in French Guiana in order to evaluate the resistance parameters under real infestation conditions. The resistance type (RT), the presence of stromata (ST) and the level of attack (AT) were observed 20-times on a 22-months period, and semi-quantitative evaluation of stromata was registered only once. The search for QTLs was performed using the Kruskal-Wallis test, Interval Mapping and the Composite Interval Mapping method. One major QTL located on linkage group g13 was detected on the RO 38 map, responsible for 36 to 89% of the phenotypic variance of resistance. This resistance QTL corresponds to one that had previously been detected under controlled conditions of infestation and we called it *M13-1bn*. Surprisingly, the effect of this QTL was larger under natural conditions of infestation than under controlled inoculation. Other minor QTLs (four on the RO38 map and one on the PB 260 map) were also detected. The type of resistance brought by *M13-1bn*, as well as its durability, are discussed. Applications for rubber tree breeding programs are considered.

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Introduction

The rubber tree (*Hevea* spp.) is the main natural rubber-producing crop, and is cultivated in numerous equatorial and tropical countries. It is an outcrossing perennial crop, vegetatively propagated by grafting, which belongs to the botanical family Euphorbiaceae. Although originating from Amazon basin, rubber production in south and central America represents less than 2% of total world production (http://apps.fao.org), due to South American Leaf Blight (SALB). This disease, caused by the ascomycete *Microcyclus ulei* (P. Henn.) v. Arx, is also endemic from Amazon forest and, until now, never reached the major-rubber producing countries in South-east Asia and Africa. SALB expansion outside the Amazon basin has already affected distant regions of the American continent, as Central America or the Brazilian state of Bahia (Dean 1987), and for all other rubber-growing countries represents an important potential threat, as the majority of cultivated clones in Asia and Africa is considered as very susceptible to this disease. This fact has been recognized by the United Nations, by including *M. ulei* in a list of plant pathogens for expert controls in order to prevent its use as a biological weapon (http://www.unodc.org/unodc/terrorism_weapons_mass_destruction_page005.html). Infection occurs only on young leaves, and results in repeated defoliation, die-back of the canopy and, in most severe cases, may lead to the death of trees (Chee and Holliday 1986). Biology of *M. ulei*, disease cycle, epidemiology and variability of its pathogenicity have been described by several authors (Holliday 1970; Chee et al. 1985; Junqueira et al. 1986; Hashim and Almeida 1987), as well as genetic resistance of rubber tree and its utilization in breeding strategies (Darmono and Chee 1985; Junqueira et al. 1990; Rivano 1992). At least two distinct forms of resistance were identified. A complete resistance defined by rubber phytopathologists as the absence of sporulating lesions is often encountered in *Hevea benthamiana* (Junqueira et al. 1988) and *Hevea pauciflora* (Pinheiro et al. 1984) species, or in particular clones of the cultivated *Hevea*

brasiliensis species (Gonçalves 1968). Partial resistance has also been described (Rivano 1997a), which is characterized by a susceptible infection type and a reduced rate of epidemic development. One same clone can show complete resistance in a particular place, and partial resistance in another, depending on the presence of different strains of *M. ulei* (Junqueira et al. 1988). Some resistance mechanisms developed by leaf tissues were investigated (Garcia et al. 1995) and showed histochemical reactions with the production of scopoletin.

The first genetic study on determinism of resistance to *M. ulei* was performed by Lespinasse et al. (2000b) on a controlled progeny. A F1 population for which a high-density linkage map was constructed (Lespinasse et al. 2000a), has been evaluated for several resistance traits under controlled conditions of inoculation with five different pathogen strains among which two were originated from French Guiana. This allowed to identify eight QTLs associated with resistance, distributed on seven linkage groups of the genetic map. For four of these strains the resistant parent showed a complete resistance. Before the study of Lespinasse et al. (2000b), no experiments have been carried out on heritability and genetic determinism of SALB resistance. In the lack of precise knowledge, pathologists and breeders used to retain the hypothesis that this complete resistance was a monogenic trait (Simmonds 1990) and for that reason a qualitatively inherited trait was expected. Contrary to this hypothesis, Lespinasse et al. (2000b) demonstrated that both partial and complete resistances were quantitatively expressed in the progeny, and based on four to five loci. One QTL, located on linkage group g13, had a large effect on two resistance traits for the five strains. This single QTL was also responsible for partial resistance to strain G77 (Lespinasse et al. 2000b). This study was, until now, the only one ever carried out on the genetics of resistance of the rubber tree to SALB, either under controlled conditions or in field experimentation.

The work reported here aimed to evaluate SALB resistance under natural infestation in field conditions. These studies were developed on the same F1 population which was used to detect QTLs under controlled conditions (Lespinasse et al. 2000b). Several resistance traits were evaluated, some of them which could not be measured under controlled conditions, and are specific to natural infestation.

Materials and methods

Plant material

A population of 192 F1 progeny was created through a cross between two heterozygous genotypes, PB260 and RO38 (Lespinasse et al. 2000a). RO38, alias FX3899, is a low yielding interspecific hybrid between *H. brasiliensis* and *H. benthamiana*, and has inherited from this later species a SALB resistance which proved to be very efficient under French Guiana natural conditions of infestation (Rivano 1992). PB260 is a high yielding *H. brasiliensis* clone, which is very susceptible to SALB. The 192 progenies were multiplied in the nursery by grafting and transplanted into a naturally infested area located in the Cirad Experimental Center of Combi, French Guiana. They were field-planted in January 1999 according to the following statistical design: an elementary plot made of four grafted trees of the same clone, two completely randomized blocks constituted by all the 192 tested clones and a high-density plantation (2,500 trees per hectare). Due to experimental constraints, it was not possible to include the parents of the family in the experiment.

Pathogen population

Until now, studies of variability of *M. ulei* were performed by artificial inoculation on a differentiated set of rubber-tree clones. Natural populations of *M. ulei* already proved to be extremely variable (Junqueira et al. 1986), with more than 50 distinct physiological races identified in the same place (Mattos et al. 1999). In French Guiana, previous studies also demonstrated a highly polymorphic pathogen population (Rivano 1992). The majority of the strains isolated in Combi does not develop any sporulating lesion on parent RO38 and only one isolate (G77) resulted in weakly sporulating lesions when inoculated on RO38 (Lespinasse et al. 2000b).

Disease evaluation

A precise evaluation of SALB disease in field experimentation is more complicated than under standardized controlled conditions of an inoculation chamber, for various reasons: (1) the favorable foliage stage for infection and detection of sporulation only lasts a few days, and therefore symptoms of disease may be observed only on a small portion of the progeny individuals on each observation date; (2) the rubber tree being a tropical perennial crop, infestation may occur throughout the year and a good disease evaluation needs to take into account all seasonal variations; (3) being impossible to know exactly the inoculation date of a foliar cluster, the intensity of sporulation and the lesion diameter which are highly time-correlated parameters (Rivano 1992), may be influenced by the duration between infection and observation. For all these reasons, it was decided to repeat the observations during nearly 2 years, in order to be able to obtain valuable data on a sufficient number of progeny individuals.

Four parameters were observed: an assessment of the severity of attack (AT) which can be related to the abundancy of the natural inoculum; the reaction type (RT) which is an assessment of the type of lesion caused by the inoculum (the necrotic, chlorotic or sporulating lesion); a qualitative assessment of the presence of stromata (ST) and a semi-quantitative measurement of stromata (STQT). AT was scored according to quantities of lesions and lamina deformation, on a 0–4 scale adapted from Chee (Chee and Holliday 1986). Score 0 corresponded to less than four lesion spots per leaflet, in average. Score 4 corresponded to an area affected by leaf necrosis larger than 30%, with severe lamina deformation, and eventually die-back. The RT was based on a visual determination of dominant symptoms on a 0 to 6 scale modified from Junqueira (Junqueira et al. 1986). Score 0 corresponded to the absence of lesions. Scores 1 and 2, respectively, corresponded to necrotic and chlorotic non-sporulating lesions. Scores 3, 4 and 5 corresponded to sporulating lesions, with slight, moderate and heterogeneous, high and homogeneous sporulation, respectively. Score 6 corresponded to abundant sporulation on upper and lower surfaces of leaflets. These two types of symptoms, AT and RT, can be detected only in a particular foliar development stage, corresponding to approximately 12 to 16 days after emergence of the foliar cluster.

The presence of stromata on the upper surface of leaflets, which corresponds to the sexual phase of the fungus, can be detected as long as mature leaves are attached to the tree. The ST was based on a 0–3 scale: 0 corresponding to total absence of stromata, 1 to few stromata on few leaves, 2 to a rather large quantity of stromata on

several leaves and 3 to abundance of stromata on a majority of leaves. The STQT notation relied on a numeration of stromata in a sample of five leaves per tree (15 leaflets), and a classification into four classes: 0 stromata per leaflet (class c 0), 1 to 10 stromata per leaflet (class c 1), 11 to 50 stromata (class c 11), and more than 50 stromata (class c 50). This measurement was carried out only once, in November 1999, corresponding to the eighth observation of the AT, RT and ST parameters, when a majority of plants exhibited a maximal number of symptoms. An index was calculated for each individual progeny, as follows:

$$\mathrm{STQT} = \frac{(\text{number leaflets in c1}) + 2 \times (\text{number leaflets in c11}) + 5 \times (\text{number leaflets in c50})}{(\text{total number leaflets})}.$$

Thus, STQT is a continuous variable which can take values from 0 (no stromata encountered in any of observed leaflets in the clone), to 5 (every leaflet of the clone classified in c50). The choice of 2 and 5 for weighting factors was made in order to obtain a final index with the same range of values as for other three traits. Modifying these factors only affects the range of values, the further QTL detection being slightly modified. The ST notation can be considered as a visual estimation of the quantity of stromata in the entire plant, whereas STQT is a variable based on the numeration of stromata on a determined sample of leaflets.

AT, RT and ST were measured for all living trees 20-times between July 1999 and December 2000, on a basis of one measurement every 2 weeks except for two periods, one of 40 days without measurements in January 2000 (heavy rainy season unfavourable for spore dispersion) and another 4-months period from July to September 2000 during the dry season. For each observation date, and if the presence of the accurate foliar stage allowed it, a global notation was determined per tree and per trait. At the end of the 20th observation, a maximum of 80 data were then available per trait and per progeny for each block. Further analyses were then carried out on overall average values for these three traits. These averages were simply computed taking into account the total numbers of available data on all living trees of the progeny for the period of observation.

Map construction

In the present study, we used exactly the same segregation and linkage data of the two parental core maps built and used in Lespinasse et al (2000b). The two pseudo-testcross core maps used for QTL analysis, derived from the RO38 and PB260 saturated map, encompass 231 and 158 markers respectively. Linkage analysis was performed on segregation data from 195 progenies, and core markers are distributed every 10–15 cM.

Statistical analysis and QTL detection

QTL analysis was performed using the computer software MAPQTL version 4.0 (Van Ooijen and Maliepaard 1996). Different analysis methods were employed in order to detect QTLs. The rank-sum test of Kruskal-Wallis (Van Oijen and Maliepaard 1996), which presents the advantage of being a non-parametric one and therefore does not need any assumption on the normality of distribution, was first applied individually to each segregating locus with a threshold value of P=0.001. For interval mapping, permutation tests (Churchill and Doerge 1994) carried out on the whole genome, led to the conclusion that an overall 5% significance level, which is a reasonable risk (Van Ooijen 1999), corresponded to a 2.9 LOD-score empirical-threshold value, either on the PB260 map or on the RO38 map. Interval mapping (Lander and Botstein 1989) as well as composite interval mapping (Jansen 1993) were applied on both parental maps, using these LOD-score values.

Another set of analyses was then conducted, considering only susceptible progeny individuals, as already done by Lespinasse et al. (2000b) and for blast resistance in rice (Wang et al. 1994). Individuals were separated on the basis of RT values, discarding those with an RT value inferior or equal to 2, which do not sporulate on average. All progeny individuals satisfying this criterion were thus included in this new set of analyses carried out on all parameters.

Results

SALB disease-evaluation data

The total number of registered data from the 20 observation dates are very different for AT and RT on one hand, and ST on the other hand, due to the above-mentioned problem of the presence of the accurate foliar stage. For this reason, the average number of observed data per clone for AT and RT was 16.7, with a standard deviation of 5.9, on a potential number of 160 data per clone; whereas these numbers were respectively 137.0 and 28.6 for ST. For AT and RT parameters, individual progenies with less than ten observations were not taken into account for the further genetic analyses.

The distribution of variables generated from AT, RT, ST, as well as STQT values, are presented in Fig. 1. The shape of these various diagrams does not look like that of a normally distributed trait, which was confirmed by the

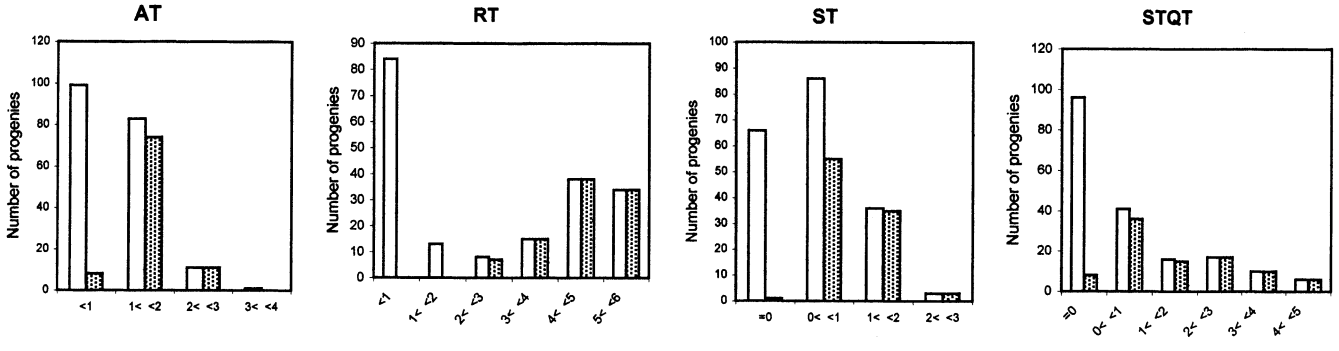

Fig. 1 Distribution of attack (*AT*), reaction type (*RT*), stromata (*ST*) and quantitative stromata (*STQT*)

Table 1 Matrix of correlations between resistance traits. All correlation coefficients are highly significant ($P<0.0001$)

Trait	AT	RT	ST
RT	0.88		
ST	0.81	0.82	
STQT	0.70	0.70	0.91

Table 2 Results of the QTL analysis for ST, RT, AT and STQT. Output from MapQTL for Kruskal-Wallis analysis (non-parametric analysis) on the RO38 genetic map

Trait	Linkage group	Marker	Nb[a]	K[b]	P[c]
ST	g13	EM36/14	191	114.31	<0.0005
ST	g8	MX692	137	12.34	<0.0005
RT	g13	EM36/14	172	111.00	<0.0005
AT	g13	EM36/14	171	106.80	<0.0005
STQT	g13	EM36/14	186	109.83	<0.0005
ST[d]	g14	EM22/9	94	19.50	<0.0005
STQT[d]	g14	EM22/9	94	21.61	<0.0005

[a] Nb is the number of progeny individuals with available data for QTL detection
[b] K is the value of the Kruskal-Wallis test, to be compared with chi-square threshold values with 1 *df*
[c] *P* is the probability associated with the K value
[d] Analysis made on a sub-sample of susceptible individuals (RT>2)

normality test of Shapiro and Wilks. Strong correlations ($P>0.0001$) were found between resistant traits (Table 1).

The RO38 map

As none of the observed parameters showed a normal distribution, we first tried to analyze the data with the non-parametric Kruskal-Wallis method. For a genome-wide significance level of 5%, calculations showed that either for RO38 or for PB260, the linkage group-wide significance level to be considered was 0.0005. Results for all four resistance traits are presented on the RO38 genetic map in Table 2. Two molecular markers were found to be associated with resistance QTLs, located on linkage group g8 and g13. The QTL on g13, located in the chromosomal region of marker EM36/14, was detected with a highly significant chi-square value, and for all tested parameters. The one near-marker MX692 on linkage group g8 was detected only for the ST trait.

The Interval Mapping procedure was then applied for QTL analysis to all four parameters. This analysis confirmed the remarkable fact that in all cases the same QTL was involved, located on linkage group g13 (peak at marker EM36/14) (Table 3). The LOD score for this QTL varied from 16 for quantitative observation of stromata (STQT) to 26 for qualitative observation of stromata (ST), 38 for the level of attack (ST) and 76 for the reaction type (RT). Such high levels of the LOD score strongly suggested the presence of a major resistance gene. In a previous work (Lespinasse et al. 2000b), RO38 and its parents were genotyped simultaneously, thus identifying the origin of the alleles, and to be sure that for this particular locus the allele responsible for resistance comes from the *H. benthamiana* parent. Owing to the huge effect of the resistance allele at this locus, we decided to name it *M13-1bn* (*M* for *Microcyclus*, *13* for linkage group g13, *1* is the first QTL detected on this linkage group and *bn* for *benthamiana*).

The QTL previously detected with the Kruskal-Wallis method for RT on linkage group g8 was no more revealed with the interval mapping method. Conversely, a new QTL was detected on linkage group g14 near the marker EM22/9 for the trait STQT. Due to its probable influence for stromata formation, we decided to name it *M14-bn*, according to the same designation as previously described.

A total of 94 progeny individuals (on 192 observed individuals) exhibited average-sporulating lesions (i.e. RT>2), significantly characteristic of a 1:1 segregation. These individuals were considered separately for a new set of interval-mapping analyses on all studied parameters (see Fig. 1). Results (presented in Table 3) clearly showed three interesting points: (1) the *M13-1bn* QTL was not detected any more, suggesting that elimination of resistant individuals resulted in a related elimination of a resistant allele at this locus; (2) *M14-bn* appeared for both ST and STQT with increased values of the LOD score (5.3 and 5.0 respectively); (3) a new significant QTL was detected for RT on linkage group g2, with a LOD score of 3.1, whereas no more QTLs were detected for AT. These

Table 3 Results of the QTL analysis for ST, RT, AT and STQT on the RO38 map. Output from MapQTL for interval mapping

Trait	Linkage group	Marker[a]	Pos[b]	Nb[c]	LOD	R^2 (%)[d]
ST	g13	EM36/14	+5	191	25.5	55
RT	g13	EM36/14	+5	172	76.3	91
AT	g13	EM36/14	0	171	38.0	65
STQT	g13	EM36/14	0	186	16.0	33
STQT	g14	EM22/9	−11	186	2.9	7
ST[e]	g14	EM22/9	−1	94	5.3	24
RT[e]	g2	EM22/10	0	94	3.1	15
STQT[e]	g14	EM22/9	0	92	5.0	23

[a] Marker is the name of the closest molecular marker from the LOD peak
[b] Pos is the genetic distance (in cM) between Lod peak and marker
[c] Nb is the number of progeny individuals with available data for QTL detection
[d] R^2 is the proportion of the explained phenotypic variance
[e] Analysis made on a sub-sample of susceptible individuals (RT>2)

Table 4 Results of the QTL analysis for ST, RT, AT and STQT. Output from MapQTL for MQM mapping, with EM36/14 as a cofactor on the RO38 genetic map

Trait	Linkage group	Marker[a]	Pos[b]	Nb[c]	LOD	R^2 (%)[d]
ST	g13	EM36/14	+5	191	25.6[f]	55[f]
ST	g14	EM22/9	+5	191	4.4	7
RT	g13	EM36/14	+5	172	76.4	91
AT	g13	EM36/14	0	171	38.1	65
AT	g3	V430	0	171	3.1	3
STQT	g13	EM36/14	0	186	16.0	33
STQT	g14	EM22/9	+5	186	4.4	8
ST[e]	g14	EM22/9	+5	94	5.5	29
RT[e]	g2	EM22/10	0	94	3.0	14
STQT[e]	g14	EM22/9	0	92	5.2	24

[a] Marker is the name of the closest molecular marker from the LOD peak
[b] Pos is the genetic distance (in cM) between Lod peak and marker
[c] Nb is the number of progeny individuals with available data for QTL detection
[d] R^2 is the proportion of the explained phenotypic variance
[e] Analysis made on a sub-sample of susceptible individuals (RT>2)
[f] EM36/14 was the peak marker used as a cofactor for MQM analysis. The LOD and R^2 values given here for this marker correspond to results of simple interval mapping as in Table 3.

results were confirmed with non-parametric Kruskal-Wallis analysis (Table 2).

Composite interval mapping analysis (MQM procedure of MAPQTL, version 4.0) was also performed both upon the whole-progeny population and on selected susceptible individuals (Table 4), taking as a cofactor the EM36/14 marker, located close to the *M13-1bn* resistance gene. The minor QTL *M14-bn* appeared with a LOD score slightly higher than with interval mapping. A new QTL for AT was revealed with this MQM procedure, located on linkage group g3 with a LOD score value of 3.1.

Considering *M13-1bn* as a 1:1 segregating genetic marker, its position was tentatively determined, identifying the allelic form present at the EM36/14 marker, both for resistant individuals (i.e. with RT inferior or equal to 2) and susceptible ones (i.e. with RT>2). From a total of 192 observed progeny individuals, there were 16 missing data for marker EM36/14. Recombination between the resistance status and the marker was observed for three individuals out of 176, which corresponded to a 1.7% recombination rate, and a 1.7-cM genetic Kosambi distance. Comparisons made with other genetic markers on linkage group g13 led to the conclusion that *M13-1bn* was most probably located between markers EM36/14 and EM29/3.

The PB 260 map

The same QTL analyses were performed on the PB 260 genetic-linkage map. According to the LOD-score threshold, empirical values were determined with a permutation test, and no QTLs were found for any resistance parameter using the interval-mapping method. On the other hand, the non-parametric Kruskal-Wallis analysis allowed us to detect for the ST trait, one minor QTL located on linkage group g9 near marker EM17/5, with a significance level inferior to 0.0005. This QTL did not appear for other parameters and its participation to the global resistance of the studied progenies may be rather weak.

Discussion

Relevance of observed resistance parameters

The AT, RT and ST traits may be considered respectively as indicators of the intensity of damages caused by an epidemic of SALB, the multiplication rate of the fungus and its capacity in completing its biological cycle (Rivano 1997a, b). Under controlled conditions, it has been noticed that AT was influenced by conidia-concentration in the inoculum (Lespinasse 1999). It should therefore be worthwhile to examine the relevance of this parameter for our purpose, considering that in a field experimentation, there is no way to control the quantity of conidia which possibly infects a plant. Both non-parametric and interval mapping-tests revealed solely the effect of the *M13-1bn* allele for AT. However, the composite interval mapping-procedure allowed us to also detect a new QTL on linkage group g3 for this trait (Table 4.). This could signify that the major effect of *M13-1bn* masked the expression of a minor QTL on g3, which only could be revealed considering the genetic marker closest to *M13-1bn* as a cofactor. In the same way, other specific minor QTLs were detected for RT, ST and STQT in addition to *M13-1bn* (on g2, g8 and g14 linkage groups), either with non-parametric interval mapping or composite interval mapping tests. Thus, every measured trait contributed to the detection of original low-effect resistance QTLs, in spite of the major effect due to *M13-1bn.* The strategy of measuring different resistance parameters seems therefore to have been a suitable choice for this particular situation.

Comparison with QTLs detected in the inoculation chamber

The previous mapping study on the resistance of the rubber tree to *M. ulei*, carried out under controlled conditions (Lespinasse et al. 2000b), resulted in the detection of eight QTLs associated with resistance. Among these eight QTLs, two had a major effect, especially the one located on linkage group g13 near the marker EM36/14 (the one named *M13-1bn*), whose huge-effect on the two observed parameters (reaction type and lesion diameter) was observed for all tested fungus strains. It was concluded that complete and partial resistance of RO38 could share a common mechanism, and that partial resistance may correspond to a complete resistance that had partially failed. However, this hypothesis already formulated for other pathosystems has been questioned (Zeigler et al. 1994), and the alternative hypothesis of partial and complete resistance-genes co-located in a same cluster can not be discarded.

Conditions of field experimentation in Combi appeared quite similar to those for the controlled inoculations used previously (Lespinasse et al. 2000b). No strain of *M. ulei* with a high degree of aggressiveness on RO38 was known in Combi, in the same way as only one out of five tested strains in the inoculation chamber had shown virulence (and hardly a weak one) on RO38. We thus expected to detect some QTLs already identified under controlled conditions, especially *M13-1bn*. The LOD scores at *M13-1bn* for all parameters of resistance measured in the field, even for those which were not studied under controlled conditions, were unexpectedly large. A QTL whose contribution to phenotypic variance can reach values as high as 91%, with a LOD score of 76, can be considered as a locus of huge effect on the whole resistance. In the light of these new results, the type of the resistance mechanism conferred by the *M13-1bn* gene has to be further investigated.

The second important QTL detected under controlled conditions by Lespinasse et al. (2000b), and located on linkage group g15, was not found in field evaluation. The authors succeeded in detecting this QTL for the three Brazilian strains which were inoculated, but not for the two Guyanese ones. For two of the three Brazilian strains, the LOD score and R^2 were even-greater for "*M15-bn*" than for *M13-1bn*. This suggests that for *M15-bn*, the lack of QTL-detection should not be due to statistical effects but to the non-efficiency of this QTL against the natural fungus-population of French Guiana. This hypothesis would be confirmed by a field evaluation of the progenies in Brazil. A third important QTL located on linkage group g12 and detected under controlled conditions (Lespinasse et al. 2000b), was not found in the field experiment. This QTL was detected only for the Lesion Diameter trait, and for the five inoculated *Microcyclus* strains (Lespinasse et al. 2000b). For technical reasons, it was not possible to measure this trait under field conditions, due to very frequent coalescence of close lesions and lack of knowledge on the duration of the inoculation period. The question of the usefulness of the Lesion Diameter trait as a good predictor for SALB resistance under field conditions, remains unanswered.

In our study, a nearly complete resistance was observed in the progeny, illustrated by the fact that for all parameters almost half of individuals did not exhibit symptoms of evolution of the disease (see Fig. 1). This virtually 1:1 distribution, characteristic of a monogenic segregation for an either dominant or codominant resistance allele, was confirmed by the genetic analysis which showed the tremendous contribution of the *M13-1bn* QTL to the phenotypic variances. However, QTLs of minor importance (on linkage group g2, g3, g8 and g14) were also detected, which may modulate the expression of *M13-1bn*.

This hypothesis of a QTL with major effect, modulated by few other minor QTLs, was strengthened by the analyses carried out on a selected sub-population made exclusively of progeny individuals on which sporulating lesions were detected. These analyses led to the following conclusions: (1) *M13-1bn* was no-more detectable in susceptible progeny individuals; (2) the *M14-bn* QTL previously detected on the entire segregating population reached higher LOD score threshold values; (3) one QTL never encountered until now (on linkage group g2), appeared with a significant LOD score value on this sub-population. In this particular example of host-pathogen relationship, it appears that the efficiency of *M13-1bn* is best under field infestation than under controlled inoculation.

Another category of relevant information brought by this study concerns the quantity of stromata produced on adult leaves, a character that has not been observed under controlled conditions. This parameter is under the influence of *M13-1bn* as for all other resistance parameters, but also under the influence of *M14-bn*, which seems to have a small influence exclusively on stromata. This statement re-inforces the previous conclusion that measuring different parameters of resistance proved to be useful in detecting various QTLs of resistance.

As for resistance to *Xanthomonas axonopodis* in cassava, another Euphorbiaceae (Jorge et al. 2000, 2001), our work has revealed an absence of consistency for minor QTLs. There is no common minor resistance QTL between controlled inoculations (Lespinasse et al. 2000b), and those revealed by field observations. This could be explained by the fact that only two Guyanese strains had been previously tested in the inoculation chamber. Nevertheless, in our case on SALB analyses, the presence of *M13-1bn*, a gene with major effect (either considered as one of the QTLs of resistance or as a complete-resistance gene), and the absence of virulent strains in sufficient number in the natural inoculum, reduced the statistical power of minor QTL detection, which could be made only on half of the progenies, i.e. the sporulating progenies lacking the *M13-1bn* allele. This is illustrated by the QTL on g2 which was significantly detected only on the 94 sporulating progenies. On the other hand, studies developed for resistance

to *Venturia inaequalis* in apple-tree under both field and glasshouse conditions, led to very similar results on detection and location of *Vf* the major resistance gene (King et al. 1998). As in our case for *M13-1bn*, a gene with large effect on resistance was detected, both under artificial and natural-inoculation conditions.

Breeding for resistance

Our knowledge of the genetic determinism of resistance to SALB is just initiating. This type of study is not very frequent on perennial crop, being well-developed mainly for resistance to *Venturia inaequalis* in apple-tree (Yang et al. 1997; Patocchi et al. 1999), resistance to *Uncinula necator* in grapevine (Pauquet et al. 2001; Donald et al. 2002) or resistance to *Melampsora* spp. in poplar (Cervera et al. 1996; Newcombe et al. 1996; Villar et al. 1996; Tabor et al. 2000). These genetic studies are even more uncommon on tropical perennial crops. In the rubber-tree, the only major gene whose genetic determinism was identified until now, is that of resistance to *Phyllachora huberi* (Le Guen et al. 2000). For SALB resistance, the defence mechanism of *M13-1bn* has to be more investigated, trying to elucidate why this resistance locus was considered as one of the QTLs for partial resistance in the inoculation chamber, and as a gene for complete resistance under field conditions. Comparing this situation with that of resistance to *Magnaporthe grisea* in rice (Wang et al. 1994), it will be important to determine whether *M13-1bn* represents: (1) always the same allele at the same resistance locus, but showing differential reactions to different isolates of the fungus in various environmental conditions, or (2) a cluster of co-located partial and complete resistance genes. Obtaining this information will be important for predicting the durability of resistance conferred by this locus.

The efficiency of the allele *M13-1bn* under field conditions in French Guiana is so high that the trunk diameter of individual progenies having this allele is significantly higher than that of progenies with the alternative allele (data not shown), thus showing the pleiotropic effect of a resistance allele on an agronomical trait. The most-promising follow up of this study should be the development of a Marker-Assisted Selection program, aiming at selecting with a high-level of confidence at the nursery stage individual-progenies with the resistance allele *M13-1bn*. However, the progeny from the cross PB260×RO38 was until now only checked against five fungus strains under controlled conditions (Lespinasse et al. 2000b), and in one location for natural infestation in the present study. Testing with other strains of *M. ulei*, coming from various origins, and establishing the segregating population at least in one or two other field trials, possibly in rubber-tree growing regions, would permit us to have a more definite opinion on the geographic extent of the efficiency of *M13-1bn* and thus on its predictable durability.

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