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Selection of Rubber Clones for Resistance to South American Leaf Blight and Latex Yield in the Germplasm of the Michelin Plantation of Bahia (Brazil)

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South American Leaf Blight (SALB), caused by an ascomycete fungus, Microcyclus ulei *Henn. is one of the most serious disease in* Hevea *spp. Although it is not found in South-East Asia and Africa, it is nevertheless a potential threat for these regions which account for over 98% of world rubber production. SALB resistance and latex yield were measured in a rubber tree clonal trial planted at Ituberá (Bahia, Brazil), on 36 different clones coming from the germplasm of the Michelin plantation of Bahia. The clones were evaluated for their resistance to SALB during the first two years after planting. Inoculation tests and trials of the resistance during collection were carried out to confirm the trial observations. Latex yield was estimated on five-year-old rubber trees and architecture of the trunk and the canopy, structural traits of the laticiferous system were taken into consideration for the ultimate selection. This study indicated that a few clones which were high yielding were also SALB resistant. The clones MDX 624, FDR 5788, CD 1130, MDX 607, CDC 312, FDR 5665, FDR 5802, CD 1174 were selected and will be studied in a large-scale clonal trial.*

Key words: *Microcyclus ulei*; disease; *Hevea brasiliensis*; SALB; breeding; resistance; latex yield; Brazil; germplasm

Microcyclus ulei (P. Henn.) von Arx is the causal agent of South American Leaf Blight (SALB), a leaf disease which limits *Hevea* expansion in Central and South American inter-tropical regions. In spite of intensive research on chemical protection of the rubber tree against leaf blight, suitable fungicide could be applied economically only in the nursery and during the first two years after planting[1,2]. Even if alternative protection methods such as plant management (crown budding[1,3]), biological control by an hyper-parasite, *Dycima pulvinata*

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(Berk & Curt) Huges[4,5], or mycorhized plants demonstrate positive effects on resistance, they have never been commercially adopted. Since 1928, several companies have participated in breeding for SALB resistance[6–8]. Until 1945, the Ford Motor Company tried hard to plant 9500 ha of rubber plantations in Fordlandia and Belterra, in Brazil. In spite of considerable investment, successive SALB epidemics forced the Ford Company to surrender unsuccessful plantations to the Brazilian government. From 1947 to 1983, Firestone participated actively in SALB resistance breeding programs and germplasm exchanges with other institutes in Latin America. From these two South-American breeding programs, perhaps because of relatively low average latex productivity of genetic material as compared to Asiatic clones, or because of *Microcyclus* resistance breakdown, few clones could be issued and recommended for large-scale planting in Latin America[9–11].

The seventy years of rubber breeding activities resulted in approximately 960 original clones being planted at the Fazenda Três Pancadas (nowadays Plantações Michelin da Bahia – PMB). For 13 years now, Michelin and CIRAD have been improving these clones to achieve good yield and strong SALB resistance levels.

This paper describes some of these clones, and identifies the most promising ones for future crosses, as well as for further studies in large-scale clonal trials.

MATERIALS AND METHODS

Small Scale Clonal Trial

The experimental trial involved 36 clones planted in August 1997, at the Michelin Plantation of Bahia (PMB), Ituberá, Brazil, an area subjected to high SALB pressure. The statistical design consisted of eight randomised blocks with six trees per clone for each elementary plot.

Field Evaluation Resistance

The resistance of the trees to SALB was evaluated for two years in the small-scale clonal trial (SSCT).

The observed parameters were:

- *The reaction type.* It was scored from 1 to 6 on chlorophylian and drooping leaflets (stage C) with the following specifications : 1, necrotic lesion without spores; 2, chlorotic lesion without spores; 3, partial sporulation around the lesion on the lower surface of the leaf; 4, partial sporulation on the lower surface of the leaf; 5, abundant sporulation on the lower surface of the leaf; 6, abundant sporulation on the lower and upper surface of the leaf.

- The stromata density on mature leaves was scored from 0 to 4 with the following specifications : 0, absence of stromata; 1, less than five stromata per leaflet; 2, between five and ten stromata per leaflet; 3, between ten and thirty stromata per leaflet; 4, more than thirty stromata per leaflet.

The visual scores were taken monthly. In order to obtain strong selection criteria, only maximum values for the two parameters were considered on each tree. The same evaluations were carried out in conservation budwood gardens, from 1996 to 2002, on regularly pruned trees.

Inoculation Tests

Complementarily, the most resistant clones in SSCT were tested in controlled conditions with nine *Microcyclus ulei* polyvirulent strains. *Microcyclus ulei* isolates used for the tests were taken from lesions on *Hevea* leaflets in the plantation. They were cultivated *in vitro* on medium M4 (modified potato sucrose agar-PSA) in the dark[12] and induced to sporulate on a modified medium[13] with a 12 h photo period. The primary inoculum, consisting of a few spores, was deposited with a paint brush on the abaxial surface of leaflets of highly susceptible plants. Spores were collected 8 days after the inoculation using a wet sterile brush. An inoculum spore suspension consisting of 2×10^5 conidies mL^{-1} was prepared in demineralised sterile water added with Tween 80® (0.05%).

Inoculations were performed in an air-conditioned chamber maintained at 24°C ± 2 with relative humidity above 95% and a 12 h photo period (2000 lux). The inoculum was sprayed on the abaxial surface of 6 to 8-day-old anthocyanic and drooping leaflets. The reaction type was observed 12 days after inoculation. Only the maximum value obtained with the nine *Microcyclus* strains is retained to define the clone resistance.

Tree Architecture

Twisted trunk, bark bust, unbalanced canopy or etiolated and branchless trees were considered negatively.

Latex Yield

The average yield was determined by registering latex production. The tapping panel was opened at one meter height for all trees with girth superior to 25 cm (five years after planting). Tapping in downward half-spiral extended from July to December 2002. Trees were tapped every three days and stimulated after the fourth cut every two months with ethephon 2.5% a.i. (Ethrel PT, 10% ethephon). Coagulum from all tapped trees of each elementary plot were combined and weighed monthly. Monthly values per clone per plot were added and divided by the number of tapped trees and by the number of tapping days. The analysed data represent the average of dry coagulum production per tree per tapping.

Anatomical Observations

Bark samples were taken from the trunk, at 1.2 meter height on the opposite side of the tapping panel. Samples of two trees per plot and per clone were analysed. Longitudinal and transverse sections of the samples were made and stained with Sudan III and osmic acid. The following characters were determined:

- Number of latex vessel rings determined from longitudinal-radial cuts (units);
- Average density of latex vessels per ring per five millimeters of bark. The second ring was chosen on the radial longitudinal section (units);
- Average diameter of latex vessels, observed through the longitudinal-radial section (μm);
- Average distance between two consecutive vessels rings (μm); and
- Virgin bark thickness in mm was measured using an awl (steel hits) at one meter height.

The trunk girth was measured at 1.20 meter height.

Statistical Analyses

The analysis of variances was processed by the SAS system[14] (SAS Institute).

RESULTS

Microcyclus ulei Resistant Clone

Table 1 shows resistance of 36 rubber clones tested in the small-scale clone trial, in controlled inoculation and in a collection garden of the same plantation (Ituberá, Bahia, Brazil). The best clones were those with reaction type strictly below score 5 and stromata density strictly lower than score 2. Eventually, clones presenting reaction type of 5 with stromata density less or equal to score 1 could be retained. Following this selection criteria for resistance, 17 clones were eliminated *i.e.* FDR 5953, CDC 429, FDR 4461, GU 507, IT 537, CD1161, MDX 50, CDC 871, FDR 5145, MDX 496, CDC 555, CDC 689, MDX 495, CDC 557, Fx 4049, CDC 547 and CDC 965. Seventeen clones were retained: MDX 624, FDR 5788, CD 1130, MDX 607, CDC 312, FDR 5283, FDR 5665, FDR 5856, FDR 5217, FDR 5802, CD 1169, FDR 4459, CDC 1534, CD 1174, FDR 5162, FDR 5175 and FDR 3376. Clones TP 749 and MDF 180 were used as control clones for productivity and for resistance.

Selection Based on Productivity, Growth, Architecture and Structural Traits of Laticiferous System

The characteristics of clones based on latex yield, girth and tree architecture are shown in *Table 2*. FDR 5283 was discarded because of unbalanced canopy. FDR 5856 was discarded because of the fasciations of its trunk and bark bursting. FDR 5217 was discarded because of bumpy bark. CD 1169 exhibited a thin canopy with an etiolate architecture. Moreover, the trunk was fasciated. FDR 4459 was discarded because of its poor growth. FDR 5175 and FDR 3376 yielded low production. Thus, out of the 17 most resistant clones, clones MDX 624, FDR 5788, CD 1130, MDX 607, CDC 312, FDR 5665, FDR 5802, CDC 1534, CD 1174 and FDR 5162 were retained.

Observations on the structural traits of the laticiferous system of 36 clones are indicated in *Table 3*. Analysis of variance showed significant differences between some clones. This study confirmed previous results showing a narrow relationship between the number of latex vessel rings and latex productivity in populations of Amazonian clones[15] (*Table 4*). The other structural traits of latex vessels did not significantly correlate with latex production in these populations. Clone TP 749 (the control clone for latex production) and FDR 5217 exhibited the highest number of latex vessel rings. Clones FDR 5217, FDR 5283, FDR 5856, CD 1169, CDC 1534 and FDR 5162 despite their poor trunk or crown characteristics could be used as parents in crosses with quick-starter or medium-starter clones such as PB 260 or GT1. Only the clone MDX 624 possessed the most favourable traits *i.e.* a thick bark, an elevated number of latex vessel rings and latex vessels, an elevated diameter of vessels and a low average distance between rings. In addition, MDX 624 stood out among the other clones for growth and yield. Finally, the clone indicated partial resistance to *Microcyclus ulei* with low sporulation and an absence of stromata formations on the leaves. The clone was suitable to be cultivated in association with ombrophylic species such as the cocoa-tree due to its light crown.

TABLE 1. RESISTANCE TO *MICROCYCLUS ULEI* OF 36 RUBBER TREE CLONES TESTED IN SMALL-SCALE FIELD TRIAL, CONTROLED CONDITIONS OF INOCULATION AND IN A COLLECTION GARDEN IN ITUBERÁ (BAHIA, BRAZIL)

Clone	Parents (Female × Male)	Origin country	Field resistance evaluation Reaction type	Field resistance evaluation Stromatas density	Test inoculation Reaction type	Collection resistance evaluation Reaction type	Collection resistance evaluation Stromatas density
MDX 624	AVROS 1581 × MDF?	Guatemala	3–4	0	3	4	0
FDR 5953	Harbel 78 × IAN 6491	Liberia	3–4	**0**[a]–3	5	6	3
FDR 5788	Harbel 8 × MDF 180	Liberia	3–4	0–1		5	1
CD 1130	Avros 308 × MDF 315	Guatemala	3–4	0	3	4	0
TP 749	Fx 3846 × ?	Brazil	4–6	**0**–3	5	6	3
MDX 607	AVROS 1581 × MDF?	Guatemala	3–4	0	3	4	1
CDC 312	AVROS 308 × MDX 40	Guatemala	3–4	**0**–1	3	4	1
CDC 429	RRIM 600 × MDX 17	Guatemala	3–5	0–4	3	6	3
FDR 5283	Harbel 68 × TU 42-525	Liberia	3–4	0	4	4	0
FDR 4461	Harbel 11 × MDF 363	Liberia	3–5	**0**–3		6	3
FDR 5665	Harbel 62 × MDX 25	Liberia	3–4	0	5	3	**0**–2
FDR 5856	IAN 2920 × P122	Liberia	3–4	0	3	4	0
GU 507	?	Guatemala	3–4	0–2	3	6	3
FDR 5217	Harbel 68 × TU 42-525	Liberia	3–4	0	4	4	0
IT 537	PR 107 × F 6395		3, **4**, 5	0–3	4	6	3
FDR 5802	Harbel 67 × CD 47	Liberia	3–4	0	3	4	0
CD 1161	AVROS 1581 × MDF 72	Guatemala	3–4	0		6	2
CD 1169	AVROS 1581 × MDF 180	Guatemala	3–4	0	4	4	0
FDR 4459	Harbel 11 × MDF 363	Liberia	3–4	0		4	1
MDX 50	AVROS 337 × MDF?	Guatemala	3–**4**	**0**–1		5	3
CDC 1534	?	Guatemala	3–4	0–1	4	4	1
CD 1174	?	Guatemala	3–4	0–1	4	5	1
FDR 5162	Harbel 68 × TU 42-525	Liberia	3–4	0	3	4	1
CDC 871	?	Guatemala	**4**–5	**0**–2	5	6	2
FDR 5145	?	Liberia	**3**, **4**, 5	**0**–1	4	4	2
MDX 496	?	Guatemala	3–4	**0**, **1**, 2	3	6	3
CDC 555	Av1581 × *H. spruceana*	Guatemala	4–5	0–1		6	3
CDC 689	?	Guatemala	4–5	0–1	5	6	1
FDR 5175	Harbel 68 × TU 42-525	Liberia	3–4	**0**–1	5	4	1
MDX 495	?	Guatemala	3–4	**0**–1	5	5	2
CDC 557	Av1581 × *H. spruceana*	Guatemala	**4**, **5**, 6	2–3		6	3
Fx 4049	F 4542 × PB 86	Guatemala	4, **5**, **6**	0–3	6		
MDF 180	Primary clone	Peru	3–4	0	5	4	0
CDC 547	Av1581 × *H. spruceana*	Guatemala	4–5	0–3	4	6	3
CDC 965	?	Guatemala	4–5	0–2	5	6	3
FDR 3376	Harbel 12 × IAN 3890	Liberia	**2**, **3**, 4	0	3	4	0

AVROS : Algemene Vereniging Rubberplanters Oostkust Sumatra; CD and CDC : Clones derived from hand pollinations between SALB (*Dothidella*) resistant and SALB susceptible clones, final C designates Clavellinas; FDR : Firestone Dothidella Resistance ; Fx : Ford Cross; IT : Ford clones from Belterra ; MDF : Madre de Dios Firestone; MDX : Madre de Dios Cross; RRIM : Rubber Research Institute of Malaysia; TP : Três Pancadas; TU : Turrialba, Costa Rica.

[a] The number in bold indicates the most frequent note observed.

TABLE 2. PRODUCTION, GIRTH AND ARCHITECTURE OF 36 RUBBER TREE CLONES TESTED IN SMALL-SCALE FIELD TRIAL IN ITUBERÁ (BAHIA, BRAZIL)

Clone	Dry rubber production (g/tree/tapping)			Girth (cm)		Tree architecture	
	Trees number	Mean	Duncan Grouping	Trees number	Mean	Trunk	Canopy
MDX 624	37	70.4	a	42	40.1	+	+
FDR 5953	17	63.5	a	22	28.7		
FDR 5788	26	50.5	b	26	37.4	+	+
CD 1130	27	47.2	bc	29	31.8	+	+
TP 749	33	44.1	bcd	37	30.4	+	+
MDX 607	31	41.2	bcde	33	34.3	+	+
CDC 312	28	39.5	bcdef	29	34.2	+	+
CDC 429	34	38.7	bcdef	42	28.9		
FDR 5283	31	38.0	cdefg	32	34.2	–	–
FDR 4461	25	37.9	cdefg	26	31.3		
FDR 5665	25	36.0	cdefg	26	36.2	+	+
FDR 5856	24	32.8	defgh	24	35.1	–	+
GU 507	24	32.4	defgh	27	32.0	+	+
FDR 5217	20	31.6	defghi	24	26.5	–	+/–
IT 537	28	30.8	efghi	29	31.2		
FDR 5802	24	29.5	efghijk	28	35.7	+	+
CD 1161	28	29.1	efghijk	28	35.5	–	
CD 1169	31	29.1	efghijk	33	36.8	–	–
FDR 4459	32	28.4	efghijkl	35	31.1	–	–
MDX 50	19	27.4	fghijkl	23	26.8		
CDC 1534	29	25.1	ghijklm	36	27.2	+/–	+
CD 1174	36	25.0	ghijklm	38	31.5	+	+
FDR 5162	19	24.6	ijklmn	24	26.4	–	–
CDC 871	31	23.6	ijklmno	35	33.8	+	+
FDR 5145	24	23.2	ijklmno	28	29.0		
MDX 496	29	22.4	ijklmnop	32	28.2		
CDC 555	33	20.4	ijklmnop	36	31.0		
CDC 689	23	18.5	jklmnop	27	28.5		
FDR 5175	25	17.0	klmonp	32	26.5		
MDX 495	32	16.1	lmnop	34	34.4		
CDC 557	23	15.7	mnop	38	25.4		
Fx 4049	30	13.0	nop	33	31.5		
MDF 180	8	12.7	nop	16	28.2		
CDC 547	31	12.3	nop	34	33.1		
CDC 965	39	10.8	op	40	33.8		
FDR 3376	16	9.8	p	17	31.7		
F value		12.81			5.97		
P > F		0.0001			0.0001		

TABLE 3. STRUCTURAL TRAITS OF THE LATICIFEROUS SYSTEM OF 36 RUBBER TREE CLONES TESTED IN SMALL-SCALE FIELD TRIAL IN ITUBERÁ (BAHIA, BRAZIL)

Clone	BT (mm)	NR (Unit)	DV (Unit)	DL (μm)	AD (μm)
MDX 624	**5.6**[a]	**14**	**10.5**	**20.2**	**0.203**
FDR 5953	4.2	**13**	9.8	**20.4**	0.214
FDR 5788	4.9	11	9.0	17.9	**0.204**
CD 1130	4.4	11	9.9	16.2	0.207
TP 749	4.8	**16**	**10.3**	17.6	**0.174**
MDX 607	**5.3**	12	9.9	19.3	**0.202**
CDC 312	4.0	10	9.3	18.6	0.248
CDC 429	4.6	11	9.5	17.9	0.209
FDR 5283	4.9	**14**	**10.3**	18.6	0.246
FDR 4461	**5.1**	11	9.5	17.9	0.258
FDR 5665	4.9	13	9.6	**20.2**	0.251
FDR 5856	4.9	12	9.9	19.5	0.236
GU 507	4.8	**13**	9.8	19.2	0.218
FDR 5217	4	**16**	**10.3**	17.8	**0.172**
IT 537	3.8	11	9.4	**19.5**	0.208
FDR 5802	4.4	12	9.9	18.2	0.255
CD 1161	**5.3**	10	9.9	**19.5**	0.255
CD 1169	4.8	11	**11.7**	17.2	0.212
FDR 4459	**5.2**	10	9.6	**20.2**	0.256
MDX 50	3.9	10	10.0	16.7	**0.187**
CDC 1534	4.1	12	9.6	19.5	**0.189**
CD 1174	5.0	**14**	9.9	17.2	0.240
FDR 5162	4.4	11	**10.3**	16.9	0.246
CDC 871	**5.1**	12	8.8	18.2	0.255
FDR 5145	4.5	11	9.4	17.2	0.210
MDX 496	3.9	9	8.8	18.9	0.259
CDC 555	**5.2**	**13**	10.1	18.6	0.246
CDC 689	4.0	11	9.0	17.6	0.214
FDR 5175	4.1	11	9.5	17.2	0.231
MDX 495	**5.2**	11	**10.5**	18.9	0.302
CDC 557	4.0	10	9.6	17.2	0.214
Fx 4049	4.1	11	9.3	18.2	0.236
MDF 180	5.0	10	**10.3**	16.6	0.256
CDC 547	4.3	11	9.8	18.9	**0.205**
CDC 965	5.0	12	9.9	**19.9**	0.228
FDR 3376	5.0	12	9.8	**20.4**	0.262
F value	8.47	8.12	2.22	2.51	2.39
P > F	0.0001	0.0001	0.0002	0.0001	0.0001

[a] In bold, the 8 best clones classified on the trait studied

BT: Virgin bark thickness; NR: Total number of latex vessel rings; DV: Overall density of latex vessel per ring; DL: Average diameter of latex vessels; AD: Average distance between consecutive latex vessel rings.

TABLE 4. MULTIPLE REGRESSION OF DEPENDENT VARIABLES OF BARK SAMPLES TAKEN FROM 36 RUBBER TREE CLONES

Independent variable	Intercept	R^2
2.45*NR (0.45)	0.985	0.0946
2.20*NR – 24.37^{ns}AD (0.46) (18.59)	9.428	0.1001
2.07*NR – 27.89^{ns}AD + 0.82^{ns}BT – 0.51^{ns}NR + 0.20^{ns}DL (0.56) (19.55) (1.68) (1.04) (0.43)	9.223	

R^2 = coefficient of determination; *$P<0.001$; ns = not significant. Figures within brackets are the standard error of respective regression coefficients.

AY: Average yield; BT: Bark thickness; NR: Total number of latex vessel rings; DV: Overall density of latex vessel per ring; DL: Average diameter of latex vessels; AD: Average distance between consecutive latex rings.

DISCUSSION

Poor information was found in the earlier literature of the 36 studied clones. Only the resistance of clone MDF 180 was tested by others researchers[16–18]. Junqueira found this clones resistant to *Microcyclus ulei* strains from Bahia, Espiritu Santo, Mato Grosso, Roraima, Minas Gerais states and susceptible to strains from Amazonas and Acre states. Chee, using the lesion size of infected leaf discs as criteria to define the resistance, found clone MDF 180 susceptible for four to five tested races. Our own experience of this clone in Bahia plantations and in various states of Brazil indicates a very stable partial resistance[18], enabling a good refoliation even during strong epidemics. One peculiarity of this clone, as with several others, is the absence of stromata, which contains the ascospores. Previous studies have shown that intense lignin accumulation around lesions may stop pathogen development in some partially resistant clones[19,20]. The absence of the sexual cycle of the fungus would be a break to fungus diversity. Consequently, the selection and the recommendation for planting of stable partial by resistant clones (characterised by low sporulated lesions and no stromatas) may be stabilised in the long-term with the diversity of the fungus population. For this reason, some clones such as MDF 180, in spite of their yield or architectural deficiencies, are preferentially used as genitor in crosses with high yielding clones. The objective is to increase yield while maintaining the same resistance level as the resistant parent. However, Quantitative Trait Loci Analysis reveals the complex genetic determination of resistance including some major resistant genes[21]. It is therefore a challenge to succeed in building new genotypes by accumulating favourable genes for resistance and for yield together, in the same genetic combination. For that, since 1995, CIRAD had developed a genetic map approach of the rubber tree, followed up by research on molecular markers of resistance and production.

From the evaluation of latex production in SSCT, it would be highly speculative to extrapolate on future productions in commercial plantings. However, we can note that yield of clones MDX 624, FDR 5788, and CD 1130 observed in SSCT were 60%, 15% and 7% higher, respectively of that of the control clone TP 749 which produced as high as 2000 kg/ha/year of dry rubber on 15-year-old plots.

In agreement with previous studies showing high correlation between the number of latex vessel rings and yield[15,22,23], these anatomical characteristics could usefully be employed as a culling criteria to select the most productive plants between semi-wild clones or progenies. Significant differences exist between the clones and MDX 624 is the only clone which display several favourable anatomical characteristics for latex yield. These observations promise equal or superior yield of MDX 624 to TP 749. After the yield potentials are confirmed, the selected clones could be as competitive with Asiatic clones and could provide an alternative measure to the risk of *Microcyclus ulei* in Africa and Asia[24]. Therefore, it would be advantageous to test these clones in those areas.

CONCLUSION

At present the Cirad-Michelin-Brazil SALB resistance breeding program is the only research program in the world trying to associate SALB resistance and latex yield in some rubber clones. The first step was to identify it in a semi-wild population of 960 clones; some cultivars could be recommended directly for replanting projects. From one initial trial, eight SALB resistant and latex yielding clones were selected: FDR 5597, FDR 4575, PMB 1, CDC 312, CDC 308, CDC 56, FDR 5240 and FDR 5788. The present results increased from 8 to 14 the number of clones to be tested in large-scale clonal trials. Moreover, 64 resistant clones of the germplasm would be used as parents in crosses in order to transfer their resistant characteristics to new clones with higher yield and growth. The Cirad Michelin Brazil clonal series could be used for large-scale experimentation around 2011.

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