INDUCED POLYPLOIDY POTENTIAL FOR IMPROVING RESISTANCE IN *HEVEA* CLONES TO RUBBER TREE LEAF BLIGHT[1]

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(Accepted for publication on 01/08/92)

JUNQUEIRA, N.T.V., MORAES, V.H.F., LIEBEREI, R. & GASPAROTTO, L. Induced Polyploidy Potential for Improving Resistance in *Hevea* Clones to Rubber Tree Leaf Blight. Fitopatol. bras. 18:12-18. 1993.

ABSTRACT

Components of resistance that reduce the epidemic rate of rubber tree leaf blight, have been evaluated on colchicine-induced polyploids (CIP) *Hevea* clones and on their respective natural diploids after inoculation of virulent *Microcyclus ulei* isolates to the diploid clones. Some CIP clones have presented high level of resistance in comparison with their susceptible diploid clones, whereas other CIP clones were susceptible. The polyploid plant inherent factors controlling the resistance to *M. ulei* and the induced polyploidy potential for rubber tree breeding aiming at resistance to leaf blight are discussed.

Key words: Rubber tree, *Microcyclus ulei,* induced resistance, physiological changes.

RESUMO

Potencial da poliploidia induzida para o melhoramento da seringueira visando resistência ao mal-das-folhas

Avaliaram-se componentes de resistência que reduzem a taxa de progresso do mal-das-folhas, em clones de seringueira poliplóides induzidos com colchicina e em seus respectivos diplóides naturais, após inoculação de vários isolados de *Microcyclus ulei* virulentos para os clones diplóides. Alguns clones poliplóides apresentaram alto nível de resistência quando comparados com seus respectivos diplóides susceptíveis, ao passo que outros foram susceptíveis. São discutidos os fatores que podem controlar a resistência dos clones poliplóides ao *M. ulei* e o potencial da poliploidia induzida para o melhoramento da seringueira visando resistência ao mal-das-folhas.

INTRODUCTION

Rubber tree leaf blight, caused by *Microcyclus ulei* (P. Henn.) Arx, causes drastic economic losses in rubber plantation in Brazil.

The chemical control of this disease is restricted to nurseries, clonal gardens and very young plantations. In spite of intensive research for chemical protection of rubber against leaf blight, no suitable economic technique for large-scale fungicide application has been developed as yet for mature plantations. Planting of productive and resistant rubber tree clones could be the most efficient method to control leaf blight. However, the high physiological variability of the pathogen (Junqueira *et al.*1986) and the difficulties to transfer the "horizontal" resistance to the productive clones through conventional

[1] Part of this work was carried out during the research stay of the senior author at the Botanical Institute of the Tecnical University of Braunschweig, Germany, receiving financial support from DGF/GTZ, IBAMA and EMBRAPA-CPAA, AM.

breeding (Junqueira *et al.* 1989b) have not yield productive rubber clones which are resistant to leaf blight.

Treatment of *Hevea* clones with colchicine, according to Moraes (1982a, 1982b), has allowed the production of different rubber tree polyploid clones with high potencial for latex production. However, the level of resistance of these colchicine-induced polyploid (CIP) clones to leaf blight disease is not yet known. The objective of this study was to show the differences between CIP rubber clones and their respective natural diploid clones concerning the resistance to leaf blight.

MATERIAL AND METHODS

Production of polyploids rubber tree clones was done in accordance with Moraes (1982a; 1982b) using solution composed of colchicine (0,25%) + sucrose (5,0%) + 0,1% of dimethyl sulfoxide (DMSO). Apical bud of grafted young plants were eliminated and when the lateral buds became swollen, the petiole of each leaf were cut, and 7,5 microliters of this solution were injected in it by using a micropipette.

The polyploid material was identified by apical meristem organogenesis analysis, chromossome number, and presence of sectorial chimeras in leaves. Mixoploid material was eliminated based on frequency, class and size of the stomata, on leaf thickness and on sieve tube caliber.

Colchicine-induced polyploid (CIP) clones and their respectives natural diploids were cultivated in black polyethylene bags containing 10 kg of substrate (40% of organic compost + 60% of yellow latossol) mantained under local ambient conditions at CPAA, Manaus, AM. Inolucations were carried out after the third leaf flush, with $2x10^5$ conidia/ml of *M. ulei*, according to Junqueira *et al.* (1986). The leaf flushes were firstly inoculated with two highly virulent isolates of *M. ulei* to the diploids clones, previously tested for virulence (Junqueira *et al.* 1989b).

Assessment of the resistance was done by determination of the *M. ulei* incubation period (IP-time from inoculation until the development of macroscopic visible lesions), the *M. ulei* generation period (GP-time from inoculation until the production of conidia), number of lesions per 9 cm^2 of leaf surface (NL), diameter of lesions (DL) sporulation (EP), and leaf fall tolerance (LFT). The NL, DL, and EP were determined 12 days after inoculation in accordance with a diagramatic scale proposed by Junqueira *et al.* (1986). The LFT was determined through inoculation of different *M. ulei* inoculum concentrations. After the first assessment, all susceptible CIP plants of each clone were eliminated whereas the resistant CIP plants were pruned above the first leaf flush and fertilized to get new leaf flush for the second inoculation. After the second inoculation, which was done on the third leaf flush emitted after the first prune, all susceptible polyploid plants were eliminated whereas the resistant plants were again pruned, fertilized and inoculated when they had emitted the third leaf flush. After the third inoculation, the resistant plants were transferred to a new substrate and, five plants of each clone, were inoculated with 13 *M. ulei* isolates from different regions of Brazil.

RESULTS

Polyploid clones as the Fx 985 P_1, IAN 6158 P_1, MDF 180 P_1 and CNS AM 7704 P_1 presented high percentage of *M. ulei* resistant plants in comparison with their respective natural diploids (Table 1). The polyploid IAN 873 P_1 (IAC 222) presented only 13,0% of resistant plants. The other CIP clones such as IAN 717 P_1, Fx 3925 P_1, IAN 6323 P_1, Fx 4098 P_1 and CNSAM 7665 did not present resistant plants.

All CIP clones, with the exception for the CNSAM 7704 P_1, have shown some susceptible plants even after the three inoculations cycles (Table 2). The higher percentage of susceptible CIP *Hevea* plants, which showed resistance during thr first *M. ulei* inoculation, was observed among the IAN 873 P_1 (IAC 222) and MDF 180 P_1 plants (see Table 1). These results suggest that the character for *M. ulei* resistance is not yet fixed in the CIP *Hevea* clones, having variable level of mixoploid tissue among those clones.

The resistant polyploid plants selected (Table 2), when reinoculated on the third leaf flush emitted after the second prune, maintained their resistance, except IAC 222 (Table 3). The results indicate the probable fixation of the character for resistance to *M. ulei* or elimination/reduction of the mixoploidy level after the third inoculation.

The components of resistance that reduce the leaf blight severity on CIP clone and on their respective natural diploids are listed in Table 4. The IP and NL were similar to CIP plants and natural diploids clones, whereas the DL, GP and EP were different among clones and within clones. Resistant CIP clones as Fx 985 P_1, IAN 6158 P_1, MDF 180 P_1, CNSAM 7704 P_1 and IAN 873 P_1 (Table 3), presented hypersensitive reactions with no spores formation, and lesions diameter at least, twice smaller than the lesions that occurred on their respective natural diploids, showing, nevertheless, higher resistance level than the diploid clones.

Susceptible CIP clones (Table 4) as IAN 717 P_1, Fx 3925 P_1, IAN 6323 P_1, Fx 4098 P_1 and CNSAM 7665 P_1, have presented lesions with larger diameter and the same sporulation level than their diploids; however, they were more tolerant to leaf fall than their diploids.

The reactions of CIP rubber clones and respective diploids to several *M. ulei* isolates from different regions of Brazil, are listed in Table 5. The selected CIP Fx 985 P_1, IAN 6158 P_1, MDF 180 P_1, IAN 873 P_1 (IAC 222), and CNSAM 7704 P_1 (Table 3), were resistant or highly resistant to all the *M. ulei* isolates, whereas their respective diploide were susceptible to some isolated groups and resistant to others, according to the host specificity (Junqueira *et al.* 1986). The susceptible CIP rubber clones (Table 5) such as IAN 717 P_1, Fx 3925 P_1, IAN 6323 P_1, Fx 4098 P_1 and CNSAM 7665 P_1 presented, according to specificity group, either susceptibility or resistance similar to their respective natural diploids.

TABLE 1. Performance of colchicine-induced polyploid *Hevea* clones to virulent *Microcyclus ulei* isolates to their natural diploid clones, after inoculations done on the third foliar flush*.

CLONES	Genetic Origin	Tested plants	Resistant plants**	Resistant plants (%)
Fx 985N	*H. brasiliensis*	93	0	0,0
Fx 985P$_1$	*H. brasiliensis*	76	58	76,3
IAN 6158N	*H. benthamiana* x *H. brasiliensis*	125	0	0,0
IAN 6158P$_1$	*H. benthamiana* x *H. brasiliensis*	83	61	73,5
MDF 180N	*H. brasiliensis*	38	0	0,0
MDF 180P$_1$	*H. brasiliensis*	22	12	54,5
IAN 873N	*H. brasiliensis*	105	0	0,0
IAN 873P$_1$(IAC 222)	*H. brasiliensis*	123	16	13,0
CNSAM 7704N	*H. benthamiana*	26	0	0,0
CNSAM 7704P$_1$	*H. benthamiana*	39	26	66,7
IAN 717N	*H. benthamiana* x *H. brasiliensis*	96	0	0,0
IAN 717P$_1$	*H. benthamiana* x *H. brasiliensis*	25	0	0,0
Fx 3925N	*H. benthamiana* x *H. brasiliensis*	43	0	0,0
Fx 3925P$_1$	*H. benthamiana* x *H. brasiliensis*	32	0	0,0
IAN 6323N	*H. benthamiana* x *H. brasiliensis*	67	0	0,0
IAN 6323P$_1$	*H. benthamiana* x *H. brasiliensis*	25	0	0,0
Fx 4098N	*H. brasiliensis*	117	0	0,0
Fx 4098P$_1$	*H. brasiliensis*	16	0	0,0
CNSAM 7665N	***	87	0	0,0
CNSAM7665P$_1$	***	36	0	0,0

N = Natural diploid; P$_1$ = Colchicine-induced polyploid *Hevea* clone
* = Corresponding to third foliar flush emitted after the planting of budded stumps.
** = Plants that did not allow the *M. ulei* conidia and stromata formation on the infected tissue were considered resistant to the pathogen.
*** = Probably natural hybrid between *H. brasiliensis* x *H. camporum*.

TABLE 2. Performance of colchicine-induced polyploid *Hevea* plants, which have presented resistance to the same *Microcyclus ulei* isolates (Table 1), after reinoculations done on the third foliar flush emitted after the first prune*.

Polyploid Clones	Tested plants	Resistant plants	Resistant plants (%)
Fx 985P$_1$	58	47	81,0
IAN 6158P$_1$	61	59	96,7
MDF 180P$_1$	12	10	83,3
IAN 873P$_1$ (IAC 222)	16	11	68,3
CNSAM 7704P$_1$	26	26	100,0

* The first prune was done above the first foliar flush when the plants had emitted the third foliar flush.
** Plants that did not allow the *M. ulei* conídia and stromata formation on the infected tissue were considered resistant to pathogen.

TABLE 3. Performance of colchicine-induced polyploid *Hevea* clones, which have presented resistance to the same *Microcyclus ulei* isolates (Table 2), after reinoculations done on the third foliar flush, emitted after the second prune*.

Polyploids Clones	Tested plants	Resistant plants	Resistant plants (%)
Fx 985P$_1$	47	47	100
IAN 6158P$_1$	59	59	100
MDF 180P$_1$	10	10	100
IAN 873P$_1$ (IAC 222)	11	9	82
CNSAM 7704P$_1$	26	26	100

* The second prune was done when the foliar flush emitted after the first prune had emitted the third foliar flush.
** Plants that did not allow the *M. ulei* conídia and stromata formation were considered resistant to pathogen.

TABLE 4. Components of resistance in colchicine-induced polyploid *Hevea* clones and their respective natural diploids to virulent *Microcyclus ulei* isolates to the diploid *Hevea* clones.

Clones	*M. ulei* incubation period-IP*	*M. ulei* generation period-GP*	Lesion number NL*	Lesion diameter DL* (mm)	*M. ulei* sporulation type-EP*	Leaf fall tolerance LFT* (conidia/ml)	Reaction type RT*
Fx 985N	3,4+	6,4	12,5+	3,3	9	6×10^5	HS
Fx 985P1	3,5	**	13,8	1,3	2	***	R
IAN 6158N	3,5	7,5	11,6	1,4	6	1×10^6	S
IAN 6158P1	3,5	**	12,5	0,7	2	***	R
MDF 180N	3,0	6,0	14,4	3,0	9	6×10^5	HS
MDF 180P1	3,5	**	12,6	1,5	2	***	R
IAN 873N	3,0	5,2	11,8	3,5	10	4×10^5	HS
IAN 873P1(IAC 222)	3,5	**	12,6	0,9	2	***	R
CNSAM 7704N	3,0	8,0	15,3	2,0	6	1×10^6	S
CNSAM 7704P1	3,0	**	12,8	0,8	2	***	R
IAN 717N	3,5	5,0	12,6	3,6	10	4×10^5	HS
IAN 717P1	3,5	5,0	13,0	4,1	10	6×10^5	HS
Fx 3925N	3,0	5,0	13,3	3,5	10	4×10^5	HS
Fx 3925P1	3,0	5,0	12,6	4,3	10	6×10^5	HS
IAN 6323N	3,3	5,5	12,4	3,6	10	6×10^5	HS
IAN 6323P1	3,0	5,0	11,3	4,0	10	6×10^5	HS
Fx 4098N	3,3	6,4	12,5	2,0	9	4×10^5	HS
Fx 4098P1	3,0	6,0	12,0	2,4	9	6×10^5	HS
CNSAM 7665N	3,0	5,0	12,0	3,5	10	8×10^5	HS
CNSAM 7665P1	3,0	5,0	13,3	3,8	10	8×10^5	HS

*N : Natural diploid; P1 = colchicine-induced polyploid; GP = time from inoculation until the production of conídia; IP = time from inoculation until appearance of visible lesions.
*NL : Number of *M. ulei* lesions observed on $9cm^2$ of leaf area;
*EP : Scale 0-10 according to Junuqueira *et al.* (1986); numbers refer to resistant or susceptible plants of each clone; 2 - necrotic leisons with 1,0-2,0 mm diameter with no spores; 6 = lesions less than 1,5 mm diameter with high spores production only on abaxial leaf surface; 9 = lesions more than 2,5 mm diameter with very high spores production only on abaxial leaf surface; 10 = lesions more than 2,5 mm diameter with very high spores production on both leaf surface.
*LFT: Maximum *M. ulei* conidia concentration supported by *Hevea* leaves after inoculations under controled environment condition
*RT : R = resistant; S = susceptible; HS = highly susceptible
** No sporulation
*** No inoculations
+ Not significantly differences ($P < 0,05$)

DISCUSSION

According to Tal (1978), the polyploidy consists in duplication of the chromossome number (conventional polyploidy) or in the increase of DNA proportion per chromossome (cryptic polyploidy) and it is an important factor in plant evolution (Lawrence, 1968).

Ploidy level of rubber tree is not yet well known, but Bouharmont (1960) and Ong (1975), mentioned by Soleille (1984) think of it as amphidiploid whereas Soleille (1984) thinks of it as autotetraploid.

It is shown in this study that the polyploidy may alter the rubber tree resistance to *M. ulei*. In potential, it is an important factor for rubber tree breeding aiming at obtaining productive and leaf blight resistant clones. Among the CIP rubber clones with resistance for all *M. ulei* isolates tested, the most presented complete resistance characterized by incompatible hypersensitive reaction.

These hypersensitive reactions, represented by small necrotic lesions, when observed under ultraviolet light (U.V.) at 365 mm, presented a blue fluorescent halo around the lesions. This blue fluorescent substance was defined by Lieberei *et al.* (1989) as scopoletin, a coumarin derivative phytoalexin. The compatible *M.ulei* lesions on either susceptible diploid or polyploid *Hevea* clones did not present blue fluorescent substances, or had small quantities of it, rarely visible under U.V. light. According to Lieberei *et al.* (1989) the scopoletin production by susceptible *M. ulei* infected leaves is totally inhibited by HCN released in larger quantity during the pathogenesis process. On resistant clones the HCN, which acts as a susceptibility factor, is slowly released in small quantities without inhibiting the defense reactions of the plant against *M. ulei*. Slow release of HCN by resistant rubber clones may be due to high β - cyanoalaninesynthase activity. These results are indications of genome changes in the CIP clones.

TABLE 5. Reactions of colchicine-induced polyploid *Hevea* clones and of their respective natural diploide to thirteen *Microcyclus ulei* isolates belonging to different groups.**

CLONES	*M. ulei* ISOLATES												
	GROUP I**				GROUP II**				GROUP III***				GROUP IV**
	11*	22*	33*	54*	10*	31*	52*	58*	53*	55*	56*	57*	4*
Fx 985N	R***	HR***	R	R	HS	HS	S	S	R	R	R	R	HR
Fx 985 P_1	R	HR	R	HR	R	R	R	R	R	HR	HR	R	HR
IAN 6158N	MR	S	MR	S	R	R	HR	MR	R	R	S	S	HR
IAN 6158P_1	HR	R	R	HR	HR	HR	HR	HR	HR	HR	HR	S	HR
MDF 180N	R	R	R	HR	S	HS	HS	S	MR	MR	HR	HS	HR
MDF 180P_1	R	R	R	R	R	R	R	R	R	R	HR	R	HR
IAN 873N	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HR
IAN 873P_1(IAC 222)	R	R	R	R	R	R	R	R	R	R	R	R	HR
CNSAM 7704N	MR	MR	MR	R	R	R	R	R	MR	MR	MR	MR	R
CNSAM 7704P_1	HR	R	R	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
IAN 717N	HS	HS	HS	HS	R	R	R	R	HS	HS	HS	HS	HR
IAN 717P_1	HS	HS	HS	HS	R	R	R	R	HS	HS	HS	HS	HR
Fx 3925N	HS	HS	HS	HS	R	R	R	R	S	S	HS	HS	HR
Fx 3925P_1	HS	HS	HS	HS	R	R	R	R	HS	HS	HS	HS	R
IAN 6323N	HS	HS	HS	HS	S	HS	S	S	S	S	HS	HS	HR
IAN 6323P_1	HS	HS	HS	HS	HS	HS	HS	S	HS	HS	HS	HS	R
Fx 4098N	R	R	R	R	HS	HS	MR	HS	MR	MR	MR	S	R
Fx 4098P_1	R	R	R	R	HS	HS	MR	HS	MR	MR	MR	S	R
CNSAM 7665N	R	R	R	R	MR	MR	R	MR	R	R	R	MR	HS
CNSAM 7665P_1	R	R	R	R	MR	MR	R	MR	R	R	R	MR	HS

* *M. ulei* isolates collection kept at EMBRAPA/CPAA, Manaus, AM. Brazil.

** According to Junqueira *et al.* (1986, 1989a) the group I *M. ulei* isolates are more specific for clones carrying genes of *Hevea benthamiana* whereas group II isolates are more specific for *H. brasiliensis* clones. The group III *M. ulei* isolates are able to produce conidia as on *H. brasiliensis* clones as on *H. benthamiana* derivative clones. The group IV *M. ulei* isolates are highly specific for clones carrying genes of *H. camporum*.

*** HR = Highly resistant; R = Resistant; MR=Moderately resistant; S = Susceptible; HS = Highly susceptible.
N = Natural diploid; P1 = Colchicine-induced polyploid clone.

Several investigators have mentioned physiological and biochemical alteration in naturally or colchicine-induced polyploids plants (Levy, 1976, Lewis, 1979: Kohli & Denford, 1977). Alterations in the content of phenolic or flavonoid compounds between diploid and CIP plants have occurred (Kohli & Denford, 1977). These compounds which are responsible for the inhibition of microbial growth in resistant plants, may act as phytoalexins (Mansfield, 1983: Kuc, 1982). They react forming hypersensitive reaction as the result of the oxidation and polymerization of pre-existent phenois (Clark, 1983). In rubber tree resistant plants, the presence and the effect of toxic substance to *M. ulei* as the Kaempferol-3 rhamnodiglycoside (Martins *et al.* 1970), quercetin (hashim *et al.* 1980), scopoletin (Lieberei *et al.* 1989) and other phenolic compounds (Figari, 1965) have been related.

Increasing in the activities of peroxidase, esterase and alcohol dehydrogenase in naturally or CIP plants has also been related (De Maggio & Lambrukos, 1974; Yoshikawa, 1983; Lewis, 1979). According to these investigators, the peroxidase may be associated with the plant resistance to disease and its activity increase in direct proportion to increase of the genome.

It is, thus, evident that natural or induced polyploid plants may express novel "non parental" chemical compounds as flavonoids and novel heteromeric enzymes, besides quantitative alterations in the alkaloid and phenolic content and increase in the activity of some enzymes (Lewis, 1979). Some alloploids, besides presenting higher phenolic compounds than their parental diploids, have a tendency to produce novel chemical compounds which associated with phenolic compounds, increase the plant's defense capacity to disease (Mansfield, 1983).

De Maggio & Lambrukos, (1974) and Lewis (1979) have reported that qualitative and quantitative alterations in the chemical constituent of naturally or colchicine-induced polyploid plants are due to the derepression or disrupture of the gene regulation. This derepression is due to the genome duplication, which increases the gene dosage, thus, increasing the global rate of transcription and resulting in an increase of enzymatic activity. Yoshikawa *et al.* (1977) and Tani & Yamanoto (1978) have shown, respectively, in studies with soybean x *Phytophthora megasperma* var. *sojae* and oats crown rust, that the increase of the transcription rate may increase the phytoalexin biosyntesis and the plant's resistance to disease. The "*De novo* DNA

transncription and translaction" is necessary for phytoalexin production and for plant resistance expression.

The presence of CIP rubber clones, which were resistant to several *M. ulei* isolates highly virulent to their respective diploid clones, indicates that alterations in the chemical constituents or plant physiological changes, after the colchicine application, have occurred.

The resistance of some CIP *Hevea* clones in comparison with their respective diploids could also be explained by increase of content or production of new toxic substances to *M. ulei* due to the increase in the transcription rate or increase in the enzymatic activity. The activation or derepression of genes able to induce production of novel toxic chemical compounds to *M. ulei*, as pre-existent phenolic compounds or other phytoalexin, may also have occurred.

The resistance of the induced polyploid rubber clones may also be explained by interference of insertion sequences, which are discrete units of DNA able to transpose from one site in the genome to another site in the same genome, independent of the homologal recombination system in the host changing, thus the plant's resistance to the pathogen (Calos & Miller, 1980). Staskawicz (1983) has related that these discrete units of DNA (Transposing elements) are responsible for virulence alterations of several plant pathogenic bacteria genera. In relation to CIP rubber clones, genome duplication and the derepression or desrupture of the gene regulation, under a possible mutagenic action of the colchicine, could facilitate the transposition of discrete units of DNA in the genome, inducing the resistance of these plants to leaf blight.

It is important to consider that, even among the resistant polyploid rubber clones, susceptible plants from resistant plant buds were found. This observation suggests that the character for *M. ulei* resistance is not yet well fixed, indicating a probable presence of pathogen susceptible mixoploid tissue. This problem can be overcame by a selection process through successive *M. ulei* inoculations.

This study revealed that the induced polyploid technique is potentially important for selecting productive and *M. ulei* resistant rubber clones. Therefore, the polyploidization of a larger number of plants per clone to increase the probability of originating resistant polypolid clones such as IAN 6158 P_1, Fx 985 P_1, CNSAM 7704 P_1 and MDF 180 P_1 is recomended. To achieve further genetic progress, the *M. ulei* resistant polyploid material shall be submitted to higher pressure of *M. ulei* inoculum either in field conditions or in the laboratory by inoculating several virulent *M. ulei* isolates to the diploid clones. The selected material must be tested for yield and used as sources of resistance for crossing with highly productive and susceptible clones.

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