

**FACTORS AFFECTING DISEASE TOLERANCE OF RUBBER TREE AND
RESEARCH NEEDS FOR DEVELOPING DISEASE – TOLERANT
GENOTYPES FOR THE SUSTAINABILITY OF RUBBER INDUSTRY**

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Resistance of *H. brasiliensis* against fungal pathogens

As rubber tree is also a member of plant kingdom, it also serves as a nutritional source for some animals, insects, and microbes. Although rubber plant inherent natural resistance, some pathogens are capable on colonising living rubber tissues and obtaining nutrients by damaging or weakening the plant with toxins or by weakening or blocking its defence mechanism.

Before the establishment, the pathogen has to break down the cuticle and the cell wall, which protects the rubber plant cells from the environment. In addition, as a result of microbial invasion, rubber plant tissues produce a number of anti-microbial phenolic compounds such as coumarins, flavonoids, isoflavonoids, triterpenes, terpenes *etc.*, which either completely or partially inhibit microbes. Non-microbial elicitors, such as salicylic acid can also induce the production of these compounds in rubber (unpublished data of Plant Pathology and Microbiology Department).

At early stages, the wild rubber tree in the Amazon basin, was infected with only few fungal pathogens. However, with the introduction of high yielding rubber genotypes during the last 50 years, many new fungi have been reported to infect the rubber tree. This has been attributed to the natural development of more virulent strains from the existing population, which subsequently caused more damage to new clones. These strains were capable of attacking even the resistant genotypes, causing more difficulties to the growers. In addition, during the recent past, a number of new pathogens [*Thanatephorus cucumeris* (Frank) Donk (Syn: *Corticium solani*) (Jayasinghe, 1993), *Geotrichum* sp. (Jayasinghe & Wettasinghe, 1996), *Sclerotium rolfsii* Sacc. (Jayasinghe *et al.*, 1988), *Corynespora cassicola* (Berk & Curt.) Wei (Liyanage *et al.*, 1986)] were also found as the causes of new diseases on rubber. Therefore, it is of a great interest to investigate the mechanisms of altering the inherent characters of pathogens making them more aggressive against the existing genotypes.

Some workers in their effort to study the defence mechanisms of rubber against fungal pathogens identified a few defence responses in rubber, such as cellular hypertrophy and hyperplasia, cambium activity stimulation, lignification and suberification of certain cell walls as some rapid reactions of *H. brasiliensis* against *Rigidoporus microporus* (Syn *R. lignosus*) and *Phelinus noxius* (Nicole *et al.*, 1985). In addition, an increase of isoperoxidase activity in both infected and reaction tissues were also detected. Nicole *et al.* (1991) also reported an elicitation of root defence responses of *H. brasiliensis*, when mycelial cell wall extract of *R. lignosus* was

injected into root system of 1-month-old seedlings. Fifteen days after injection, they observed stimulation of cinnamyl-alcohol dehydrogease activity, which is involved in lignin synthesis and callose depositions. In leaf tissues, blue fluorescent compound, which accumulated in response to *C. gloeosporioides* has been reported by Tan & Low (1975). This compound was later induced following infection by *Microcyclus ulei* (P. Henn.) v. Arx, and identified as scopoletin (Giesmann, *et al.*, 1986). Garcia *et al.* (1995a,b) suggested that the level of resistance of some *H. brasiliensis* clones against the leaf pathogen, *M. ulei*, should be related to the rate of appearance and accumulation of scopoletin a coumarin. According to Garcia *et al.* (1995a), the speed of appearance of a blue fluorescent compound in water drops was related to the resistance level.

Phenylalanine ammonia-lyase (PAL) is the key enzyme in the plant phenyl propanoid pathway catalysing the synthesis of phenolics, lignin and phytoalexin from *L*-phenylalanine (Jones, 1984). Increase in the activity or concentration of PAL could often be considered as an indicator of resistance in the host plant (Nicholson & Hammerschmidt, 1992). In addition, oxidases and peroxidases are also believed to be actively involved in polymerisation of phenolic compounds to form lignin. Since peroxidase has been related to initiation of hypersensitive cell collapse (Simons & Rose, 1971), it could be important in the resistance mechanisms. An increased PAL enzyme activity in *P. meadii* infected petiole tissues and a higher concentration of sugar in latex serum of tolerant rubber genotypes has been observed recently (Jayasuriya, 2002). In addition, 4-hydroxy-3-methoxybenzaldehyde (vanillin) has been identified as a key anti-fungal compound in petiole extracts of clone RRIC 100 (Jayasuriya *et al.*, 2003), which is tolerant to most of the rubber pathogens.

Similarly, the synthesis of pathogenesis-related (PR) proteins [acidic, low-molecular-weight proteins, soluble at low pH (Stintzi *et al.*, 1993) is one aspect of defence response of rubber towards pathogens (Narasimhan *et al.*, 2000). Polyacrylic, acetyl salicylic (aspirin), and salicylic acids also induce the synthesis of PR proteins, and their availability was known as related to increased resistance to pathogens (Gianinazzi, 1984). Fig. 1 expresses other possible responses of rubber against a pathogen.

Variable responsive levels of different rubber genotypes against diseases

Disease tolerance level of the majority of presently available high yielding genotypes varies from one to another. Some clones are resistant to most of the diseases, being susceptible for few diseases, whereas some clones are tolerant to few diseases, although susceptible to many diseases. However, there are very few clones, which tolerate most of the diseases in the field. A recent field survey on 16 rubber clones has revealed that, the severity of *Oidium* or *Colletotrichum* leaf disease in the field was mild on 31% or 12.5% of the clones respectively. However, *Oidium* or *Colletotrichum* leaf disease severity was found moderate to severe on 68% of the

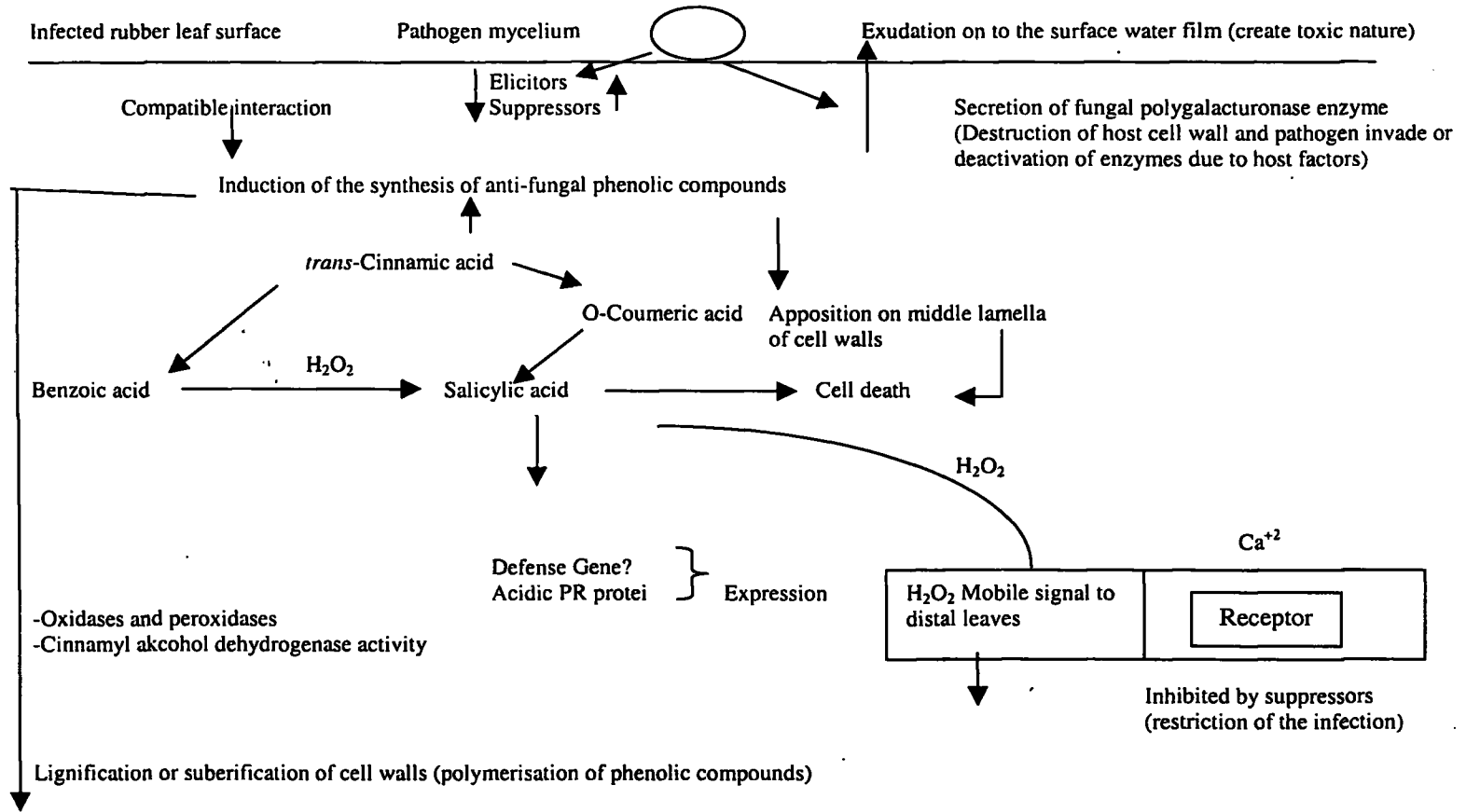


Fig. 1. Hypothetical schematic defense reactions of *Hevea brasiliensis* upon leaf infection with *Phytophthora*. Adopted from Drapper, 1997; Camp *et al.*, 1998; John *et al.*, 1997; Blumwald *et al.*, 1998; Raskin (1992).

clones (Jayasinghe, 2000). In addition, unconfirmed results from a field assessment of about 30 rubber clones including RRISL 200 series indicate that, about 30% of the tested clones are prone to *Corynespora* leaf disease. All this show how difficult it is to generate disease tolerance among the new highbred clones.

Development of aggressive strains of pathogens

Irrespective of the disease tolerance of clones, due to biotic or abiotic factors, pathogens also produce new strains, which can be more aggressive against rubber clones, which are already recommended for large-scale cultivation. Mutation may be the most prominent mechanism causing variability in pathogens changing the sequence of bases in the nuclear DNA, either through substitution, addition or deletion of one or many base pairs. Once a new factor for virulence appear in a mutant's DNA, it will take part in the sexual or para-sexual processes of the pathogen and may produce recombinants possessing virulence, quite different in degree or nature from those existing in the parental strains. In addition, as pathogenic organisms also have DNA outside the cell nucleus, mutations can also occur therein. For example through mutations in extra-chromosomal DNA, many pathogens acquire the ability to carry out a physiological process that they could not perform before. Pathogens may also acquire the ability to tolerate previously toxic substances, to utilise as new substances for growth, and to change their virulence towards host plant (Agrios, 1988). Recombination is also a process that occurs during the sexual reproduction of fungi whenever two haploid (n) nuclei, containing slightly different genetic material, unite to form a diploid (2n) nucleus called a gamete. During the meiotic division of the gamete, genetic factors crossover forming two gamete forming genetically different groups of relatively homogeneous individuals. Heterocaryosis, parasexualism and heteroploidy are other natural mechanisms which produce genetic variability (Agrios, 1988).

Organisms such as fungal spores, which are exposed to natural environmental factors such as ultraviolet radiation of sunlight, X-rays, α particles and neutrons, can also lead to genetical differentiation. The UV spectrum of the radiation has the practical biological application for utilising its erythematous effects, its ergosterol activation and its inactivating and mutational effects on bacteria, fungi and viruses. The UV affects the growth and respiration of organisms such as moulds and yeast. Slight doses may stimulate the growth of the same (Agrios, 1988). The direct effects of UV radiation on chromosomes are identified as terminal deletions, chromatic translocations and terminal aberrations. Chemicals such as mustard gas, 20-methylcholanthreneendosuccinic acid, camphor, acenaphthene, NaNO_2 , ninhydrin, chloramine, T, KI, organic peroxides, H_2O_2 , KCN, caffeine, methylxanthines, diazomethane, Colchicine and acriflavine are also effective mutagens (Agrios, 1988).

Research needs for development of resistant clones

Early reports of the US Department of Agriculture (Weir, 1926) have reported some organisms, which were originally recorded as potentially pathogenic to

H. brasiliensis in the Amazonian basin. Since then, as a result of heavy exploitation of this species, the rubber plantations world over have been affected with many new pathogens causing difficulties to growers. This has become a common phenomenon with the introduction of high yielding clones to the industry in order to maintain its sustainability. Moreover, after a considerable period, some clones became susceptible to some pathogens such as *C. cassiicola*, indicating the nature of wide range of the host specificity of such pathogens. Therefore, the disease incidence in rubber has become a crucial factor for the industry.

Crown budding of susceptible clones with a resistant canopy has been successful in managing leaf diseases, indicating the importance of this technology to be continued irrespective of the costs involved in the practice. Perhaps, a computer model specially developed for the benefit of researchers would be useful to determine the most suitable parentage combinations for the production of new clone lines. In breeding programs, in which artificial hand pollination is involved, the use of computer models may enable the breeder to combine a higher number of characteristics of individual parental clones. For the development of a suitable computer model, the following characteristics of known clones at a certain predetermined growth stage may also be important;

- a) Yield characteristics: average area of a single leaf including three leaflets in different whorls, average length of petioles, angle of leaflets to the sun, increase of the average leaf biomass from planting to the end of the immature phase, rate of girth increment of the trunk, average light absorbance capacity of canopies *etc.*
- b) Disease resistance characteristics such as; total average content of phenolic compounds in leaves of different whorls and bark tissues, the activity of enzymes such as PAL, peroxidases, density of stomata on leaves.

However, a constrain often breeders have to overcome would be non-formation of the expected genetic forms due to uncontrolled genetic crossing over causing new combination of chromatids or linked genes during the metaphase and anaphase of the mitotic division. However, this uncontrolled gene crossing over also results in production of new genetic material with high polymorphism, which is highly valued by breeders.

To generate knowledge on the host-pathogen interactions, research on pathogens has to be diverted to a new direction, using biotechnology. Although the available facilities and resources are limited for such research in most individual organizations, constrains could be resolved by collaborating the work with the organizations where the resources are available. The following are areas that need further research efforts:

Induction of disease tolerance of susceptible rubber clones

A few rubber genotypes generally, are resistant to a majority of rubber pathogens. However, a majority of rubber genotypes are tolerant to one or few

pathogens, while susceptible to other pathogens. To overcome this ad-hoc phenomenon, maintaining multi-clonal stands of rubber is considered as an acceptable technique for maintaining sustainability of plantations. Thereby, the disease incidence on susceptible genotypes will be reduced due to reduced-host-density and the availability of resistant hosts in a unit area. In contrary, the tolerance of a particular rubber clone to fungal pathogens may be improved to some extent by inducing the resistance. This may be operated through some techniques such as using physical (eg. hot water), chemicals (eg. salicylic acid) or using pathogen based elicitors, which are either proteins (peptides or glycopeptides) or carbohydrates, extractable from culture filtrates or from cell wall fragments of fungal mycelia of pathogens.

The role of elicitors in the establishment of diseases is to generate the signal to the host plant to respond to the infection *via* a signal transduction pathway. In response to the signal which has been transmitted from cell walls of an incompatible race of a fungal pathogen, plant cells perceive the signals by receptors on plasma membrane and suppresses the invasion of the pathogen at a certain point, possibly by increasing the responsive enzymatic activities of metabolism such as ATPase (Kiba *et al.*, 1996). Suppressors (glycopeptides) secreted by the same pathogen may or may not be able to suppress the host's defence mechanism. For a signal perceived by a host from a compatible fungal pathogen, plant does not activate the defence mechanisms allowing the pathogenic invasion.

Purified elicitor proteins can be characterised using gel filtration or other techniques such as affinity chromatography (Mithöfer *et al.*, 1996). Elicitor preparations obtained from pathogen grown liquid culture filtrate or pathogen cell walls could be used for disease screening purposes with certain modifications. Host responses to the pathogen elicitors could be analysed with regard to the resistance level of the host as hosts' responses to fungal suppressors which are known to be species specific (Kiba *et al.*, 1997). For example, PAL or peroxidase enzyme activities may be increased depending on the host's response. Hence, theoretically, the elicitors obtained from any fungal pathogen could be used to screen new rubber clones against their diseases. Therefore, using this method, it may be possible to screen rubber clones which are produced in Sri Lanka against the South American Leaf Blight (SALB) pathogen *Microcyclus ulei* in local conditions as a prerequisite because the SALB is a significant threat for the majority of rubber growing countries in Asia. However, the resistance induced artificially may retain only for a short period, restricting the effective use of such techniques in practice for field application. Repeated induction may therefore be useful in prolonging the induced period.

Reducing the virulence of pathogens

Development of new pathogenic strains is a common phenomenon in nature. It could be due to some reasons such as natural mutations caused by environmental factors (UV or near-UV radiation of the sun) or due to continuous use of pesticides.

The organisms, which are resistant to the above factors tend to survive, while others get eliminated. Rubber leaf disease pathogen *Colletotrichum acutatum* is resistant to fungicides such as benomyl, while *C. gloeosporioides* is susceptible to the same. As a result of the continuous use of such fungicides to manage leaf diseases in rubber nurseries or other agricultural systems, the *C. gloeosporioides* population would have been reduced, while the *C. acutatum* population increase due to tolerance to those fungicides. *C. acutatum* was found as the main causal agent of the *Colletotrichum* leaf disease on rubber during the recent past, but it may have existed on rubber for a longer period without our notice at a low population level. Therefore, it would be worth while to explore possibilities of converting the *C. acutatum* to a non-pathogenic form or minimise the virulence producing hypo-virulent strains which may be fast growing but less aggressive. This could perhaps be achieved by using as hyphae anastomosis of higher fungi in which two compatible hyphae fuse with one another at points of contact and exchange the nuclei and cytoplasm.

Some fungi loose virulence because of the development of mycoviruses in its population, although the evidence is too little. Much stronger evidence has been obtained to implicate mycoviruses in loss of virulence of *Endothia parasitica* and *Rhizoctonia solani*, of which hypovirulent strains consistently contain ds-RNA, although they may not necessarily contain identifiable virus particles.

Hypovirulent *Endothia* was reported to be responsible for the natural control of chestnut blight in Europe and currently being exploited to control the same disease in North America. A significant damage has been caused by a myco-virus in *Agaricus bisporus* lowering the growth and deforming fruit bodies (Deacon, 1988).

DNA techniques or mutagenesis could be used to develop some biotypes, which have variable characteristics different from the original culture. Biotypes, which are responsive for different stress conditions such as extreme hot or cold temperatures, high water potential or salt stress in the medium, UV or other extreme radiation, may have different characteristics, which are useful for a study. In horticulture, colchicine has been successfully used for production of new phenotypes, which produced flowers in different colours (Seneviratne *et al.*, 2002). Similar techniques may be adapted in research work on rubber too for production of new mutagenic populations. Young seedlings, bud patches for grafting (cambium tissues), callus materials, or seeds may be exposed to mild concentrations (*ca.* 0.04%) of mutagenic chemicals or other mutagenic factors and investigate the progress of the newly generating tissues.

Change of virulence on continuous re-infection to different hosts

The re-infection of pathogens among different host genotypes or alternative hosts would be another cause of induction of new virulent pathogenic strains, which are more tolerant to resistant factors. Previous studies of the Plant Pathology & Microbiology Department of the Rubber Research Institute of Sri Lanka revealed that in a collection of *Phytophthora meadii* isolates, the average degree of virulence was highest in those isolates which were obtained from a clone which is susceptible to *P.*

meadii. Therefore, the need for research on the factors causing formation of virulent strains is obvious. It has to be investigated for rubber pathogens such as *Corynespora cassiicola* using a series of few rubber clones by continuously and/or alternatively cross-inoculating with a single strain and assessing the virulence of new pathogen forms isolated from re-infections. Biotechnology can also be used to evaluate any variation that has occurred during the experiment.

Research on all aspects discussed in this review would be of important once in terms of a whole global knowledge although it may not purely or directly contribute to the industry. Such knowledge would certainly contribute to the sustainability of the rubber industry in the world.

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