Breeding for Disease Resistance in *Hevea* spp. - Status, Potential Threats, and Possible Strategies

Chaendaekattu Narayanan[1] and Kavitha K. Mydin[1]

Abstract

Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg., a forest tree native to the tropical rain forests of Central and South America, has only been recently domesticated outside its natural range of distribution. Almost all of the commercially cultivated clones of *H. brasiliensis* represent a very narrow genetic base since they originated through hybridization or selection from a few seedlings of so called Wickham germplasm. Hence, the commercial rubber cultivation, due to their genetic vulnerability, is under a constant threat of attack by native as well as exotic diseases and insects. Climate change, which is clearly felt in the traditional rubber growing regions of India, may possibly alter the host-pathogen interactions leading to epidemics of otherwise minor diseases.

Pathogenic fungal diseases including *Phytophthora*-caused abnormal leaf fall (ALF) and shoot rot, pink disease caused by *Corticium salmonicolor*, *Corynespora*-caused leaf disease, and powdery-mildew (*Oidium* sp.) are challenging diseases posing epidemic threats to rubber cultivation. South American leaf blight (SALB) is a devastating disease caused by *Microcyclus ulei* (=*Dothidella ulei*) which has prevented large-scale planting of rubber in Brazil due to epidemic outbreaks. The SALB is a looming threat to other rubber growing areas. Hence, it is essential that a global SALB resistance breeding program be implemented to tackle such future threats of epidemics. *Hevea* clones clearly exhibit variable levels of susceptibility to pathogenic diseases. *Hevea* clones have been tested for their capacity to produce phytoalexins; a strong correlation was observed between phytoalexin accumulation and clone resistance. More lignin accumulation was also often associated with clone resistance. Attempts have been made to identify possible disease resistance gene analogues in rubber. The role of *M13-1bn* marker (a putative quantitative trait locus) in screening for resistance to SALB had been investigated through genome mapping, but needs further validation. Earlier selection and breeding of *Hevea* clones resistant to *M. ulei* and *Phytophthora* sp. in Brazil led to screening of resistant clones. Most of the resistant material had been derived from *H. benthamiana* "F4542." Few other attempts for inter-specific hybridization have been made, particularly for SALB resistance (*H. camargoana* x FX 4098), but they did not follow large-scale evaluations for field resistance.

Many man hours of labor and enormous quantities of fungicidal chemicals are required every year for management of above diseases in vast areas of rubber plantations in India and other rubber growing countries. The cost of fungicides and their long-term effect on environment justify the need for breeding disease resistant trees. There are several theories for genetic basis for disease resistance (horizontal/vertical) in *Hevea*. Nevertheless, there is every possibility for breakdown of resistance due to ever-evolving pathogenic races coupled with climate change, which is exemplified by evolving SALB races. A multidisciplinary breeding program for development of disease resistant clones would have to continuously utilize Wickham resource as well as wild germplasm, in addition to other *Hevea* spp., in order to have sustainable rubber production.

Key words: *Hevea brasiliensis*, fungal diseases, South American leaf blight, *Microcyclus ulei*, disease resistance breeding

Introduction

Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg. (family, Euphorbiaceae; diploid, 2n=36), the Para rubber tree, is a forest species native to the tropical rain forests of Central and South America. Though the tree has only been recently domesticated outside its natural range of distribution, it has great commercial and socio-economic significance in many countries (Narayanan and Mydin 2011).

[1] Rubber Research Institute of India, Kottayam 686 009 Kerala, India.
Corresponding author: cnarayanan@rubberboard.org.in.

Hevea brasiliensis is monoecious, entomophilic, and predominantly out-crossing, and continues to be the major source of natural rubber in the entire plant kingdom. Latex (essentially cytoplasmic fluid) is collected from laticifer cells located in the bark tissue through systematic manual incision (the entire process referred to as 'tapping') for various uses. Most of the cultivated clones of *Hevea* have been derived either through selection or breeding among the few selected seedlings (or their progenies) which were reportedly collected from a small geographic location in Boim, near the Tapajos river in Brazil (Allen 1984, Schultes 1977, Wycherley 1968). Commercially cultivated clones of *H. brasiliensis* represent a very narrow genetic base possibly originating from few selected seedlings collected by Henry Wickham in 1876 (Baulkwill 1989).

Rubber cultivation is under a constant threat of attack by native as well as exotic pathogenic fungal diseases due to genetic vulnerability of the *Hevea* clones. Leaves, stems, and roots of *Hevea* are susceptible to fungal pathogens. Leaf diseases are caused by *Oidium heveae*, *Colletotrichum* spp., *Phytophthora* spp., *Corynespora cassiicola*, and *Microcyclus ulei*. The above pathogens cause abnormal leaf fall or leaf spot of young as well as mature leaves of *Hevea*. Among stem infections, pink disease, caused by *Corticium salmonicolor*, is the most important, capable of infecting young as well as mature trees. Dry rot caused by *Ustulina deusta*, patch canker caused by *Phytophthora palmivora*, and black stripe caused by *P. palmivora*, *P. meadii*, or *P. botryose*, are other important diseases affecting the stem. White root rot caused by *Rigidiporus lignosus*, brown rot caused by *Phellinus noxius*, and red rot caused by *Ganoderma philippii* are notable diseases of roots. Among the above diseases, South American leaf blight (SALB), caused by *M. ulei* (=*Dothidella ulei*), is the most devastating. This disease caused several serious epidemics, almost leading to cessation of planting of *Hevea* in Brazil.

The change in weather parameters due to the increasing trend in climate change, which is clearly felt in the traditional rubber growing regions of India, may possibly alter the host-pathogen interactions. This will possibly lead to epidemic outbreaks of otherwise minor diseases. Besides, there is every possibility that hitherto unreported exotic pathogens may be favored by the altered weather parameters. Emergence of leaf disease caused by *C. cassiicola* as a major pathogen is a classic example. This pathogen is rapidly progressing into new areas, thus highlighting the need for a stronger and advanced resistance breeding approach.

Clonal Variation and Breeding for Disease Resistance

Hevea breeding primarily aims at developing clones with potential to produce more latex. Introduction, ortet (plus tree) selection, and hybridization followed by clonal selection, are the major methods of crop improvement in rubber. During various stages of evaluation, observations are made on various pest and disease incidences (Mydin and Saraswathyamma 2005). Better growth vigor, smooth and thick bark, good bark renewal after tapping, and tolerance to major diseases are considered as good secondary characteristics (Varghese 1992, Varghese and Mydin 2000).

In *Hevea*, the breeding strategy follows the conventional method of cyclical 'generation-wise assortative mating' (GAM), where superior genotypes of one generation form parents for subsequent breeding programs (Simmonds 1989). Most of the cultivated clones have been developed through selection or hybridization from among few selected high-yielding popular clones. Since yield remained the primary trait for breeding, there had been limited selection for resistance genes and their transmission among the hybrid progenies, and hence, many of the *Hevea* clones exhibit variable levels of susceptibility to the pathogenic fungal diseases in the field.

Although systematic breeding in *Hevea* started in early 1900s, studies on genetic parameters were initiated after 1970 (Tan and Tan 1996). Studies on genetic parameters, however, aimed at major economic traits like yield and vigor and there were very limited attempts to understand the genetic basis of disease resistance in *Hevea*. Subsequent to major disease epidemic outbreaks of SALB, efforts were made to understand the genetic basis of disease resistance in *Hevea* and specific resistance breeding programs were implemented to identify and breed resistant clones.

Understanding the pattern of genetic inheritance of disease resistance is crucial for developing successful resistance breeding programs.

In a genetic analysis study, two populations viz. (i) 18 selected genotypes of various degrees of resistance, and (ii) 15 randomly selected progenies of a five-parent diallel cross, were screened for leaf disease resistance after artificial inoculation with *Colletotrichum* and natural infection of *Corynespora* and *Oidium* (Tan and Tan 1996). The results revealed that *Colletotrichum* and *Corynespora* resistance had higher heritability estimates than *Oidium* resistance. Hence, selection for genotypes with *Colletotrichum* and *Corynespora* would be more effective than the selection for *Oidium*-resistant genotypes. A considerable part of major genetic variation of *Colletotrichum* disease resistance was attributed to additive gene control; *Corynespora* and *Oidium* resistance was ascribed to non-additive gene control. There were high proportions of general combining ability (GCA) effects for the above three diseases. However, specific combining ability effect was significant only for *Corynespora* and *Oidium*. Earlier, degrees of resistance to *Colletotrichum*, *Corynespora,* and *Oidium*, and continuous form of variation, had indicated operation of polygenic inheritance of disease resistance in *Hevea* (Lim 1973, Tan et al. 1992, Wastie 1973). Since no adverse genetic associations have been found between disease resistance traits and latex yield or growth vigor, breeding for durable resistance contributed by polygenic inheritance has been recommended as the best resistance breeding strategy in *Hevea* (Ho 1986; Simmonds 1983, 1985, 1986, 1989, 1990; Tan 1987).

Oidium-Caused Leaf Disease

Leaf fall resulting from *Oidium* infection is capable of causing extensive defoliation, particularly when trees refoliate after wintering, leading to serious retardation of growth and considerable loss in yield (Liyanage and Jacob 1992). Although one low-yielding clone from Sri Lanka, LCB 870, has been reported to possess resistance to the disease, most of the high-yielding clones are susceptible to the disease. While clones PB 86, GT 1, GL 1, PR 107, PB 5/139, RRIM 703, RRII 208, and PB 310 show limited levels of resistance, other clones viz., Tjir 1, PB 5/51, and RRIM 605 are highly susceptible. RRII 105, the hybrid clone extensively cultivated for several years in India, PB 235, PB 280, as well as RRII 118 and RRII 300 are also susceptible to the disease. Interestingly, RRII 105 is a hybrid between Tjir 1 and Gl 1 and the male parent has been reported to show some level of resistance.

A study on *Oidium*-caused leaf fall sensitivity of 25 *Hevea* clones developed from India, Indonesia, China, Thailand, and Malaysia revealed that clones SCATC 93-114, RRIM 703, Haiken 1, RRII 208, RRII 5, and PB 310 had comparatively stable tolerance towards *Oidium*-caused leaf fall disease. These clones have been suggested for use in breeding for disease resistance (John et al. 2001, Rajalakshmy et al. 1997). In another study carried out in a plantation with 20 clones, PB 86, RRIC 52, AC/S/12 42/186, PR 261, RO/CM/10 44/7, RRIM 703, AC/S/12 42/59, and IAN 45-873 exhibited low levels of infection (John et al. 2000). High resistance in the wild genotypes viz., AC/S/12 42/59, AC/S/12 42/186, and RO/CM/10 44/7 has already been reported (Quiong 1993). Of the RRII 400 series clones developed by Rubber Research Institute of India (RRII), almost all recommended clones were affected by the disease. However, RRII 422 followed by RRII 414 showed comparatively less infection (Varghese et al. 2009).

Based on disease intensity in the field, 3,561 wild germplasm accessions of *Hevea* were screened for tolerance to *Oidium* leaf infection (Mydin et al. 2011). The above study, which was carried out for 3 consecutive years, identified two potential accessions which had less than 10 percent disease incidence. Such tolerant accessions could be used in future resistance breeding programs. Similar field screening studies carried out in Tripura (north east state of India) could identify seven wild accessions viz., MT 4859, MT 5136, RO 3794, RO 5055, RO 5087, RO 5160, and RO 5365 tolerant to *Oidium* leaf infection. Similarly, in another north east state of Assam (India), field screening

revealed stable disease tolerance in three wild accessions viz., RO 1737, AC 587, and AC 5302 (Mydin et al. 2011).

Phytophthora Leaf Diseases

Although *Hevea* clones that evolved in various rubber growing regions show variable levels of susceptibility to abnormal leaf fall disease caused by *Phytophthora* spp., most of the high-yielding clones are susceptible. High yielding clones such as RRII 105, GT 1, Gl 1, PB 86, PB 217, PB 235, PB 260, PB 311, PB 28/59, RRIM 600, RRIM 628, RRIM 703, PR 255, PR 261, and Tjir 1 are reported to be affected by the disease. Some of the above clones showed higher leaf retention under prophylactic fungicidal spraying (Liyanage and Jacob 1992). Clones susceptible to abnormal leaf fall disease are also highly susceptible to shoot rot caused by *Phytophthora* spp. In India, among the RRII 400 series clones, RRII 414 and RRII 430 exhibited low incidences of abnormal leaf fall due to *Phytophthora* spp.

Two trials consisting of 13 clones each were assessed for tolerance to the disease under prophylactic fungicidal spray (Mushrif et al. 2004). High leaf retention was observed in RRII 105 and RRII 5. Clones RRIM 600, PB 260, and PB 280 along with RRIM 703 showed low leaf retention. It may be noted that RRIM 703 had been identified as a moderately tolerant clone in Malaysia (RRIM 1975). Interestingly, another clone, PR 255, showed significantly more leaf retention although its parents, namely Tjir 1 and PR 107, are susceptible. Since stomatal entry of the pathogen had been observed, attempts were made to identify anatomical factors, particularly petiolar stomata, influencing disease development in various clones (Premakumari et al. 1979, Premakumari and Panikkar 1984, Thankamma et al. 1975). Subsequent studies using various clones also indicated the frequency of petiolar stomata and the aperture index as potential criteria for selection of disease-resistant clones (Premakumari et al. 1988). The above study showed that 68 percent variation in leaf retention after the incidence of *Phytophthora*-caused leaf fall disease could be explained by the characteristics of petiolar stomata.

About 2,691 accessions of wild germplasm of *Hevea*, originally collected from provenances viz., Mato Grasso, Acre and Rondonia were assessed for field level abnormal leaf fall disease (Mydin et al. 2011). Based on percent leaf retention after infection, 257 accessions were found to retain more than 75 percent of leaves, while more than 1,900 accessions shed leaves indicating varying levels of disease resistance operating among the wild germplasm accessions. Detached leaves of more than 100 wild accessions were subjected to artificial laboratory inoculation using zoospore suspensions. Based on lesion size as an indicator of resistance, 18 accessions were rated as tolerant compared to check clone RRII 105. The tolerant accessions include one (RO 4423) from Rondonia, three (AC 2016, AC 3146, and AC 462) from Acre, and 14 (MT 1617, MT 4494, MT 1631, MT 4436, MT 1581, MT 1715, MT 2219, MT 2233, MT 3707, MT 4252, MT 1027, MT 900, MT 4702, and MT 4874) from Mato Grosso (Mydin et al. 2011).

Corynespora-Caused Leaf Disease

Leaf disease caused by *Corynespora* infection was first reported from India in seedling nurseries (Ramkrishnan and Pillay 1961). Subsequently, the disease has been reported from Malaysia, Nigeria, Indonesia, Sri Lanka, and Thailand. The disease has now spread to almost all rubber growing regions (Chee 1990). In Thailand, several cultivated clones were screened for disease resistance in the laboratory as well as the field using 24 isolates of *C. cassiicola* (Rodesuchit and Kajornchaiyakul 1996). For laboratory bioassay 10- to 12-day- old juvenile leaflets were used and for field assessment 1-year-old budwood were inoculated with virulent isolates. Based on the laboratory bioassay and field inoculations, the study grouped clones into three classes viz., highly susceptible to susceptible, moderate, and resistant to highly resistant. While clones GT 1, RRIM 600, and KRS 225 were rated susceptible to highly susceptible in laboratory and field assays, clone KRS 226 which was rated as

resistant to highly resistant in the laboratory assay was found to be highly susceptible after field inoculation. Similarly, clone PR 261 which was rated as highly susceptible to susceptible after laboratory inoculations later proved to be resistant to highly resistant based on field inoculations. In Malaysia, high variability for resistance to *C. cassiicola* races have been reported in clones (Hashim 2011). Clones PB 260, PB 350, PB 359, RRIM 929, and RRIM 2025 were highly susceptible to race 2, but resistant to race 1. In contrary, clones RRIM 2009 and RRIM 2026 were resistant to race 2, but highly susceptible to race 1. It was also found that clone PB 355 was resistant to both the races. Also, clones RRIM 600, RRIM 928, and RRIM 2001 were highly susceptible to both the races. The above variability for resistance indicated possible operation of horizontal as well as vertical resistance in *Hevea*. While few other clones including RRIM 2024 and RRIM 2025 have been reported to be mildly infected by the pathogen (Murnita 2011), the disease reaction needs further long-term evaluation in multiple locations. These studies once again reiterate the need for a careful approach in breeding for disease resistance to *Corynespora* leaf infection in *Hevea* where laboratory assays should always be correlated with field-level resistance. In addition, multilocational field trials under different eco-climatic conditions are also needed before finally rating a clone as resistant or susceptible.

In Malaysia, more than 90 clones and 47 wild germplasm accessions were screened for resistance under laboratory and field conditions (Chee 1988). This and other studies (Othman et al. 1996, Tan et al., 1992) showed that clones which were earlier rated as resistant subsequently became susceptible, possibly due to more virulent pathotypes of the pathogen. Specifically, clone RRIM 600 which was relatively resistant to the disease eventually showed severe infection. Othman et al. (1996) hypothesized allelic heterozygosity for *Corynespora* leaf disease in *Hevea,* since controlled crosses between susceptible parents produced moderately or less susceptible progenies. Also, they observed that RRIM 600 (Tjir 1 x PB 86) was severely infected; however, both the parental clones of RRIM 600 were almost free from fungal infection.

In India, almost all clones were reported to be affected by the disease (Jacob 1997, Mathew 2006). While clone RRIC 103 has been reported to be highly susceptible, GT 1 is fairly tolerant. Sri Lankan clones viz., RRIC 104, RRIC 110, and RRIC 133 have been reported to be susceptible (Jayasinghe and Silva 1996). The disease has already been reported in many Malaysian clones including RRIM 600, PB 5/51, PB 235, and the Indonesian clone Tjir 1 (Tan 1990). AVROS 2037 (Indonesia), BPM 24 (Indonesia), and RRIC 100 (Sri Lanka) have been reported to show tolerance to the disease under Indonesian conditions (Azwer et al. 1993). In India, most of the high-yielding clones, including RRII 105, RRIM 600, PB 260, and PR 107 are susceptible (Jacob 1997). Among RRII 400 series clones developed by RRII, RRII 414, and RRII 430 have been found comparatively less infected by the pathogen under the assistance of prophylactic fungicidal spray (Varghese et al. 2009). In order to screen tolerant genotypes, wild germplasm accessions were artificially inoculated with spore suspensions and monitored for lesion development compared to GT 1 and RRII 105 (Mydin et al. 2011) and tolerant accessions were shortlisted for further evaluation.

Molecular techniques have been used to study genetic variability in *Corynespora* affecting *Hevea* (Atan and Hamid 2003, Darmano et al. 1996, Philip et al. 2004, Saha et al. 2000, Silva et al. 1998, Romruensukharom et al. 2005) using RAPD, rDNA-RFLP, etc. In general, studies revealed considerable levels of genetic variability among the isolates, which indicates the need for a molecular marker-assisted approach in breeding *Hevea* for resistance to *Corynespora*. Attempts have been made to develop *Hevea* clones resistant to the pathogen through genetic transformation techniques (Sunderasan et al. 2011) using anti-cassicolin-specific scFv gene.

Colletotrichum-Caused Leaf Disease

Studies have shown that cultivated clones viz. PB 86, RRII 5, RRII 105, RRII 118, RRII 208, and RRII 300 are susceptible to the disease. Clones PB 217, PB 260, and RRIM 600 have been shown to have comparatively more tolerance. Preliminary studies on intensity and severity of *Colletotrichum*-

caused leaf disease in wild germplasm accessions helped in identification of accessions with varying levels of disease reaction (Mydin et al. 2011). While 161 accessions were almost disease free, more than 700 accessions showed severe infection in the form of spots and leaf fall.

South American Leaf Blight

South American leaf blight (SALB), caused by *M. ulei*, is the most devastating disease of *Hevea* so far reported from the entire rubber growing region, capable of causing up to 90 percent loss in yield of rubber. The disease occurred in epidemic proportions in Brazil and adjacent regions leading to almost complete failure of rubber cultivation. The leaf blight is an obligate pathogen reported only in *Hevea* spp. and it occurs only in tropical America (Chee and Holliday 1986, Lieberei 2007). The pathogen has been recorded from four species of *Hevea* viz*., H. brasiliensis*, *H. benthamiana*, *H. guianensis*, and *H. spruceana*. Breeding for resistance to SALB is the only long-term strategy for disease management. Crown budding, a process where a resistant clone like FX 516 is budded on otherwise susceptible clone, has also been suggested as an interim strategy for management of the disease. Clones of *Hevea* have been screened for susceptibility to the SALB pathogen (Chee 1976). Several other trials are also underway in order to assess resistance of clones to SALB (Omokhafe 2011).

Hevea clones were tested for their capacity to produce a phytoalexin named scopoletin and to produce lignins in their infection sites (Garcia et al. 1999). A strong correlation was observed between scopoletin accumulation and clone resistance. Moreover, strong lignin accumulation was often associated with a longer stromatic generation period. These two physiological reactions could interfere by limiting fungal development in several clones. However, neither scopoletin nor lignin accumulation could individually explain the behavior of all clones (Garcia et al. 1999). Studies carried out in several accessions of wild germplasm using microsatellite markers indicated correlation between genetic resistance and geographical distribution (Guen 2011).

The earliest breeding program concentrated on breeding resistant genotypes using surviving trees identified from severely diseased plantations at Belterra and Fordlandia in Brazil (ANRPC 1995). The strategy involved crossing resistant genotypes like F 170, F 315, F 351, F 1425, and F 4542 with high-yielding clones viz., AVROS 49, AVROS 193, AVROS 363, PB 86, PB 186, and Tjir 1 imported from Indonesia and Malaysia. Later, resistance breeding was continued in Brazil by crossing clones, including RRIM 600 and RRIM 501 (introduced from Malaysia, Sri Lanka, and Indonesia) with the primary Ford clones and also progenies of the Ford crosses. Although there have been several other reports about breeding for SALB resistance, none of the reported clones have been found resistant to the pathogen, possibly due to rapidly evolving pathogenic races. Another collaborative breeding program carried led to development and selection of 13 CMS clones with promising horizontal resistance to SALB (Mattos 2011). The resistant clones viz., CD 1174, CDC 56, CDC 312, MDX 607, MDX 624, PMB 1, FDR 4575, FDR 5240, FDR 5283, FDR 5597, FDR 5665, FRD 5788, and FDR 5802 were tested in multi-regional trials in Brazil, Ecuador, Colombia, Guatemala, and Peru. The above clones were also found to possess putative resistance to selected isolates of *C. cassiicola* (Murnita et al. 2011). However, further studies are required to assess the *C. cassiicola* resistance of the above clones through large-scale field testing.

The mechanism of resistance of *Hevea* to *M. ulei* has not yet been fully understood, but two possible types of resistance viz., vertical (race specific) and horizontal (race non-specific), have been proposed (Hashim and Pereira 1989a, 1989b) based on field observations and laboratory assays. At least 11 physiological races of the pathogen have been reported so far. Hitherto, resistant clones were susceptible to new races of the pathogen. Among the species of *Hevea*, *H. benthamiana* clone F 4542 was found resistant to Race 1 (wild race) of *M. ulei*. However, most of the progenies of F 4542 were susceptible to Race 2 and Race 3.

Vertical resistance has been attributed to host cell death around the site of infection in clones which are resistant to some races of the pathogen. Breeding for vertical resistance led to clones

which were resistant only to few races. Clones with vertical resistance subsequently succumbed to new races of the pathogen. Horizontal resistance has been suggested as more durable since it confers resistance to almost all races of the pathogen. Clones with horizontal resistance allowed fungal penetration, but prolonged the rate of spread, reduced the size of lesion, and minimized spore production. In order to simplify the field assessment method for SALB disease, Rivano et al. (2010) tested the resistance of eight rubber tree clones to *M. ulei* in Ecuador in a Fisher block design with four replicates per treatment and concluded that assessing the resistance of rubber tree clones to SALB in large-scale clone trials can be optimized to reduce the number of observation times by 50 percent.

In the lines of conventional breeding for disease resistance, intra- and inter-specific hybridizations were attempted for developing SALB resistance. While the initial intra-specific breeding utilized clones of *H. brasiliensis*, subsequent inter-specific hybridization involved another species, *H. benthamiana* (clone F 4542). Further search for resistance in other species led to the identification and use of *H. pauciflora* (clone P 10) since this species was free from SALB infection. Thus, *H. pauciflora* was hybridized with *H. brasiliensis* and *H. benthamiana* (Pinheiro and Libonati 1971). Subsequently, controlled crosses were also made between *H. camargoana* and *H. brasiliensis* (*H. camargoana* x Fx 4098); Fx 4098 is a hybrid of PB 86 x FB 74, both primary clones of *H. brasiliensis* (developed in Malaysia and Brazil, respectively) and the resultant hybrid progenies were selected for resistance to SALB (Goncalves et al. 1982). However, the resistant hybrids were apparently not high-yielding. As indicated in several studies using wild genotypes as well as cultivated clones, durable resistance is possibly present in wild accessions of *H. brasiliensis* as well as other allied species (Priyadarshan and Goncalves 2003).

Stem Diseases

Pink disease, caused by *C. salmonicolor*, mainly affects young trees, but of late, trees up to 7-years-old are also affected. Repeated infection of young branches lead to retardation of growth, thereby extending the period before the trees can be utilized for extraction of rubber through tapping. Clones PB 217, PB 311, and RRII 105 are highly susceptible to the disease. Among the other cultivated clones, Tjir 1, LCB 1320, RRIM 501, RRIM 701, etc., were also found affected. Another study indicated less susceptibility to the disease in clones viz., PB 86, RRIM 513, Gl 1, PR 107, GT 1, and PB 260 (Ramakrishnan and Pillay 1962, RRIM 1992). Among the RRII 400 series clones, RRII 430 is least affected while RRII 429 is highly susceptible (Varghese et al. 2009).

With regard to other minor stem diseases, clones RRIM 600, PB 235, PB 311, and PB 28/59 are severely affected by black stripe due to *Phytophthora* spp.; PB 217 is moderately affected. With reference to patch canker caused by *Phytophthora* spp., clone PB 260 is highly susceptible (Kothandaraman and Idicula 2000).

Disease Resistance Genes, Molecular Mapping, and Marker-Assisted Selection

Attempts have been made to identify markers linked to disease resistance in *Hevea*. Chen et al. (1994, 2003) used RAPD markers to identify *Oidium* leaf fall resistance gene and subsequently sequence one RAPD marker ($OPV\text{-}10_{390}$) possibly linked to gene conferring resistance to *Phytophthora*. Studies have been carried out to identify possible disease resistance gene analogues in rubber (Licy et al. 2000). Eighteen primers, designed based on homologies between known resistance genes, were used in various combinations to amplify sequences from rubber cultivar FX 516, which is resistant to *Phytophthora*-caused leaf fall disease and cultivar RRII 105, which is tolerant. Although none of the clones obtained had high homology to resistance gene sequences, the putative protein encoded by one sequence had some homology to *hem* N gene. Studies are being carried out at RRII on resistant gene analogues (RGAs) and their relationship with functional RGAs

in response to *Corynespora* infection (Saha et al. 2010). Using degenerated primers based on conserved motifs of NBS domains of known *R*-genes, a PCR-based approach was followed. RGAs of NBS-LRR class were cloned from *H. brasiliensis* and *H. benthamiana* and 22 transcriptionally active diverged RT-RGAs were identified. Characterization of these RGAs is required for better understanding plant reaction to *Corynespora* infection.

Using a population of 192 progeny individuals derived from a cross between a resistant clone (RO 38; original name is FX3899, a low yielding inter-specific hybrid between *H. brasiliensis* and *H. benthamiana*) and a susceptible cultivated clone (PB 260), Guen et al. (2003) identified a major QTL named as *M13-1bn*, located on linkage group g13, that is responsible for 36 to 89 percent of the phenotypic variance of resistance. The role of *M13-1bn* in screening clones for resistance to SALB needs validation through large-scale multilocational field testing.

Discussion and Conclusion

Field evaluation through visual observation and laboratory assays through excised leaf inoculation have led to screening of putatively resistant Wickham clones and wild germplasm. However, laboratory bioassays are preliminary and may not ensure actual field-level resistance as observed for diseases of other forest trees. *Hevea* clones have also been tested for their capacity to produce phytoalexins; a strong correlation was observed between phytoalexin accumulation and clone resistance. More lignin accumulation was also often associated with clone resistance. With regard to wild germplasm, earlier studies indicated considerable variation in growth and disease resistance among and within genotypes collected from various provenances of Brazil.

Many man hours of labor and enormous quantities of fungicidal chemicals are required every year for management of the above diseases in vast areas of rubber plantations in India and other rubber growing countries. The cost of fungicides and their long-term effect on the environment justify the need for breeding disease-resistant trees. There are several theories for genetic basis for disease resistance (horizontal/vertical) in *Hevea*. Nevertheless, there is every possibility for breakdown of resistance due to ever-evolving pathogenic races coupled with climate change, which is exemplified by evolving SALB races. Multidisciplinary breeding programs for development of disease-resistant clones would have to continuously utilize the Wickham resource, as well as wild germplasm, in addition to other *Hevea* spp. in order to have sustainable rubber production. The present germplasm resource of *Hevea* is predominantly constituted by the domesticated clones and more than 4,000 wild germplasm accessions collected from Acre, Rondonia, and Mato Grosso.

Disease resistance in *Hevea*, like other forest trees, is apparently polygenic, as indicated by varying levels of resistance in domesticated and wild germplasm. Elaborate studies are needed to understand the molecular basis of resistance and mechanisms of inheritance of disease resistance in *Hevea*. Molecular markers, wherever feasible, should be integrated into breeding programs to accelerate selections. Once the germplasm accessions are characterized for disease resistance, association mapping strategies could be employed for identifying markers linked to resistance which would ultimately help in molecular-assisted selection/breeding (MAS/MAB) in *Hevea*.

Acknowledgments

The first author expresses his sincere thanks to: Chairman, Rubber Board, Government of India and Director, Rubber Research Institute of India, India, for granting sponsorship; Department of Science and Technology, Government of India, for the travel grant; Richard Sniezko, Geneticist and Workshop Chair, Dorena Genetic Resource Center, Oregon; Katie Palmieri and Janice Alexander, California Oak Mortality Task Force, University of California; and many others at the workshop for all their help.

Literature Cited

Allen, P.W. 1984. Fresh germplasm for natural rubber. Span. 27 (1): 7–8.

ANRPC [Association of Natural Rubber Producing Countries]. 1995. South American leaf blight (*Microcyclus ulei*) of rubber – a training manual. Kuala Lumpur: Association of Natural Rubber Producing Countries. 36 p.

Atan, S.; Hamid, N.H. 2003. Differentiating races of *Corynespora cassiicola* using RAPD and internal transcribed spacer markers. Malaysian Journal of Rubber Research. 6(1): 3.

Azwer, R.; Daslin, A.; Anzwar, R. 1993. Performance of 1974 multilateral exchange rubber clones at various locations in Indonesia. International Journal of Crop Science. 8(1): 11–22.

Baulkwill, W.J. 1989. The history of natural rubber production. In: Webster, C.C.; Baulkwill, W.J., eds. Rubber. Harlow, UK: Longman Scientific and Technical. 56 p.

Chee, K.H. 1976. Assessing susceptibility of *Hevea* clones to *Microcyclus ulei*. Annals of Applied Biology. 84 (2): 135–145.

Chee, K.H.; Holliday, P. 1986. South American leaf blight of *Hevea* rubber. Monograph No. 13. Kuala Lumpur: Malaysian Rubber Research and Development Board. 50 p.

Chee, K.H. 1988. Sporulation, pathogenicity and epidemiology of *C. cassiicola* on *Hevea* rubber. Journal of Natural Rubber Research. 3(1): 21–29.

Chee, K.H. 1990. Present status of rubber diseases and their control. Review of Plant Pathology. 69: 423–430.

Chen, S.; Shao, H.; Hu, D.; Lin, S.; Zheng, X. 1994. Identification of mildew resistance from *Hevea* tree by RAPD technique. Chinese Journal of Tropical Crops. 15(2): 26.

Chen, S.; Shao, H.; Hu, D.; Wu, K.; Zheng, X.F. 2003. Use of RAPD marker to screen mildew-resistance germplasm from *Hevea brasiliensis*. In: Chen, Q.; Zhou, J., eds. Proceedings of the IRRDB Rubber Conference, 1999. [Place of publication unknown]: [Publisher unknown]: 345–350.

Darmano, T.W.; Darussamin, A.; Pawirosoemardjo, S. 1996. Variation among isolates of *Corynespora cassiicola* associated with *Hevea brasiliensis* in Indonesia. In: Darussamin, A.; Pawirosoemardjo, S.; Basuki, Azwar, R.; and Sadaruddin. eds. Proceedings on the workshop on *Corynespora* leaf fall disease of *Hevea* rubber. Indonesian Rubber Research Institute, Indonesia: 79–91.

Garcia, D.; Troispoux,V.; Grange, N.; Rivano, F.; D'Auzac, J. 1999. Evaluation of the resistance of 36 *Hevea* clones to *Microcyclus ulei* and relation to their capacity to accumulate scopoletin and lignins. European Journal of Forest Pathology. 29 (5): 323–338.

Goncalves, P. de. S.; Fernando, D.M.; Rossetti, A.G. 1982. Interspecific crosses in the genus *Hevea*. Pesquisa Agropecuária Brasileira (Brasilia). 17(5): 775–781.

Guen, V.; Le Lespinasse, D.; Oliver, G.; Rodier-Goud, M.; Pinard, F.; Seguin, M. 2003. Molecular mapping of genes conferring field resistance to South American leaf blight (*Microcyclus ulei*) in rubber tree. Theoretical and Applied Genetics.108 (1): 160–167.

Guen, V. 2011. Characterization of genetic structure of Amazonian rubber tree populations with microsatellite markers. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Hashim, I.; Pereira, J.C.R. 1989a. Lesion size, latent period and sporulation on leaf discs as indicators of resistance of *Hevea* to *Microcyclus ulei*. Journal of Natural Rubber Research. 4: 56–65.

Hashim, I.; Pereira, J.C.R. 1989b. Influence of resistance of *Hevea* on development of *Microcyclus ulei*. Journal of Natural Rubber Research. 4: 212–218.

Hashim, I. 2011. South American leaf blight and *Corynespora* leaf fall: challenging diseases of *Hevea* rubber. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Ho, C.K. 1986. Rubber, *Hevea brasiliensis*. In: Breeding for durable resistance in perennial crops, FAO Plant Production and Protection Paper No. 70. Rome: FAO: 85–114.

Jacob, C.K. 1997. Diseases of potential threat to rubber in India. Planters Chronicle. 92: 451–461.

Jayasinghe, C.K.; Silva, W.P.K. 1996. Current status of *Corynespora* leaf fall in Sri Lanka. In: Darussamin, A.; Pawirosoemardjo, S.; Basuki, Azwar, R.; and Sadaruddin, eds. Proceedings of the workshop on *Corynespora* leaf fall disease of *Hevea* rubber. Indonesia. 3–5.

John, A.; Annakutty, J.; Meenakumari, T.; Saraswathyamma, C.K.; Varghese, Y.A. 2000. Clonal variation in the intensity of powdery mildew (*Oidium heveae* Steinm.) disease of *Hevea*. Indian Journal of Natural Rubber Research. 13 (1&2): 64–68.

John, A.; Nair, R.B.; Rajalakshmy, V.K.; Saraswathyamma, C.K.; Varghese, Y.A. 2001. Sensitivity relationship of *Hevea* clones to the stress of powdery mildew (*Oidium heveae* Steinm.). Indian Journal of Natural Rubber Research. 14 (2): 88–92.

Kothandaraman, R.; Idicula, S.P. 2000. Stem diseases. In: George, P.J.; Jacob, C.K., eds. Natural rubber: agromanagement and crop processing. Rubber Research Institute of India, India. 297–308 .

Licy, J.; Dickinson, M.J.; Bligh, F.J.; Power, B.J.; Saraswathyamma, C.K.; Davey, M.R. 2000. An approach to identify disease resistance gene analogues in *Hevea*. Indian Journal of Natural Rubber Research. 13 (1&2): 79–85.

Lieberei, R. 2007 South American leaf blight of the rubber tree (*Hevea* spp.): New steps in plant domestication using physiological features and molecular markers. Annals of Botany. 100 (6): 1125–1142.

Lim, T.M. 1973. A rapid laboratory method of assessing susceptibility of *Hevea* clones to *Oidium heveae*. Experimental Agriculture. 9: 275.

Liyanage, A.D.S.; Jacob, C.K. 1992. Disease of economic importance in rubber. In: Sethuraj, M.R.; Mathew, N.M. eds. Natural rubber: biology, cultivation and technology. Developments in Crop Science 23. The Netherlands: Elsevier Science Publishers: 324–369.

Mathew, J. 2006. Clonal resistance of *Hevea brasiliensis* to *Corynespora* leaf fall disease. In: Jacob, C.K.; Srinivas, P.; Roy C.B., eds. *Corynespora* leaf disease of *Hevea brasiliensis* - strategies for management. Rubber Research Institute of India: 83–101.

Mattos, C. 2011. CMB (CIRAD-MICHELIN-BRAZIL) Project for the genetic improvement of rubber trees in Brazil. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Murnita, M.M. 2011. Major leaf diseases distribution, severity and clonal susceptibility in peninsular Malaysia. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Murnita, M.M.; Nasaruddin, M.A; Othman, R. 2011. Susceptibility of South American leaf blight tolerant clones to *Corynespora cassiicola* infection. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Mushrif, S.K.; Joseph, A.; Johan, A.; Jacob, C.K. 2004. Evaluation of *Hevea brasiliensis* clones against abnormal leaf fall disease caused by *Phytophthora*. Natural Rubber Research. 17 (1): 74–78.

Mydin, K.K.; Saraswathyamma, C.K. 2005. A manual on breeding of *Hevea brasiliensis*. Rubber Research Institute of India. 97 p.

Mydin, K.K.; Jacob, J.; Reghu, C.P.; Sankariammal, L.; Nair, D.B.; Mercy, M.A.; Joseph, A.; Saha, T.; Das, K.; Sreelatha, S.; Mondal, G.C.; Das, G. 2011. Conservation, characterization, evaluation and utilization of the 1981 IRRDB wild *Hevea* germplasm collection in *Hevea*. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Narayanan, C.; Mydin, K.K. 2011. Heritability of yield and secondary traits in two populations of Para rubber tree (*Hevea brasiliensis*). Silvae Genetica. 60 (3-4): 132–139.

Omokhafe, K. 2011. Strategy for trials of the SALB resistant clones in *Hevea brasiliensis* in Nigeria. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Othman, R.; Benong, M.; Ong, S.H.; Hashim, I. 1996. Strategies and development of resistant *Hevea* clones against *Corynespora* leaf fall. In: Darussamin, A.; Pawirosoemardjo, S.; Basuki, Azwar, R.; and Sadaruddin. eds. Proceedings of the workshop on *Corynespora* leaf fall disease of *Hevea* rubber. Indonesian Rubber Research Institute, Indonesia: 177–193.

Philip, S.; Joseph, A.; Abraham, T.; Zacharia, C.A.; George, J.; Manju, M.J.; Jacob, C.K. 2004. ERIC PCR based genomic finger printing of *Corynespora cassiicola* isolates infecting rubber (*Hevea brasiliensis*) plantations. Journal of Plantation Crops. 32: 301–305.

Pinheiro, E.; Libonati, V.F. 1971. O emprego do *Hevea pauciflora* M.A. como fuente genetica de resistencia ao mal das folhas. Poliméros. 1:31–39.

Premakumari, D.; Annamma, Y.; Bhaskaran Nair, V.K. 1979. Clonal variability for stomatal characters and its application in *Hevea* breeding and selection. Indian Journal of Agricultural Sciences. 49 (6): 411–413.

Premakumari, D.; Panikkar, A.O.N. 1984. Organographic variability of stomatal characters in *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. and it possible significance in clonal susceptibility to leaf fall disease. In: Proceeding of the International Rubber Conference. Sri Lanka: Rubber Research Institute of Sri Lanka: 317–324.

Premakumari, D.; Panikkar, A.O.N.; Sethuraj, M.R. 1988. Correlations of the characters of petiolar stomata with leaf retention after the incidence of *Phytophthora* leaf fall disease in *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. Indian Journal of Natural Rubber Research. 1(1): 22–26.

Priyadarshan P.M.; Goncalves, P. de. S. 2003. *Hevea* gene pool for breeding. Genetic Resources and Crop Evolution. 50 (1): 101–114.

Quiong, H.D. 1993. Identification and evaluation by major characteristics of part of the 1981 Amazon collection of wild *Hevea* germplasm in China. In: Proceedings of the IIRDB Joint Meeting. Hartford, England: International Rubber Research and Development Board: 47–59 .

Rajalakshmy, V.K.; Annakutty, J.; Varghese, Y.A.; Kothandaraman, R. 1997. Evaluation of *Hevea* clones against powdery mildew caused by *Oidium heveae* Steinm. Indian Journal of Natural Rubber Research. 10 (1 and 2): 110–112.

Ramakrishnan, T.S.;. Pillay. P.N.R. 1961. Leaf spot of rubber caused by *Corynespora cassiicola* (Berk. & Curt.) Wei. Rubber Board Bulletin. 5: 52–53.

Ramkrishnan, T.S.; Pillay, R. 1962. Pink disease of rubber caused by *Pellicularia salmonicolor* (Berk. and Br.) Dastur (=*Corticium salmonicolor* Berk. and Br.). Rubber Board Bulletin. 5(3): 120–126.

Rivano, F.; Martinez, M.; Cevallos, V.; Cilas, C. 2010. Assessing resistance of rubber tree clones to *Microcyclus ulei* in large-scale clone trials in Ecuador: a less time-consuming field method. European Journal of Plant Pathology. 126(4): 541–552.

Rodesuchit, A.; Kajornchaiyakul, P. 1996. Screening *Corynespora* resistant clones of rubber in Thailand. In: Darussamin, A.; Pawirosoemardjo, S.; Basuki, Azwar, R.; and Sadaruddin, eds. Proceedings of the Workshop on *Corynespora* Leaf Fall Disease of *Hevea* Rubber. Indonesian Rubber Research Institute, Indonesia. 163–176.

Romruensukharom, P.; Tragoonrung, S.; Vanacichit, A.; Toojinda, T. 2005. Genetic variability of Corynespora *cassiicola* populations in Thailand. Malaysian Journal of Rubber Research. 8(1): 3.

RRIM [Rubber Research Institute of Malaysia]. 1975. Enviromax planting recommendations, 1975-1976. Planters' Bulletin. 137: 27–50.

RRIM [Rubber Research Institute of Malaysia]. 1992. Planting recommendations, 1992-1994. Planters' Bulletin 211: 31–50.

Saha, T.; Kumar, A.; Sreena, A.S.; Joseph, A.; Jacob, C.K.; Kothandaraman, R.; Nazeer, M. A. 2000. Genetic variability of *Corynespora cassiicola* infecting *Hevea brasiliensis* isolated from the traditional rubber growing areas in India. Indian Journal of Natural Rubber Research. 13(1and 2): 1–10.

Saha, T.; Roy, C.B.; Ravindran, M. 2010. Characterization of a family of disease resistant gene analogues (RGAs) in rubber (*Hevea brasiliensis*) and their relationship with functional RGAs in response to *Corynespora* infection. In: Proceedings of the national symposium on molecular approaches in management of fungal diseases of crop plants. Bangalore: Indian Institute of Horticultural Research. 101 p.

Schultes, R.E. 1977. Wild *Hevea* – an untapped source of germplasm. Journal of Rubber Research Institute of Sri Lanka. 54: 1–31.

Silva, W.P.K.; Deverall, B.J.; Lyon, B.R. 1998. Molecular, physiological and pathological characterization of *Corynespora* leaf spot fungi from rubber plantations in Sri Lanka. Plant Pathology. 47(3): 267–277.

Simmonds, N.W. 1983. Strategies for disease resistance breeding. FAO Plant Protection Bulletin v. 31(1): 2–10.

Simmonds, N.W. 1985. A plant breeder's perspective of durable resistance. FAO Plant Protection Bulletin. 33: 13–17.

Simmonds, N.W. 1986. Strategies for disease resistance breeding in tropical perennial crops. In: Breeding for durable resistance in perennial crops. FAO Plant Production and Protection Paper No. 70: 3–15.

Simmonds, N.W. 1989. Rubber Breeding. In: Webster, C.C.; Baulkwill, W.J., eds. Rubber. England: Longman Scientific and Technical: 85–124.

Simmonds, N.W. 1990. Breeding horizontal resistance to South American leaf blight of rubber. Journal of Natural Rubber Research. 5(2): 102–113.

Sunderasan, E; Kadir, R.A.; Valerie, P.R.; Malik, A.; Badarudin, B.E.; Yeang, H.Y.; Nathan, S. 2011. *Hevea* genetic transformation: A molecular approach to enhance resistance to *Corynespora* leaf disease. Paper presented at IRRDB-CIRAD plant breeders seminar, 4-7 April 2011. Bahia: Brazil.

Tan, H. 1987. Strategies in rubber tree breeding.. In: Abbott, A.J.; Atkin, R.K., eds. Improving vegetatively propagated crops. London: Academic Press. 27–62.

Tan, A.M. 1990. Survey of *Corynespora* leaf fall disease. Planters Bulletin. 204: 80–85.

Tan, A.M.; Loo, T.P.; Vadivel, G.; Bachik, M.R.; Yoon, K.F. 1992. Survey of major leaf disease of rubber in Peninsular Malaysia. Planters Bulletin. 211: 51–62.

Tan, H.; Tan, A.M. 1996. Genetic studies of leaf resistance in *Hevea*. Journal of Natural Rubber Research. 11(2): 108–114.

Thankamma, L.; Rajalakshmy, V.K.; Radhakrishna Pillai, P.N. 1975. Mode of entry of *Phytophthora* in *Hevea brasiliensis*. In: Proceedings of the International Conference, vol. III. Kuala Lumpur: Rubber Research Institute of Malaysia: 213–216.

Varghese, Y.A. 1992. Germplasm resources and genetic improvement. In: Sethuraj, M.R.; Mathew, N.M. eds. Natural rubber: biology, cultivation and technology. Developments in Crop Science 23. The Netherlands: Elsevier Science Publishers: 88–115.

Varghese, Y.A.; Mydin, K.K.; Meenakumari, T. 2009. Performance of the RRII 400 series and certain Prang Besar (PB) clones in various locations in India. Rubber Research Institute of India, India. 63 p.

Varghese, Y.A.; Mydin, K.K. 2000. Genetic improvement. In: George, P.J.; Jacob, C.K., eds. Natural rubber: agromanagement and crop processing. Rubber Research Institute of India: 297–308.

Wastie, R.L. 1973. Nursery screening of *Hevea* for resistance to *Gleosporium* leaf disease. Journal of Rubber Research institute of Malaya. 23 (5): 339.

Wycherley, P.R. 1968. Introduction of *Hevea* to the Orient. Planter (Kuala Lumpur). 44: 1–11.