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Review paper

This paper is dedicated to the memory of Dr. Richard Evans Schultes, the then Jeffrey Professor and Director Emeritus, Botanical Museum, Harvard University, Cambridge, Massachusetts. Dr. Schultes rendered classical contributions to the botany of Hevea till he passed away on April 10, 2001.

Hevea gene pool for breeding

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Abstract

The conservation and utilization of allied gene resources is vital for the improvement of crop species. Rubber has been an undeniably beneficial commodity for the past 100 years. Progress in yield improvement over 70 years resulted in primary and hybrid clones with exceptional yielding abilities. The extension of *Hevea* to marginal areas necessitated breeding of new clones with resistance to environmental constraints. India, China, Brazil, Thailand, Côte d'Ivoire and Vietnam have marginal environments with single or multiple constraints such as low temperature, wind, higher altitude, moisture deficit and diseases. Allied species and accessions from the Brazil Amazonia can be integrated into such breeding programmes. Many of these clones are resultant of natural interspecific hybridizations. Also, allied species are excellent resources of timber. Molecular interventions revealed mtDNA of modern clones are highly conserved contributed by only two clones (PB 56 and Tjir 1). Nuclear DNA is highly divergent due to breeding and selection under varied geo-climates. The adaptation of allied species and wild accessions under a wide range of environments of Brazil is promising as a source of variation for breeding, and enables selection of clones for specific marginal areas.

Introduction

Rubber has been an undeniably beneficial commodity for the past 100 years. It is synthesized in over 2000 plant species confined to 300 genera of seven families *viz.*, Euphorbiaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Moraceae, Papaveraceae and Sapotaceae (Cornish et al. 1993). The para rubber tree, *H. brasiliensis* Willd. ex A. L. Juss.) Muell.-Arg. is the chief producer of natural rubber (Greek 1991). Guayule (*Parthenium argentatum* Gray, Asteraceae), an alternate rubber source, a shrub native to Chihuahuan desert of Texas and north Mexico provides only 10 percent of the world's natural rubber. Guayule can withstand a temperature range of −18 to 49 °C and can grow in well drained soils with an annual rainfall as low as 230 to 400 mm. The yield potential of guayule is only 600 to 900 kg/ha (Estilai and Ray 1991). However, guayule latex is useful for hypoallergenic latex products (Cornish and Siler 1996). A few other rubber sources are: Ceara rubber (*Manihot glaziovii*), India rubber (*Ficus elastica* Roxb.), Panama rubber (*Castilla elastica* Cerv.), Lagos rubber (*Funtimia elastica* Stapf.) and Madagascar rubber (*Cryptostegia grandiflora* R. Br.) (George and Panikkar 2000). However, these species are not commercially exploited. Rubber is a hydrocarbon polymer constructed of isoprene units, and natural rubber is a secondary metabolite (cis 1,4-polyisoprene) chiefly originating in the secondary phloem of the tree. No other synthetic substitute has comparable elasticity, resilience and resistance to high temperature (Davies 1997). The concerted efforts of various institutions such as Rubber Research Institute of Malaysia (RRIM), Rubber Research Institute of India (RRII), Rubber Research Institute of Sri Lanka (RRISL) and Centre de Coopération Internationale en Researche Agronomique pour le Développement (CIRAD), France, resulted in gradual increment in yield from 650 kg/ha with unselected seedlings during the 1920s to clones yielding 2000 kg/ha (RRIM 700 series) during the 1950s and then to 2500 kg/ha (RRIM 2000 series) during the 1990s. During these 70 years of meticulous breeding and selection, notable clones such as 'RRIM 501', 'RRIM 600', 'RRIM 712', 'RRIM 2001', 'RRIM 2015', 'RRIC 100' and 'RRII 105' were derived (Tan 1987; Saraswathyamma et al. 1993; Ong et al. 1994). Some of the primary clones such as 'PB 56', 'Tjir 1', 'Pil B84', 'Pil D65', 'Gl 1', and 'PB 86' selected during this period later became parents of improved clones.

Allied Species

The genus *Hevea* has 10 species, which are intercrossable (Clément-Demange et al. 2000). Schultes (1977) and Wycherley (1992) refer the readers to excellent reviews on the subject. The taxonomic considerations from 1874 to 1970 delineated the genus with several species at different occasions. Though even 24 species were considered during 1906, the species concept crystallized with nine species in 1970 (Schultes 1977). A tenth species, *H. camargoana* Pires was added during 1971 (Schultes 1987). Brazil considers 11 species including *H. paludosa* Ule (Pires 1973; Gonçalves et al. 1990). Three botanists are considered principal workers on species delineation – Baldwin, Seibert and Schultes – who during their classical exploratory studies contributed significantly towards the botany of *Hevea*. A Harvard University Gazette says "Schultes' field work, conducted mostly in the Colombian Amazon beginning in 1941, made him a leading voice in the field and one of the first in the 1960s to warn about destruction of the rainforests and disappearance of their native people" (see www.harvard.edu).

A summary of the salient features of different species of *Hevea* is presented in Table 1. All species have 36 chromosomes ($2n = 36\ x = 9$). *H. brasiliensis* behaves as an amphidiploid (Ong 1979). However, this contention is disputed at the molecular level. *In situ* hybridization studies revealed two distinct 18S-25S r DNA loci and one 5S rDNA locus (Leitch et al. 1998), suggesting a possible allotetraploid origin with the loss of 5 S rDNA during the course of evolution. Hence, as long as a potential ancestor with $2n = 18$ is unknown, the rubber tree will be considered as an amphidiploid. The genus *Hevea* could eventually be considered as a species complex, due to the absence of barrier to recombination between species.

Hevea species are widely distributed among the countries of South America (Figs. 1A & B). *Hevea* species are indigenous to Bolivia, Brazil, Colombia, French Guiana, Guyana, Peru, Surinam and Venezuela. All species, except *H. microphylla* occur in Brazil, the center of origin. Four species have been found in Colombia and three occur in Venezuela. Two occur in Bolivia, French and British Guyana. *H. guianensis* is the most widely adapted species (Fig. 1A). Temperate type rubber thrives up to 2500–3000 m in the Andes Mountains (Senyuan 1990). These species of *Hevea* were evolved in Amazonian forests over 100 thousand years ago (Clément-Demange et al. 2000). Species are adapted to a particular area with specific climatic and edaphic requirements. *H. camporum*, *H. paludosa* and *H. rigidifolia* show restricted adaptation to specialized environments compared to *H. guianensis* and *H. brasiliensis*. *H. camporum*, *H. camargoana* and *H. nitida* have xeromorphic characters. The specific adaptation these species needs to be closely studied, with reference to climatic and edaphic factors, when clones are to be developed for new environments especially for marginal areas. It is worthwhile to note that except *H. benthamiana* (F 4512, F 4542 - at Instituto Agronomico du Norte, Brazil), none of the other species has been actively utilized for the improvement of rubber tree.

Growing Conditions

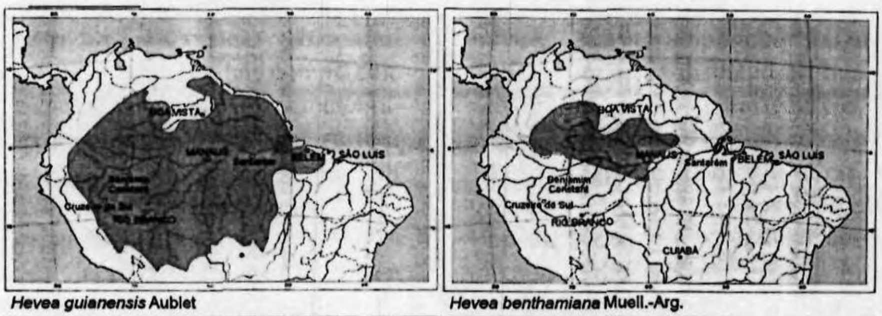
Ideal environment

Hevea brasiliensis is native to the rain forests of the Tropical region of the Great Amazon basin of South America. This area, between equator and 15° south is distinctly characterized by its flat land with altitudes not exceeding 200 m with a wet equatorial climate (Strahler 1969). The mean monthly temperature is 25

Table 1. Occurrence and features of *Hevea* species

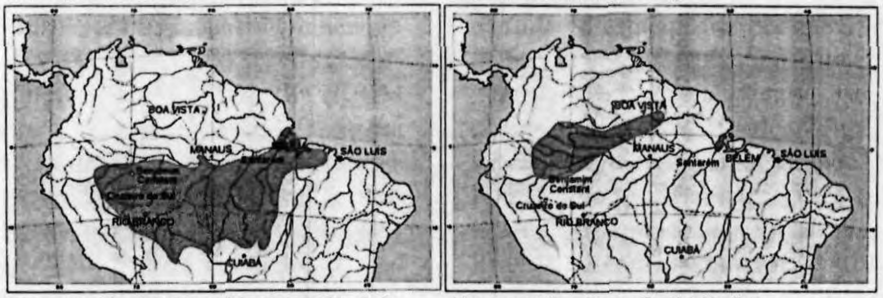
Species	*Occurrence*	Notable features[1]
H. benthamiana Muell.-Arg.	North and West of Amazon forest basin, upper Orinoco basin (Brazil)	Complete seasonal defoliation Medium size tree. Habitat: swamp forests
H. brasiliensis (Willd. ex A. L. Juss.) Muell.-Arg.	South of Amazon river (Brazil, Bolivia, Ecuador, Peru)	Complete defoliation. Medium to large tree size. Habitat: well drained soils
H. camargoana Pires	Restricted to Marajo island of Amazon river delta (Brazil)	Possibility of natural hybridization with *H. brasiliensis*. 2 m to 25 m tree height Habitat: seasonally flooded swamps
H. camporum Ducke	South of Amazon between Marmelos and Manicoré rivers tributaries of Madeira river.	Retains old leaves until new leaves appear. Maximum 2 m tall. Habitat: dry savannahs
H. guianensis Aublet	Throughout the geographic range of the genus (Brazil, Venezuela, Bolivia, French Guyana, Peru, Colombia, Surinam, Ecuador)	Retains old leaves until new leaves and inflorescences appear. Grows at higher altitudes (1100 m msl) Medium size tree. Habitat: well drained soils
H. microphylla Ule	Upper reaches of Negro river in Venezuela. It is not found in other region of geographic range of the genus	Complete defoliation. Small trees. They live on flooded area (igapós). Habitat: sandy soils
H. nitida Mart. ex Muell.-Arg.	Between the rivers Uaupes and Icana tributaries of the upper Negro river (Brazil, Peru, Colombia).	Inflorescences appear when leaves are mature. Small to medium size trees (2 m).
H. pauciflora (Spr. ex Benth.) Muell.-Arg.	North and West of Amazon river (Brazil, Guyana, Peru). Distribution discontinuous due to habitat preferences.	Retains old leaves until new leaves and inflorescences appear. No wintering. Small to big size trees. Habitat: well drained soils, rocky hill sides.
H. rigidifolia (Spr. ex Benth.) Muell-Arg.	Among Negro river and its effluents. Uaupes and Içana rivers (Brazil, Colombia and Venezuela)	Retains old leaves even after inflorescences appear. Small tree from savannahs. Sometime tall, with small crown on the top. Habitat: well drained soils
H. spruceana (Benth.) Muell.- Arg.	Banks of Amazon, Rio Negro and lower Madeira (Brazil)	Retain old leaves until new leaves and inflorescences appear. Flowers reddish purple. Medium size tree Habitat: muddy soils of islands
H. paludosa Ule [2]	Marshy areas of Iquitos, (Peru)	Small leaflets, narrow and thin in the fertile branches; Habitat: marshy areas

After Wycherley (1992), Schultes (1977) Gonçalves et al. (1990), Pires (1973) and Brazil, (1971). [1]Deciduous characteristics mentioned here has a bearing on the incidence of fungal diseases especially through secondary leaf fall (Oidium) since retention of older leaves may make the tree 'escape oidium'. Dwarf types are desirable for the possible wind tolerance. All species are diploid ($2n = 36$) (Majumder 1964), and are inter-crossable (Clément-Demange et al. 2000) [2]Pires (1973) considered 11 species including H. paludosa; Brazil, (1971) considers 11 species.



Hevea guianensis Aublet

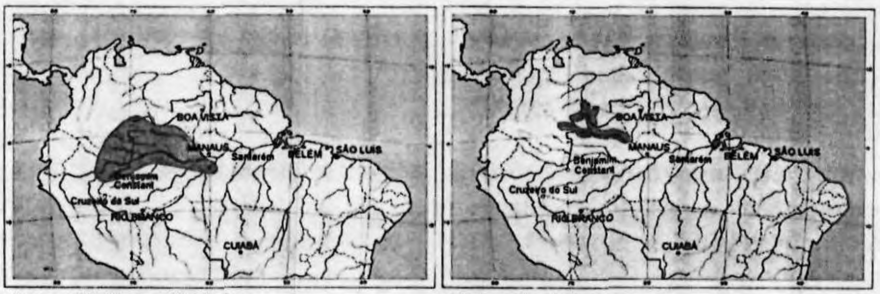
Hevea benthamiana Muell.-Arg.



Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell.-Arg.

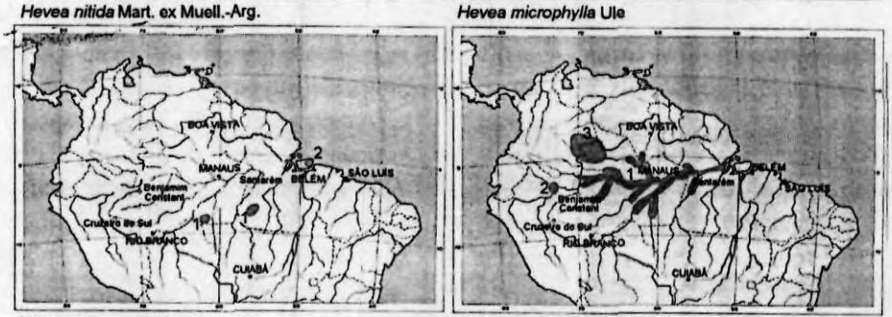
Hevea pauciflora (Spr. ex Benth.) Muell.-Arg.

Figure 1a. Distribution of *Hevea* species in the Amazon valley (adapted from Brazil, 1971)



Hevea nitida Mart. ex Muell.-Arg.

Hevea microphylla Ule



[1] *Hevea camporum* Ducke
[2] *Hevea camargoana* Pires

[1] *Hevea spruceana* (Benth.) Muell.-Arg.
[2] *Hevea paludosa* Ule
[3] *Hevea rigidifolia* (Spr. ex Benth.) Muell.-Arg.

Figure 1b. Distribution of *Hevea* species in the Amazon valley (adapted from Brazil, 1971)

to 28 °C with abundant rainfall of more than 2000 mm/year, without any real dry season. The amount of precipitation is at least twice the evaporation losses on a yearly basis (Pushparajah 2001). The climatic conditions of Brazil delineate four distinct zones: the Amazonian Basin, the Brazilian plateau, the coastlands within the tropics and the southern states. Tropical temperatures (27–32 °C) make the environment on the Brazilian plateau different compared to other areas. Sao Paulo state of the Brazilian plateau is ideal for rubber. However, in the southern states, rubber is not prominent. The basic climatic requirements for rubber are: a) 2000–4000 mm rain fall distributed over 100–150 rainy days/ annum (Watson 1989); b) mean annual temperature around 28 + 2 °C with a diurnal variation of about 7 °C (Barry and Chorley 1976) and c) sunshine hours of about 2000 h/year at the rate of 6 h per day in all months (Ong et al. 1998). In a study with hydrothermal index, the Senai area of Malaysia (1° 36′ N; 103° 39′ E) appeared to be the most suitable area for rubber cultivation and production (Rao et al. 1993). The environmental suitability for rubber and degree of limitation were amply reviewed by Pushparajah (2001). The increased global demand for rubber and competition by other agricultural crops prompted the countries outside the hitherto traditional areas to attempt the cultivation of rubber (Pushparajah 1983). The decision to utilize rubber as a source of income for the economically backward classes also resulted in the cultivation of rubber under marginal conditions. Thus, breeding new clones suitable for marginal environments became necessary.

Marginal area:

The mean annual temperature decreases when moving away from the equator with more prominent winter conditions either during November – January (towards the north) or June to August (towards the south). Northeastern states of India, south China, north and north east Thailand, north Côte d'Ivoire and highlands and coastal areas of Vietnam although satisfy of the basic requirements for rubber, present one stress situations such as low temperature, typhoons, dry periods and higher altitude (Priyadarshan et al. 2001; Zongdao and Yanqing 1992; Watson 1989; Tuy et al. 1998; Dea et al. 1997). Similarly, the southern plateau of Brazil (450–500 m MSL) especially Sao Paulo (23° S) is being tested for rubber cultivation (Costa et al. 2000). Areas of Brazil on the south of the equator offer entirely a different climate for rubber inflicting considerable phenological changes (Priyadarshan et al. 2001). A climatic comparison of India, China, Brazil, Malaysia, Vietnam, Thailand, Indonesia, Nigeria, Sri Lanka and Côte d'Ivoire shows a range of climatic conditions for rubber cultivation (Table 2). Many of them offer marginal environments with one or more limitations such as moisture stress, low temperature, wind, high altitude and disease epidemics. The aforesaid range of climatic attributes are noteworthy and deserve special attention while deriving adaptive clones, evolving agro-management strategies and rescheduling exploitation systems. These areas are away from the equator, where a higher annual input of radiation energy would facilitate greater potential for dry matter production (Oldeman and Frere 1982). However, this is not reflected in yielding potential of clones under these environments.

Areas that fall between 15–20° N experience multitude of stress factors. For instance, the Konkan region of western India experience long dry periods, high temperatures, low atmospheric humidity and zero rainfall between September and May. Daytime temperatures range at 38–41 °C and summer months with occasional days getting as hot as 47 °C. Although the area receives good rainfall (2430 mm), the distribution is uneven (Devakumar et al. 1998). The atmosphere during summer results in high vapor pressure deficit. Almost an analogous situation prevails in the eastern India, northeastern Thailand, and the highlands of Vietnam (450–900 m MSL).

The strategies to identify rubber clones for a new area are: evaluation of prominent clones, evaluation of polyclonal seedlings, recombination breeding & selection and evaluation of Brazilian germplasm. Variation in breeding population is the key attribute that governs the success of any of these strategies, since genotype-environment interaction is to be identified in the intended growing areas. Plant breeders normally just use only available genetic diversity in cultivated germplasm. However, the case of *Hevea* is exceptional since the cultivated clones are bred from only 22 seedlings of Wickham's collection for the past 80 years (Baulkwill 1989). The breeding involved intensive generation-wise assortative mating and selection, where the best suitable genotype is taken as a parent for the next generation. This causes erosion of genetic variation, obviously prompting breeders to look for genetic diversity in wild populations of Brazil Amazonia. Ladizinsky (1989) observed that rational exploitation of wild relatives is based on three principles: *a*) identification of wild gene pool, *b*) availability of sufficient material for screening and *c*) evaluation and the use of appropriate methods for gene transfer. Of these, the former two were accomplished partly, but the last is crucial that requires more attention.

Wild Germplasm

Since the introduction of rubber to south east Asian countries by Wickham and Cross in 1877 through

Table 2. Climatic features of rubber growing countries

Country	General climate
Brazil	Range: equatorial, tropical, semi arid, high land tropical and sub tropical. Annual average temperature in the Amazon region is 22–26 °C. Brazil is in the south of the equator, seasonal changes are vice - versa compared to north of the equator. Plateau of Sao Paulo is non-traditional area for rubber.
China	Extremely diverse, tropical in south to sub arctic in the north, with great climatic differences resulting from the monsoon, the expanse of the land mass, and the considerable differences in altitude. Typhoons are prudent in southeast China between July and September. China is a non-traditional zone for rubber.
Côte d'Ivoire	Tropical along coast, semi arid in far north; three seasons – warm and dry (November to March), hot and dry (March to May), hot and wet (June to October); Three main climatic regions: the coast, the forest and the savannah. Low rainfall areas in north (less than 1300 mm) are non-traditional experimental zone for rubber.
India	Tropical monsoon type with winter (November to January), Summer (March to May), southwest monsoon season (June to Sept.) and post monsoon or northeast monsoon season (Oct. to Dec.). Most of the rainfall brought by southwest monsoon. Because of the geographical diversity of India, regional climate conditions in the extreme north, east and west vary from the general conditions given here. Specific areas of west, east and northeast are non-traditional for rubber.
Indonesia	Tropical, climate even all year around. Heavy rainfall usually between Dec. and Jan. The equatorial position of the country makes opposite climates in the north and the south.
Liberia	Tropical; hot, humid; dry winters with hot days and cool to cold nights; wet, cloudy summers with frequent heavy showers.
Malaysia	Tropical, annual southwest (April to October) and northeast (October to February) monsoons.
Nigeria	Varies; equatorial in south, tropical in center, arid in north. Two principal wind currents affect Nigeria; the *harmattan*, from the northeast, is hot and dry and carries reddish dust from the desert and causes high temperatures during the day and cool nights. The southwest wind brings cloudy rainy weather.
Sri Lanka	Tropical monsoon; northeast monsoon (December to March); southwest monsoon (June to October).
Thailand	Tropical; rainy, warm, cloudy southwest monsoon (mid-May to September); dry, cool northeast monsoon (November to mid March); southern isthmus always hot and humid. North and northeast areas are non-traditional for rubber.
Vietnam	Tropical in south; monsoonal in north with hot, rainy season (mid-May to mid September) and warm, dry season (mid-October to mid March). Diverse range of latitude, altitude and weather patterns produces enormous climatic variation. North Vietnam like China has two basic seasons: a cold humid winter from Nov. to April, and warm, wet summer for the reminder of the year. The northern provinces share the climate of the north, while the southern provinces share the tropical weather of the south. South Vietnam is relatively warm. Central highlands and the coastal regions are non-traditional areas for rubber.

sources: www.britannica.com; www.worldatlas.com; www.wmo.ch; www.usda.gov; www.iwmi.org

Kew Botanic Gardens, there have been attempts to collect new material and increase the genetic diversity. Between 1945 and 1982, at least 10 collections from Brazil (mostly Rondonia) were undertaken (Concalves et al.). During 1951–52, 1614 seedlings of five *Hevea* species (*H. brasiliensis*, *H. guianensis*, *H. benthamiana*, *H. spruceana and H. pauciflora*) were introduced in Malaysia (Tan 1987). Seeds of different *Hevea* species were also imported from the Schultes Museum at Belem, Brazil in 1966 to Malaysia. In Sri Lanka, 11 clones of *H. brasiliensis* and *H. benthamiana* and 105 hybrid materials were imported during 1957–59, through collaboration of USDA, IAN (Brazil) and Liberia. Many of these clones were later given to Malaysia which were used for further breeding programmes at RRIM (Tan 1987).

IRRDB explorations

With the initiatives taken up by the IRRDB (International Rubber Research and Development Board), 64,734 seeds, 1413 m of bud wood from 194 high yielding trees and 1160 seedlings were collected during 1981 from Acre, Rondonia, and Mato Grosso states of Brazil (Tan 1987). Of this, 37.5% of the seeds went to Malaysia and 12.5% to Côte d'Ivoire while half of the collections were retained in Brazil. The clonal selections were brought to Malaysia and Côte d'Ivoire after quarantine measures for South American Leaf Blight (SALB - *Microcyclus ulei*). IRRDB supports germplasm centers based in Malaysia and Côte d'Ivoire to conserve these materials. Malaysia alone established 8900 seedlings and 109 clones from this exploration (Pushparajah 2001). Crosses between Wickham and Amazonian accessions could introduce more variation. Breeding at IRCA (Institut de Recherches sur le Caoutchouc en Afrique), Côte d'Ivoire, under the auspices of CIRAD, involve utilization of Amazonian accessions (Clément-Demange et al. 1998).

Diseases

Diseases, especially SALB (South American Leaf Blight – *Microcyclus ulei* P. Henn von Arx.) that is singularly devastating is yet another stress factor limiting the yield of rubber. It is noteworthy that unlike other clonally multiplied species, *Hevea* is not affected by viral diseases (Simmonds 1989). Other diseases of economic importance are the *Gloeosporium* leaf disease (*Colletotrichum gloeosporioides* Pen. Sacc.), powdery mildew (*Oidium heveae* Stein.), minor leaf spot (*Corynespora cassiicola* Ber. et Curt. Wei.) and the *Phytophthora* leaf fall (*Phytophthora* sp.). Clonal and location specificity is evident towards resistance to these diseases (Wycherly 1969). A study with *Gloeosporium* showed that clones from Malaysia and Indonesia are resistant while clones from Sri Lanka and China are less resistant. Ho (1986) gives a good narration of the phenomenon of local adaptation that is more evident in the case of minor leaf spot. Malaysian clones exhibited good horizontal resistance (HR), while clones from Thailand and Malaysia were susceptible. The case of SALB is evidently different. The resistance exhibited by the wild relatives *H. benthamiana*, *H. pauciflora* and *H. spruceana* have been exploited through crosses with *H. brasiliensis* but was shown to be vertical resistance (VR), and the crosses became susceptible to newly evolved pathotypes (Ho 1986). This is due to wide pathogenicity of the fungus, the unknown genetic determination of resistance and the ability of new strains to overcome specific resistances (Clément-Demange et al. 1998). Since the wild relatives own only VR, the breeding programmes need to start from a very low level of genetic variability. On the other hand, achieving HR would imply several cycles of selections under epiphytotic conditions. Since HR is polygenic, a fairly high heritability (h^2) would be evident through additive inheritance, where advanced generations produce more resistant progenies (Simmonds 1990). In French Guiana, approximately 200 *M. ulei* strains have been collected from isolates taken from local experimental plantations (Clément-Demange et al. 2000). This diversity explains the rapid development of the pathogen, which is also capable of sexual reproduction. An immediate remedy to SALB is to practice crown budding (Tan 1979). This is based on the assumption that a vigorous, wind-fast, disease resistant crown would provide good flow of photosynthate (Simmonds 1982). However, such exercises need to be done at the field level, where the infection of SALB largely depends on climatic conditions of the location. *M. ulei* requires at least 10 consecutive hours of relative humidity above 95%, with optimum average daily temperatures of 24–26 °C with intermittent rains that are most favourable for germination and infection (Watson 1989). It is imperative that the search for genes conferring HR involves allied species, especially those that defoliate but retain older leaves (see Table 1). Recent research work has been oriented towards identifying sources of resistance at molecular level. This should help in deriving clones with HR.

QTLs and disease resistance

Quantitative trait loci (QTLs) for resistance to SALB were mapped using 195 F_1 progeny derived from crosses between PB 260 (susceptible) and RO 38 (resistant) clones (Lespinasse et al. 2000b), which was done in continuation to a genetic analysis done earlier (Seguin et al. 1996). Eight QTLs were identified for resistance in RO38 map (Lander and Botstein 1989). The F_1 consensus map confirmed results obtained in parental maps. Lespinasse et al. (2000b) further rationalized that the resistance alleles of RO 38 have inherited from a wild grandparent (*H. benthamiana*) and no favourable alleles came from AVROS 363, the Wickham parent. Eight different QTLs for five strains of fungus were available in RO 38, with specificity of resistance to different strains. Resistance that is more durable is likely to be available in other allied species and wild accessions of *Hevea*. Darmono and Chee (1985) while studying lesion size on leaf discs identified SIAL 263, an illegitimate progeny of RRIM 501 as resistant to SALB, indicating thereby that a systematic search in relatives of RRIM 501 would be profitable for SALB resistance (Simmonds 1990).

Molecular Diversity

The association between DNA sequence variation and heritable attributes has helped to define variations in plants at the molecular level. However, identification and utilization of recombinants with desirable traits is time consuming and laborious in rubber due to long generation time and larger size of the crop. With the advent of DNA markers, localization of desirable traits has become routine. The molecular marker systems can be broadly classified into three *viz.*, first generation (RFLPs, RAPDs and modifications); second generation (simple sequence repeats–SSRs, Amplified Length Polymorphism-AFLPs) and third generation markers (Expressed Sequence Tags–ESTs, Single Nucleotide Polymorphism – SNPs) (Gupta et al. 2001). Of these, SNPs are the new generation markers used for Marker Assisted Selection (MAS). All marker systems, except SNPs have been applied in Hevea to facilitate identification and characterization of genes. Recently, a saturated linkage map of *H. brasiliensis* has been accomplished (Lespinasse et al. 2000a). Efforts were on for breeding *Hevea* at the molecular level ever since Low and Bonner (1985) characterized nuclear genomes containing 48% of most slowly annealing DNA (putative single copy) and 32% middle repetitive sequences with remaining highly repetitive or palindromic ones. Also, the whole genome size was calculated as 6×10^8 base pairs.

Nuclear vs. cytoplasmic genes

Further, Besse et al. (1994), using 92 clones of Amazonian origin and 73 Wickham clones did an assessment of RFLP profiles. Interestingly, accessions of Brazil Amazonia could be categorized into genetic groups according to their geographic origin (Acre, Rondonia, Mato-Grosso) (Figure 2). On the other hand, cultivated clones conserved relatively high level of polymorphism, despite narrow genetic base and continuous assortative mating and selection. As expected, polymorphism is very prudent among allied species of *Hevea*. A comparison of isozyme analysis (Lebrun and Chevallier 1990) with that of DNA markers showed much similarity (Besse et al. 1994). Identification of all Wickham clones could be done with 13 probes associated with restriction enzyme *Eco* RI (Besse et al. 1993). The cultivated clones are genetically close to the Mato-Grosso ones. Rondonia and Mato-Grosso clones are more polymorphic as per RFLP data (Besse et al. 1994). A Rondonia clone (RO/C/8/9) showed eight specific restriction fragments and a unique malate dehydrogenase (MDH) allele, indicating this clone is of interspecific origin. Such molecular markers are useful in rubber tree breeding since no distinct morphological traits exist. Mitochondrial DNA (mtDNA) polymorphism was analysed in 345 Amazonian accessions, 50 Wickham clones and two allied species (*H. benthamiana*, *H. pauciflora*) (Luo et al. 1995). While the variation in wild accessions was considerable, the cultivated clones formed only two clusters.

Potentiality of mtDNA

The aforesaid observations amply indicate that the selection was indirectly towards nuclear DNA polymorphism, while evolving modern clones. Luo et al. (1995) argue that the geographic specificity towards nuclear and mtDNA polymorphisms are due to great level of genetic structuring among natural populations in the Amazon forests in relation to hydrographic network. In wild accessions, seed dispersal and selection are as per the environmental conditions. If this is true, we observe that much of the variations produced in the natural habitat are being lost due to selection pressure of environmental factors. This is a matter of concern since the wild accessions have not rendered much contribution in evolving high yielding clones so far, after introduction to other parts of the globe. On the other hand, Wickham clones exhibited high nuclear DNA polymorphism, perhaps due to breeding under different climates. It is presumable that the nuclear genome has been forced to enhance variation to suit the diverse hydrothermal situations of newly introduced areas, resulting in selection of rightly adapted clones under a given environment. mtDNA of Wickham clones has lesser variation because their female progenitors are all primary clones, naturally bred under the similar environmental conditions of Malaysia and Indonesia. These clones were introduced later into India and Sri Lanka for further breeding programmes. Moreover, cytoplasmic donors for most of the improved clones are either PB 56 or Tjir 1 (Figure 3). While the cytoplasm of PB 56 is transferred through PB 5/51, the cytoplasm of Tjir 1 was through RRII 105, RRIM 600 and RRIM 605. In conventional breeding systems followed in rubber, the best parents of one generation are used as parents for the next cycle of breeding (Simmonds 1989). Obviously, this is the reason for the mtDNA profile showing only two clusters. A possible explanation for greater polymorphism in mtDNA in wild accessions is that they must have been evolved through interspecific hybridization. mtDNA polymorphism in wild accessions needs to be exploited fully. A molecular survey of available Amazon accessions and isolation of competent molecular variants in their progeny are the possible exercises that would give meaningful results.

Latex and Timber Clones

Estimation from RRIM shows that a hectare of rubber

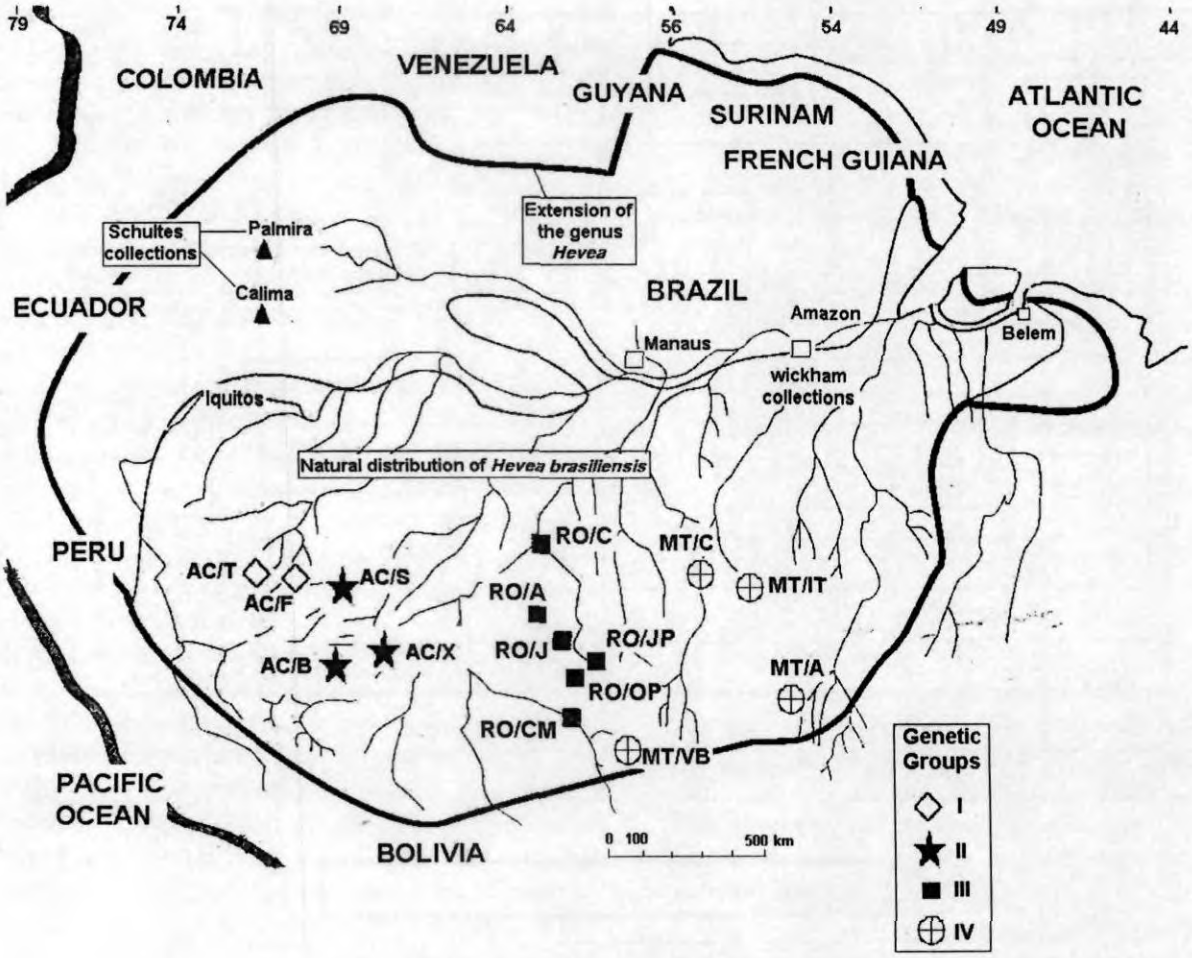
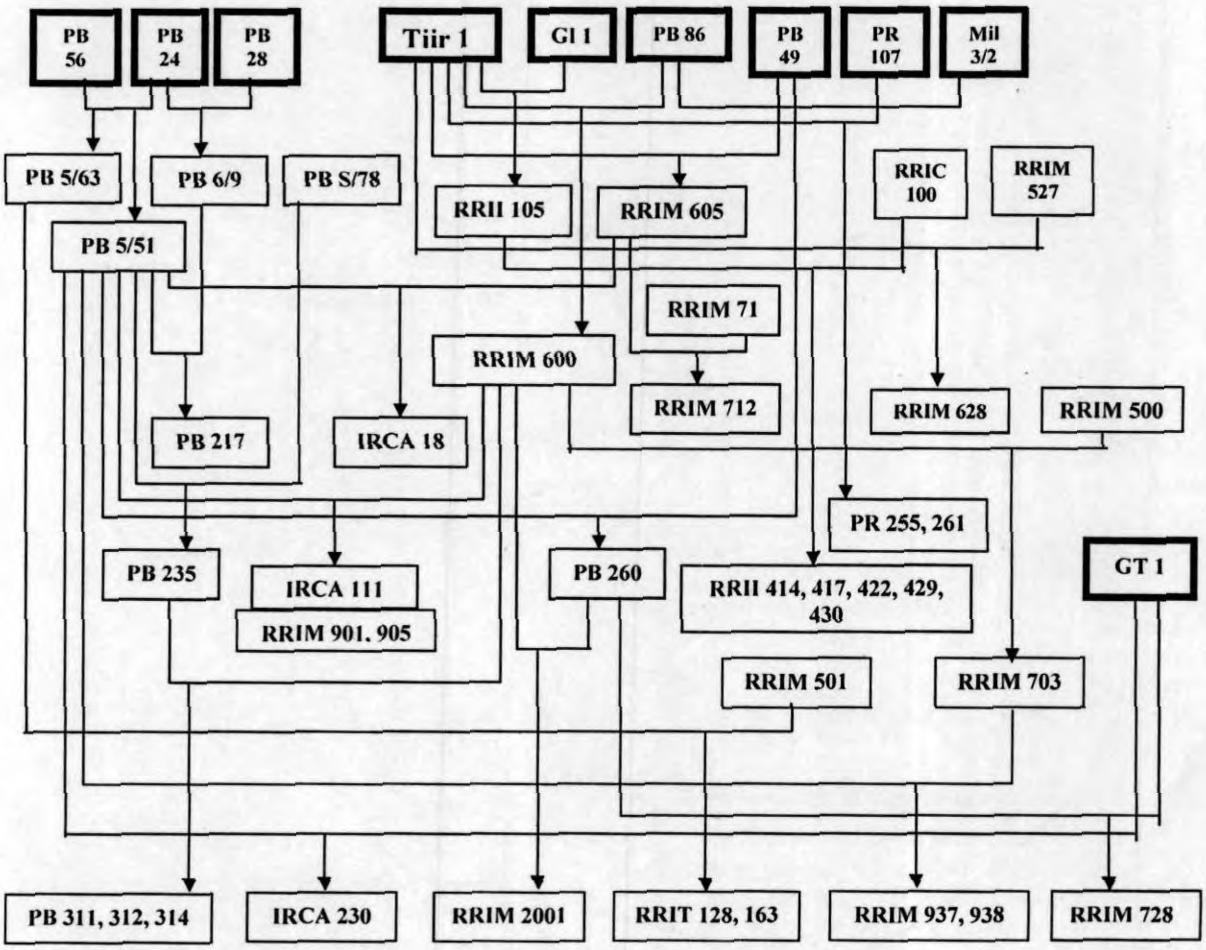


Figure 2. Genetic grouping of wild *Hevea* germplasm

plantation can yield 190 m^3 of rubber wood and by 2000, 2.7 million m^3 of *Hevea* wood would be available from Malaysia (Arshad et al. 1995). This is used for chip logs (for the production of cement board, chip board, band medium density fireboard) and saw logs (for plywood and veneer). Nearly 741 million m^3 of wood must be available from 89,27,000 ha worldwide. The demand is expected to increase by 2012. RRIM, RRIT, and RRII have been making concerted efforts in deriving latex timber clones (Table 3). PB 235, PB 260, RRIM 2008 and RRIM 2014 are promising because they also yield higher. A few accessions of allied species like *H. pauciflora, H. guianensis and H. nitida* also yielded wood volume in the range of 1.14 to 3.64 m^3/tree, with *H. guianensis* being the highest yielder. Nearly 20 clones of 1981 Amazonian collection were also selected for timber yield by the RRIM yielding at a range of 1.438 to 2.518 m^3/tree at the age of 13 years. The African center of CIRAD identified 50 Amazonian clones best adapted for timber use (Clément-Demange et al. 1998). In 1995, RRIM and Forest Research Institute of Malaysia (FRIM) collaborated in an expedition to Brazil to collect seeds from mother trees with vigorous tall straight boles. More than 4,00,000 seedlings were secured to form a nucleus of ortets for future breeding programmes (Pushparajah 2001). It is pertinent to increase production of rubber wood due to constant decline in area under both smallholdings and estates. However, this attribute needs to be complimented with latex yield probably through intercrossing and selection.

Abiotic Stresses

Major attributes that need more variation in the breeding populations are wind, cold and drought tolerance. While China experiences cold and severe winds, Tripura and north Côte d'Ivoire experience cold and



Clones in darkened boxes are primary clones

Abbreviations:

Gl = Glenshiel, Malaysia; GT = Gondang Tapen, Indonesia;

IRCA = Institute de Recherches sur le Caoutchouc en Afrique, (Côte d'Ivoire);

Mil = Milakande, Sri Lanka;

RRIC = Rubber Research Institute of Ceylon (currently Sri Lanka);

RRII = Rubber Research Institute of India; RRIM = Rubber Research Institute of Malaysia;

RRIT = Rubber Research Institute of Thailand;

Tjir = Tjirandji, Indonesia; PB = Prang Besar, Malaysia;

PR = Profestation voor Rubber, Indonesia

Figure 3. Inheritance of PB 56 and Tjir 1 cytoplasm for derivation of clones

dry seasons respectively. China over the years evolved a breeding strategy to integrate both yield and secondary attributes like resistance to wind and cold. It has developed five new clones incorporating both higher yield and stress tolerance (Huasun et al. 1998). Two clones, 'SCATC 7-33-97' and 'SCATC 8-333' gave 1977 kg/ha and 2187 kg/ha, that is 69.7% and 81.5% more than 'RRIM 600' respectively. They are

Table 3. Estimated wood volume from clones with good potential, accessions of Brazil Amazonia and allied species.

Clone	Parentage	Age(year)	Clear bole volume m^3/tree	Canopy wood volume m^3/tree	Total wood volume m^3/tree
RRIM 910	PB 5/51 × RRIM 623	22	0.76	0.57	1.33
RRIM 912	-do-	22	0.75	0.75	1.50
RRIM 931	PB 5/51 × RRIM 713	20	0.68	0.68	1.36
PB 235	PB 5/51 × PB S/78	20	0.80	0.80	1.60
PB 355	PB 235 × PR 107	22	0.93	2.32	3.25
RRIM 2008	RRIM 623 × PB 252	14	0.33	0.99	1.32
RRIM 2014	RRIM 717 × PR 261	14	0.53	0.80	1.33
Clones of Brazil Amazonia:					
RO/OP/4-20/125	–	13	1.259	1.159	2.518
AC/F/5-21/197	–	13	1.403	1.052	2.455
MT/C/5-12/137	–	13	1.054	1.318	2.372
AC/F/21-64/221	–	13	1.137	1.364	2.501
Allied species:					
H. pauciflora	–	24	1.13	0.41	1.14
H. guianensis	–	24	1.45	2.18	3.64
H. nitida	–	24	1.04	1.04	2.08

After Arshad et al. (1995)

directly or indirectly derived from either 'RRIM 600' or 'PR 107'. Primary clones such as 'HAIKEN 1' and 'PR 107' were used in deriving wind fast clones 'Xuyu 141–2', 'Wenchang 217' and 'Wenchang 33–24'. Of these, 'Wuyu 141–2' could withstand typhoons of < 12 on the Beaufort scale (Huasun et al. 1998). China proposes to integrate allied species and Brazilian accessions into their breeding programmes (Senyuan 1990). Three Chinese clones (SCATC 88–13, SCATC 93–114 and Haiken 1) are being evaluated in Tripura along with other clones developed in India. Of these, 'HAIKEN 1' can be adjudged as higher yielder with wind endurance and 'SCATC 93-114' exhibits better girth increment during cold period, but a low yielder (Priyadarshan et al. 1998a, 1998b). The clones being evaluated in Côte d'Ivoire are different from India and China since many of its new experimental areas experience moisture deficit in addition to severe wind. The growth of rubber is significantly influenced by rainfall (Dea et al. 1997).

Conclusions

The extension of *Hevea* to marginal environments has prompted the search for variation. The distribution of allied species with more adaptability holds promise to derive clones with specific adaptation. Clones derived from crosses involving *H. benthamiana* such as IAN 6158 and FX 2784 (Wickham × Amazonian hybrid) are seen to be very resistant to SALB. Amazonian accessions found to be resistant to SALB are: AC/F/6A/36/376, AC/F/6A/36/485, AC/S/8/40, RO/JP/3/22/374, RO/JP/3/22/418, and RO/JP/3/22/44, indicating that genes for resistance are available in the Amazonian clones, so a cross between Wickham × Amazonian would be a good compromise between yield and resistance (Clément-Demange et al. 1998). Three isolated breeding orchards have been created at Côte d'Ivoire, for Amazonian × Amazonian crosses. Such crosses are promising since many of these accessions must be the result of interspecific hybridization, evidenced by existence of morphological variants e.g. in flower colour (Jayashree et al. 1997). First seeds from such crosses were made available in 1996, which will form the basis for intense selection for yield (Clément-Demange et al. 1998). This programme will ensure the reduction of gap between Wickham and wild accessions as far as yield is concerned, and widen the gap with regard to disease resistance. Hence, Clément-Demange et al. (1998) propose that the data collected from accessions of Brazil Amazonia on agronomic attributes, molecular markers and isozymes are to be evaluated following the 'Principal Component Scoring Strategy' of Noirot et al. (1996). Such exercises must ensure systematic selection of genotypes with both yielding and resistance capabilities at the juvenile stage. Clones resulting from these selections need to be evaluated in International Multi-environmental Trials, involving sites of Latin America for SALB and at ecological sites affected by *Colletotrichum gloeosporioides* and *Corynespora cassiicola*. Two such trials have already been set up in Cameroon. Crosses between allied species and *H. brasiliensis* must also hold promise for deriving clones resistant to diseases inflicting damage during refoliation. Some of the allied species never defoliate completely and show the tendency of retaining old leaves that would help to circumvent the infection.

Wind resistance and cold tolerance are the two attributes that deserve ample attention. Breeding for these attributes requires variation in the population that can be had from either allied species or accessions of Brazil Amazonia. An attribute that helps to render wind resistance is short stature, available in allied species. Artificial pruning, raising high-density plantations and delaying of tapping are some of the criteria followed in China and Côte d'Ivoire (Zongdao and Xuequin (1983), Clément-Demange–personal communication). China indeed has made good progress in breeding for cold tolerance and wishes to utilize more variants in their programmes (Huasun et al. 1998). At least three allied species have high timber yield awaiting exploitation.

Early seedling selection is one of the prime strategies for the improvement of *Hevea*, since it takes almost 35 years to develop a new clone. Parameters studied include girth, height, bark thickness, latex vessel number, latex vessel and sieve tube diameters and rubber hydrocarbon in bark and petiole, but the relationships with eventual yield were inconsistent. This inconsistency calls for development of suitable isozyme / molecular markers to exercise early selection from seedlings.

Though identification and screening of allied resources could be accomplished, the gene transfer mechanism needs to be streamlined so that clones can be evolved as soon as possible. The aforesaid discussion amply proves the need to integrate allied species and accessions of Brazil Amazonia into the *Hevea* breeding programmes.

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