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Use of *Hevea* Gene Pool in Rubber Tree (*Hevea brasiliensis* Muell.-Arg) Breeding

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The conservation and utilisation of allied gene resources is vital for the improvement of crop species. Rubber has been an undeniable beneficial commodity for the past 100 years. Progress in yield improvement over 70 years resulted in primary and hybrid clones with exceptional yielding abilities. But the extension of Hevea *rubber 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 like low temperature, wind, higher altitude, moisture deficit and diseases. Allied species and accessions of Brazilian Amazonian* Hevea *can be integrated into breeding programmes. Many of these clones are resultant of natural interspecific hybridisations. Also, allied species are excellent resources of timber. Molecular interventions revealed mt DNA 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 promise a source of variation for breeding and selection of clones for specific marginal areas.*

Keywords: *Allied species, gene resources,* Hevea *breeding, molecular diversity, marginal areas, cytoplasm, latex-timber clones, stress factors.*

Plants are useful to the world as food, feed, fiber or fuel. Among them, rubber tree (*Hevea brasiliensis* Willd. ex Adr. de Juss.) Muell.-Arg. owes an exceptional position as the chief source of natural rubber (Greek, 1991). Rubber has been an undeniable beneficial commodity from more than 100 years ago. Rubber latex is synthesised in over 2 000 plant species confined to 300 genera of seven families *viz.*, Euphorbiaceae, Apocyanaceae, Asclepiadaceae, Asteraceae, Moraceae, Papaveraceae and Sapotaceae (Cornish *et al.*, 1993). At least two fungal species are also known to make natural rubber (Stewart *et al.*, 1955). Guayule (*Parthenium argentatum* Gray - Asteraceae), an alternate rubber source, and a shrub native to Chihuahuan desert of Mexico provides only 10 per cent of the world's natural rubber. Guayule can withstand temperature range of –18 to 49°C and can

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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.*

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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 per hectare (Estilai & Ray, 1991). However, guayule latex is useful in developing hypoallergenic latex products (Cornish & Siler, 1996). 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).

Progress in yield improvements in *Hevea* rubber worldwide through concerted efforts of various institutions like Rubber Research Institute of Malaysia (RRIM), Rubber Research Institute of India (RRII), Rubber Research Institute of Sri Lanka (RRISL) and Centre de Coopération Internarionale en Researche Agronomique pour le Développement (CIRAD), France, resulted in gradual increment of yield from 650 to 2 000 kg per hectare. This was further improved to 2 500 kg per hectare (RRIM 2000 series) during late 90s. During these 70 years of rigorous breeding and selection, notable clones such as 'RRIM 501', 'RRIM 600' and 'RRIM 712' were derived (Tan, 1987). Some of the primary clones like 'PB 56', 'Tjir 1', 'Pil B84', 'Pil D65', 'Gl 1', and 'PB 86' selected during this period later became parents of improved clones. Modern clones of the RRIM 2000 series also have their ancestry in these clones.

HEVEA AS A SPECIES COMPLEX

The genus *Hevea* has 10 species, which are inter crossable (Clement-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 in 1906, the species concept crystallised with nine species in 1970 (Schultes, 1977). A tenth species, *H. camargoana* was added during 1971 (Schultes, 1987). *H. paludosa* has been identified in Brazil as eleventh species (Pires, 1973; Goncalves *et al.*, 1990). Three botanists shall be 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 Archives 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."

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* hybridisation 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 5S rDNA during the course of evolution. Hence, as long as a potential ancestor with $2n = 18$ is unknown, rubber tree will be considered as an amphidiploid. The genus *Hevea* could eventually be considered as a species complex.

DISTRIBUTION OF ALLIED SPECIES

The distribution of allied species of *Hevea* is wide among the countries of South America . *Hevea* species are indigenous to Bolivia, Brazil,

TABLE 1

ALLIED SPECIES OF THE GENUS *HEVEA* - OCCURRENCE AND FEATURES

Species	*Occurrence*	*Notable features*[1]
H. benthamiana Muell -Arg	North and West of Amazon forest basin, upper Orinoco basin (Brazil)	Complete defoliation of leaves. Medium size tree. Habitat: swamp forests.
H. brasiliensis (Willd. ex. A de Juss.) Muell. - Arg	South of Amazon river (Brazil, Bolivia, Ecuador, Peru)	Complete defoliation of leaves. From medium to big tree size. Habitat: well drained soils.
H. camargoana Pires	Restricted to Marajo island of Amazon river delta (Brazil)	Possibility of natural hybridisation *H. brasiliensis* from 2 m to 25 m height. Habitat: seasonally flooded swamps, xeromorphic.
H. camporum Ducke	South of Amazon between Marmelos and Manicore rivers tributaries of Madeira river.	Retain old leaves until new leaves appear. Maximum 2 m tall. Habitat: dry savannahs, xeromorphic.
H. guianensis Aublet	Throughout the geographic range of the genus (Brazil, Venezuela, Bolivia, French Guyana, Peru, Colombia, Surinam, Ecuador)	Retain 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 of leaves. Small trees. They live on flooded area (igapos). Habitat: sandy or lateritis 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 (2m). *Habitat: quartzitic soils, xeromorphic.*
H. pauciflora (Spr ex Bth) Muell - Arg	North and West of Amazon river (Brazil, Guyana, Peru). Distribution discontinuous due to habitat preferences.	Retain 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 Bth) Muell - Arg	Among Negro river and its affluents. Uaupes and Icana rivers (Brazil, Colombia and Venezuela).	Retain old leaves even after inflorescences appear. Small tree from savannahs. Sometime tall, with small crown on the top. Habitat: well drained soils.
H spruceana (Bth) 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; up to 30 m height. Habitat: marshy areas.

after Wycherley (1992); Schultes (1977); Goncalves *et al.* (1990); Pires (1973); Brazil (1971) and Pushparajah (2001)

[1] Wintering characteristics mentioned here has a bearing on the incidence of fungal diseases especially secondary leaf fall (Oidium) since retention of older leaves may make the tree 'oidium escape'. Dwarf types are desirable of the possible wind fastness. All species are diploid (2n = 36) (Majumder, 1964), and are crossable among themselves (Clement-Demange *et al.*, 2000).

[2] Pires (1973) considered 11 species including *H paludosa*; Brazil (1971) considers 11 species.

Colombia, French Guiana, Guayana, Peru, Surinam and Venezuela. All species except *H. microphylla* occur in Brazil, the center of origin (Goncalves *et al.*, 1990). Four species have been found in Colombia and three occur in Venezuela. Two occur in Bolivia, French and British Guyanas. *H. guianensis* is the most widely adapted species (Pushparajah, 2001). Temperate type rubber thrives up to 2 500-3 000 m in the Andes Mountains (Senyuan, 1990). These species of *Hevea* were evolved in Amazonian forests over 100 thousand years ago (Clement-Demange *et al.*, 2000). It is pertinent that species adaptation to a particular area is as per climatic and edaphic requirements. The center of diversity lies within the constantly humid equatorial zone where the amount of precipitation is at least twice the evaporation losses on a yearly basis (Pushparajah, 2001). Species like *H. camporum*, *H. paludosa* and *H. rigidifolia* shows only limited adaptation. The specific adaptation 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), none of the other species has been utilised for the improvement of rubber tree.

ENVIRONMENTS FOR RUBBER

Hevea brasiliensis is native to the rain forests of the Tropical region of the Great Amazonian basin of South America. This area, between equator and 15° south is distinctly characterised by its flat land with altitudes not exceeding 200 m with a wet equatorial climate (Strahler, 1969). The mean monthly temperature is 25 to 28°C with abundant rainfall of more than 2 000 mm per year, without any real dry season. The climatic conditions of Brazil delineate four distinct zones *viz.*, the Amazonian Basin, the Brazilian plateau, the coastlands within the tropics and the southern states. Tropical temperatures (27-32°C) make the environment in Brazilian plateau a different one, where some of the areas like Sao Paulo are found to be ideal for rubber. However, in southern states, rubber is not prominent. The three attributes ideal for rubber cultivation are:

a) 2 000 - 4 000 mm rainfall distributed over 100-150 rainy days per annum (Pushparajah, 1977; Yew, 1982; Watson, 1989),

b) mean annual temperature around 28 + 2°C with a diurnal variation of about 7 °C (Barry & Chorley, 1976) and

c) sunshine hours of about 2 000 h per year at the rate of 6 h per day in all months (Pushparajah, 1977; Yew, 1982; Ong *et al.*, 1998). In a study with hydrothermal index, Rao *et al.* (1993) rationalised Senai of Malaysia (1° 36 N'; 103° 39' E) to be the most suitable area for rubber cultivation and production.

The increased global demand for rubber as also the extension in cultivation of other agricultural crops prompted the countries outside the hitherto traditional zone to focus their attention on the cultivation of rubber (Pushparajah, 1983). The decision by countries to utilise 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 inevitable.

The mean annual temperature decreases when one moves away from the equator with more prominent winter conditions either during November – January (towards north) or June to August (towards south). Northeastern states of India, south China, north and north east Thailand, north Côte d'Ivoire and highlands and coastal areas of Vietnam though satisfy some of the basic requirements for rubber, having stress situations like low temperature, typhoons, dry periods and higher altitude (Priyadarshan *et al.*, 2001; Zongdao & Yanqing, 1992; Watson, 1989; Tuy *et al.*, 1997; Dea *et al.*, 1997). Similarly, southern plateau of Brazil (450-500 m MSL) especially the state of Sao Paulo (23 °S) is being experimented for rubber cultivation (Costa *et al.*, 2000). Brazil, being on the west of the Greenwich Meridian offers entirely different climate for rubber causing 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 reveals a spectrum of climatic conditions over which rubber is being grown. Many of them offer marginal environments (*Table 2*). A multitude of limitations *viz.*, moisture stress, low temperature, wind, high altitude and disease epidemics, apart from altered soil physical factors make these areas marginal. 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 & Frere, 1982). However, this is not reflected in yielding potential of clones under these environments.

As aforesaid, the 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 during summer months with occasional days getting as hot as 47° C, and although it receives rainfall of 2 430 mm; 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 part of India, northeastern Thailand, and the highlands of Vietnam (450-900 m MSL). Areas of China, Northeastern India, Thailand and highlands of Vietnam experience cold temperature resulting in yield depression.

Wind is another abiotic stress influencing establishment and growth of rubber. Wind speeds of 2.0 - 2.9 m per second retards rubber growth and latex flow and that of 3.0 m per second and above severely inhibit normal growth. Wind over Beaufort force 10 (more than 24.5 m per second) prevalent in China, during June to October play havoc with branch breaks, trunk snaps, and uprooting of trees (Zongdao & Xueqin 1983; Watson 1989). In Tripura, wind speeds of about 125 km per hour often accompanied with hails had been experienced inflicting bark wounds and damages to the tree either partially or wholly. Studies in China reveal clones 'PR 107' and 'Haiken 1' can be considered wind tolerant and in addition, 'PB 5/51' and 'PR 107' appears to be wind enduring in Tripura (Priyadarshan *et al.*, 1998a). Establishment of shelterbelts consisting of fast growing and wind resistant species is one remedial measure being followed in China to circumvent wind damages. Alternatively, adoption of judicial pruning of branches and dwarfing techniques are shown to reduce wind damage from 25.3 to 13.7 per cent in a study conducted in China. Raising high-density plantations in wind prone areas is another viable alternative being adopted in Cote

TABLE 2
SPECTRUM OF CLIMATIC FEATURES OF RUBBER GROWING COUNTRIES

Country	*General climatic features*
Malaysia	Tropical, annual southwest (April to October) and northeast (October to February) monsoons.
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.
India	Tropical monsoon type with winter (November to January), Summer (March to May), southwest monsoon season (June to September) and post monsoon or northeast monsoon season (October to December). 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 varies from the general conditions given here. Specific areas of west, east and northeast are non-traditional for rubber.
Sri Lanka	Tropical monsoon; northeast monsoon (December to March); southwest monsoon (June to October).
Indonesia	Tropical, climate even all year around. Heavy rainfall usually between December and January. The equatorial position of the country makes opposite climates in the north and the south.
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.
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 November to April, and warm, wet summer for the remainder 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.
Cote 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.
Nigeria	Varies; equatorial in south, tropical in centre, 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.
Liberia	Tropical; hot, humid; dry winters with hot days and cool to cold nights; wet, cloudy summers with frequent heavy showers.
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.

d'Ivoire (A Clement-Demange, personal communication). Tapping in such areas should commence after the trees attaining 65 cm girth.

The strategies feasible for a new area for rubber cultivation would be: evaluation of potential clones, evaluation of polyclonal seedlings, recombination breeding and evaluation of germplasm. Variation in the breeding population is the key attribute that governs the success of any of these strategies, since the environment of a particular location and the interaction of genotypes necessarily helps the breeder to evolve clones with better adaptation. Plant breeders normally use only available genetic diversity in the cultivated germplasm. However, the case of *Hevea* is exceptional since the cultivated clones are bred from only 22 seedlings of Wickham collection (Baulkwill, 1989) for the past 70 years. The breeding intensively involved generation wise assortative mating and selection, where the best suitable genotype will be taken as a parent for the next generation. This ensured erosion of genetic variability, obviously prompting breeders to look for genetic diversity in wild Brazilian Amazonian germplasm. 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 and deserves ample attention.

CLONES OF BRAZILIAN AMAZONIA

Since the introduction of *Hevea* rubber by Wickham, through Kew Botanic Gardens to south east Asian countries, there had been a few attempts to collect new material and increase genetic diversity. Between 1945 and 1982, at least 10 collections from Brazil (mostly Rondonia) were undertaken (Goncalves *et al.*, 1983). During 1951-52, 1 614 seedlings of five *Hevea* species (*H. brasiliensis, H. guianensis, H. benthamiana, H. spruceana* and *H. pauciflora*) were introduced to 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, H. benthamiana* and 105 hybrid materials were imported during 1957-59, through triangular collaboration of USDA, *Instituto Agronomico do Norte* (IAN), Brazil, and Liberia. Many of these clones were later given to Malaysia which were used for further breeding programmes at RRIM (Tan, 1987).

With the initiatives taken up by the IRRDB (International Rubber Research and Development Board), 64 734 seeds from 194 high yielding trees and 1 160 seedlings were collected during 1981 from Acre, Rondonia, and Mato Grosso states of Brazil (Tan, 1987). Of this, 37.5 per cent of the seeds went to Malaysia and 12.5 per cent to Côte d'Ivoire and 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. Crosses between Wickham and Amazonian accessions could introduce more variation. Breeding in IRCA (*Institut de Recherches sur le Caoutchouc en Afrique*), Côte d'Ivoire, under the auspices of CIRAD, involve utilisation of Amazonian accessions (Clement-Demange *et al.*, 1998).

BIOTIC STRESSES

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 *Hevea*. 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* (Penz) Sacc.), powdery mildew (*Oidium heveae* Stein.), minor leaf spot *(Corynespora cassiicola)* (Berk et. Curt.) Wei. and the Phytophthora leaf fall (*Phytophthora* sp). A study with *Gloeosporium* showed that clones from Malaysia and Indonesia are resistant while clones from Sri Lanka and China are less resistant. But clones from South America are seen to be highly resistant indicating local adaptation rather than breeding is the cause for the resistance (Simmonds, 1989). Ho (1986) gives a good narration of the breeding implications of diseases in *Hevea*. It is imperative that too much susceptible genotypes are rejected at the first instance and the survivors are seen to be moderately resistant. The phenomenon of local adaptation is more evident in the case of minor leaf spot. While Malaysian clones exhibited good horizontal resistance (HR), clones from Thailand were susceptible. The case of SALB is evidently different. The resistance exhibited by wild relatives like *H. benthamiana, H. pauciflora* and *H. spruceana* has been exploited through crosses with *H. brasiliensis* but were turned to be vertical resistance (VR), and were susceptible to newly evolved pathotypes (Ho, 1986). This is due to wide pathogenicity of the fungus, the unknown genetic determination of resistance and ability of new strains to overcome specific resistances (Clement-Demange *et al.*, 1998). On the other hand, achieving HR would imply several cycles of selections under epiphytotic conditions. Research work of late, has been oriented towards identifying sources of resistance at molecular level. This should amply help in deriving clones with HR.

Quantitative trait loci (QTLs) for resistance to SALB were mapped using 195 F_1 progeny derived from cross 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 & Botstein, 1989). The F_1 consensus map confirmed results obtained in parental maps. Lespinasse *et al.* (2000b) further rationalised that the resistance of RO 38 was contributed by the wild parent (*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 shall be available in other allied species and wild accessions of *Hevea*. However, the selection of clones with durable resistance is also of much importance while undertaking such studies. 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).

NUCLEAR AND CYTOPLASMIC GENES

The association between DNA sequence variation and heritable attributes has helped to define variations in plants at the molecular level. Identification and utilisation 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, localisation 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 Fragment 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 characterisation of genes. Recently, a saturated linkage map of *H. brasiliensis* has been reported (Lespinasse *et al*., 2000a).

Efforts were on for breeding *Hevea* at the molecular level ever since Low and Bonner (1985) characterised nuclear genome containing 48 per cent of most slowly annealing DNA (putative single copy) and 32 per cent middle repetitive sequences with remaining highly repetitive or palindromic. Also, the whole genome size was calculated as 6 x 10^8 base pairs. Further, Besse *et al*. (1994), using 92 clones of Amazonian prospection and 73 Wickham clones did an assessment of RFLP profiles. Interestingly, accessions of Brazilian Amazonian could be categorised into genetic groups according to their geographic origin (Acre, Rondonia, Mato-Grosso). On the other hand, cultivated clones conserved relatively high level of polymorphism, despite narrow genetic base and continuous assortative mating and selection. Similar results were reported by Varghese *et al*. (1997) while evaluating the applicability of RAPD markers for genetic analysis in *Hevea* using 42 informative primers in a set of 24 clones from Rubber Research Institute of India. As expected, polymorphism is very prudent among allied species of *Hevea*. A comparison of isozyme analysis (Lebrun & 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 near to Mato-Grosso. Rondonia and Mato-Grosso clones are more polymorphic as per RFLP data (Besse *et al*., 1994). A Rondonia clone (RO/C/8/9) shows eight specific restriction fragments and a unique malate dehydrogenase (MDH) allele, indicating this clone is of interspecific origin. 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.

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 natural habitat are being lost due to selection pressure of environmental factors. On the other hand, Wickham clones exhibited high nuclear DNA polymorphism, perhaps, due to breeding under differential geo-climates. mtDNA of

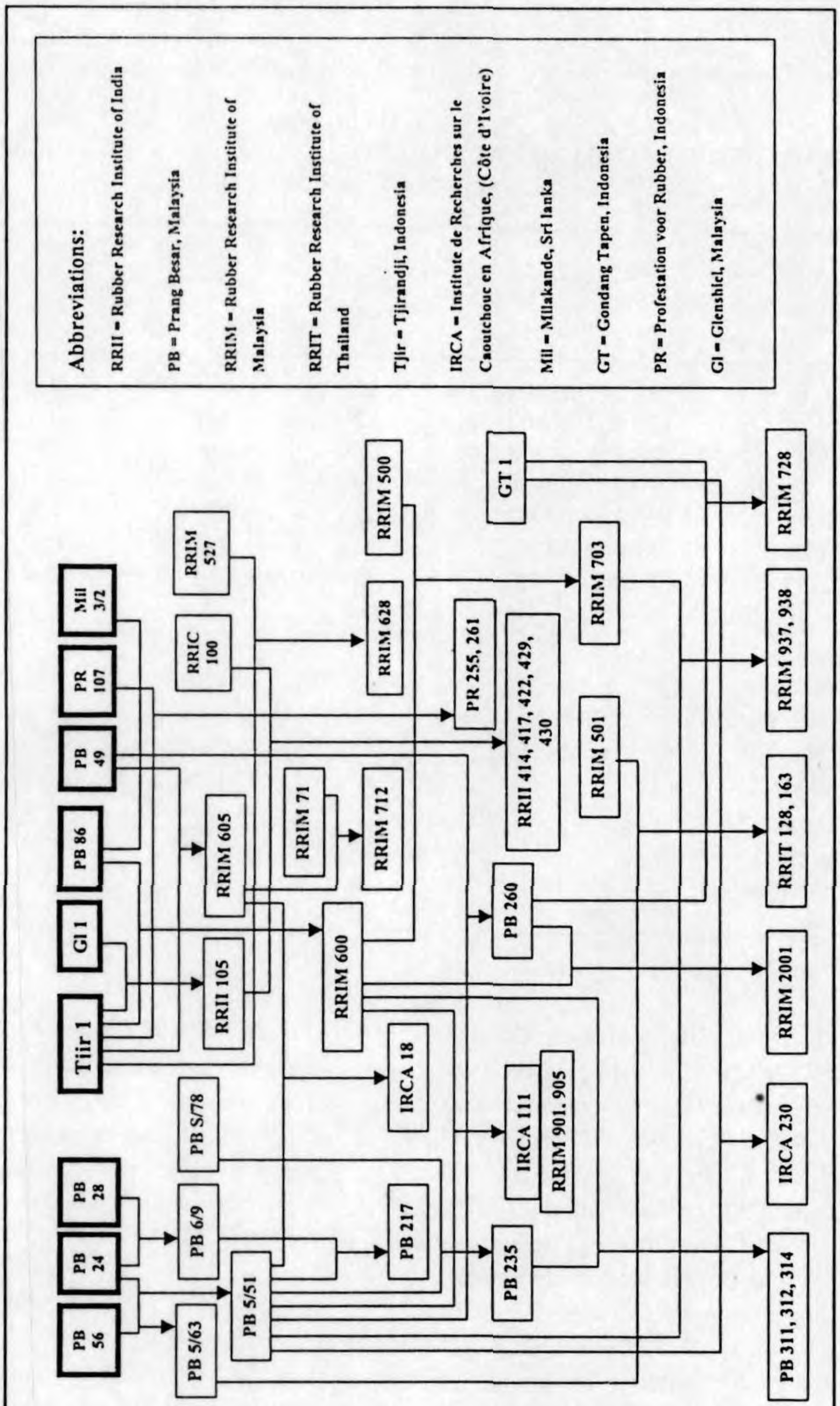
Wickham clones has lesser variation for their female progenitors are all primary clones, naturally bred under the similar environmental conditions of Malaysia and Indonesia. These clones were introduced later into India and Sri Lanka for further breeding programmes. Moreover, cytoplasmic donors for most of the improved clones are either PB 56 or Tjir 1 directly or indirectly through other clones that were used as female parents (*Figure 1*). 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 system followed in rubber, the best parent of one generation is used as parent 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 hybridisation. mtDNA polymorphism in wild accessions needs to be exploited fully.

LATEX TIMBER CLONES

Estimation from RRIM shows that a hectare of rubber plantation can yield 190 m^3 of rubberwood and by 2000, 2.7 million m^3 of rubberwood 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 operations). Theoretical estimations indicate that India is expected to have 48 million m^3 of growing stock from 518 000 ha (Anon, 1996). Hence, nearly 741 million m^3 of wood must be available from 8 927 000 ha worldwide. The demand for wood is expected to increase by 2012. The Rubber Research Institute of Malaysia has been making concerted efforts in deriving latex timber clones (*Table. 3*). PB 235, PB 260, RRIM 2008 and RRIM 2014 are promising because they are complimented with higher rubber yield also. 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 per tree, with *H. guianensis* being the highest yielder (*Table 3*; After Arshad *et al.*,1995). 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 per tree at the age of 13 years. IRCA (*Institute de Recherches sur le Caoutchouc en Afrique*, Côte d'Ivoire) identified 50 Amazonian clones best adapted for timber use (Clement-Demange *et al.*, 1998). It is pertinent to increase production of rubberwood 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.

WIND FASTNESS AND COLD ENDURANCE

Two major attributes that need more variation in the breeding population are wind fastness and cold endurance. While China experiences cold and severe wind, Tripura and north Côte d'Ivoire experience cold and moisture deficit respectively. China, over the years evolved a breeding strategy to integrate both yield and secondary attributes like wind fastness and cold endurance. 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 1 977 kg per hectare and 2 187 kg per hectare, that is 69.7 and 81.5 per cent more than RRIM 600 respectively. They are derived



Abbreviations:

RRII = Rubber Research Institute of India

PB = Prang Besar, Malaysia

RRIM = Rubber Research Institute of Malaysia

RRIT = Rubber Research Institute of Thailand

Tjir = Tjirandji, Indonesia

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

Mil = Milakande, Sri lanka

GT = Gondang Tapen, Indonesia

PR = Profestation voor Rubber, Indonesia

Gl = Glenshiel, Malaysia

Figure 2. Inheritance of PB 56 and Tjir 1 cytoplasm for derivation of clones

TABLE 3
ESTIMATED WOOD VOLUME FROM POTENTIAL CLONES, ACCESSIONS OF BRAZILIAN AMAZONIAN 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 x RRIM 623	22	0.76	0.57	1.33
RRIM 912	PB 5/51 x RRIM 623	22	0.75	0.75	1.50
RRIM 931	PB 5/51 x RRIM 713	20	0.68	0.68	1.36
PB 235	PB 5/51 x PB S/78	20	0.80	0.80	1.60
PB 355	PB 235 x PR 107	22	0.93	2.32	3.25
RRIM 2008	RRIM 623 x PB 252	14	0.33	0.99	1.32
RRIM 2014	RRIM 717 x PR 261	14	0.53	0.80	1.33
Clones of Brazilian 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

From Arshad *et al.* (1995)

directly or indirectly from either RRIM 600 or PR 107. Primary clones like 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. Three Chinese clones (SCATC 88-13, SCATC 93-114 and Haiken 1) are being evaluated in Tripura along with other clones developed in India, Malaysia and Indonesia. 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.*, 1998 a,b). 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 there is significantly influenced by rainfall (Dea *et al.*, 1997).

CONCLUSIONS

The extension of *Hevea* cultivation to marginal environments has prompted the search for variation. The distribution of allied species under differential geo-climates with wider adaptability holds promise to derive clones with specific adaptation. Clones derived from crosses involving *H. benthamiana* like IAN 6158 are seen to be very resistant to SALB. FX 2784 (Wickham x Amazonian hybrid) is an example. 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. The rationale is that genes for resistance are available in the Amazonian clones and a cross between Wickham x Amazonian would be a good compromise between yield and resistance (Clement-Demange *et al.*, 1998). Three isolated breeding orchards had been created at Côte d'Ivoire, for Amazonian x Amazonian crosses. Such crosses hold promise since many of these accessions must be the result of interspecific hybridisation, as evidenced by existence of morphological variants *viz.*, flower colour (Madhavan *et al.*, 1997). First seeds from such crosses were made available in 1996, which will form the basis for intense selection for yield (Clement-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, Clement-Demange *et al.* (1998) proposes that the data collected from accessions of Brazilian Amazonian accessions on agronomic attributes, molecular markers and isozymes are to be evaluated following a 'Principal Component Scoring Strategy' of Noirot *et al.* (1996). Such exercises must ensure systematic selection of genotypes owning both yielding and resistance capabilities at the juvenile stage. Clones emanating from these selections need to be evaluated under 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 infection of diseases, especially powdery mildew (*Table 1*).

Wind fastness and cold endurance are the two attributes that deserve ample attention. Breeding for these attributes needs variation in the population that can be had from either allied species or Accessions of Brazilian Amazonian. An attribute that helps to render wind fastness is short stature that is available in allied species. Artificial pruning, raising high-density plantations and delaying tapping are some of the criteria followed in China and Côte d'Ivoire (Zongdao & Xueqin, 1983; Clement-Demange – personal communication). China indeed has gone far ahead in breeding for cold tolerance and wish to utilise more variants in their programmes (Huasun *et al.*, 1998). At least three allied species own high timber yield that needs to be exploited fully.

Though identification and screening of allied resources could be accomplished, the gene transfer mechanism needs to be streamlined so that clones can be evolved within minimum time permissible. The aforesaid discussion amply proves the need to integrate allied species and accessions of Brazilian Amazonian into the *Hevea* breeding programmes.

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