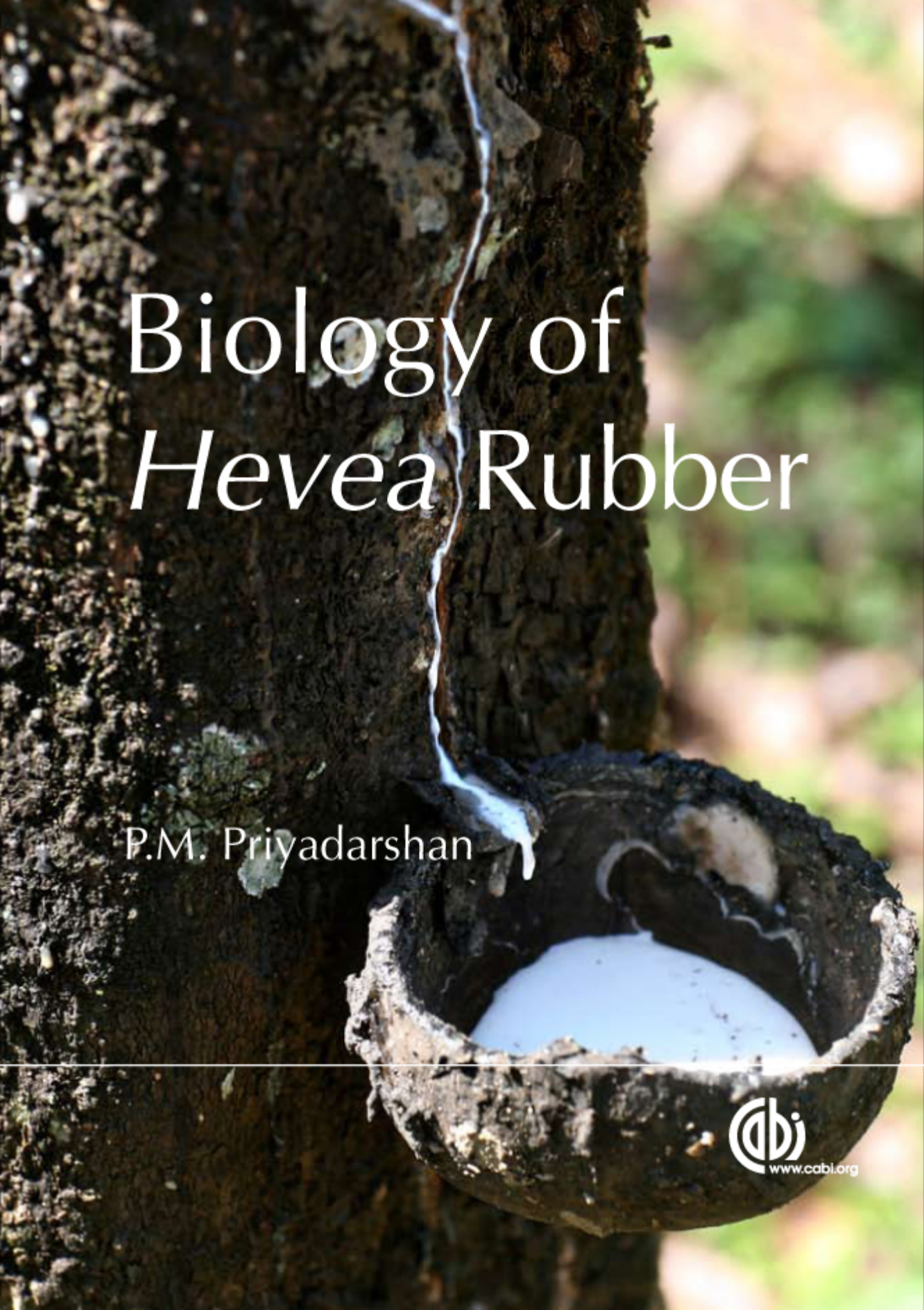

Biology of *Hevea* Rubber

P.M. Priyadarshan

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BIOLOGY OF *HEVEA* RUBBER

adhas co rdhvam prasrtas tasya sakha
gunapravrddha visayapravalah
adhas ca mulany anusamtatani
karmanubandhlni manusyaloke

Bhagavad Gita, Chapter 15, verse 2

Translation

Its branches spread below and above, nourished by *Gunas* (the qualities of nature), with objects of the senses as the sprout/shoots and below, its roots stretch forth in all directions, binding the soul according to the actions performed in the human body.

I dedicate this book to the memory of my beloved parents.

Biology of *Hevea* Rubber

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Contents

Preface

This is a purposeful and wholehearted attempt to narrate the biology of *Hevea* rubber. Few books had been published in the past on the subject. *Hevea – Thirty Years of Research in the Far East* authored by M.J. Dijkman in 1951 and a comprehensive edition on *Rubber* by C.C. Webster and W.J. Baulkwill in 1989 remained authentic reference sources for years. Updating these is an uphill task. But I have been nurturing the idea of writing the biology of *Hevea* for several years. I do not claim that every section of this book is up to date, yet every effort has been made to make them as comprehensive as possible.

While writing this, I always strived to have a balance between two vital aspects: (i) to provide as much information as possible for a beginner; and (ii) to provide the established researcher with a reference source. In doing so, justice could be observed only to relevant publications. This book can be helpful to students, teachers, researchers and planters as well. Though *Biology of* Hevea *Rubber* will be useful to estate managers, it may not work as an exclusive reference book for them.

I am indebted to my wife Bindu and daughter Sandra for their unflinching support. Finally, I thank CABI for agreeing to publish this book.

P.M. Priyadarshan

1 Introduction

Rubber is an elastic substance obtained from the exudates of certain tropical plants (natural rubber) or derived from petroleum and natural gas (synthetic rubber). Because of its elasticity, resilience and toughness (Table 1.1), rubber is the basic constituent of tyres used in automotive vehicles, aircraft and bicycles. The same properties make it useful for machine belting and hoses of all kinds. Rubber is also used in electrical insulation, and, because it is waterproof, it is a favoured material for shoe soles. From mere rubber bands to catheters, condoms and latex threads, rubber makes more than 50,000 products. A car has almost 30% of its components made of rubber.

Natural rubber is produced from over 7500 plant species (Compagnon, 1986), confined to 300 genera of seven families, namely the Euphorbiaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Moraceae, Papaveraceae and Sapotaceae (Archer and Audley, 1973; Heywood, 1978; Backhaus, 1985; Lewinsohn, 1991; John, 1992; Cornish *et al*., 1993) (Table 1.2). At least two fungal species are also known to make natural rubber (Stewart *et al.*, 1955). *Hevea brasiliensis* (Willd. Ex. A. de. Juss. Müll-Arg.) is the almost exclusive contributor towards natural rubber produced worldwide (Greek, 1991). *Hevea* trees descended from seedlings transplanted from Brazil to South and South-east Asia that have undergone several cycles of breeding are now the prime source of the modern world's natural rubber. Natural rubber is produced in South-east Asia (92%), Africa (6%) and Latin America (2%). The main producing countries are (by descending order): Thailand (3.09 million t in 2008), Indonesia, Malaysia, India, China, Vietnam, and also Sri Lanka, Brazil, Liberia, Côte d'Ivoire, the Philippines, Cameroon, Nigeria, Cambodia, Guatemala, Myanmar, Ghana, Democratic Republic of Congo, Gabon and Papua New Guinea.

The latex found in the inner bark of *H. brasiliensis* is obtained by tapping – shaving the bark with a sharp knife – and collection of latex in cups (Fig. 1.1). Addition of acid, such as formic acid, will solidify rubber. The solidified rubber can then be pressed between twin rollers to remove excess water to form sheets.

Table 1.1. Properties of natural rubber (source: UNCTAD secretariat, 2011).

Item	Attribute	Properties
Molecular behaviour	Glass transition temperature	–70°C
	Melting temperature	25°C
	Hardness range	30–100 Shore A
	Maximum tensile strength	4000 psi at 70°F
	Maximum elongation	750% at 70°F
Advantages	Physical resistance	Excellent resilience
		Excellent tear strength
		Excellent abrasion resistance
		Excellent impact strength
		Excellent cut growth resistance
		Good compression set
	Environmental resistance	Excellent water resistance
		Good low temperature flexibility
		Good oxidation resistance
	Chemical resistance	Good resistance to alcohols and oxygenated solvents
		Good resistance to acids
Limits	Environmental resistance	Poor ozone resistance
		Poor sunlight resistance
		Very little flame retardance
	Chemical resistance	Poor oil and petrol resistance
		Poor resistance to (aliphatic and aromatic) hydrocarbon solvents

The sheets are commonly packed in bales for shipping. Rubber is also commonly transported in the form of concentrated latex. The strip of latex coagulated on the tapping panel (lace) and the lump left out in the cup (cup lump) that form the 'scrap' of commerce also fetches income to the planter. Despite the competition of synthetic rubber, natural rubber continues to hold an important place; its resistance to heat build-up makes it valuable for tyres used on racing cars, trucks, buses and aircraft.

Hevea rubber is depicted in ancient religious documents from Mexico dating back to AD 600 (Serier, 1993). Columbus gave the first description of rubber in 1496, and astronomer de la Condamine was the first to send samples of the elastic substance called 'caoutchouc' (the French word meaning 'weeping wood') from Peru to France in 1736 with full details about habit and habitat of the trees and procedures for processing (Dijkman, 1951; Baker, C.S.L., 1996). Natural rubber was first scientifically described by C.-M. de la Condamine and François Fresneau of France following an expedition to South America in 1735. The English chemist Joseph Priestley gave it the name rubber in 1770 when he found it could be used to rub out pencil marks. As a botanist, Fusée Aublet described the genus *Hevea* in 1775. Charles Macintosh in 1818 discovered waterproofing and Thomas Hancock in the 1820s invented mastication by developing a 'prickle' masticator, which gave a homogeneous ball of rubber. But raw rubber

Table 1.2. Selected rubber-yielding species (other than *Hevea*). See Chapter 5 for allied species of rubber.

Scientific name	Common name	Distributional range
Castilla elastica Sessé	Panama rubber tree	AMERICA (Mexico; Central America; western South America); widely naturalized in tropics
Ficus vogelii (Miq.) Miq.	West African rubber tree	AFRICA (Micronesia; north-east tropical Africa; east tropical Africa; west-central tropical Africa; west tropical Africa; south tropical Africa; South Africa; western Indian Ocean)
Funtumia africana (Benth.) Stapf	Lagos silk rubber tree	AFRICA (east tropical Africa; west-central tropical Africa; west tropical Africa; south tropical Africa)
Manihot glaziovii Muell.Arg.	Ceara rubber	
Holarrhena floribunda (G. Don) Durand & Schinz	False rubber tree	AFRICA (west-central tropical Africa; west tropical Africa)
Funtumia elastica Stapf	Lagos silk rubber	AFRICA (north-east tropical Africa; east tropical Africa; west-central tropical Africa; west tropical Africa); also cultivated elsewhere
Ficus elastica Roxb.	Indian rubber plant	ASIA-TROPICAL (India; China; Malaysia); widely cultivated elsewhere
Parthenium argentatum Gray	Guayule	NORTH AMERICA (south-central USA; Mexico)
Taraxacum kok-saghyz Rodin	Russian dandelion	ASIA-TEMPERATE (former Soviet Union; China)
Cryptostegia grandiflora R. Br.	Palay rubber	AFRICA, AUSTRALASIA, NORTH AND SOUTH AMERICA

did not withstand the extreme changes in temperature and this prompted Charles Goodyear (Fig. 1.2) to discover vulcanization in 1839 (heating rubber with sulfur), which gave explosive advancements in product manufacturing.

Research on the chemistry of natural rubber in the 19th century led to the isolation of isoprene, the chemical compound from which natural rubber is polymerized. Polymerization, the process by which long chain-like molecules are built up from smaller molecules, attracted continued research in the early 20th century. Rubber derived from *H. brasiliensis* is predominantly constituted of *cis*-1,4 polyisoprene $(C_5H_8)_n$ where *n* may range from 150 to 2,000,000. Carbonyl groups were also detected which significantly help the degree of cross-linking and storage hardening (Pushparajah, 2001). The possible roles of latex in plants, though unclear so far, have been suggested as: (i) to provide protection from predation; (ii) to provide a source of stored carbon and moisture; and (iii) to counteract ozone injury (Hunter, 1994). However, further detailed research

Fig. 1.1. Obtaining latex from the inner bark by (a) tapping (shaving the bark with a sharp knife) and (b) collecting the latex in a cup.

Fig. 1.2. Charles Goodyear (source: http://www.historycentral.com).

will only give an insight into the phenomenon of the functions of latex, which is essentially an extensive subject.

The rubber available in the 19th century was of varying quality and of uncertain supply when the demand was only for waterproofing of fabric and making of shoes. However, during the second half of the century circumstances

changed in favour of extension of rubber culture. The widespread adoption and improvement of vulcanization since 1850, coupled with growing demand for mechanical rubber devices, resulted in the expansion of the rubber industry both in Europe and in North America. The increase of population and the rising standards of living created vast new markets for rubber footwear and clothing. The discovery of the pneumatic tyre by John Boyd Dunlop in 1888, the ensuing cycling craze of the 1890s and development of the motor car resulted in greater demand for rubber, compelling the sources of supply to be widened. In the USA, great efforts were made to tap scrap rubber as a supply source, and indeed the US consumption of reclaimed rubber equalled that of the natural product. The British with a global empire tried to manage the short supplies through imports from Africa and by transplanting rubber seeds from the Amazon valley to their colonies in the East. At the centre of this shift of the rubber supply from West to East, as Professor Woodruff reports, was a group of British botanists working with Kew Botanic Gardens (see Chapter 2, 'Genesis and Development').

During World War I, German scientists produced a crude synthetic rubber, and during the 1920s and 1930s several polymerizing processes were developed in Germany, the Soviet Union, Britain and the USA. World War II threatened to shift the rubber wealth. Japan occupied prime rubber-producing areas in South-east Asia and the USA feared it would run out of the vital material since every tyre, hose, seal, valve and inch of wiring required rubber. Hence, the USA sought out other sources including establishing a rubber programme that saw explorers going to the Amazon with the ultimate goal of establishing rubber plantations close to home. Also, extensive work on synthetic rubber yielded a product that could replace natural rubber. By 1964, synthetic rubber made up 75% of the market. The situation changed drastically with the Oil Producing and Exporting Countries (OPEC) oil embargo of 1973, which doubled the price of synthetic rubber and made oil consumers more conscious of their petrol mileage, prompting them to own radial tyres. Radial tyres replaced the simple bias tyres (which had made up 90% of the market only 5 years earlier). Within a few years, virtually all cars were fitted with radials. Synthetic rubber did not have the strength for radials; only natural rubber could provide the required sturdiness. By 1993, natural rubber had recaptured 39% of the US market. Today, nearly 50% of every auto tyre and 100% of all aircraft tyres in the USA are made of natural rubber. Of this rubber, 85% is imported from South-east Asia.

Rubber plantations in Asia were seized by the Japanese in World War II; hence, the Allies frantically tried to establish New World plantations and to invent synthetic rubber. During the war, the US Congress passed the Emergency Rubber Project Act to solve the rubber shortage problem. With this, government used lands in the western states for the production of rubber from another rubber-producing plant, the shrubby guayule, *Parthenium argentatum*. Much rubber was produced from guayule during the war. Guayule is still preferred as an alternate source of natural rubber (Mooibroek and Cornish, 2000). However, after World War II, production levels of both *Hevea* rubber and guayule dropped, because US chemists had developed (in 1944) synthetic rubber by polymerizing butadiene and styrene. Nowadays, much of the rubber that we use is synthetic.

But, because natural rubber has different polymer lengths and side chains and therefore has different characteristics from synthetic rubber, some natural rubber is still added to products. Car tyres have 12.5–28% natural rubber (higher in radial tyres), truck and bus tyres 50–75%, and aircraft tyres 90–100%. The world consumes about 4 million t of natural rubber every year.

2 Genesis and Development

Since the early 20th century, the chief source of latex has been *Hevea brasiliensis* (Greek, 1991), though there are several other tropical and subtropical species that yield rubber from their laticifers (latex vessels) – small tubes found in the inner bark. As its botanical name suggests, *H. brasiliensis* is native to tropical regions of South America, especially Amazonia and adjoining areas.

2.1 The Amazon River Basin

During the latter half of the 19th century, the Amazon River and its major tributaries were inhabited by relatively dense, sedentary populations of indigenous peoples who practised intensive root-crop farming, supplemented by fishing and hunting of aquatic mammals and reptiles. The higher areas away from the rivers and their flood plains were (and still are) inhabited by small, widely dispersed, semi-nomadic tribes of Indians living on hunting animals and on wild fruits, berries and nuts with some small-patch agriculture of low yield. Rainforest covers the largest part of the Amazon region, most of the Guyanas, southern and eastern Venezuela, the Atlantic slopes of the Brazilian Highlands, and the Pacific coast of Colombia and northern Ecuador (Fig. 2.1). The huge Amazon region is the largest and probably the oldest forest area in the world; it also ascends to the slopes of the Andes until it merges with subtropical and temperate rainforest. On its southern border it merges with the woodlands of the Brazilian state of Mato Grosso, with galleries of its trees extending along the rivers.

The Amazon basin consists of enormous trees, some exceeding a height of 100 m, with an incredible number of species growing side by side in the greatest profusion arranged in different strata. For example, in Manaus (Brazil), 1652 plants belonging to 107 species in 37 different families were found in about 630 m^2. There are about 2500 species of Amazonian trees (Ducke, 1941) and as many as 100 arboreal species have been counted on a single acre of forest with hardly

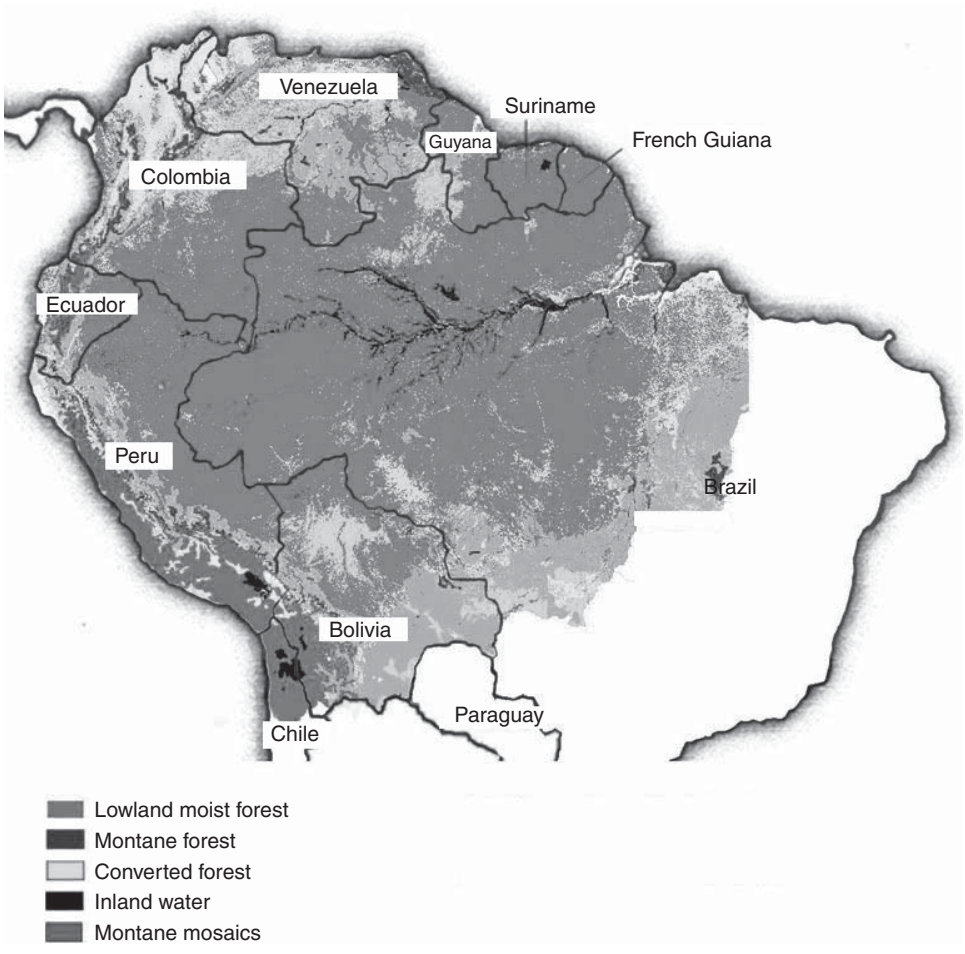


Fig. 2.1. Amazonia – geographic and vegetation potential (based on Eva *et al.* (1999)).

any one of them occurring more than once. Papers of Seibert (1947) and Schultes (1945) further confirm this enormous diversity. The Amazon forest has a strikingly layered structure. The canopy of sun-loving giants, soar to as much as 40 m above the ground and a few, known as emergents, rise beyond such canopies, frequently attaining heights of 70 m. Their straight, whitish trunks are covered with lichens and fungus. A characteristic of these giant trees is the buttresses, or basal enlargements of their trunks, which presumably help stabilize the top-heavy trees during infrequent heavy winds. Further characteristics of the canopy trees are their narrow, downward-pointing 'drip-tip' leaves that easily shed water. Flowers are inconspicuous. Among the canopy species, prominent members include the rubber tree (*H. brasiliensis*), the silk cotton (*Ceiba pentandra*), the Brazil nut (*Bertholletia excelsa*), the sapucaia (*Lecythis*) and the sucupira (*Bowdichia*). Many creatures, including monkeys and sloths, spend their entire lives in this sunlit canopy.

Table 2.1. Top 20 carbon-emitting countries (source: Marland *et al.*, 2004).

Country	Total emissions (1000 t of carbon)	Per capita emissions (t per capita)	Per capita emissions (rank)
USA	1,650,020	5.61	(9)
China (mainland)	1,366,554	1.05	(92)
Russian Federation	415,951	2.89	(28)
India	366,301	0.34	(129)
Japan	343,117	2.69	(33)
Germany	220596	2.67	(36)
Canada	174,401	5.46	(10)
UK	160,179	2.67	(37)
Republic of Korea	127,007	2.64	(39)
Italy (including San Marino)	122,726	2.12	(50)
Mexico	119,473	1.14	(84)
South Africa	119,203	2.68	(34)
Iran	118,259	1.76	(63)
Indonesia	103,170	0.47	(121)
France (including Monaco)	101,927	1.64	(66)
Brazil	90,499	0.50	(118)
Spain	90,145	2.08	(52)
Ukraine	90,020	1.90	(56)
Australia	89,125	4.41	(13)
Saudi Arabia	84,116	3.71	(18)

The Amazon basin covers a surface area of 4,100,000 km^2 (1,583,000 square miles), of which around 3.4 million km^2 (1.3 million square miles) are presently forested (Schroth *et al.*, 2004). Accounting for parts of the Amazon outside Brazil, the total extent of the Amazon is estimated at 8,235,430 km^2 (3,179,715 square miles); by comparison the land area of the USA (including Alaska and Hawaii) is 9,629,091 km^2 (3,717,811 square miles). In total, the Amazon River drains about 6,915,000 km^2 (2,722,000 square miles), or roughly 40% of South America (Schroth *et al.*, 2003).

Amazonian evergreen forests account for about 10% of the world's terrestrial primary productivity and 10% of the carbon stores in ecosystems (Melillo *et al.*, 1993) – of the order of 1.1×10^{11} t of carbon (Tian *et al.*, 2000). Amazonian forests are estimated to have accumulated 0.62 ± 0.37 t of carbon ha^{-1} $year^{-1}$ between 1975 and 1996 (Tian *et al.*, 2000). Fires related to Amazonian deforestation have made Brazil one of the top greenhouse-gas producers. Brazil produces about 300 million t of CO_2 a year; 200 million of these come from logging and burning in the Amazon. Despite this, Brazil is listed as one of the lowest per capita (rank 118) in CO_2 emissions according to the US Department of Energy's Carbon Dioxide Information Analysis Center (CDIAC) (Table 2.1).

Currently, *Hevea* rubber is planted in compact areas as rubber plantations that cover vast tracts in Indonesia, Malaysia, Thailand, India, Vietnam, China, Sri Lanka (erstwhile Ceylon) and Nigeria. How a wild plant of the Amazon jungles was domesticated and trained to be the producer of a pre-eminent industrial raw material is the central saga in the history of the so-called indispensable rubber industry. A crucial episode in that narrative is the transport of *Hevea* seeds from Brazil to England and from there to South and South-east Asia as described in the 14th edition of *Encyclopedia Britannica* by William Woodruff, professor of economic history and author of *The Rise of the British Rubber Industry During the Nineteenth Century* (1958) and later by many authors (Tan, 1987; Simmonds, 1989; Clément-Demange *et al*., 2000; Priyadarshan, 2003a, 2007; Priyadarshan and Clément-Demange, 2004). A brief account of the history of *Hevea* domestication is given here.

2.2 History of Domestication

History recapitulates the names of five distinguished men: (i) Clement Markham (of the British India Office); (ii) Joseph Hooker (Director of Kew Botanic Gardens); (iii) Henry Wickham (naturalist); (iv) Henry Ridley (Scientific Director of Singapore Botanic Gardens); and (v) R.M. Cross (Kew gardener), with Kew Botanic Gardens playing the nucleus for rubber procurements and distribution. As per directions of Markham, Wickham (Fig. 2.2) collected 70,000 seeds from the Rio Tapajoz region of the Upper Amazon (Boim district) and transported the collection to Kew Botanic Gardens during June 1876 (Wycherley, 1968; Schultes, 1977b; Baulkwill, 1989). Of the 2899 seeds germinated, 1911 were sent to the Botanic Gardens, Ceylon (now Sri Lanka), during 1876, and 90% of them survived. During September 1877, 100 *Hevea* plants specified as 'Cross material'

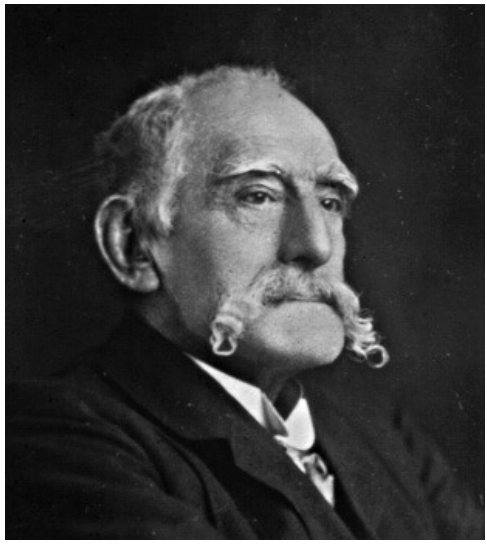


Fig. 2.2. Sir Henry Wickham.

were also sent to Ceylon. Earlier, in June 1877, 22 seedlings not specified either as Wickham or Cross, were sent from Kew to Singapore, which were distributed in Malaya and formed the prime source of 1000 seedling tappable trees found by Ridley during 1888. An admixture of Cross and Wickham materials might have occurred, as the 22 seedlings were unspecified (Baulkwill, 1989). One such parent tree planted during 1877 was available in Malaysia even after 100 years (Schultes, 1987). Seedlings from the Wickham collection of Ceylon were also distributed worldwide. Rubber trees covering millions of hectares in South-east Asia are believed to be derived from very few plants of Wickham's original stock from the banks of the Tapajoz (Imle, 1978). After reviewing the history of rubber tree domestication in East Asia, Thomas (2001) drew the conclusion that the modern clones have invariably originated from the 1911 seedlings sent to Ceylon during 1876. Also, Charles Farris could transport some seedlings to Kolkata in India (erstwhile Calcutta) during 1873 (Fig. 2.3). Hence, the contention that the modern clones were derived from '22 seedlings' is debatable. Moreover, if the modern clones are derived from 1911 seedlings, then the argument that they originated from a 'narrow genetic base', as believed even now, needs to be reviewed (Thomas, 2002). A chronology of events is given in Table 2.2.

The first introduction of rubber to India was during 1873 from Ceylon (now Sri Lanka) when 28 *Hevea* plants were planted in the Nilambur Valley of Kerala state in South India (Haridasan and Nair, 1980). During the period 1880–1882, plantations on an experimental scale were raised in different parts of South India and the Andaman islands. *Hevea* was first introduced to Vietnam in 1897 by the French, but was rejuvenated only after 1975 because of the long-lasting war (Priyadarshan, 2003a).

Developments in domestication of rubber after 1880 commenced in Singapore Botanic Gardens, one of the world's finest in terms of both its aesthetic appeal and the quality of its botanical collection. Approximately 3000 species of tropical and subtropical plants and a herbarium of about 500,000 preserved specimens are the hallmark of this garden. Under the direction of Henry N. Ridley (Fig. 2.4), who took over as superintendent in 1888, the garden became a centre for research on *H. brasiliensis*. Ridley developed an improved method of tapping rubber trees that resulted in a better yield of latex. His innovation revolutionized the region's economy. His persistence resulted in the first rubber estate in 1896 using his seeds and thereon the rubber industry grew into one of the economic mainstays of the Malay states.

Significant development on the propagation of *Hevea* rubber occurred after 1910. In particular the contribution to propagation and breeding of *Hevea* made by P.J.S. Cramer (Bogor, Indonesia) during the period 1910–1918 is noteworthy. He made a trip to the Amazon and succeeded in getting seeds of *Hevea spruceana* and *Hevea guianensis*. Cramer also conducted experiments on variations observed in 33 seedlings imported from Malaysia in 1883 from which the first clones of the East Indies were derived (Dijkman, 1951). Along with van Helten, a horticulturist, he could standardize vegetative propagation by 1915. The first commercial planting with bud-grafted plants was undertaken during 1918 in Sumatra's east coast. Ct3, Ct9 and Ct38 were the first clones identified by Cramer (Dijkman, 1951; Tan *et al*., 1996). Commercial ventures gradually spread to

Fig. 2.3. The voyage of rubber to East Asia (source: *Indian Rubber Journal*).

Table 2.2. Events in the history of rubber.

Year	Event
1735	Rubber samples sent to Europe by Charles-Marie de la Condamine
1763	French (François Fresneau) found caoutchouc could be dissolved in naphtha; suggested use in waterproofing clothing but it became tacky when warm
1770	Joseph Priestly discovered that the material would rub out paper marks, hence the name India rubber, and now simply 'rubber'
1803	The first rubber factory was established near Paris
1823	Macintosh manufactures waterproof raincoats by coating fabric with rubber dissolved in naphtha
Early 1820s	Hancock invented the masticator, a machine that shredded rubber scraps, allowing rubber to be recycled after being formed into blocks or rolled into sheets
1824	Hancock suggested plantation growing of rubber
1839	Goodyear and/or Hancock discovered vulcanization; when rubber was heated with sulfur, rubber retained physical properties from 0° to 100°C. This led to rubber boom
1830s	Interest in rubber with vulcanization process led to increased demand and exploitation of wild *Hevea* trees (*Hevea* was the native word)
1845	The first patent for a pneumatic tyre was issued to Robert William Thomson in England
1858	The first patent on an integral pencil and eraser was issued in the USA to Joseph Rechendorfer of New York City
1870	Sir Clements Markham of the British India Office suggested that rubber along with cinchona (source of quinine) be obtained from tropical America and grown in Asia
1872	James Collins reviewed rubber-producing plants and published a monograph entitled *Caoutchouc of Commerce*
1873	Seeds from Brazil sent to Kew Botanic Gardens; 12 plants raised and sent to Calcutta, but failed
1875	Second consignment of seed failed to germinate
1876	Markham sends Robert Cross to Para, Brazil where he obtained 1000 plants of *Hevea*, but no plants reach the East. At this time H.A. Wickham, an Englishman residing at Manaus (centre of the rubber boom in Brazil) sent 70,000 seeds from the Central Amazon basin (he received £10 per 100 seeds). This provided the basis for the world's rubber industry. The seeds were sent to Kew. The seeds had short viability but produced 2899 plants. Seedlings were sent to Ceylon and Singapore, and a few to Java
1888	In Singapore, there were nine trees of the original introduction, 21 5-year-old trees and 1000 seedlings. Ceylon had 20,000 seeds
1888–1911	H.N. Ridley, Scientific Director of Singapore Botanic Gardens, demonstrated that *Hevea* was the superior rubber-bearing plant, discovered the excision method of extracting latex and devised a method for coagulating latex
1888	John Boyd Dunlop, a veterinary surgeon of Belfast, obtained patents on a pneumatic tyre for bicycles
1898	Dunlop rediscovers pneumatic tyres (motor cars were invented in 1885). Today, 70% of rubber is used in transportation, 6% for footwear, 4% for wire and cable

(*Continued*)

Table 2.2. *Continued*

Year	Event
1898	First planting in Malaysia by a Chinese grower named Tan Chan Yoy. At this time, coffee prices slumped and there was interest in establishing a new industry
1910	Rubber boom; rubber reaches US$3 a pound
1956	Ridley dies at the age of 101
After 1956 to the present day	*Hevea* rubber popularized as a cash crop all through East Asia and many countries of Africa. Agencies such as the Association of Natural Rubber Producing Countries (ANRPC), the International Rubber Study Group (IRSG) and the International Rubber Research and Development Board (IRRDB) were constituted. Development extended to suboptimal climates of various countries

Fig. 2.4. Sir Henry Ridley.

Table 2.3. Area of rubber in East Asia.

Country	Area (in 1000 ha)
Indonesia	3414
Thailand	2434
Malaysia	1229
China	776
India	635
Vietnam	549
Sri Lanka	120
Myanmar	295
The Philippines	82
Total	9543

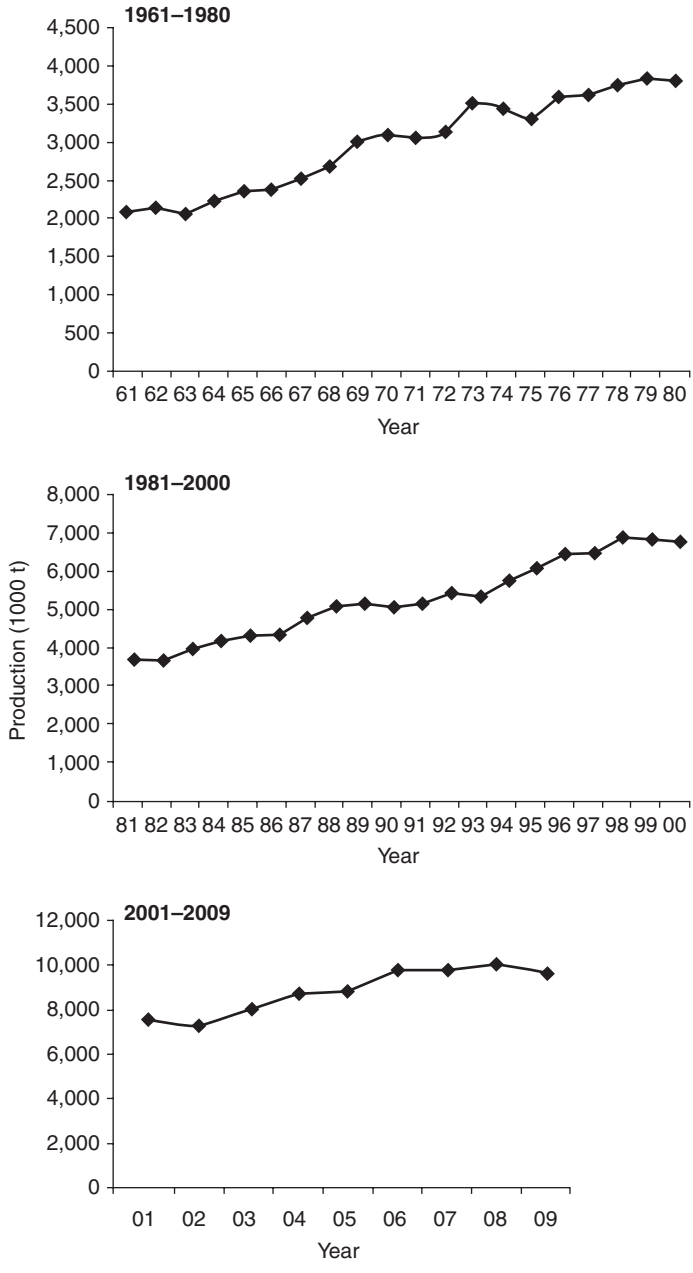


Fig. 2.5. World production (in '000 t) of rubber from 1961 to 2009.

Sri Lanka, China, Thailand, Nigeria and Vietnam and rubber became an integral part of the economy of South-east Asia towards the latter half of the 20th century.

Synthetically speaking, yield improvement through breeding was initiated with a very strict mass selection among the trees at the beginning of 20th century.

With the introduction of bud grafting, 'generative' and 'vegetative' selection methodologies were simultaneously used that resulted in seedlings and grafted clones (Dijkman, 1951). Around 1950, the advantages of grafted clones proved to be overwhelming for yield potential compared to genetically improved seedlings, and the focus shifted to derivation of clones for latex productivity. With all these cultural developments, *H. brasiliensis* soon ousted many other rubber-producing species including *Castilla*, *Manihot glaziovii* (ceara or manicoba rubber tree), *Ficus elastica*, *Landolphia* and *Clitandra* vines (African rubber).

Once the *Hevea* tree had been successfully transplanted to South-east Asia, the development of the rubber plantation industry was rapid and considerable quantities of the commodity were in the market by 1910. Factors such as availability of labour and favourable soil and climate contributed to this development. With the growth in world demand increasing, the total area of plantation in the East in 1900 amounted to 5000 acres. In 1910, it was 1 million acres and in 1920, 4 million acres. After the end of World War II in 1945, the total acreage exceeded 9 million, and by the mid-1960s it was 11.5 million. According to the Food and Agriculture Organization of the United Nations (FAO), the total land area harvested of natural rubber in Asia in 1996 amounted to some 15.6 million acres. Rubber produced from *Hevea* in Asian countries, ranging from the Philippines to Sri Lanka, accounted for almost 95% of the world's natural rubber supply – 9.2 million t from 9.5 million ha (Table 2.3) (IRSG, 2008). Worldwide, there is a 37% increase in yield from 1995 to 2007 (Fig. 2.5) (IRSG, 2008). There has been a constant correlation in the prices of oil and natural rubber. World economic recessions also have always experienced a downfall in the prices of natural rubber. An extensive survey of the history and development of natural rubber is beyond the scope of this book. Readers interested in such details may refer to Baulkwill (1989) for an extensive account.

3 Plant Structure and Ecophysiology

Hevea rubber is a quick-growing, erect tree with a straight trunk and bark which is usually grey and fairly smooth, but varies somewhat in both colour and surface. It is the tallest species of the genus and in the wild may grow to over 40 m and live for over 100 years (Fig. 3.1), but in plantations they rarely exceed 25 m (Priyadarshan and Clément-Demange, 2004) since growth is limited by tapping and hence plantations are usually replanted after 25–35 years when yields fall to an uneconomic level. Yet another reason for this short stature is that the plantations consist of bud-grafted trees, and the shoot that is seen above ground is virtually the primary branch. On both large estates and smallholdings, rubber trees are either grown as clones resulting from bud grafting on to seedling rootstocks or as seedlings. Seedlings, mainly of polycross origin, are the result of natural crossings between several selected clones planted in an isolated clonal garden (see Chapter 5 for details).

3.1 Reproductive Biology and Botany

Rubber is synthesized in over 7500 plant species, confined to 300 genera of seven families: Euphorbiaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Moraceae, Papaveraceae and Sapotaceae (Backhaus, 1985; Lewinsohn, 1991; Cornish *et al*., 1993) (see Chapter 1 for other sources of rubber). At least two fungal species (*Lactarius deceptiva* and *Peziza* sp.) are also known to make natural rubber (Stewart *et al*., 1955). Of late, scientists have started exploring the possibility of generating microorganisms that can produce rubber (Steinbüchel, 2003). The *Euphorbiaceae* family is extremely diverse and considered to be polyphyletic (Webster, 1994). Webster and Paardekooper (1989) give a comprehensive account of the botany of *Hevea*.

Fig. 3.1. Oldest rubber tree in Malaysia, the result of nine seeds planted in 1877.

3.1.1 Flowering

Similar to other tropical trees, *Hevea* normally takes 4–5 years to attain the reproductive stage – a phase called ripeness to flower (Kramer and Kozlowski, 1979). Though the capacity to flower is retained thereafter, the periodicities as well as the quantitative importance of flowering vary from clone to clone, as in other tropical trees (Owens, 1991). Precocious flowering is rarely observed in rubber seedlings (Sasikumar, 2000). The rubber tree is monoecious, with lateral inflorescences (branched panicles) bearing both staminate and pistillate flowers that appear in the last phase of the defoliation–refoliation process during wintering (the dry season: March–April in the northern hemisphere and September–October in the southern hemisphere) (Priyadarshan *et al*., 2001) (Fig. 3.2a and b).

Hevea shows seasonal flowering in response to alteration of the seasons. In the northern hemisphere, March–April is the main flowering season, and a short spell of secondary flowering prevails in August–September in many areas. Over much of Malaysia, the main flowering season occurs in February–April, following wintering in January–February, and there is a lesser flowering season during September–October (Yeang, 2007). It seems reasonable to presume that geographic location has a bearing on whether the trees flower during the secondary season. While it flowers and sets seeds during both the seasons in Malaysia, the southern parts of India experience flowering in March and April only. In north-east India, Tripura state experiences flowering and seed set during both seasons, but the viability of seeds is reduced during the secondary season. This prompts hand-pollination experiments to be centred on March and April when a substantial number of clones undergo flowering for a short span of 10–15 days. The shift in flowering coincides with the latitudinal changes. Flower emergence occurs towards

Fig. 3.2. Precocious flowering (a), flowering (b), fruits (c) and seeds (d) of *Hevea*.

mid-February. However, towards mid-March, the emerging flowers remain apparently dormant until the onset of favourable environmental conditions. The appearance of female flowers takes 10–12 days more than male flowers (dichogamy), and, due to incomplete protrandry, some of the male flowers emerge after the appearance of female flowers (Webster and Paardekooper, 1989). In Manaus and São Paulo (Brazil), which are located south of the equator, *Hevea* flowers only during September–October (Priyadarshan *et al*., 2001; Yeang, 2007).

Hermaphroditism and the occurrence of bisexual flowers have also been reported in the clones PR 107, AVROS 1328, GT 1 and Tjir 16 (Cuco and Bandel, 1994, 1995). However, this could not be confirmed in the north-eastern states of India, perhaps due to altered environmental conditions. GT 1 is a male-sterile clone, and the impediment to the normal process of gametogenesis has been characterized at a histological level (Leconte, 1983). Some other clones having GT 1 as the female parent, such as IRCA 41 and IRCA 319, are also proven to be male sterile, indicating that cytoplasmic genes might be responsible for male sterility (Nouy, B. and Leconte, A., 1985, unpublished observations). BPM 24 is also

reported to be male sterile in Thailand. Male sterility can be visually observed by the fact that stamens remain small and flat and produce no pollen.

Inflorescences are borne in the axils of the basal leaves of the new shoots that grow out after wintering. The inflorescence is a many-branched, shortly pubescent panicle bearing flowers of both sexes. The larger female flowers are borne at the end of the central axis and main branches while the smaller and more numerous male flowers appear on other parts of the panicle. Flowers are greenish yellow, with a bell-shaped calyx having five triangular lobes but no petals. Such pentamerous flowers and a tricarpelar ovary are typical of the Euphorbiaceae and the alternation of vegetative and reproductive phases with the formation of inflorescences at the end of the dry season implies a tight control of flowering time (Dornelas and Rodriguez, 2005). Staminate flowers have ten anthers arranged over a staminal column in rows of five each. The pistillate flower consists of a three-celled ovary with three short sessile stigmas. For each pistillate flower, about 70 staminate flowers are found. One floral-meristem-identity gene (*HbLFY*) has been isolated from the rubber tree and the highest level of *HbLFY* expression occurs during the time of flower meristem formation and declines as the organs expand. *HbLFY* works as a functional orthologue of *FLORICAULA/LEAFY* found in other dicot species such as *Arabidopsis*, grape vine and kiwi fruit (Dornelas and Rodriguez, 2005). *HbLFY* seems to be responsible for male/female floral inductions. Non-synchronous flowering is a restriction to genetic recombination between available genitors. Interestingly, high solar radiation induces synchronous anthesis and blooming in *Hevea* around the time of spring and autumn equinoxes on the equator (Yeang, 2007). This opens the possibility of conducting hand-pollination experiments at the equator which would ensure the seed-set success in desired combinations. The possibility of pollen storage used to counter non-synchronous flowering (Hamzah *et al.*, 1999) cannot be easily mastered for practical use.

Hevea appears to be obligatorily insect pollinated (Rao, 1961) and predominantly cross-fertilized (Simmonds, 1982). The strongly scented flowers of mature inflorescence attract insects (pollinators) that are mostly midges and ants of the families Heleidae and Ceratoponoidae. Wind appears to play little or no part since no pollen was collected in spore traps placed within 15 m of heavily flowering trees, and inflorescences enclosed in insect-proof bags did not set seeds. In Malaysia, over 30 species of insects have been seen to visit the flowers, but it is likely that pollination is almost entirely effected by midges and, to a minor extent, by thrips. In Puerto Rico, Brazil and Malaysia, Ceratopogonoid midges, which are very small and hairy with a capacity for sustained flight, were found to be the most important pollinators, while several species of thrips, which are not very active fliers, probably play a minor role (Warmke, 1952; Rao, 1961). As per a logarithmic model, pollen grains can travel 0.3–1.1 km (Yeang and Chevallier, 1999).

The pollen grains are triangular, measuring about 35–40 µm on each side, and their surface is sticky. The viability of pollen grains can be as high as 90%, but on average is only about 50% (Gandhimathi and Yeang, 1984; Sowmyalatha *et al.*, 1997). In tests on artificial media, Majumder (1964) found no differences between a large number of clones in percentage germination of healthy,

well-filled pollen grains. He observed that pollen taken from male flowers during or soon after rain gave a smaller germination percentage than pollen from dry flowers.

3.1.2 Fruit set

Fertilization occurs within 24 h after pollination and unfertilized female flowers quickly wither (Majumder, 1964). Clones vary greatly in flowering, fertility and fruit set. This ranges from near sterility to prolific fertility. There is no evidence of self-incompatibility (Webster and Paardekooper, 1989). The mature fruit is a large three-lobed capsule, 3–5 cm in diameter, having a woody endocarp and a thin, leathery mesocarp, and contains three seeds (Fig. 3.2c and d). The fruit reaches its maximum size in about 80–90 days and the endocarp becomes woody in about 110 days. The endosperm matures in about 130 days and the cotyledons get pressed to the endosperm. Thereafter, the moisture content of the capsule wall declines when the fruit is about 140 days so that the dry capsules dehisce explosively into six pieces with dispersal of seeds up to 15 m from the tree (Husin *et al*., 1981).

Low fruit set and its variation among clones, notably in the case of self-pollination, may be regarded as a general characteristic of the reproductive biology of *H. brasiliensis* and are not confined to specific incompatible crosses (Hamzah *et al*., 2002). This is a major limitation to genetic recombination in rubber breeding. It affects the number of full-sib families that can be evaluated, the size and the balance in sizes of these families, the cost of hand-pollination campaigns, and the quality of mating designs that can be established for genetic analysis. Pollen fertility, which varies from 50 to 98%, does not seem to be a limitation. The development of flowers to fruits is estimated to be very low, around 5% (Husin, 1990), and non-fertilized flowers soon wither. In contrast, most of the young fruits that are able to initiate their growth will produce viable seeds. At the time of maturation of fruits, a secondary shedding may occur as a result of infection by *Phytophthora* and *Oidium*. Low fruit set is not due to natural pollination deficiency (Warmke, 1952). While in Puerto Rico 5% or less of the female flowers bear fruit (Warmke, 1952), in Malaysia it happened to be only 0.3–1.6% (Rao, 1961), one of the pertinent reasons being the non-occurrence of pollination in all female flowers. In fact, fruit-set success rate assessed by controlled pollination varies widely, depending on the pollinated clones, from no success at all to a maximum of 5–10% for the more fertile clones such as PB 5/51 or PB 260. This is because the pollinator ensures the pollination of the highest number of female flowers (Maas, 1919). The success rate varies from year to year with a coefficient of variation of 45% (Clément-Demange *et al*., 1995). When all the female flowers of an inflorescence are hand pollinated, the fruit set is 3–8% (Gandhimathi and Yeang, 1984; Sowmyalatha *et al*., 1997).

Paiva *et al.* (1994) indicated cross-pollination to be 64% through isozyme studies. A clear example is PB 5/51, which is heterozygous for a recessive yellow gene, where open pollination led to an estimation of 16–28% self-pollination (Simmonds, 1989). Since many allozymes are produced at different development

stages (Adams and Joly, 1990), it is always reliable to use DNA analysis as a means to spell out the proportion of cross-pollination. Dijkman (1951) argued that a clone like LCB 510 (PR 107) is practically self-sterile, and that over 3000 self-pollinations yielded only one seed, indicating clonal variation towards self-incompatibility. As a matter of fact, the self-pollination rate in open pollination is strongly assumed to be much influenced by the specific context of the female trees and of the possible neighbouring cross-pollinators. By comparing eight hand-pollinated full-sib families, including two self-pollinated crosses, Leconte (1983) found no difference in the share of pollinated flowers with pollen tubes growing from stigmas to ovules (an average of 77%). Leconte (1984) and Sedgley and Attanayake (1988) confirm that there is no difference in pollen-tube growth between different clones and between self- or cross-pollinated flowers, so indicating that low fruit set and poor success in self-pollination are not due to incompatibility between the pollen and the stigma. A pre-zygotic or post-zygotic control exerted by the same incompatibility alleles and/or the inbreeding effects due to accumulation of homozygous loci in the embryo appear to be the reasons for low fruit set in self-pollination (Priyadarshan and Clément-Demange, 2004).

All three ovules of a fruit need to be fertilized for fruit setting (Gandhimathi and Yeang, 1984; Sedgley and Attanayake, 1988). Rubber fruits mostly have three carpels (sometimes four, and very rarely five), and fruits with only one or two seeds are almost never observed. Hamzah *et al.* (2002) confirmed that fruit-set success was clone characteristic of the seed parent and those pistillate flowers of PB 5/51 have a greater propensity for successful fruit set, while PR 107 is a poor seeder. Another study showed that the abortion of ovules never precludes fruit-wall formation in the early stages (Sowmyalatha *et al*., 1997). A fruit-load compensation phenomenon can be assumed, where fruit set ceases when an optimum number of fruits are formed. Only 25% of total pistillate flowers form fruits, and of these only about 25% attain maturity. The maternal parent might selectively abort genetically inferior progeny. Abortion of fruits is seen even 80 days after pollination. When seed set of PB 5/51, RRIM 600 and PR 107 were studied, flowers with no ovules penetrated were greatly over-represented (non-random distribution), and one explanation for this is the existence of 'receptive' flowers that favour successful fertilization (Hamzah *et al.*, 2002). Hence, understanding the genetics of the female flower is vital in increasing seed set.

Though meagre, studies on the effect of environmental attributes over the fruit set during hand pollination demonstrated that fruit-set success could be negatively correlated with evaporation (Yeang *et al*., 1986). Maximum temperature and relative humidity (RH) of post-pollinated days are also found to influence fruit set. The distribution of fruits on the floral shoots was found to conform to a negative binomial distribution that supports aggregated distribution, indicating that some of the shoots are favoured for fruit setting (Yeang and Ong, 1988). Leconte (1983) found that most of the fruits were borne by flowers from the buds located at the base of assimilatory leaves rather than from the buds of the scale leaves; consequently, he suggested focusing hand pollination on this type of flower.

3.1.3 Post-fertilization events

Ovule abortion decreases seed production and is a crucial factor for the survival of remaining ovules. On the other hand, the abortion of ovules never precludes fruit-wall formation in the early stages, indicating that the control mechanisms of these two processes are not interdependent (Sowmyalatha *et al.*, 1997). However, due to fruit-load compensation, when the optimum number of fruits is set, the pistillate flowers arising later may not fulfil their reproductive role. Only 25% of total pistillate flowers form fruits, and of these only about 25% attain maturity. The total number of fruits set will only be < 5% (Fig. 3.3). Since *Hevea* is an outbreeding taxon, reproductive success is expected to be low, as demonstrated in other outbreeding species (Weins *et al.*, 1987). Selfing, or the crossing of related parents, of an outbreeding taxon could result in inbreeding depression,

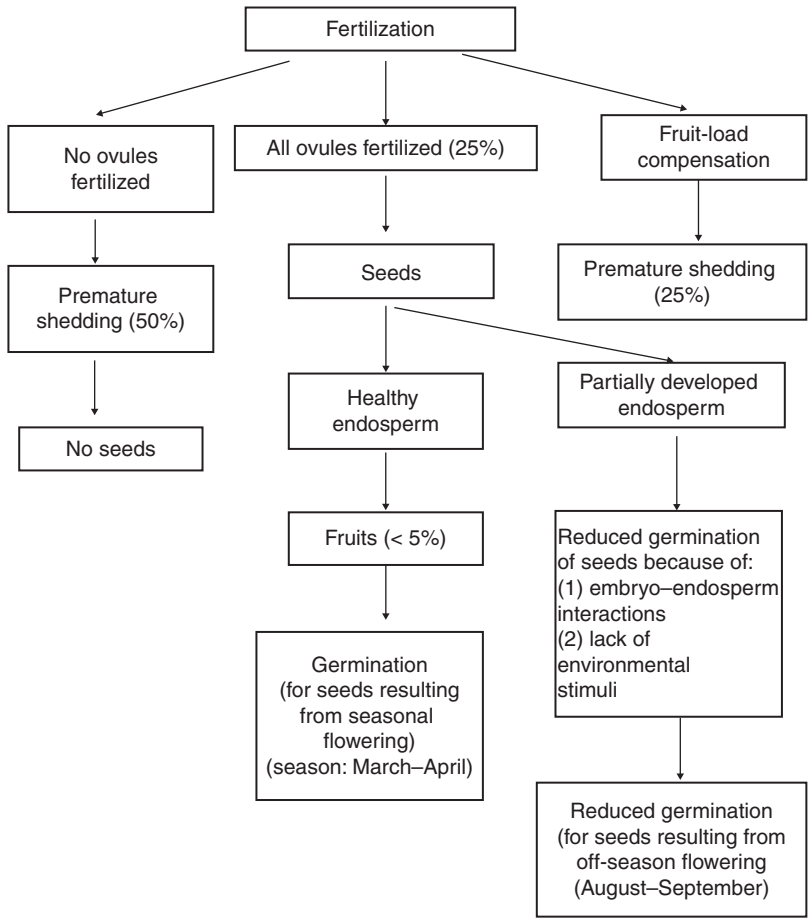


Fig. 3.3. Probable post-fertilization events behind seed maturity and germination in *Hevea* (after Priyadarshan and Clément-Demange, 2004).

and, since there are no conspicuous incompatibility barriers, it can very well be presumed that an inbred progeny would bring together deleterious recessive alleles largely contributing to reduced seed set. Here, the maternal parent can selectively abort genetically inferior progeny. Such preferential abortion is prevalent in many tree species (Stephenson, 1981). The abortion of fruits is seen even 80 days after pollination.

3.1.4 Seed

Seeds are large (3.5–6.0 g) and ovoid with the ventral surface slightly flattened. The seed coat, or testa, is hard and shiny, brown or grey-brown with numerous darker mottles or streaks on the dorsal surface, but few or none on the ventral side (Fig. 3.2d). The female parent of a seed could be identified by its markings and shape since the testa is a maternal tissue and the shape of the seed is determined by the pressure exerted by the fruit capsule during its development. The hilum can be seen as a shallow, approximately circular depression on the ventral surface and the micropyle is adjacent to it. A papery integument lines the inner testa and encloses the endosperm, which fills the seed. The embryo is situated in the middle of the endosperm with the radicle pointing towards the micropyle. The two white, veined cotyledons are pressed against the endosperm and enclose the plumular end of the axis of the embryo. The endosperm, which forms 50–60% of the weight of the seed, contains semi-drying oil which can be used as a rather poor substitute for linseed oil. If seeds are not sown in 10–15 days, they lose viability on storage as a result of the production of hydrocyanic acid (HCN). Seed filling with ultimate growth of the endosperm ensures the germination of the seed.

Hevea seeds are recalcitrant (Roberts, 1973). For storage, seeds can be mixed with sawdust and kept at 7°C to have vigour for up to 4 months (Ang, 1976). Treatment with polyethylene glycol 1500 improved storage up to 6 months with 25% germination (Normah and Chin, 1995). As mentioned above, *Hevea* seeds lose viability due to production of HCN during storage. But over 90% of the cyanogenic material is consumed to form non-cyanogenic compounds during seedling development. The cyanogenic glucosides are believed to be transported and metabolized in the young growing tissues (Lieberei *et al*., 1985). Protein profiling of dry and germinated seeds revealed that in both cases, though common proteins are profiled, unique protein profiles are also seen (Wong and Abubakar, 2005).

Seed germination is hypogeal and commences within 3–5 days after sowing. The radicle breaks through the testa at the hilar depression and very soon produces a ring of primordia which rapidly grow out as lateral roots. The radicle grows rapidly to form the primary taproot. The emerging plumule is bent in a 'u'-shape, but it soon withdraws its tip from within the seed, straightens up and grows vigorously. The endosperm remains inside the testa. The plumule produces the first pair of leaves in about 10 days after commencement of germination. Subsequently, an internode grows and the first flush of three leaves is produced above it. At the same time further lateral roots grow out on the primary taproot, which will continue rapid growth and will be well provided with root hairs near its tip (Gomez, 1982).

3.1.5 Vegetative growth

Growth in the length of stem is discontinuous, with rapid elongation of an internode towards the end of which a cluster of leaves is produced. This will be followed by a rest period for the scale leaves to develop around the terminal bud. This sequence is repeated and leaves are produced in whorls separated by bare stem. Young scions of bud grafts elongate internodes for 2–3 weeks followed by a rest period. Although the elongation of stems is intermittent, their girth increases continuously. New flushes in the mature tree appear at any time of the year.

The spirally arranged trifoliate leaves hang downwards approximately parallel to the petioles and are reddish or bronze in colour and gradually become green. The angle with the petioles will now be increased to 180°, in which position they remain until they senesce. The mature laminae are shiny dark green on their upper surfaces and a paler, glaucous green below. Leaves are trifoliate and glabrous, arising on petiolules with long petioles (about 15 cm) bearing extrafloral nectaries at the point where petiolules merge. Nectar is secreted only on the new flush of leaves during flowering. The leaflets are elliptic or obovate with the base acute and the apex acuminate; they have entire margins and pinnate venation. Clones can be identified through a closer examination of the architecture of leaves (Mercykutty *et al*., 2002).

The upper epidermis and palisade parenchyma of the leaflets are single layers of cells below which there are several layers of spongy parenchyma and the single layer of the lower epidermis. The latter has many ridge-like appendages and a reticulate cuticle (Rao, 1963). Stomata are only present in the lower epidermis. Senanayake and Samaranayake (1970) examined 25 clones and found that these showed a wide variation in stomatal density from 22,000 to 38,000 cm^{-2}, but they found no significant relationship between stomatal density and latex yield. Gomez and Hamzah (1980) investigated variation in leaf morphology and anatomy in 11 clones. They found significant differences between clones in: (i) stomatal density (which ranged from 28,000 to 37,000 cm^{-2}); (ii) cell number in the upper epidermis; and (iii) thickness of palisade and spongy layers.

3.1.6 Wintering

Trees of more than 4 years exhibit defoliation or 'wintering', a term used to describe the annual shedding of senescent leaves which renders the trees wholly or partly leafless for about 15–20 days. Defoliation is followed by terminal bud bursting in 15 days and in a week the expansion of new leaves occurs. There used to be a yield depression during defoliation and more markedly during refoliation. In areas experiencing a dry period, the duration of wintering tends to be short and refoliation is completed fast, thus minimizing yield reduction. Most of the non-traditional rubber-growing areas above 15°N fall under this category. For instance, trees of north-east India tend to be completely leafless for 10–15 days. There are marked differences between clones in wintering behaviour. A few tend to shed and replace part of their foliage simultaneously over a relatively

long period and may thus show no very obvious signs of wintering, while at the other extreme some become completely leafless for a time. The majority are intermediate between these extremes. Clones also vary considerably in the extent to which they suffer yield depression during refoliation.

3.1.7 Root system

Trees grown as seedlings, or as bud grafts on seedling rootstocks, develop a strong taproot and extensive lateral roots, the whole root system forming about 15% of the total dry weight of a mature tree. In a study of rooting habit on a range of soils, which revealed no marked differences between trees of nine clonal seedling families, it was found that, on deep soils without impediments to root growth, 3-year-old trees had taproots about 1.5 m long and laterals 6–9 m long, while at 7–8 years the taproots were about 2.4 m and the laterals over 9 m. The laterals normally extend well beyond the spread of the branches so that in plantations at the usual spacing they commonly grow through the adjacent planting rows. The roots of neighbouring trees intermingle and some may become grafted together. The major lateral roots almost invariably arise from the taproot in a whorl within 30 cm of the soil surface and grow horizontally, or only slightly downwards. Further laterals are commonly produced at a depth of 40–80 cm, but do not extend horizontally as far as those nearer the surface. All the laterals ultimately give rise to unsuberized, yellow-brown roots of about 1 mm diameter, possessing root hairs, and these are known as feeder roots since they are mainly responsible for absorption of nutrients and water. While the feeder roots are mostly in the top 30 cm of the soil, a proportion arises from the deeper laterals and there is no reason to believe that these are less efficient absorbers than those nearer the surface.

An investigation of the distribution of the feeder roots of clonal seedling trees aged 1–22 years, planted in rows 6.1 m apart on a range of soils, showed that up to 3 years after planting the roots were concentrated near the trunks; at 4 years the feeder roots of trees in adjacent rows met; and at 5–7 years the feeder root density in the centre of the inter-rows was significantly greater than close to the trees. After the roots of neighbouring trees had met, ramification occurred nearer the trunk with the result that in the mature plantation there was little variation in the concentration of feeder roots across the inter-rows, except where the roots branched prolifically on entering a patch of particularly well-aerated, moist or nutrient-rich soil.

While the development of the root system always follows the general pattern described above, considerable variations occur due to: (i) soil type; (ii) soil aeration and moisture content; (iii) cultivation; (iv) nature of the ground vegetation; and (v) mode of fertilizer application. Soong (1976) investigated the influence of several factors on the density and distribution of feeder roots to a depth of 45 cm by augur sampling the roots of mature trees of four clones, all bud grafted on Tjir 1 clonal seedlings and growing on seven different soil series. He found that feeder root development was markedly influenced by the scion clone: for example, the vigorous clone RRIM 605 had about 80% more feeder roots by weight than the slower-growing RRIM 513. Soil texture had a marked effect. On sandy soils the weight of feeder roots was significantly greater than on clayey soils,

probably due to the plant's reaction to the lower moisture retention, or better aeration, of the former soils. Over a range of soil types, feeder root densities were positively and significantly correlated with fine sand content and negatively correlated with clay content. However, this did not apply on some clayey soils which possessed a good structure due to their high sesquioxide content. On most soils about 50% of the feeder roots in the 0–45 cm layer were in the top 7.5 cm and the proportion decreased rapidly with depth, only about 10% occurring between 30 and 45 cm below the surface. Exceptions to this were found on soils with a compact or poorly structured subsurface layer, where 70% or more of the feeder roots could occur in the top 7.5 cm, and also on uncompacted soils with little or no profile differentiation, where distribution was fairly uniform throughout the 0–45 cm layer. In the 7.5–45 cm soil layer the lowest feeder root density occurred at the same time as in the surface soil but peak root development was about 3 months later than it was in the upper layer.

The presence of mycorrhizae on the roots of rubber trees was recorded by Park in Ceylon in 1928 (Dijkman, 1951). In Malaysia, the occurrence of endotropic, vesicular arbuscular mycorrhizae of the endogone type on the roots was found to be general on rubber trees of all ages and on a variety of soils (Wastie, 1965). Spores of several species of endomycorrhizal fungi have been identified in soil samples from rubber plantations examined by Jayaratne (1982) in Sri Lanka and by Ikram and Mahmud (1984) in Malaysia. It is not known whether the mycorrhizae are of significance in the nutrition of the tree. Young rubber trees grow well from sterilized seed in sterile sand if supplied with the requisite nutrients, but mycorrhizae may be of value on soils high in organic matter or of low phosphate content.

3.1.8 Juvenile and mature characteristics

When grown from seed, the rubber tree passes through a juvenile stage to a mature stage, the latter normally beginning with the formation of the branches. Throughout its life a seedling tree exhibits certain juvenile characteristics, in that its bark is somewhat rough, and with increasing height the trunk tapers, the thickness of the bark decreases and the number of latex vessel rings declines. The 'mature-type' bud grafting used commercially is formed by bud grafting seedling rootstocks with buds which are mature-stage tissue. The scion does not pass through a juvenile phase and therefore grows into a trunk which lacks juvenile characteristics; it does not taper but is almost cylindrical, the bark is smoother than that of a seedling and both its thickness and the number of latex vessel rings within it remain virtually constant with increasing height. Virtually a primary branch is being tapped in bud-grafted trees.

3.1.9 Growth studies

Templeton (1968, 1969) studied the growth before and during tapping of two clones, RRIM 501 and RRIM 513, bud grafted in the field on seedling stocks

planted at the normal spacing of 9.15 × 2.44 m (30 × 8 ft), giving 444 trees ha^{-1}. The determination of the dry weight of the different parts of the plants, including the roots, was done by destructive sampling of complete trees at 9, 15, 21, 27, 39, 55, 63 and 81 months after bud grafting. The total dry weight per tree increased approximately exponentially up to 39 months from bud grafting, after which the rate was slower. Trees of RRIM 501 exceeded 300 kg within 81 months, a figure agreeing well with that obtained by Shorrocks (1965) for the same clone. The rate of girth increment rose to a maximum of 1.0 cm $month^{-1}$ between 27 and 39 months and then declined. The relative growth rate (RGR; the rate of increase in dry weight per unit of dry matter present per unit of time) declined steadily from 0.04 g $week^{-1}$ at 9 months to 0.005 g $week^{-1}$ at 81 months.

The leaf area ratio (LAR; leaf area per unit of dry weight) was naturally low at first with a small scion on a 1-year-old rootstock, but rose to 12 cm^2 g^{-1} by 9 months from bud grafting and remained at about this level until 39 months, after which it declined steadily, reflecting the increasing proportion of plant weight in the non-photosynthetic tissues of trunk and branches. The leaf area index (LAI; the area of leaf laminae per unit area of ground) increased rapidly to reach a maximum of 5.8 m^2 m^{-2} between 50 and 60 months, when a complete canopy over the ground was achieved, and continued at this level up to 81 months. The net assimilation rate (NAR; the rate of increase in dry weight per unit area of leaf) declined slowly from 0.0032 g cm^{-2} $week^{-1}$ at 9 months to 0.0013 g cm^{-2} $week^{-1}$ at 81 months due to increased self-shading of the foliage as the LAI increased, resulting in a lower photosynthetic rate per unit leaf area. The crop growth rate (CGR; the rate of dry matter production per unit area of ground per year, which is proportional to LAI × NAR) increased to a peak of 35.5 t ha^{-1} $year^{-1}$ by 55 months, after which it declined. The cumulative dry weight for clone RRIM 501 at 81 months was 135 t ha^{-1}, which is close to the figure for the same clone quoted by Shorrocks (1965).

Templeton (1968) considered that the RGR, LAR, NAR and CGR would all decline slowly after canopy closure, but that the LAI, which had levelled off at 5.8 m^2 m^{-2} after 63 months, would remain near this value for many years. However, Shorrocks (1965), who reported a similar LAI value (6.3 m^2 m^{-2} at 6 years) for RRIM 501 to that of Templeton, found that the LAI rose to 14 m^2 m^{-2} at 10 years and was still about 9 m^2 m^{-2} at 24 years. Premature senescence and fall of lower leaves is frequently observed in plantations with dense canopies. It is also common for some of the lower branches of mature stands to die back and be shed. Both these features suggest that the LAI is high enough for self-shading to reduce the light intensity to below the compensation point low down in the canopy. Templeton's data show that the CGR of RRIM 501 fell off after 4–5 years, while the LAI rose to 5.8 m^2 m^{-2} at 5 years and remained at this level. Similarly, Shorrocks's figures indicate that the CGR fell from the sixth to the tenth year, while the LAI rose over the same period. Thus, there is some evidence to suggest that the LAI may be above the optimum for dry matter production (but not necessarily for latex production) over much of the life of the plantation.

Templeton (1969) estimated the efficiency of the rubber tree from the maximum recorded CGR of 35.5 t ha^{-1} $year^{-1}$ of dry matter which, at 4800 cal g^{-1}, is

equivalent to 1704×10^8 cal ha^{-1}. The average solar radiation in Malaysia is 420 cal cm^{-2} day^{-1} and, assuming 40% utilization of this energy for photosynthesis (including 10% loss of radiation energy to non-photosynthetic pigments), the available energy amounts to about $61{,}320 \times 108$ cal ha^{-1} $year^{-1}$, so that the efficiency of utilization of solar radiation by the closed canopy of the rubber plantation is about 2.8%.

3.1.10 Root heterogeneity and stock–scion interactions

A major part of rubber breeding efficiency can be attributed to the grafting technique which enables the multiplication of elite genotypes at the level of the bud-grafted part (aerial part of the tree) and which determines the use of clones as the almost exclusive varietal type in rubber cropping. Unfortunately, cloning the whole tree (aerial part and roots) for the development of single-component clonal trees by the cutting technique (self-rooted marcots and mist-propagated cuttings) generates a high ratio of uprooting due to lack of taproot and inadequate anchorage. Notwithstanding, a bud-grafted population has a high level of homogeneity and should exhibit intraclonal variation in yield to a minimum, barring factors such as: (i) soil heterogeneity; (ii) difference in juvenility of buds; and (iii) variable seedling rootstocks. However, our own experience with RRII 105 monoclonal population spelled a difference of 10–310 ml in the total volume of latex per tap and a range of 28.1–43.9% in dry rubber content (drc) during the peak yielding period (October–January). An estimate showed 20% of the trees yielded more than 150 ml per tree per tap and 20% showed higher drc (more than 38%). In another experiment with RRII 105, the total volume of latex and dry rubber yield were 5.0–325.0 ml and 1.8–144.0 g, respectively (Chandrashekar *et al.*, 1997). Such variation was reported from countries such as Malaysia (Hardon, 1969), Indonesia (La Rue, 1921) and Sri Lanka (Philpott, 1946). The differences exhibited are significant and refutable for a homogeneous population.

Two factors may affect these results. The first factor is soil heterogeneity, a key attribute manoeuvring the overall yield of a stand and one that can be geared at will through observance of appropriate agronomic practices. Soil can be tested for any deficiencies, and a fertilizer dosage can be followed in cognizance with the soil test data. The second factor is the difference of juvenility in bud-grafted plants. This factor has actually not been assessed so far. However, this should not make a significant difference, since bud-grafted plants of a given population would normally arise from the same bud-grafting generation.

The effect of rootstock has been most intriguing. Studies conducted in the past have proved there are reliable and marked effects of rootstock on the yield of scion when bud grafting was made on to illegitimate seedlings, where variation in terms of yield and girth was significant (Buttery, 1961; Abbas and Ginting, 1981). Further, it has been demonstrated that monoclonal or selfed seedlings from monoclonal blocks of bud-grafted plants showed marked differences in yield (Ng *et al.*, 1982). From these results, it was suggested that vigorous hybrid seedlings issued from polycross seed gardens would provide better rootstocks

(Simmonds, 1985). Also, monoclonal seedlings of PB 5/51 and RRIM 623 were found to be significantly superior to other stocks (Ng, 1983). In an unpublished experiment (Centre National de Recherche Agronomique (CNRA)–Centre International de Recherche pour l'Agriculture et le Développement (CIRAD), Côte d'Ivoire), monoclonal seedlings of GT 1 used as the rootstock were found to be significantly better than three other monoclonal seedling rootstocks (issued from clones RRIM 600, PB 5/51 and LCB 1320). This confirms the good performance of GT 1 seedling rootstock already published earlier (Combe and Gener, 1977). This performance of GT 1 seedling rootstock is assumed to be linked with the male-sterile behaviour of clone GT 1 which displays exclusive unselfed seedling progenies, with no inbreeding effect and a better growth. Another experiment confirms that the most important part of yield variation is due to differences between the bud-grafted clones rather than between the seedling rootstocks, so mitigating the importance of the choice of seedling rootstock which is heavily dependent on the availability of monoclonal seedlings in the planting areas. It was also shown that the rootstock can affect the thickness of scion bark and the number of latex vessel rings. Babilioff (1923) made a notable observation that during the formation of new bark, when the scion cambium made 12 latex vessels, stock could make nine latex vessels, so making the superiority of the scion cambium more prominent.

The rationale enunciated indicates that, though the soil heterogeneity and juvenility of buds may not necessarily influence the yielding pattern of a bud-grafted population, the variable rootstock should exert effects over yield that are largely uncontrollable. Although poorly addressed by breeding in the lack of an efficient cloning technique, the root system directly affects: (i) soil–plant relationships; (ii) water and mineral uptake; (iii) water stress resistance (Ahmad, 2001); and (iv) resistance to uprooting by wind. Moreover, efficient breeding for growth of bud-grafted clones and the increasing use of fast-growing clones may have generated an imbalance between stock and scion, so emphasizing uprooting hazard (Clément-Demange *et al*., 1995). Consequently, cloning the root system is a major challenge for rubber tree breeding, as it would greatly facilitate growth and yield improvement as well as adaptation to various environments.

3.2 Propagation

There are different kinds of rubber seeds such as: (i) legitimate; (ii) illegitimate; (iii) ordinary; (iv) monoclonal; and (v) polyclonal seeds. Among these, monoclonal and polyclonal seeds are produced in specially raised plantations. The other types of seeds are collected from commercially established plantations. Monoclonal seeds, especially those of clone Tjir 1, were once commonly used for propagation. For this, plantations consisting of this clone alone were established. Special care was taken not to have any tree of any other clone in the garden to prevent contamination of pollen grains. This ensured that all the seeds produced were selfed seeds of Tjir 1. However, monoclonal seeds are not recommended now for raising plantations due to their inferiority compared with the modern clones.

3.2.1 Polyclonal seed generation

Polyclonal (polycross) seeds, which are hybrid seeds, are produced in plantations called polyclonal seed gardens. In these gardens, several clones are planted intermixed so as to maximize cross-pollination. Clones planted in these gardens should possess desirable characters such as: (i) high yield; (ii) disease resistance; (iii) vigour; (iv) ability to produce good seedling families; and (v) profuse production of seeds. All the clones should flower simultaneously. Genetic factors like genetic divergence and inbreeding depression also have to be taken into account while selecting clones (Mydin *et al*., 1990). Some of the clones usually planted in polyclonal seed gardens are RRIM 600, RRIM 605, RRIM 623, PB 5/51, PB 28/59, Tjir 1 and PR 107. Other clones having the required desirable attributes mentioned above could also be included. The number of clones in the seed gardens usually varies from three to seven (Simmonds, 1986). To maximize cross-pollination, special designs are adopted while planting. Selection of at least four clones enables better randomization so that trees of the same clone are not planted adjacent to each other (see Chapter 5). A wider spacing is adopted in seed gardens for proper development of the crown, essential for profuse flowering and fruit set. A spacing of 9.1×3.0 m (358 trees ha^{-1}) is considered suitable for this purpose. The stand is reduced to 247 by the sixth year by progressive thinning out.

Pollinating insects may carry pollen from other nearby rubber trees. Such contamination of pollen grains can result in production of undesirable seeds with different genetic constitutions and their mixing up with the desirable ones. To prevent this, an isolation belt, about 100 m wide, is provided around the garden. This isolation belt is planted with a non-rubber crop. If rubber has to be planted, one of the clones included in the garden has to be used. Production of seeds in a garden depends to a great extent on clones, climate and diseases. On average, a tree produces 150 seeds in well-maintained gardens.

3.2.2 Vegetative methods

Propagation through asexual (vegetative) parts such as buds, leaves and stem cuttings is termed vegetative propagation. Vegetative propagation of rubber is carried out mainly by bud grafting (budding is the colloquial term). Propagation through rooted cuttings is possible in rubber but is not generally practised due to unsatisfactory development of the root system, especially the taproot.

The principle involved in bud grafting is the replacement of the shoot system of a genotype with that of another more desirable genotype. The method of bud grafting adopted is a modified form of the Forket method of patch bud grafting. In this process, a patch of the bark of the seedling plant (stock) is replaced by a bud patch taken from the clone to be multiplied (scion). A thin film of polythene is wound over the bud patch for waterproofing. After 21 days, the polythene is taken off. The bud patch gets attached to the stock permanently and becomes a part of it. The stock is then cut off above the bud-grafted portion and the grafted bud develops into a new shoot (scion) and then into a two-part tree.

Depending on the colour and age of the buds, three main types of bud grafting are recognized. These are: (i) brown (conventional); (ii) green; and (iii) young bud grafts. In the first method, older buds having a brown colour are used while, in the other two, tender green buds are utilized (Marattukalam and Saraswathyamma, 1992). Depending on the part of the stock where bud grafting is carried out, the classification would be: (i) base bud grafting; (ii) crown bud grafting; (iii) over bud grafting; and (iv) high bud grafting.

Brown bud grafting

This was developed in 1916 in Indonesia by van Helten, a horticulturist, in collaboration with two planters, Bodde and Tass. The first handbook on this subject was published by Bodde in 1918 (Dijkman, 1951). Brown bud grafting is generally carried out by grafting brown-coloured buds taken from bud wood of about 1 year's growth on to stock plants of 10 months' age. Vigorously growing healthy stocks having a girth of 7.5 cm are ideal for grafting. Stocks should be grafted when the bark peels off very easily. Test peeling of a small patch of bark 15 cm above the base is the surest method of assessing the peeling quality of the bark.

Brown buds are usually obtained from brown budwood produced by budgrafted plants raised as source bush nurseries (SBNs). Buds found in the axils of fallen leaves are generally utilized for bud grafting. Budwood should be collected when the top whorl of leaves has fully expanded but not hardened to ensure proper peeling of the bark and high bud-grafting success. Budwood should, as far as possible, be collected in the morning or evening, and should preferably be utilized for bud grafting as soon as collected. If bud grafting is delayed, special measures should be adopted for preventing moisture loss. Budwood is harvested as per the requirement and cut into pieces of convenient length, usually 1 m (Fig. 3.4).

Bud grafting is usually carried out with a specially designed knife with two blades, called a bud-grafting knife. Taking the stock plant, two parallel vertical cuts that reach the wood are made, starting from about 2.5 cm above the collar. The cuts should be a little more than 5 cm in length and 1.5 cm apart. Then a horizontal cut joining the bottom ends of the vertical cuts is made. Latex oozes out for a few minutes through the cuts and this can be wiped off. The flap of bark separated by the three cuts is then gently lifted with the aid of the knife and peeled upwards. The practice of removing the flap completely is also adopted. The exposed region is called the bud-grafting panel.

The bud patch used for brown bud grafting has a length of about 5 cm and a width of about 1.5 cm. For preparing the bud patch, two parallel vertical cuts having a length of 5 cm are made on two sides of a bud, 1.5 cm apart. Then, two horizontal cuts are made connecting the lower and upper ends of these cuts (Fig. 3.5). Latex is allowed to ooze out and meanwhile incisions are made around neighbouring buds of the same budwood. When the oozing of latex stops, it is wiped off and the bud patch marked out by the four cuts is stripped off (Fig. 3.6). The inner side of the bud patch is examined for the presence of the core of the bud, which appears as a slight projection. If that is not present, the bud patch should be discarded. The bud patch is then gently placed in the budgrafting panel after lifting the flap (Fig. 3.7). Due care must be taken not to injure the cambium. The panel is bandaged using a polythene strip. Bandaging should

Fig. 3.4. Budwood.

Fig. 3.5. Preparing a bud patch with a bud grafting knife.

commence at the bottom and move upwards in a close tight spiral that can end with a knot (Fig. 3.8). It requires 15–20 days for the bud patch to heal and form part of the stock. The presence of green colour on the bud when the bud is scratched indicates initial success of bud grafting.

Fig. 3.6. Bud patch ready for bud grafting.

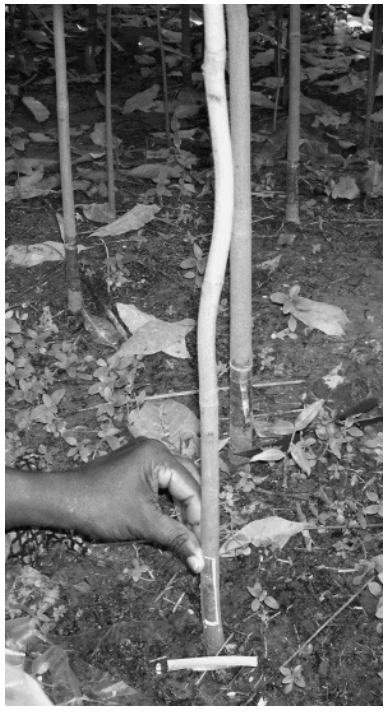


Fig. 3.7. Bud grafting: the bud patch is gently placed in the bud-grafting panel after lifting the flap on the stock plant.

Fig. 3.8. Bandaging.

Green bud grafting

Developed in Indonesia in 1960 by H.R. Hurov, this process involves a young stock plant and budwood. Stock seedlings would be 2–8 months old and the budwood 6–8 weeks old. Buds found above the scale leaves of the shoots alone are used for grafting. For proper peeling of the bud patch, harvesting should be done when the leaves are copper brown to dark green.

After cleaning the basal portion of the stock, two vertical incisions, a little more than 5 cm long and 1 cm apart, are made, starting from a point about 2.5 cm above the collar region. The lower ends of these cuts are joined by a horizontal cut and a few minutes allowed for the cessation of latex flow. The flap of bark thus separated out is then gently lifted upwards exposing the bud-grafting panel. The flap is then cut off, leaving a short 'tongue' of about 1.5 cm at the top.

The bud patch can be stripped from the budwood in the same way as in the case of brown bud grafting. The upper end of the bud patch is gently inserted under the 'tongue' and placed in the bud-grafting panel (Tinley, 1962). Then the bud patch is secured firmly by bandaging with a transparent polythene strip as in the case of brown bud grafting. This strip can be about 25 cm long and 2 cm wide. Transparent tape allows light to fall on the green bud patch, which in turn enhances the grafting success. Buds can be examined after 3 weeks. Retention of green colour is the indication of success. The final observation to check on the success of grafting is made after 10 or more days. Bud grafting can be carried out

throughout the year, but predominantly during the rainy season (de Silva, 1957). However, days with heavy rainfall are not suitable for bud grafting (Marattukalam and Premakumari, 1982).

Young bud grafting

Young bud grafting is carried out on plants of less than 2 months old (Ooi *et al*., 1976). Stocks are raised in small bags of 33 × 15 cm size. The plants are given intensive nursing such as foliar application of fertilizers and fungicides twice weekly (Leong *et al*., 1986) and soil application of an NPKMg mixture weekly. Four weeks after bud grafting, plants are cut back to a height of 20–25 cm (Yoon *et al*., 1987). When the scion develops two or three whorls of leaves, it is transplanted in the field just like other bag plants. Using this technique, bag plants could be produced within 7 months after the planting of germinated seeds in the bags during August–September. These plants will also have a well-developed root system (Seneviratne, 1995).

Crown grafting

Susceptibility to diseases and wind are the undesirable characters of modern clones. An undesirable crown can be replaced by a desirable one through crown bud grafting. Crown bud grafting was first adopted in Indonesia (Java) in 1928 and in South America in the 1930s to prevent the damage caused by South American leaf blight (SALB). The tree produced by crown bud grafting is a three-part tree comprising: (i) the root system of the stock plant; (ii) the trunk of one clone; and (iii) the crown of another clone (Yoon, 1973).

Crown grafting is ideally carried out when the scion has attained a height of 2.4–3.0 m; 1–2 years are usually required for the plants to attain such a growth. The height of the plant is more important than the age. Grafting is carried out at a height of 2.1–2.4 m on the inter-whorl region below the top whorl of leaves. Grafting should be done only when the top flush of leaves is fully expanded and hardened and the stem tissue should be green or dark green. Plants having a height up to 4.5 m can also be used for crown grafting. For crown grafting, the green grafting technique is followed. If grafting fails, re-grafting is done on the opposite side of the stem, 5 cm above or below the first attempt. Successfully grafted plants are cut back, leaving a snag of about 5 cm. Treating the cut ends of the stem with some wound-dressing compound is desirable. About 9 months after cutting back, when the crown–trunk union is firmly established, the trunk shoots are pruned. The crown shoot later on fully establishes itself and in due course develops to be the crown of the three-part tree (Yoon, 1973). Similarly, crown grafting can be done on to plants grown in polythene (poly) bags, which can grow into a full three-part tree.

Rooting of cuttings

Terminal cuttings with one whorl of mature leaves are used for rooting. Rooting is done in a mist-propagation chamber, in raised beds filled with rooting media under artificial mist-generating systems. Proper shade and coverage are provided to protect the cuttings from intense sunlight as well as to prevent mist drift (RRIM, 1959). Shoots of about 30 cm length are planted in the beds and mist is

applied continuously during the daytime. After 5–9 weeks, the cuttings produce roots. Subsequently, they are transferred to poly bags and kept in hardening beds (RRIM, 1962). In these beds they are initially provided with continuous mist for 1 week, and thereafter the duration of misting is gradually reduced. Shade also is progressively removed. Within 3–6 weeks the process of hardening is over and the plants are ready for field planting.

Layering

Development of roots on a stem while it is still attached to the parent plant is known as layering. The type of layering adopted in the case of rubber is air layering. Young branches are used for this purpose (RRIM, 1960). The stem is first girdled by removing the bark around it to a width of about 2–5 cm. The cambium of the exposed wood is completely removed by scraping and growth hormones are applied to this region to enhance rooting. Then this portion is completely covered with a ball of any moist rooting medium (such as sphagnum moss, soil containing plenty of organic matter, coconut husk–soil mixture) and finally covered with polythene sheet to prevent loss of moisture (Yoon and Ooi, 1976). Within a few weeks, roots develop from the upper edge of the girdle and grow into the rooting medium. When the roots are properly developed, the layer is separated from the plant by cutting the branch below the ball. The layer is then planted after careful removal of the polythene cover without damaging the rooting medium. Studies conducted by the Rubber Research Institute of India (RRII) have proved that sphagnum moss is far superior to other rooting media (Sobhana *et al.*, 1995).

Micropropagation

Micropropagation is a technique of producing plants *in vitro* from small (micro) pieces of plant tissues (see Chapter 6 for further details).

Root trainers

Planting materials of *Hevea* have traditionally been raised in poly bags. However, a drawback of poly-bag plants is that coiling of the taproot and lateral roots lead to slow growth and poor wind endurance (Sharma, 1987; Josiah and Jones, 1992; Ginwal *et al*., 2001).

An alternative to raising plants in poly bags is using root-trainer containers. These are made of polypropylene and have an inner diameter of 7.5 cm at the top, tapering and ending with a drainage hole at the bottom, are 30 cm in depth and have a holding capacity of 800 cm^3. The growth medium used in root-trainer containers is sieved coir pith that has been previously dipped in water for 2 weeks to remove tannin. The dried coir pith can be mixed with powdered charcoal (in a ratio of 9:1) and rock phosphate (200 g) and neem cake (100 g) can be added. Appropriate quantities of fungicides and pesticides can also be added. One-third of the container is filled with this mixture and green bud-grafted stumps are planted into the container and the container is topped up with growth medium.

Plants raised in root trainers (Fig. 3.9a) showed better sturdiness (height: diameter ratio) than poly-bag plants (Soman *et al*., 2002). The lateral roots were

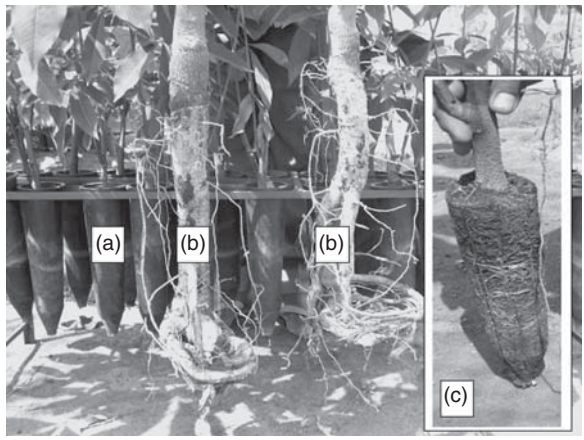


Fig. 3.9. (a) Root-trainer plants; (b) roots of poly-bag plants; (c) root core of root-trainer plants.

also found to be significantly higher in root-trainer plants compared with poly-bag plants (Soman and Saraswathy Amma, 2005). In poly bags, the taproot reached the bottom of the bag in 6–7 weeks after planting the budded stumps and it started coiling thereafter leading to root strangling and distortion (Soman and Saraswathy Amma, 1999) (Fig. 3.9b). Compared with poly-bag plants, root-trainer plants showed uniform distribution of roots (Fig. 3.9c).

Preparation and packing of propagation materials

The propagation materials handled by rubber growers are: (i) fresh seeds; (ii) germinated seeds; (iii) seedling stumps; (iv) brown bud wood; (v) green bud shoot; (vi) brown-bud grafted stumps; (vii) green-bud grafted stumps; (viii) poly-bag plants; (ix) stumped bud grafts; and (x) three-part stumps. During storage and transit, they are likely to get damaged by loss of moisture.

Fresh and healthy seeds collected from the field can be kept under shade without much loss of viability for about 7 days and storing fresh seeds in water at ambient temperature increases their water content, which in turn prolongs viability (Mercykutty *et al*., 1996). By packing seeds loosely in well-aerated containers with powdered charcoal that has 40% moisture, 70% viability could be retained for up to 30 days (Eikema, 1941). The viability of seeds can be prolonged to 2 months by mixing them with an equal volume of sawdust (10% initial moisture content) in perforated poly bags (RRIM, 1966). Storage of seeds at 4°C in sealed poly bags is also considered to be a reliable method for retaining viability for up to 4 months (Wycherley, 1971).

Germinated seeds are collected from germination beds when the radicle just comes out. To prevent rot damage, beds are inspected every day and germinated seeds are picked up. For transporting short distances and immediate use, germinated seeds are carried in water. For transporting long distances, they are packed in boxes between layers (2.5 cm thick) of aged sawdust, charcoal powder

or damp coconut fibre (Subramaniam, 1980). They should be laid in such a manner that the radicle points downwards.

Brown bud wood is cut into pieces of 1 m length. For use on the same day and transporting over short distances, brown bud wood is kept wrapped in wet sacking. For longer storage and transport, their cut ends are sealed with molten wax and each piece covered with a banana sheath, wet sacking, coconut fibre or grass leaves. Viability can be retained up to 3 days.

While collecting green bud shoots, the leaf-bearing top portion is cut off. The leafless lower part with scale leaves alone is used for taking buds. Since green bud shoots are tender it is better to use them for bud grafting immediately. In the seedling nursery, they are carried in trays or buckets containing water. Storage is possible up to 6 days with their cut ends sealed with wax (Subramaniam, 1980).

Seedlings prepared to a convenient size by pruning the stem and roots are called seedling stumps. They should have a minimum girth of about 7.5 cm at the base and brown colour up to a height of 45 cm or more. For stumping, the seedlings are cut back at some point between 45 and 60 cm, where the brown colour ends. Pruning is always done with a slanting cut, preferably above a whorl of buds. While cutting back, green or partially brown stem should not be retained on the stump as transpiration can take place through such regions and the resulting loss of water may lead to the drying of the stumps after planting. The plants are left in the nursery for 7–10 days. During this period, a few buds below the cut end become activated and swell. At this stage the decapitated plants are pulled out without causing much damage to the roots and bark of the stem. If it is difficult to pull out the plants due to drying of soil or extensive development of the root system, the lateral roots can be loosened by digging the soil on one side of the taproot or all around the plant, to make it easier. Once it is pulled out, the taproot is pruned to the maximum possible length, but not more than 60 cm and not less than 45 cm. After preparing the seedling stumps by proper pruning of roots and stem, the cut end is sealed with molten paraffin wax. For storing overnight, they should be kept in fresh water. For longer storage, the procedure followed for brown bud wood can be replicated (RRIM, 1968).

Brown-bud grafted plants with pruned stem and roots are known as brown-bud grafted stumps. To prepare a brown-bud grafted stump, the plant is cut at a height of 7.5 cm above the upper end of the bud patch. The cut should have a downward slant of around 45° from the side of the bud to the opposite side. The cutting back is done about 10 days before pulling out. During this period the bud becomes activated, which in turn will speed up the establishment of the bud-grafted stump after planting. The plants are then pulled out and the taproot is pruned to a length of 45–60 cm and the laterals to a length of 10–15 cm. Where it is difficult to pull out the plant, it can be lifted before cutting back and then pruned. If the bud-grafted stumps are intended for planting in poly bags, the tap root should be pruned to a length about 15 cm less than the depth of the soil core and laterals to around 5 cm length. The cut end of the stem is sealed with wax and the bud patch is protected by covering with a small piece of banana sheath. The viability of brown-bud grafted stumps can be retained for up to 30 days by packing them in boxes with wet sawdust (Premakumari and Nair, 1974).

Poly-bag plants are raised from green bud grafts or brown bud grafts. The poly-bag plants are field planted usually at the two- to three-whorl stage. While transporting poly-bag plants, utmost care should be given to prevent any damage to the soil core. If the soil core is damaged, casualties may arise. Three-part stumps are produced by proper pruning of the stem and roots of crown-bud grafted plants raised in the nursery. To produce a three-part stump, a bud-grafted plant in the nursery is first cut back above the bud patch as in the case of stumped bud grafts. When the scion grows to a height of 240 cm, crown bud grafting is done below the top whorl of leaves. Then the scion (trunk shoot) is cut back at about 7.5 cm above the crown bud. The crown bud grows and produces the new crown shoot.

Stock–scion interaction

A grafted plant comprises a root system contributed by the stock plant and the shoot system by the scion. Stock–scion interaction is obvious since both these coexist as they exert mutual influence. Vigorous stocks can increase the vigour and yield of the scion (Dijkman, 1951; Ooi *et al*., 1980). Stocks raised from monoclonal seeds of clones like PB 5/51 are found to favourably influence the growth and yield of several scion clones, while some other stocks like RRIM 600 affect the performance of the scion negatively. Polyclonal seeds are generally more vigorous and impart better growth to the scions (Ng *et al*., 1981). Stocks produced from vigorous clones like GT 1 also enhance growth of the scion, resulting in reduction of the immaturity period (Combe and Gener, 1977). Similarly, vigorous scions induce more growth in the root system (Dijkman, 1951). However, of the two parts of a bud-grafted tree, namely the stock and scion, it is primarily the scion that determines the performance of the plant.

3.3 Ecophysiology

Rubber planters are under permanent pressure to raise land productivity while protecting the environment as a result of: (i) the decrease in the amount of cultivatable land; (ii) the change in human lifestyles increasing the demand for rubber; and (iii) environmental issues generating a demand for natural rubber over synthetic rubber. During the past decade this increasing demand has prompted rubber cultivation to be rapidly expanded into non-traditional rubber-growing areas. The principal way of achieving high productivity has been the development of high-yielding clones with desirable characteristics through rigorous breeding and selection. Supplementary approaches to achieve greater productivity have been determination of an optimum tapping schedule and soil management to maintain fertility. All these parameters affect the overall growth and physiology of trees. In this section, aspects of ecophysiology are considered in some detail.

Before the rubber tree can produce latex it needs to attain physiological maturity and the first step in this development is maturation of the leaves, the organs of photosynthesis. Leaves attain maturity in around 35–40 days after emergence. In addition to phytohormonal equilibrium, leaf maturity can be

regarded in terms of CO_2 balance (Samsuddin and Impens, 1979) and the maturity leads to a series of characteristics such as leaf expansion, chlorophyll accumulation and formation of photosynthetic apparatus (photosystems I and II and carboxylative enzymes), stomata, cell wall and supporting structures. Knowledge about phenological behaviour is especially relevant when one intends to determine the required time to start leaf net photosynthesis (Senevirathna *et al*., 2003). Studies on leaf ontogeny, relative to CO_2 balance, even done under greenhouse conditions (Bergonci, 1981; Pita, 1984; Schwob *et al*., 1998) are scarce under field conditions. The dry matter increase in rubber-tree leaves is directly related to the balance between CO_2 assimilation from photosynthesis and its release by respiration. During the juvenile phase, the CO_2 balance in the rubber tree is especially affected by significantly higher respiration. The higher respiration rates that occur in the juvenile phase seems to indicate a higher metabolic activity (growth respiration), during which energy released is required to synthesize structural compounds and chlorophyll. To reach net photosynthesis, the young leaves need to increase the concentration of CO_2 (Miguel *et al.*, 2007).

The dynamic aspects of the competition between latex and wood production and the spatial distribution of radial growth rates around the tapping cut have been studied by Silpi *et al*. (2006). In untapped control trees, radial growth started with the onset of the rainy season and lasted until the onset of the dry season, with no growth during the driest and winter periods. Also, on reaching the flowering stage, the annual girth increment is seen to be significantly greater and after the trees are tapped the girth increment is drastically reduced (Priyadarshan and Clément-Demange, 2004). While the former is due to attainment of physiological maturity, the latter is due to source–sink adjustments and the cumulative growth was about half that of untapped trees. When the latex production increased over the years, establishment of a latex sink becomes a long-term process probably involving many aspects of metabolism.

3.3.1 Photosynthetic efficiency

As *Hevea* rubber is a perennial crop requiring over 6 years' growth before latex can be harvested and another 7 years to assess yield, breeding programmes take more than 20 years to produce a suitable clone. Based on the principle that plants produce basic compounds for growth and yield through photosynthesis, photosynthetic efficiency, together with associated factors such as water vapour diffusion, was coined as the early determinant of high-yielding genotypes (Nugawela and Aluthhewage, 1985; Nugawela *et al*., 1995a). Knowing the high variability of the instantaneous rate of photosynthesis due to its dependency on the incident radiation levels, key parameters of the light response curve (LRC) of photosynthesis (i.e. light-saturated level of photosynthesis (A_{max} – the photosynthetic rate at which the LRC tends to plateau) and apparent quantum yield (ϕ_{app} – initial slope of the LRC)) could be used in such studies (Rodrigo, 2007). The rate of A_{max} denotes the maximum expected rate of photosynthesis under the ambient temperature and CO_2 levels (light saturation stage) while ϕ_{app} demonstrates the photosynthetic performance under low light levels (light limiting stage).

In general, an equation of either a rectangular hyperbola or quadratic function is used to construct the LRC and the latter provides a more accurate measure of A_{max} and ϕ_{app} and also provides an additional parameter on the convexity (θ) of the LRC which represents the transition stage between light limitation and saturation (Hay and Walker, 1989; Thornley and Johnson, 1990). Radiant energy (P_{sat}), carboxylation efficiency (CE), stomatal conductance (g_s) and CO_2 compensation concentration (Γ) are the attributes that influence the biomass production and water use efficiency (WUE). A clone with high P_{sat}, high CE, low g_s and low Γ will be more dependent on mesophyll factors than stomata, producing relatively more biomass and maintaining high WUE (Nataraja and Jacob, 1999).

Attempts to use photosynthetic parameters to judge yield potential for early screening for newly bred *Hevea* genotypes were made in Sri Lanka. The daily photosynthetic integral was estimated using the Charles-Edwards equation (Charles-Edwards, 1982), where A_{max} and ϕ_{app} are the key components. Photosynthetic rates and WUE were greater in high-yielding clones and vice versa for dark respiration rates (Nugawela *et al*., 1995b). Even though the approach is unique, genotype separation is only into three broad categories of high-, medium- and low-yielding groups. While seedlings behave as discrete units exposing most of the leaves to incoming solar radiation, there is a high level of light attenuation within the closed canopies of mature rubber trees. Photosynthesis in mature trees is highly dependent on canopy architecture, which is generally represented by the light extinction coefficient (k) and the LAI. Hence, it would be unrealistic to predict the values of k and LAI of a mature crop from the assessment in seedlings (see Chapter 5 for parameters used for early selection). Attributing a fixed value for k across all genotypes and estimation of LAI of different genotypes with known differences in LAI and leaf area per whorl of seedlings were also attempted (Nugawela *et al*., 1995b). This exercise was too simplistic, offering large variation in the seedling population. Essentially, developing a simple model to predict k and LAI of mature crops would also require consideration of canopy architecture.

3.3.2 Dry matter production and water use efficiency (WUE)

As said earlier, rubber cultivation has been extended to suboptimal non-traditional rubber-growing areas. These areas experience a multitude of stresses including soil moisture and cold stresses. Trees avoid the effects of water stress by judicious maintenance of water uptake, reducing transpirational loss or by osmotic adjustment. The mechanism and the magnitude of the response depend on the genotype and its sustainability and productivity. Stomata of *Hevea* leaves are highly reactive to environmental changes (Rodrigo *et al.*, 2005b). Diffusive resistance to water vapour and CO_2 of stomata depends mainly on atmospheric vapour pressure deficit, irradiance and temperature. Trees will be responsive to internal water status as measured by leaf water potential (ψ_μ), which is highly dependent on soil water status. Therefore, clonal responses to dry conditions are generally assessed with measurements of stomatal conductance (g_s) or resistance (r_s) (each being the reciprocal of the other), ψ_μ and relative water content.

Trees grown in wet regions of Sri Lanka (annual rainfall is ~ 5000 mm) have shown a wide range of r_s, varying from 1 to 8 s cm^{-1} (Rodrigo *et al.*, 2005b). Stomatal resistance is distinct with diurnal variation commencing with high values around midday, particularly after dry spells. Under no major soil moisture deficit, the maximum r_s is ~ 4 s cm^{-1} which could increase over twofold under dry conditions. An increase in r_s can adversely affect the photosynthetic rate by limiting CO_2 transfer (Rodrigo, 1997). Similarly, trees grown under two distinct agroclimatic regions, namely Dapchari of western India (with high temperature summer stress) and Agartala of north-east India (with cold winter stress), experienced high photosynthetic photon flux density (PPFD) and severe inhibition of photosynthesis. The upper canopy leaves exposed to high PPFD fixed little carbon through the day. Photosynthetic rates were higher in the shaded leaves with low PPFD. Inhibition of photosynthesis due to high PPFD was also evident in the decreased quantum yield of CO_2 assimilation and *in vivo* photosystem II (PSII) activity in the stressed leaves (Devakumar *et al.*, 2002). Photosynthesis is one of the foremost processes that is inhibited when plants are exposed to drought and cold (Baker, N.R., 1996). High solar radiation can cause an imbalance between light–dark reactions of photosynthesis leading to increased diversion of photosynthetic electrons for the production of oxygen species that will further lead to senescence of stressed leaves (Jacob *et al.*, 1999). Under an ideal climate, leaf photosynthesis saturated at a photosynthetically active radiation (PAR) of 1000 µmol m^{-2} s^{-2} (Nataraja and Jacob, 1999). On the other hand, a higher PAR will induce imbalance between the photochemical and the biochemical reactions resulting in the over-energization of thylakoid membranes, diverting photosynthetic electrons for the production of active oxygen species (AOS) like superoxide, hydrogen peroxide and singlet oxygen (Jacob *et al.*, 1999). Photosynthetic response to higher temperatures was similar in different clones. Subjecting RRIM 600 and PB 260 to a wide range of temperatures between 10 and 45°C, Kositsup *et al.* (2007) concluded that the rate of photosynthesis stayed constant between 23 and 37°C and decreased above or below this range.

The WUE that gives the measure of how much dry matter is produced per unit of water consumed under moisture stress situations is most important. Only a limited amount of work has been conducted in this line since growth and water use assessments on *Hevea* rubber are tedious. Other studies have been dependent mainly on the instantaneous rate of CO_2 assimilation and predicted transpiration rates based on stomatal conductance without properly taking into account the boundary layer conductance at different canopy levels. Values of WUE were within the range of 1.5–3.5 g mm^{-1} m^{-2} (Rodrigo *et al.*, 2005a), while that calculated using CO_2 assimilation and stomatal conductance was below 1 µmol CO_2 $mmol^{-1}$ H_2O for water stress conditions (Dey and Vijayakumar, 2005) and 3 µmol CO_2 $mmol^{-1}$ H_2O without stress (Nugawela *et al.*, 1995b). Since *Hevea* rubber originated in the wet tropics, moisture stress situations do not favour dry matter production owing to low values of WUE. The extreme moisture stress conditions in Dapchari resulted in low plant moisture status and high plugging indices coupled with inhibition of stomatal conductance and transpiration rates. A comparative study with two clones (GT 1 and RRIM 600) revealed that attributes influencing latex flow and production, namely pre- and post-tapping

turgor (P_{lv}), latex solute potential (ψ_π), leaf water potential (ψ_μ) and stomatal conductance (g_s), significantly decline in the dry season (February–May) compared with the wet season (June–December). Water deficit during the non-rainy season can be as high as 1070 mm as against 350 mm in the traditional rubber-growing areas (Jacob *et al.*, 1999). Adequate irrigation reduced the immature period to 6 years (Devakumar *et al.*, 1998). Only a few rain-fed trees attained tappable girth even after 9 years (Devakumar *et al.*, 1998). The first-year yield for these two clones was 550 kg and 622 kg ha^{-1}, respectively (Chandrashekar *et al.*, 1990). These clones gave 672 and 681 kg ha^{-1} in the traditional rubber-growing areas of India, whereas in the cold-stressed environment of Tripura (north-east India) it was 577 kg and 1085 kg ha^{-1}, respectively (Priyadarshan *et al.*, 1998b).

Generally, the Penman–Monteith equation is used to estimate the amount of water loss through transpiration (Monteith, 1965; Monteith and Unsworth, 1990). Though data can be retrieved through sophisticated instruments, the assessment of boundary layer conductance (g_b) is time-consuming and requires continuous weighing of leaf samples in the crop canopy and calculations to find g_b values. A study with *Picea sitchensis* gives a classic example to demonstrate the tediousness of assessment of g_b where a huge tree had to be suspended in the air and provided with artificial rain (Teklehaimanot and Jarvis, 1991). Therefore, suitable ecophysiological models are required to estimate g_b. A model has been developed to estimate g_b of tree crops (Rodrigo *et al.*, 2005a) and this has been used for rubber ($g_b = 0.048 \times U \times LAI^{0.381}$ where U refers to wind speed at 2 m above the canopy, in m s^{-1}). Nevertheless, only the LAI represented the canopy architecture in this model (Rodrigo *et al.*, 2005a) and therefore future investigations should incorporate other parameters of canopy architecture such as leaf distribution. The thermocouple-based Heat pulse/Sap flow system provides direct measurements of transpiration water loss with no difficulty in assessing either g_b or g_s. This system has not been used effectively in rubber. The adaptability of trees in dry regions to conserve water on overall canopy photosynthesis and latex productivity also needs attention.

Rubber is grown in cooler climates in China and India (in areas above 21°N). *Hevea* rubber is tropical and is predominantly grown in areas where conditions are above 20°C (ideally 28 ± 2°C with a diurnal variation of about 7°C; Barry and Chorley, 1976) and at altitudes below 200 m mean sea level (MSL) (see Chapter 8). High temperatures result in high evapotranspiration and water stress, while low temperatures lead to low growth rates and cold damage. Low temperatures can cause permanent damage to the photosynthetic apparatus. Photoinhibition is the final result under such circumstances even under moderate light levels, which otherwise are beneficial under optimum temperatures. A few clones have proved to be tolerant to low temperature. For instance, GT 1 has performed reasonably well at 5°C in China and SCATC 93/114 (now named REYAN 93/114) can tolerate temperatures even below 0°C for a short period (Zongdao and Xuequin, 1983; Priyadarshan, 2003a). Though such clones survive the cold stress, overall productivity appears to be rather lower (Priyadarshan *et al.*, 1998a). However, photosynthetic efficiency can be a measure for selecting such clones. Although A_{max} provides a useful measure, assessments of Φ_{app} and

chlorophyll a fluorescence emission in terms of the ratio of variable to maximum emission (F_v/F_m) indicate the level of any damage to the photosynthetic apparatus (Ireland *et al*., 1989). Photo-inhibition of the leaf photosynthetic apparatus results in a major decrease in the F_v/F_m ratio and can be measured with a commonly available portable plant efficiency analyser. Being a rapid measurement, fluorescence emission can be assessed in a large number of plants within a short period and hence it would appear to be a suitable measurement for shortlisting suitable clones. Thereafter, analysis of the light response of photosynthesis would be appropriate in early screening. The same procedure could be adopted in genotypic evaluation to some extent under dry conditions; however, in rubber plants, no permanent damage of the photosynthetic apparatus has been recorded in such conditions. Instead, any short-term drop in the F_v/F_m ratio and Φ_{app} could be attributed to downregulation of photosynthesis. In both wet and dry climatic conditions in Sri Lanka, the F_v/F_m ratio was in the range of 0.75–0.85 (Senevirathna *et al*., 2003; Iqbal and Rodrigo, 2006). Only under sunny conditions has some level of decrease in the F_v/F_m ratio been recorded around midday (Senevirathna *et al.*, 2003). Such downregulation of photosynthesis limits the maximum capacity of photosynthesis, affecting the A_{max}, and, in addition, is more prominent in the early stages of field establishment when rubber plants are small (Iqbal and Rodrigo, 2006).

4 Latex Production, Diagnosis and Harvest

4.1 Latex

Latex is a colloidal suspension. Biochemically, latex is true cytoplasm. Latex contains most of the subcellular elements, which, besides rubber particles, include: (i) lutoids – an important vacuolysosomal compartment (Pujarniscle, 1968; Ribaillier *et al*., 1971); (ii) plastids, the Fre-Wyssling particles whose role is not clearly understood (Gomez and Moir, 1979; Hébant, 1981); and (iii) ribosomes (Coupe *et al*., 1976). However, neither the nuclei nor the mitochondria are expelled during tapping, probably because of their parietal position (Dickenson, 1965), which makes investigations on nuclear and energy metabolism difficult.

The cytoplasmic origin of *Hevea* latex, as expressed for the first time by Berthold (1886), was partly confirmed by Milanez (1951) using optical microscopy. Later, with the advent of electron microscopy, the same could be established with certainty (Andrews and Dickenson, 1961). While electron microscopy enabled *in situ* observation of the main organelles present in laticiferous tissue, the development of ultracentrifugation and biochemical analyses have contributed to extensive knowledge of the various structural elements of latex *in vitro*. Huret (1948) was the first to carry out ultracentrifugation of fresh latex using a compressed air ultracentrifuge. The Dutch school that worked in Bogor (Indonesia, 1925–1950) obtained the first fundamental knowledge of latex organelles. This school, which notably included Frey-Wyssling, used optical microscopy and centrifugation. Homans and Van Gils (1948) of the Dutch school used low-speed centrifugation (2000 rpm $\times$ 20 min) to separate latex into a white supernatant fraction and a heavier yellow fraction accounting for 15–35% of the initial volume of latex (Resing, 1955). The white fraction is colloidal, made of rubber particles, whereas the yellow fraction contains carotenoids, as pointed out by Frey-Wyssling (1929) and thus named Frey-Wyssling particles. However, the yellow fraction consists essentially of organelles discovered by Homans and

Van Gils (1948) named lutoids by Ruinen (1950). The aqueous phase of latex plays the role of the dispersal phase for these two fractions.

The use of refrigerated ultracentrifuges (50,000 $\boldsymbol{g} \times 60$ min) separated latex into four clearly distinct zones (Cook and Sekhar, 1953). However, the investigations of Moir (1959) using vital stains characterized 11 distinct fractions in centrifuged latex that was maintained at a temperature less than 5°C.

4.1.1 Rubber particles

Latex usually contains 25–50% dry matter, 90% of which is made up of rubber. Among the 11 distinct zones obtained (Moir, 1959) by means of ultracentrifugation of fresh latex (Fig. 4.1), zones 1, 2 and 3 contain the rubber particles, and the biggest particles are found in zone 1, which is by far the largest (Southorn, 1961). The size of the particles in zone 2 varies from 0.05 to 0.25 μm and the particles are frequently elliptical and sometimes connected by fragments of membrane which might be endoplasmic reticulum. The particles in zone 3 are of lower average size (0.035–0.2 μm) and often appear to be linked to each other. Molecular weight and protein content are believed to be responsible for the differences in location of particles in zones 1, 2 and 3 (Gomez and Hamzah, 1989). Rubber particles are 0.1 μm in

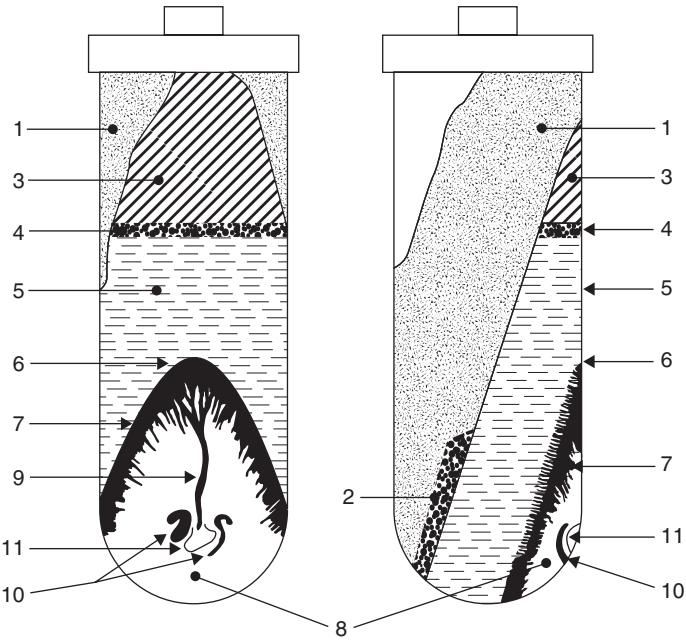


Fig. 4.1. Ultracentrifugation of *Hevea* latex. Fractions 1–3 are the white rubber phase. Fraction 4 is the Frey-Wyssling particles. Fraction 5 is the clear serum (C serum) corresponding to latex cytosol. Fractions 6–11 constitute the 'bottom fraction' of which the fraction 8 is the lutoid fraction.

diameter and contain several hundred *cis*-polyisoprene molecules. Electron microscopy reveals that rubber particles have a fully homogeneous internal structure (d'Auzac and Jacob, 1989).

The existence of a protein film surrounding rubber particles that contributes to their stability has been accepted for a very long time. Weber in 1903 gave the isoelectric pH of latex as 3.0–5.0; this is the characteristic value of many proteins (cited in Verhaar, 1959). As early as 1906, Henri showed that rubber particles of *Hevea* latex in an electrical field move towards the anode and therefore have a negative charge (Verhaar, 1959). One of the most important proteins in *Hevea* latex from the quantitative point of view has been characterized by Archer *et al.* (1963a) as being an α-globulin with a low sulfur content (0.06%), with the same isoelectric pH as latex (4.5), and easily absorbed at the surface of the rubber particles to ensure its colloidal stability. The *de novo* formation of rubber molecules occurs, at least in the very last stages, at the surface of the rubber particles. Rubber transferase is responsible for this and is normally distributed between the cytosol and the rubber particles (McMullen and McSweeny, 1966). This enzyme has been isolated and purified from cytosolic serum. It remains inactive as long as it has not been absorbed on particles of rubber, even when the latter have been purified by centrifugation and repeated washing. The reaction catalysed by this enzyme appears to be essentially a chain extension process (Archer and Cockbain, 1969).

Some 20% of the dry weight of the bottom fraction (i.e. of the lutoids) consists of soluble proteins. Hevein forms 70% of the total bottom fraction. Lutoids contain an acid serum, divalent cations such as Mg^{2+} and Ca^{2+}, and positively charged proteins that are effectively capable of provoking the formation of microflocculates and the creaming of a dilute suspension of rubber (Gomez and Tata, 1977). According to Sherief and Sethuraj (1978), a high ratio of cationic and anionic proteins in B serum may also increase plugging. Lutoids sometimes contain spring-like proteinic microhelices (Dickenson, 1969). These microhelices appear to occur more frequently in the latex of trees whose production has been stimulated by treatment with Ethrel®. Clusters of proteinic microfibrils with a double-helical structure are also seen in lutoids filling the intravacuolar space (Gomez, 1976). Purified microfibrils are thought to contain up to 4% carbohydrates (Audley, 1966). Their presence in young lutoids and their disappearance during the ontogeny of laticiferous vessels led to the suggestion that they may form nitrogen reserves which can be degraded by lutoid proteases (Dickenson, 1969).

Frey-Wyssling (1929) revealed another kind of globules – 'lipoids' – with carotenoids for yellow colouring, which later became known as Frey-Wyssling particles (Dickenson, 1965). The Frey-Wyssling complex consists of a complex system of branching single-membrane tubules associated with several concentric double-membrane lamellae. The Frey-Wyssling complex thus described is 4–6 nm in diameter and enclosed in a typical double membrane (Dickenson, 1965, 1969). Their high carotenoid content suggested that they contain the enzymes of the isoprenic synthesis pathway (d'Auzac and Jacob, 1989). Their double membrane resembles plastids, whose physiological role remains mysterious.

4.1.2 Organic non-rubber constituents

Proteins are prominent among the non-rubber constituents of latex (Tata, 1975, 1980b). The earliest report of the presence of proteins in latex was by Spencer (1908), who detected peroxidase and catalase activities in dialysed aqueous extracts of rubber sheets, and subsequently in dialysed latex. The total protein content in latex has been estimated to be about 1% (Archer and McMullen, 1961; Archer *et al*., 1963b; Tata, 1980a). While 27.2% of the total proteins were absorbed on the rubber surface, 47.5% was seen in the C serum and 25.3% in the bottom fraction (Tata, 1980b).

Proteins over the rubber particles are responsible for their colloidal stability. The existence of a protein-phospholipid layer imparting a negative charge on the surface of rubber particles contributes to the colloidal stability of these particles (Bowler, 1953). Isopentenyl pyrophosphate polymerase (Archer *et al.*, 1963a; Lynen, 1969) and rubber transferase are the two enzymes associated with the rubber particle surface (Archer *et al.*, 1963a, 1966; McMullen and McSweeney, 1966; Lynen, 1969; Archer and Cockbain, 1969). They are involved in rubber biosynthesis, and the fact that only two are associated with rubber particles needs to be explained further since several enzymes are expected to be involved in rubber biosynthesis. Proteins located in the C serum include enzymes for the glycolytic pathway and rubber biosynthesis (Archer and Audley, 1967; Bealing, 1969; d'Auzac and Jacob, 1969). Recently, 27 enzymes were separated by electrophoresis by Jacob and co-workers, of which 17 were shown to exist in multiple forms (Jacob *et al.*, 1978). Studies of Wititsuwannakul *et al*. (2004) showed the osmotically sensitive bottom-fraction membrane was found to be highly active for rubber biosynthesis, in contrast to previous reports that describe rubber biosynthesis only occurring on the rubber particle surface. It was clearly shown that washed bottom-fraction membrane was much more active than fresh rubber particles for rubber biosynthesis. Serial acetone extraction of washed bottom-fraction proteins showed a distinct profile of the fractions with different rubber biosynthesis activity, indicating that washed bottom fraction has both an enzyme system and a factor for regulation of rubber biosynthesis.

The first protein to be isolated from latex was from C serum. It was named α-globulin, the major component of C serum (Archer and Cockbain, 1955). It is readily adsorbed at a water–air or oil–water interface with a resulting fall in the interfacial tension. This led to the suggestion that α-globulin was one of the proteins on the surface of rubber particles and that it contributed to the colloidal stability of fresh latex (Archer and Cockbain, 1955). However, α-globulin was later found not to be present on the surface of the rubber particles (RRIM, 1982). With the advent of sensitive techniques like starch gel electrophoresis, Tata and Moir (1964) reported the presence of 22 protein bands in C serum. Seventeen of these were anionic at pH 8.2, while five were cationic and existed at much lower concentrations. A comparative study on the proteins in the C sera from four clones, namely RRIM 501, GT 1, Tjir 1 and Pil A44, revealed very few differences between their general electrophoretic patterns (RRIM, 1963). Later, the list of proteins in C serum was enlarged to 24 (Tata and Edwin, 1970), using the same starch gel electrophoretic technique. Using polyacrylamide gel electrophoresis, Yeang *et al.*

(1977) reported 26 protein bands from C serum at alkaline pH and 15 bands at acid pH. These workers also did not observe significant differences in the protein patterns of C sera between clones (Tjir 1, PR 107, GT 1, PB 86 and BR 2).

Proteins in the bottom fraction are essentially studied as the soluble proteins in B serum. The use of paper electrophoresis (Moir and Tata, 1960), starch gel electrophoresis (Tata and Edwin, 1970; Tata, 1975) and polyacrylamide gel electrophoresis (Yeang *et al.*, 1977) leads to the conclusion that the proteins of B serum were found to be markedly different from those of C serum. Upon electrophoresis, the B-serum proteins were usually separated into two major protein bands at the extreme anionic and cationic ends, with several minor bands in between. The major protein in B serum is hevein, which accounts for about 70% of the water-soluble proteins in the bottom fraction (Archer *et al.*, 1969). Hevein is a low molecular weight anionic protein (approximately 5000 daltons) with higher (5%) sulfur content (Archer, 1960; Tata, 1975, 1976). All the sulfur in hevein exists as eight disulfide bridges (S-S) of cystine (Archer, 1960; Tata, 1976). Because of its low molecular weight and the large number of S-S bridges, hevein is heat stable (Tata, 1975, 1976).

Subsequent analysis showed that earlier preparations of hevein were mixtures containing hevein, traces of esterase and a protein with slightly less anionic mobility termed pseudo-hevein (Archer, 1960; Karunakaran *et al.*, 1961; Tata, 1975, 1976). When pure hevein (free of pseudo-hevein) was isolated and characterized, it was found to be a single peptide chain with glutamic acid as the N terminus and, as mentioned above, a molecular weight of approximately 5000 daltons (Tata, 1975, 1976). The molecular weight of pseudo-hevein was also 5000 daltons. Later, an almost complete amino acid sequence of hevein was reported (Walujono *et al.*, 1976) that contained 43 amino acid residues in a single polypeptide chain and an estimated molecular weight of 4729 daltons.

Dickenson (1963, 1965, 1969), in his ultrastructural studies and electron microscopic investigations of lutoids, described some fibrillar components having a tightly coiled helical structure, which he named microfibrils. These structures were observed within lutoids of young latex vessels but were absent from mature vessels. These microfibrils were later shown to be proteins containing up to 4% carbohydrate, and having an isoelectric pH of about 4 (Audley, 1965, 1966). At ambient temperature (20°C), the microfibrils break up into smaller segments which reassemble on freezing (Audley, 1965, 1966). Later, Southorn and Edwin (1968) and Gomez and Yip (1975, 1976) carried out detailed investigations and reported that these zigzag structures differed from microfibrils in that they were larger in dimension and were open helices (not lightly coiled helices of the microfibrils). They were called 'microhelices' by Gomez and Yip (1975). Lowering of the ionic concentration of B serum by dialysis against water or by dilution with water resulted in the formation of microhelices (Gomez and Yip, 1975, 1976; Tata, 1975).

The presence of basic proteins in B serum was first demonstrated when B serum or an aqueous extract of freeze-dried bottom fraction was electrophoresed (Moir and Tata, 1960; Karunakaran *et al.*, 1961; Tata and Edwin, 1970). A major and a minor basic protein, which account for about 4% of the total, were found to have lysozyme and chitinase activities (Tata, 1980b; Tata *et al.*, 1983). The major basic protein has been crystallized and its molecular weight determined (approximately 26,000 daltons).

The major soluble carbohydrates in the latex are the cyclitols, sucrose and glucose (Low, 1978). Though the latex was believed to be mainly sucrose and a smaller amount of raffinose (Tupy and Resing, 1969), glucose and fructose are also present in significant quantity (Bealing, 1969). The low fructose concentration in latex sera is believed to be due to its rapid metabolism in preference to glucose (Bealing, 1969). The distribution and concentration of the major soluble carbohydrates in latex have been described (Low, 1978). The concentration of total cyclitols (i.e. quebrachitol, R- and m-inositols) appear to vary with clones (13.0–32.0 mg ml^{-1} of C serum). Like total cyclitols, the concentration of sucrose in C serum also varies with clones (4.0–10.5 mg ml^{-1}). While cyclitols and sucrose are confined largely to C serum, glucose is located mainly in the lutoids (Bealing, 1969; d'Auzac and Jacob, 1969).

Lipids and phospholipids associated with the rubber and non-rubber particles in latex play a vital role in the stability and colloidal behaviour of latex. Earlier studies (Cockbain and Philpott, 1963; Blackley, 1966) demonstrated that the rubber particles are strongly protected by a complex film of protein and lipid material. It is believed that some of the lipids are present within the rubber particles. The concentration and distribution of lipids between the rubber cream and the bottom fraction had been studied (Ho *et al.*, 1976). These lipids were isolated and divided into neutral lipids and phospholipids for further analysis. There appeared to be distinct clonal variation in the total amount of neutral lipids extractable from rubber cream and from the bottom fraction. The colloidal stability of latex was found to be related to the natural lipid content of rubber particles (Sherief and Sethuraj, 1978). Lipids from different clones, however, were qualitatively similar. Triglycerides and sterols were the main components of the neutral lipids of rubber particles, while sterols and long-chain free fatty acids are mainly made up of the neutral lipids of the bottom fraction. A furanoid fatty acid containing a methylfuran group was found mainly in the triglyceride fraction of the neutral lipids (Hasma and Subramaniam, 1978). It constituted about 90% of the total esterified acids. It was suggested that the main triglyceride in latex contained three furanoid fatty acids, hence making it a rare triglyceride known in nature. The phospholipid content of the rubber particles (approximately 1% of the dry weight of rubber) was similar between different clones. The total phospholipid content of the bottom fraction was much less (only about 10%) than that in the rubber cream. Ho *et al*. (1976) suggested that the amount of neutral lipid (especially triglycerides) associated with the rubber particles was inversely related to the plugging index (PI; this estimates the average plugging rate over the entire flow) of the clone. Lutoid stability, as indicated by the bursting index (BI), was found to be negatively correlated with the phospholipid content of the bottom fraction of latex (Sherief and Sethuraj, 1978).

4.1.3 Nucleic acids and polysomes

The presence of nucleic acids in latex was discovered by McMullen (1962). Latex contains both ribosomal RNA, soluble RNA, DNA and mRNA (Tupy, 1969). These are all present in the serum fraction of latex. Functional polysomes were

also discovered in the serum phase of latex (Coupe and d'Auzac, 1972). More recently, RNA (Marin and Trouslot, 1974) and ribosomes have been found to be located in lutoids. The lutoid ribosomes represent 11.9% of the total ribosomal content of the latex. Two high molecular weight RNA components have also been identified and their nucleotide base composition determined (Tupy, 1969). The presence of these membrane-bound ribosomes in lutoids led to the speculation that these ribosomes were transported from the cytoplasm to the lutoids (which are also lysosomes) where they are rapidly destroyed.

4.2 Latex Metabolism

Tapping causes loss of cell constituents from the laticifers (latex vessels). When flow stops as a result of the complex phenomena which lead to the coagulation of rubber particles and plugging of the wound, regeneration of the latex becomes necessary (Southorn, 1969). This involves intense metabolic activity. If there is a sufficiently long interval between two tappings, this regeneration can be complete and the intra-laticiferous metabolism then slows down (Jacob *et al.*, 1988). Although metabolism plays a major role in the production through the reconstitution of latex in the laticiferous tissue, factors regulating the latex flow also determine the amount of latex loss and the subsequent rate of catabolic activities (Sethuraj and Raghavendra, 1987). Also, transport of water and solutes to the laticiferous system requires biochemical energy (Jacob *et al.*, 1988). The biochemical composition of latex (Compagnon, 1986) shows very clearly that the 'royal metabolic pathway' of the laticiferous system is the synthesis of rubber, which forms 35–45% of the fresh weight and over 90% of the dry weight of latex. All the enzymatic processes are thus coordinated and arranged to result in the biosynthesis of rubber.

Hevea rubber is a macromolecule formed by chains of five-carbon isoprenic units (Bouchardat, 1875) – the *cis*-1,4 polyisoprene $(C_5H_8)_n$ – where *n* may range from 150 to 2,000,000 (Pushparajah, 2001). This high molecular weight polymer is formed from sequential condensation of isopentenyl diphosphate (IDP) units. IDP is a common intermediate for the production of numerous classes of isoprenoids produced in the plant kingdom. These units are the precursor of numerous other natural isoprenic substances (sterols, carotenoids, etc.). A close study of its structure has shown that the isoprenic bonds are mainly of the *cis* form; less than 0.2% is in the *trans* form and these make the first 'geranylgeranyl' links in the polyisoprene chain (Archer *et al.*, 1982; Audley and Archer, 1988). According to Kekwick (1989), the average molecular weight is between 200,000 and 800,000 daltons.

Laticifers are the major location of rubber biosynthesis (Gomez and Moir, 1979). Numerous classes of isoprenoids are produced through IDP as a common intermediate (Kekwick, 1989). The mevalonate (MVA) pathway has been the conventionally studied pathway for isoprenoid biosynthesis since the 1950s (Fig. 4.2). This cytosolic pathway of rubber formation was demonstrated through incorporation of radiolabelled pathway intermediates such as MVA and 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) (Hepper and Audley, 1969; Skilleter

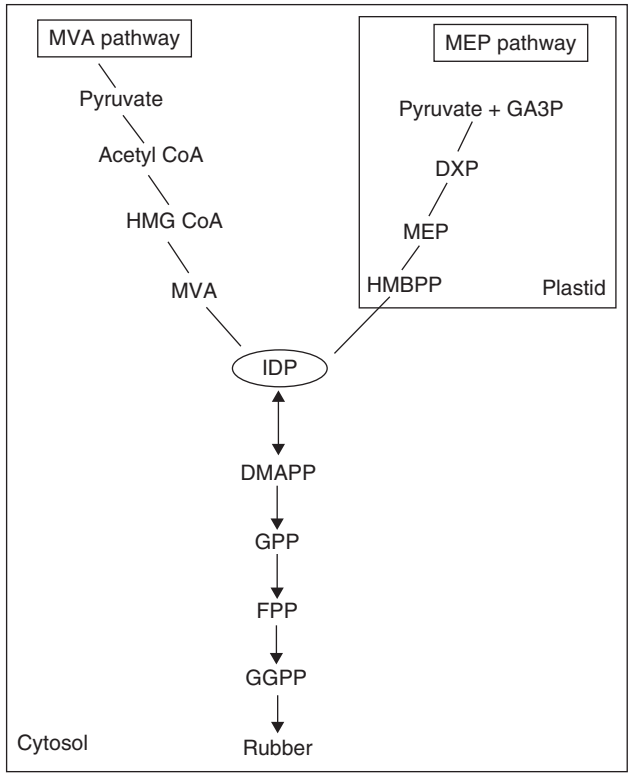


Fig. 4.2. Biosynthesis of *Hevea* rubber. Isopentenyl diphosphate (IDP) for the biosynthesis may be contributed by the mevalonate (MVA) and I-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (MEP) pathways. DMAPP, Dimethylallyl pyrophosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; FPP, farnesyl diphosphate; GA3P, glyceraldehyde-3-phosphate; GGPP, geranyl-geranyl diphosphate; GPP, geranyl diphosphate; HMBPP, 1-hydroxy-2-methyl-2-butenyl-4-diphosphate; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

and Kekwick, 1971) into rubber. Recently, the plastidic 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (MEP) pathway is being considered as a possible alternative route for rubber biosynthesis (Lichtenthaler, 1999; Rodríguez-Concepción and Boronat, 2002). The expression of 1-deoxy-D-xylulose 5-phosphate synthase (DXPS) in *Hevea* latex and leaves suggests that the MEP pathway exists in the laticifer (Ko *et al.*, 2003) and therefore could provide an alternative means of generating IDP for *cis*-polyisoprene synthesis.

The initiator molecules of rubber (short-chain allylic diphosphates) are synthesized from IDP by soluble *trans*-prenyltransferase (Archer *et al.*, 1963a; Archer and Audley, 1987). A membrane-bound *cis*-prenyltransferase or rubber transferase is thought to facilitate the condensation of new IDP units from *trans*

to *cis* configuration (Tanaka, 1989). There are numerous reports on the identification of *Hevea* rubber transferase (Archer and Cockbain, 1969; Archer and Audley, 1987; Light and Dennis, 1989; Cornish *et al.*, 1993); the involvement of *Hevea cis*-prenyltransferase in generating high molecular weight rubber molecules was more recently reported (Asawatreratanakul *et al.*, 2003). A number of other proteins have also been shown to participate in *cis*-polyisoprene biosynthesis. Initially, most attention was directed to the major membrane proteins of rubber particles, rubber elongation factor (REF) (Dennis and Light, 1989) and small rubber particle protein (SRPP) (Oh *et al.*, 1999), which share 72% protein sequence similarity. In addition, the cytosolic proteins identified were the rubber biosynthesis stimulator protein which corresponds to eIF-5A (Yusof *et al.*, 2000; Chow *et al.*, 2003) and a patatin-like inhibitor protein (Yusof *et al.*, 1998). The surface of pre-existing rubber particles are the presumed site for the synthesis of allylic diphosphate initiators and *cis*-polyisoprene (Archer *et al.*, 1963a and b; Archer and Audley, 1987). Also, Tangpakdee *et al*. (1997) and Wititsuwannakul *et al*. (2004) suggested that non-rubber particles may be the site for rubber initiation. Recently, sequencing of the expressed sequence tags (ESTs) has resulted in more precise insights into laticifer gene expression. Chow *et al*. (2007) carried out a genomic analysis of the latex transcriptome based on a collection of 10,040 latex ESTs with emphasis on genes known to be related to rubber biosynthesis. The majority of ESTs encoded proteins related to rubber biosynthesis and stress or defence responses. Both ESTs and quantitative reverse transcription-PCR (QRT-PCR) analyses revealed that REF and SRPP are the most abundant transcripts in latex. Numerous proteins with varying regulatory control and with mutual interactions are involved in the whole rubber biosynthesis machinery (Chow *et al.*, 2007).

4.2.1 Factors regulating metabolism of latex

Availability of sugar in the laticifers is the main attribute regulating the metabolism of latex, which depends on the carbohydrate loading to laticiferous tissue and its use at cell level (Tupy, 1988). Indeed, sucrose catabolism supplies the acetate molecules which initiate the isoprene chain and provide the energy necessary for the functioning of the laticifers (Jacob *et al.*, 1988). Positive, highly significant correlations have been established between sugar concentration and latex production (Jacob *et al.*, 1986). Sucrose loading of the laticifers is thus an extremely important phenomenon. Yield in *Hevea* rubber during summer tends to be low compared with other months with high sucrose content. This high sucrose concentration is demonstrated to be due to high sucrose synthase and thiol groups which are activators of sucrose synthase (Yeang *et al*., 1984). High sucrose might indicate less utilization of the same through glycolysis, thus low rubber yield. Lacrotte showed the existence of an intermembrane transport process which requires energy. It operates at laticifer plasmalemma level and involves a cotransport H^+-sucrose energized by an ATPase proton pump (Lacrotte *et al.*, 1985). Ions such as Mg^{2+} and PO^- and other products like citrate and thiols influence the activity of certain key enzymes as activators or inhibitors.

The process of active lutoid loading regulates the cytosolic concentration of certain ions (Mg^{2+}, Pi, Ca^{2+}), organic acids (citrate) and basic amino acids (Ribaillier *et al.*, 1971) and thus detoxifies the cytosol of certain ions which could be powerful enzymatic inhibitors (e.g. citrate). These transport phenomena are linked with the functioning of the lutoid membrane ATPase proton pump (d'Auzac, 1975) which, by inducing an electrochemical proton gradient, enables the cotransport of molecules with H^+ symport or antiport (Marin *et al.*, 1981). A lutoid membrane pyrophosphatase, which is also a proton pump (Prevot *et al*., 1988), probably plays a similar role to that of the ATPase.

pH is yet another essential factor in the regulation of the laticiferous metabolism which has an effect on glycolysis. Indeed, invertase and phosphoenolpyruvate carboxylase (PEPcase), the two key enzymes involved in sugar catabolism, are extremely sensitive to physiological variations in pH (Tupy, 1973; Jacob *et al.*, 1983). These organic acids are not connected with isoprenic synthesis, but may be connected with the energy-producing oxidation reactions. The pH of the cytosol and the lutoid compartment is different. While the cytosol pH corresponds to that of whole latex (neutral and varies between 6.5 and 7.4), the lutoid pH is much more acidic, ranging from 5.2 to 5.8 (Brzozowska-Hanower *et al.*, 1979). Numerous factors regulate the pH of the cytosol connected with the functioning of specific ATPases, which are expected to have a role in carbohydrate supply to the laticifers (Lacrotte *et al.*, 1985). Highly significant correlations have been found in intraclonal experiments between cytosol pH and production. Highly significant negative correlations were also seen between lutoid pH and production (Brzozowska-Hanower *et al.*, 1979). The difference between cytosol and lutoid pH values (ΔpH) has also been positively and significantly correlated with production (Marin and Chrestin, 1984).

4.3 Latex Vessels and Turgor Pressure

On tapping, initial latex flow is fast but recedes rapidly and ceases after a period that lasts from a few minutes to several hours. Subsequent tappings at regular intervals result in increased yield due to longer duration of flow and more dilute latex until it attains equilibrium. The increase in yield before reaching a state of equilibrium was termed by early workers as the wound response (Pakianathan, 1967). Regular and controlled tapping not only increases the time of flow but also enhances the biosynthesis of rubber in the drained vessels below the tapping cut. Longer latex flow is equated to higher yield provided the other circumstances and attributes remain unaffected. Ethephon (chloroethylphosphonic acid) is a popular stimulant to extend the latex flow (Abraham *et al*., 1971).

The physiological mechanisms of latex exudation and cessation of flow have been the subject of much research in the past (Southorn, 1969; Gomez, 1983; d'Auzac *et al*., 1989; Yeang, 2005). Latex accumulates in the latex vessels with the turgor pressure of 7.9–15 atmospheres (Arisz, 1920; Buttery and Boatman, 1964, 1966). Pakianathan (1967), using a vapour pressure osmometer, obtained values of 10–12 atmospheres on drop samples of latex. Diurnal turgor and

osmotic pressure measurements taken at various intervals from 0530 to 1900 h showed maximum turgor values at 0530 h whereas maximum osmotic pressure values were recorded between 1300 and 1600 h (Buttery and Boatman, 1966). The extent of dilutions, 5 min after the tapping had commenced, was 24.7%, 18.8% and 12.1%, for trees tapped at 0400 h, 0800 h and 1230 h, respectively. The diffusion pressure deficit was highest in trees tapped at 1230 h. Trees tapped at 0400 h yielded more latex than those tapped at 0830 h or 1230 h (Buttery and Boatman, 1966). Thus, it appeared that latex production was largely influenced by the internal water relations of the tapping panel. These observations showed that latex vessels behaved as a relatively simple osmotic system. Turgor pressure falls during the day as a result of withdrawal of water under transpirational stress (Buttery and Boatman, 1964; Pakianathan, 1967).

Upon tapping, the high turgor pressure expels latex from the vessels and, over the period, the loss of turgor pressure tends to stop the flow with the mechanism of latex vessel plugging (Boatman, 1966). Almost all hypotheses implicate the vacuole-like organelles called lutoids found in the bottom fraction of the centrifuged latex to be responsible for latex vessel plugging (Pakianathan *et al.*, 1966; Kongsawadworakul and Chrestin, 2003) (Fig. 4.1). The lutoidic serum (B serum) contains latex destabilizing factors and its release from damaged lutoids leads to the formation of plugs of flocculated or coagulated rubber at the cut ends of the latex vessels that lead to plugging of the latex vessels. The most commonly used measure of latex vessel plugging rate is the 'plugging index' (PI), which estimates the average plugging rate over the entire flow. The higher the PI, the lower will be the latex yield. The total solids content and dry rubber content (drc) are two other measures that give an idea of yield. The total solids content depends very much on tapping intensity and is reflected in the drc. A low drc (< 30%) is indicative of the tree getting over exploited.

Gomez (1983) made an extensive study of the events leading from the opening of the latex vessel to plugging. Through measuring flow rates at definite intervals, the exact shape of the flow curve can be determined; the total yield depends on the initial flow rate and duration of flow. Trees exhibiting different flow patterns can have more or less the same yield. The logarithmic transformation of flow rates shows linear trends except for the initial 30 min. The model of Paardekooper and Samosorn (1969) incorporates all these:

$$Y = b.e^{-at}$$

where Y is the flow rate at time t after tapping; b is the initial flow rate; e is the base of natural logarithms; and a is a constant mainly depending on the clone.

When successive tappings were conducted at 10 min intervals, the flow rate recovered markedly after each reopening so that stepped flow curves could be obtained (Boatman, 1966; Buttery and Boatman, 1966, 1967), indicating that some impediment develops at the cut ends of the latex vessels. Southorn (1968a) undertook optical and electron microscope studies on longitudinal sections of latex vessels near the tapping cut. Rubber particles and lutoids form internal plugs in some of the vessels, successfully plugging the vessels. With all this information in hand, Milford *et al*. (1969) studied clonal variations in plugging and

suggested that plugging phenomena could be explained through a PI derived from initial flow rate and total volume of latex:

$$\text{PI} = \frac{\text{Mean initial flow rate (ml min}^{-1})}{\text{Total yield volume (ml)}} \times 100$$

It is a clonal characteristic that varies with season and the tapping system and stimulation schedule adopted (Paardekooper, 1989).

The major cause of latex vessel plugging is lutoid damage. Changes in the osmotic concentration of the latex during latex flow damages the lutoids, which form aggregates with rubber particles that are found in large numbers at the bottom of the tapping cut (Pakianathan *et al*., 1966). The intensity of plugging was found to be proportional to the square root of time of latex flow in trees tapped with a half-spiral cut (medium flow), a quarter-spiral cut (short duration of flow) and trees that were stimulated (long duration of flow) (Yeang, 2005). Hence,

$$y = a + b\sqrt{x} \qquad \text{Equation 1}$$

where y = the percentage of cumulative latex vessel plugging on the tapping cut and a and b are constants. Applying this equation, the variance in cumulative plugging over the course of flow that was explained by the time lapsed from tapping exceeded 98% in each case for short-, medium- and long-flow duration trees. To confirm the relationship between cumulative plugging and time as suggested by curve fitting, a validation was performed by comparing the time during flow (x) predicted by Equation 1 with the actual time at which cumulative plugging (y) was known or could be measured. There were two such occasions during latex flow: (i) at the time of tapping where y would be 0 since all latex vessel plugs were presumed removed by tapping; and (ii) at the time of flow cessation where cumulative plugging would have just reached 100%. In Equation 1 y was substituted for $y = 0$ and $y = 100$. For the trees with short-, medium- or long-flow duration, the estimated values of x when $y = 0$ were very close to 0 in each case. Similarly, the estimated values for x when $y = 100$ were also very close to the observed time of flow cessation. Equation 1 was therefore experimentally validated. If all latex vessel plugs were deemed to be removed upon tapping the tree, then plugging would be 0 immediately the tree is tapped. Thus, the constant a may be dispensed with and Equation 1 may be simplified to:

$$y = b\sqrt{x} \qquad \text{Equation 2}$$

At the time of flow cessation, all the latex vessels would just have been plugged, and the cumulative plugging on the tapping cut, y, would therefore be 100%. Hence at flow cessation, $100 = b\sqrt{t}$ or $b = 100/\sqrt{t}$, where t is the total flow duration. Since $y = b\sqrt{x}$ (Equation 2),

$$y = (100/\sqrt{t}).\sqrt{x} = 100\sqrt{(x/t)} \qquad \text{Equation 3}$$

From Equation 3, the cumulative plugging at any point in time during latex flow, or the time taken to accrue a certain extent of plugging, can be determined. For example, to estimate the time it takes for half of the severed latex vessels at the tapping cut to be plugged, $y = 50$ is substituted in Equation 3, giving $x = t/4$.

Hence, 50% of the latex vessels are plugged at one-quarter of the way through the total flow duration. Similarly, to estimate what proportion of latex vessels would have been plugged at the midpoint of the total flow duration, $x = t/2$ is substituted in Equation 3, giving $y = 70.7$. Hence, 71% of the latex vessels are plugged at the midpoint of the total flow duration. Yeang (2005) suggested that tapping-panel turgor pressure and cumulative latex vessel plugging are major determinants regulating latex flow rate. Multiple regression models were examined. Since cumulative latex vessel plugging is proportional to $\sqrt{(x/t)}$ where t is the total duration, turgor pressure (TP) and cumulative plugging data can be fitted into a linear regression model:

$$y = a + b_1(\text{TP}) + b_2\sqrt{x/t}$$

where a, b_1 and b_2 are constants. Since t is constant for a tapping, the cumulative PI is a function of time (x) and the model can be:

$$y = a + b_1(\text{TP}) + b_2\sqrt{x}$$

Since cumulative latex vessel plugging is a function of time, latex flow can be expressed as a function of the laticifer turgor pressure and time.

Another measure of plugging, the 'intensity of plugging', calculates the cumulative plugging from the time of plugging to a given point of time of latex flow (Southorn and Gomez, 1970).

4.4 Anatomy and Latex Flow

Latex flow rate and the changes in tapping-panel turgor pressure have a direct relation to the anatomical aspects of the laticifer system. The latex vessels (laticifers) are arranged in concentric cylinders among the phloem tissue (Riches and Gooding, 1952). Elongated laticifer cells are laid down in each cylinder end to end with their end walls dissolved, thus forming sets of continuous articulated tubes (Fig. 4.3). These cylinders appear as rings in a cross section, known as 'latex vessel rings'. Lateral connections between adjacent latex vessels within the same ring occur, and the laticiferous system is thus made up of a complex network of interconnected vessels, gaining the name 'anastomosing latex vessels'. There are no connections between adjacent latex vessel rings. Hence, when the tree is tapped, the latex that exudes originates not only from the latex vessels of the trunk that are cut, but also from connected latex vessels of the same latex vessel ring that are uncut, but that lie within the proximity of the 'drainage area' of the tapping cut. Similarly, tapping-panel turgor pressure has a bearing on the changes in the drainage area as a whole.

On tapping, release of pressure occurs to a greater extent in the latex vessels than in the surrounding tissues. This results in a rapid elastic expulsion of latex flow through the vessels along the pressure gradient. The gradient is highest near the cut and becomes smaller with increasing distance away from the tapping cut. Frey-Wyssling (1952) and Riches and Gooding (1952) made extensive studies on the mechanism of latex flow and cessation of flow. Further work by Boatman (1966) and Buttery and Boatman (1967) demonstrated that flow is rapidly

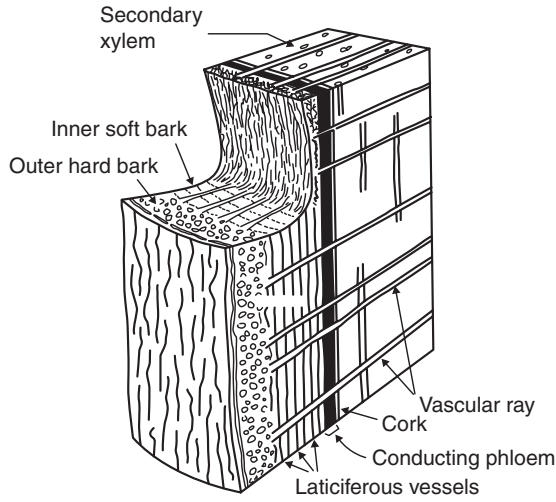


Fig. 4.3. Organization of virgin bark (after de Fay, 1981).

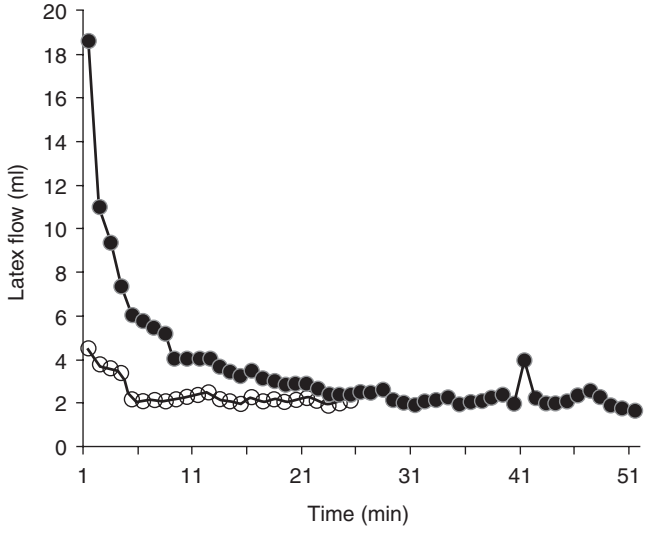


Fig. 4.4. Latex flow over time in RRIM 501 (○) and Tjir 1 (•).

restricted by plugging of the vessels at or near the cut surface and this was usually the major factor causing a decline in the flow rate (Fig. 4.4).

The collapse of latex vessel elasticity in relation to turgor pressure of the tapping panel was studied in the past in some detail. Latex vessels could contract by up to one-fifth of their diameter when cut (Frey-Wyssling, 1952). Frey-Wyssling (1952) also observed that latex was forcibly expelled when the turgid latex vessels collapsed at tapping. Pyke (1941) and Gooding (1952), who measured the minute contraction of the rubber tree trunk using a dendrometer, confirmed these observations. However, the dendrometer measurements were made against

a background of diurnal contraction and expansion of the trunk that was four to six times the magnitude of change due to tapping itself. This was not surprising since the dendrometer measured changes in the dimension of the entire tree trunk, whereas latex vessels constituted only 2% of the bark (Yeang, 2005). The measurements should be restricted to the laticifer system for a better understanding of the presumed latex vessel collapse and consequent loss of turgor. Buttery and Boatman (1964, 1966, 1967) meet this requirement as they measured the laticifer turgor pressure using a manometer that allowed latex flow into its capillary tubing. Since latex vessels are the only articulated cellular elements in the tapped bark, primarily only latex enters the glass capillary of the manometer that is visually verified (Buttery and Boatman, 1967; Yeang, 2005). Panel turgor pressure and the corresponding latex-vessel wall pressure close to the tapping cut drop immediately after tapping. This is consistent with the collapse of latex vessels after tapping envisioned by Pyke (1941) and Gooding (1952). The proportional changes in latex flow rate and the change in turgor pressure envisage a direct relationship between these attributes.

As an alternative to estimate laticifer turgor pressure, the trees were re-tapped and the manometric readings taken (Yeang, 2005). Cumulative latex vessel plugging at any point during latex flow can be eliminated by re-tapping the tree to remove all latex vessel plugs. Hence, the rate of latex flow immediately after re-tapping should reflect the laticifer turgor pressure. The change in latex flow rate upon re-tapping the tree is indeed proportional to the change in the manometer reading. In particular, turgor pressure readings in the early flow (within 15 min of tapping) are lower than expected as compared with the corresponding latex flow rate. This indicates that, though turgor pressure is primarily responsible for expelling latex from the tree when it is tapped, the manometric readings are, perhaps, underestimated during the early flow. Following the initial drop immediately after tapping, panel turgor recovers to a considerable extent before flow cessation (Buttery and Boatman, 1967). Hence, the latex flow cessation cannot be attributed entirely to turgor loss. Instead, there appear to be barriers that seal latex vessels progressively until flow ceases eventually.

It is clear that latex contains destabilizing factors normally located in the lutoid particles. Consequently, any physiological or biochemical factor which affects the stability of the lutoids would undoubtedly affect the latex flow and plugging of the vessels. By repeated reopening of the tapping cut, Boatman (1966) demonstrated that flow was restricted rather rapidly by some process occurring at or near the surface of the cut. Pakianathan *et al.* (1966) observed flocs of damaged lutoids in tapped latex and suggested that dilution of latex during flow might damage the osmotically sensitive lutoids and provide a possible mechanism of latex vessel plugging. Electron microscope observations of the ends of the tapping cut revealed both a cap of coagulum on the surface of the cut and internal plugs within the latex vessels (Southorn, 1968a). Lutoid counts taken before tapping and at various intervals during flow showed a rapid loss during the initial 30 min of flow, indicating that lutoids were trapped on the cut surface, and initial cap formation during the early stages of flow. Shear stress may play an important part in lutoid damage. Internal plugging occurs mainly during the fast initial flow whereas coagulation on the surface of the cut is effective when

the flow is slow. It seems that there is no substantial reason to suppose that the two types of sealing processes are separated in time (Southorn, 1968b).

4.5 Lutoids and Coagulation of Latex

Lutoids can destabilize the negatively charged colloidal suspension of rubber particles (Southorn, 1969). The negative charges of rubber particles can be neutralized with attributes such as the acidic pH, divalent cations (Mg^{2+} and Ca^{2+}) and entrapped positively charged proteins that are available in lutoids. In addition, some of the acid hydrolases trapped in lutoids can attack the protective coating of rubber particles. The coagulant role of intralutoid serum (B serum) has been demonstrated globally in a dilute suspension (2.7%) of rubber particles. In a few seconds the serum stops the Brownian movement that causes flocculation.

The breakdown of lutoids during or after tapping may liberate some hydrolytic enzymes able to attack the phospholipoprotein films which protect the stability of rubber particles. Among the lutoid enzymes discovered by Pujarniscle (1968) only a protease (cathepsin) displaying a very acid optimum pH ($\sim$ 3.5) was believed to be involved in this process, but no experimental proof has been proposed. Lysozyme, a quantitatively important hydrolytic lutoid enzyme, is not suited to attack the protective film of rubber particles, and in fact an exogenous lysozyme is unable to coagulate a suspension of rubber particles. Thus, proof of involvement of lutoid enzymes in latex coagulation has yet to be found. However, an exception must be made for the case of an NADH-quinone reductase originating from the lutoid membrane and which plays a role at least in bark dryness induced by overexploitation. The mechanism of action of the latter enzyme is quite different: the forms of toxic oxygen produced attacked the double bonds of the ethylenic fatty acid in the organelles and cell membranes. Leakage of the organelle components (lutoids and Frey-Wyssling particles) follows and then the destabilization of the colloidal suspension occurs. The efficiency of the NADH-quinone reductase depends on the equilibrium between the oxidizing and reducing molecules of the latex. Such equilibrium is itself related to the concentration of certain reducing molecules such as glutathione or ascorbic acid and to the activity of various protective enzymes such as catalase and superoxide dismutase or oxidizing enzymes like peroxidases and phenol oxidases. Studies by Hao *et al.* (2004) demonstrated that, during latex flow, the activities of chitinase and β-1,3-glucanase (the well-known defence proteins of lutoids) are responsible for making a protein network with rubber particles that protects wounded laticifers. They also argued that the lack of a protein network is the factor that leads to tapping panel dryness (TPD). Recently, Wititsuwannakul *et al.* (2008) demonstrated that a *Hevea* latex lectin-like protein (referred to as a *Hevea* latex lectin; HLL) present on the lutoid membrane is demonstrated to be responsible for rubber particle aggregation. A binding protein (BP) ligand counterpart for HLL was also identified. Based on protein identification by peptide mass fingerprinting, the RP-HLLBP (where RP is an abbreviation for 'rubber particle') was confirmed to be the small rubber particle protein (SRPP). Hence, an intrinsic rubber particle glycoprotein (RP-HLLBP or

SRPP) is the key component in the formation of rubber latex coagulum and hence latex vessel plugging.

4.5.1 Lutoid breakdown mechanisms

Natural coagulation, both *in situ* and *in vitro*, begins by the appearance of microflocs of degraded lutoids and rubber particles, and lutoids are the main elements responsible for stopping the flow sooner or later. The question to address now is to find out how during tapping the lutoids can release their contents into the latex, thus leading to the appearance of microflocs, which accumulate to form caps which block the tubes and stop the flow.

The duration of latex flow depends on the quantity of lutoids when they flow out of the laticiferous tubes. Biochemical analysis uses the bursting index (BI) defined by Ribaillier *et al.* (1971) which measures the ratio of lutoidic free acid phosphatase activities to total acid phosphatase activities determined after lutoids are burst with a detergent (0.1% Triton® X-100). This test reports the approximate percentage of degraded lutoids. The BI is generally negatively correlated with the yield – the more burst lutoids, the lower will be the yield and the higher will be the PI.

The fact that in *Hevea* with a high PI, the removal of a layer of bark about 1 mm thick from the tapping cut reactivates flow shows clearly that the plugging of laticiferous vessels is limited to the immediate proximity of the cut. This observation led to the hypothesis that the wound may cause an action potential at the wound itself which might lead to depolarization of cellular or intracellular membranes which had been polarized following active phenomena. Such depolarization might act as a trigger for the release or leakage of solutes across membranes. In so far as lutoid membranes are concerned, it is conceivable that the formation of microflocs may be induced near the wound site.

The yellow fraction, which is essentially lutoidic and usually viscous, may be observed under the microscope to stiffen and flocculate after the addition of water (Homans and Van Gils, 1948). The dilution of fresh latex *in vitro*, with increasing quantities of water, causes progressive damage to the bottom (essentially lutoidic) fraction, which disappears progressively (Pakianathan *et al*., 1966). With increased dilution, the damaged lutoid particles tend to aggregate with rubber particles and form clusters which are lighter than lutoids. These clusters float in the clear C serum and finally, with the highest percentages of dilution, collect just beneath the rubber particles after ultracentrifugation, and the bottom fraction disappears completely. The mechanism of lutoid degradation by dilution with water is related to the absorption of water through the semipermeable lutoid membrane, which has a negative osmotic potential (ψ_s). Further, it was demonstrated that the dilution of latex by 0.3 M mannitol buffered to pH 7 prevented the rapid degradation of lutoids (Pakianathan *et al.*, 1966). Determination of the osmolarity of whole latex performed either using the freezing point technique or with a vapour pressure osmometer (Pakianathan, 1967), gave values of ψ_s ranging from -330 to -450 mosmol kg^{-1}. An increase of +143 mosmol kg^{-1} between the beginning and the end of flow was frequently observed. These increases

were correlated with the well-known dilution of latex which occurs during tapping. All the processes described above involve the degradation of the lutoid membrane. The main reason that this occurs is probably the shear stress caused at the open extremity of the latex tubes early on in the tapping operation when the turgor pressure drops by nearly 10 atmospheres (Southorn, 1969). In addition, dilution of latex with water during tapping can increase its osmotic pressure to a certain extent, but it is not clear whether the hypotonic conditions which appear in this way are strong enough to cause much bursting of lutoids (Pakianathan *et al.*, 1966).

4.6 Harvest

One of the main reasons for the successful establishment of *H. brasiliensis* on a plantation scale in the Far East was H.N. Ridley's discovery (in 1890–1891) of an improved method of harvesting rubber from the tree, the excision method of tapping. In this method, the same cut is regularly reopened by the removal at each tapping of a thin shaving of bark from a sloping cut, a principle which is in general use today. On each occasion, a tree is tapped by means of a suitable knife, so that a channel is prepared along which the latex can flow. This method avoids wounding of the trees as the tissues of the tree can be recognized.

Hevea does not accumulate more than a certain amount of rubber in the latex vessels, and draining out the latex by tapping stimulates the production of latex to replace it. Thus, a tree can be trained by regular tapping to a continuous process of rubber regeneration leading to high cumulative yields. Regeneration of rubber and maintenance of yield are directly in response to regular tapping, requiring the continual application of manual labour. Different tapping systems modify the amount of rubber produced per unit labour or per unit capital invested in the planting and abandoning tapping means permanent loss of the crop. The importance of excision tapping lay in the fact that the method was based on the specific characteristics of the bark.

When the demand for rubber increased in the beginning of the 20th century, planters became daring and began increasing the length of the tapping cut and practised intensive tapping systems to obtain greater yields. However, experience soon taught them that with lengthening the cut the yield per unit length of the tapping cut became less and they learned that yield was not proportional to the amount of bark incised. They also noticed that, although they obtained good yield responses with intensive tapping at the beginning, the yield declined after some time. Bark renewal too became poor and the planters returned to less intensive tapping systems (Dijkman, 1951). In those days nothing was known of the physiology of latex flow so it was a matter of finding a tapping system by empirical means. This situation prevailed until 1920. The results of Dutch scientists (De Jong, 1916 cited by Dijkman, 1951; Bobilioff, 1923; Maas, 1925 cited by Dijkman, 1951) who carried out many tapping experiments and studies on anatomy and physiology of *Hevea* helped to formulate tapping systems on a scientific and rational basis. Thus the early tapping systems slowly evolved into the modern systems largely by reducing the number of cuts and the frequency of tapping with B0-1, B0-2, BI-1 and BI-2 panels (Fig. 4.5).

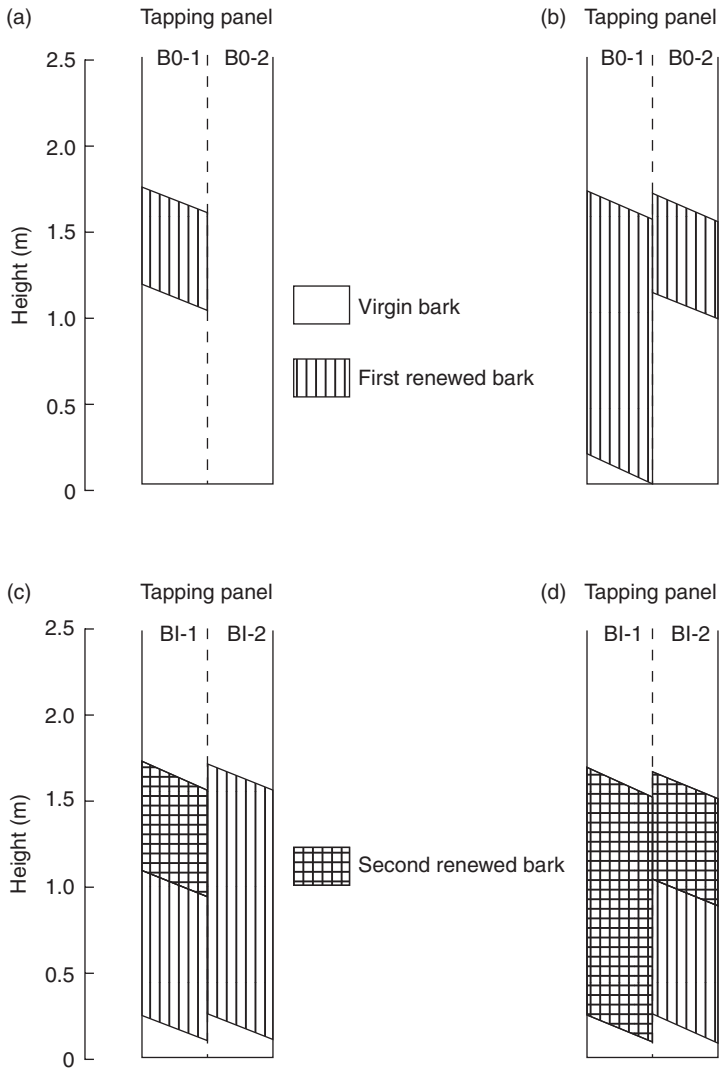


Fig. 4.5. Representation of tapping panels. The first cut of the tapping panel is shown in (a), second cut in (b), third in (c) and fourth in (d). The cutting sequence is then repeated.

4.6.1 Tapping notations

Many tapping systems evolved over the years which included complicated techniques such as the herringbone system. Local names were given for each system and this led to a lot of confusion and difficulty. Thus, the various rubber research institutes formulated a uniform method of expressing various tapping systems that are commonly used. At the initiative of the International Rubber Research

and Development Board (IRRDB), a standard international tapping notation for tapping systems was revised (Lukman, 1983). The notation consists of a set of symbols which should be used in regular sequence.

The first symbol describes the number and nature of the cuts. Four symbols, representing four types of cuts, are:

- S – a spiral cut;
- V – a V cut;
- C – a circumferential cut; this could be a V cut or a spiral extending around the entire circumference of the tree; and
- Mc – a minicut (5 cm or less in length).

The length of the cut is represented by a fraction preceding the symbol for the cut. For the minicut, the actual length is denoted in centimetres. So for example:

- S = one full spiral cut;
- V = one full V cut;
- C = one full circumference cut;
- ½ S = one-half of a spiral cut;
- ¼ S = one-quarter of a spiral cut;
- ⅓ V = one-third of a V cut;
- ¾ S = three-quarters of a spiral cut;
- ½ C = one-half of a circumference cut; and
- Mc5 = a minicut 5 cm in length.

While undertaking an upward tapping, an upward arrow (↑) is given immediately after the tapping notation. Bidirectional tapping is denoted by both upward and downward arrows. Hence:

- ½ S↓ = one-half spiral cut downwards;
- ½ S↑= one-half spiral cut tapped upwards;
- 2 × ½ S↑↓ = two half spiral cuts; one upwards and another downwards; and
- ¼ S↑ + ½ S↓ = one one-quarter spiral cut upwards and another half spiral downwards.

The unit is a day (d) for frequency of tapping and the denominator will be the actual interval between tappings. Hence, the actual frequency will be:

- d/1 – daily tapping;
- d/2 – alternate day tapping;
- d/3 – third day tapping; and
- d/4 – fourth day tapping.

If the practical frequency is broken by a day of rest or a regular day (say a holiday), the numerator will be the total days tapped and the denominator will be the total period. For example, alternate days tapping followed by 1 day of rest every week will be denoted as: d/2 6d/7. Similarly, if the tapping is every third day followed by 1 day of rest, then the notation will be: d/3 6d/7. Periodicity

consists of details of weeks (w), months (m) and years (y). For example, every third day tapping followed by 1 day of rest done for 4 weeks, followed by 1 week rest undertaken for 9 months followed by 3 months rest will be: d/3 6d/7 4w/5 9m/12. If the length of tapping cut is shortened or lengthened, a horizontal arrow will separate old and new notations. For example, one-quarter tapping downwards changed to half spiral tapping downwards will be denoted as: ¼ S → ½ S.

Panel notations were described as A, B, C, D, E and F. The latest panel notations are: B0-1 (first virgin bark), B0-2 (second virgin bark), B1-1 (first renewed bark of B0-1), BI-2 (first renewed bark of B0-2), B2-1 (second renewed bark of B0-1) and B2-2 (second renewed bark of B0-2).

While the tree is stimulated for yield, the notations are different but not separated from the tapping notation. If the tree is stimulated with 1.0% ethephon (ET) applied to the panel (Pa) with 1 g of stimulant per application on a 1 cm band with 16 applications year^{-1} at weekly intervals the notation will be: ET1.0%Pa1(1).16/y(1w).

As a modification of the above, Vijayakumar *et al*. (2009) gave the following tapping notations with the approval of the IRRDB:

S/2 d3 6d/7 .ET 2.5,% Pa2(2) 8/y(m)

i.e. half spiral cut without a rain guard tapped downwards, at a frequency of tapping every third day, 6 days tapping followed by 1 day of tapping rest, stimulated with ethephon using 2.5% active ingredient with 2 g of stimulant applied on the panel in a 2 cm band, eight applications year^{-1} at monthly intervals.

S/2(RG) d3 6d/7 95/104. ET 2.5% Pa2(2) 8/y(m) 6/8

i.e. half spiral rain-guarded cut tapped downwards at a frequency of tapping every third day, 6 days, tapping followed by 1 day rest, with 95 tappings achieved against 104 scheduled tapping days year^{-1}, stimulated with 2.5% ethephon with 2 g of the stimulant applied on the panel in a 2 cm band, eight scheduled applications year^{-1} at monthly intervals. Six stimulations could be done against the scheduled eight year^{-1}.

S/2(RG) d3 6d/7 6m(JUN–NOV)/12. ET 2.5% Pa2(2) 4/6m(6w);
S/4U d3 6d/7 6m(DEC–MAY)/12. ET 5.0% La1(–) 9/6m(3w) (6m,6m)

i.e. half spiral rain-guarded cut tapped downwards at a frequency of tapping every third day, 6 days tapping followed by 1 day of tapping rest, 6 months of tapping from June to November, stimulation with 2.5% ethephon with 2 g of the stimulant applied on the panel in a 2 cm band, four applications in 6 months at intervals of 6 weeks between applications, changed to a one-quarter spiral cut tapped upwards ('U' in the notation stands for upward tapping; if this is not shown it is downward tapping) for the next 6 months from December to May, stimulation with 5.0% ethephon with 1.0 g of stimulant applied on lace, nine applications in 6 months at intervals of 3 weeks between applications. The cycle is repeated.

S/4 d4 6d/7 9m (MAR–NOV)/12. ET 2.5% Pa 1 (2) 18/9m (2w) + S/4U d4 6d/79m (MAR–NOV) /12. ET 5% La1(–) 18/9m (2w)

i.e. two quarter spiral cuts, one tapped downwards and the other tapped upwards, at a frequency of tapping every fourth day, 6 days tapping followed by 1 day of tapping rest, 9 months of tapping from March to November followed by 3 months of rest, both cuts stimulated, the lower cut with 2.5% ethephon, 1.0 g of stimulant applied on the panel in a 2 cm band, 18 applications in 9 months at fortnightly intervals, while the upward tapped cut is stimulated with 5.0% ethephon, 1.0 g of stimulant applied on the lace, 18 applications in 9 months at fortnightly intervals. While expressing data, the number of tappings realized may be shown as a fraction of the maximum number of tapping days possible.

The aforesaid tapping notations are the latest and are approved by the IRRDB. Although they are a little complicated, these notations are to be followed at least while presenting scientific data. Planters, however, use the age-old A, B, C and D panels for their convenience.

4.6.2 Tapping techniques

There is a need to open trees for tapping as soon as the required minimum girth is attained. While the budded trees have cylindrical trunks and can be opened at a height which tappers can reach without any aid, seedling trees are conical with a bigger girth at the base of the tree and hence a lower height of opening is recommended. With conventional tapping the recommendation is to open bud-grafted trees for tapping with a girth of 46 cm and above (when 70% of the trees attain that girth at a height of 1.5 m from the ground). For seedling trees, the convention is to open them when a similar girth is reached at a height of 75 cm from the ground.

The latex vessels in the bark traverse from bottom left to top right at an angle of 30° in an anticlockwise direction. Hence, a cut from the high left to low right will sever a greater number of latex vessels, which led to the current practice of a sloping cut from high left to low right on all spiral cuts. Similarly, a 25° slope is preferred for seedling trees because it results in a smaller area of bark being lost when the cuts reach ground level without much loss of yield. Further, the presence of a thick corky layer in the bark provides a channel for the flow of latex (Fig. 4.6). Since the bark thickness is less in bud-grafted trees, the latex may overflow the sides of the tapping cut with a 25° slope; which is an additional reason for having a 30° slope in bud-grafted trees. When the tapping cut approaches the base of the tree, a new cut on the opposite panel can be similarly opened.

The yield obtained from the tree is greatly influenced by the skill of the tapper. A skilled tapper will tap to an optimum depth of within 1 mm of the cambium without wounding it. The greatest number of latex vessels is situated near the cambium so tapping as close to it as possible realizes the best yield (Fig. 4.3). This is where the skill of the tapper is critical in that he is able to tap deep without wounding the trees. Low intensity tapping systems benefit more from deep tapping than high intensity systems (Abraham and Tayler, 1967). Experiments have shown that beyond a minimum bark consumption, yield is not enhanced with increasing thickness of bark shaving (De Jonge and Warriar, 1965). Low frequency

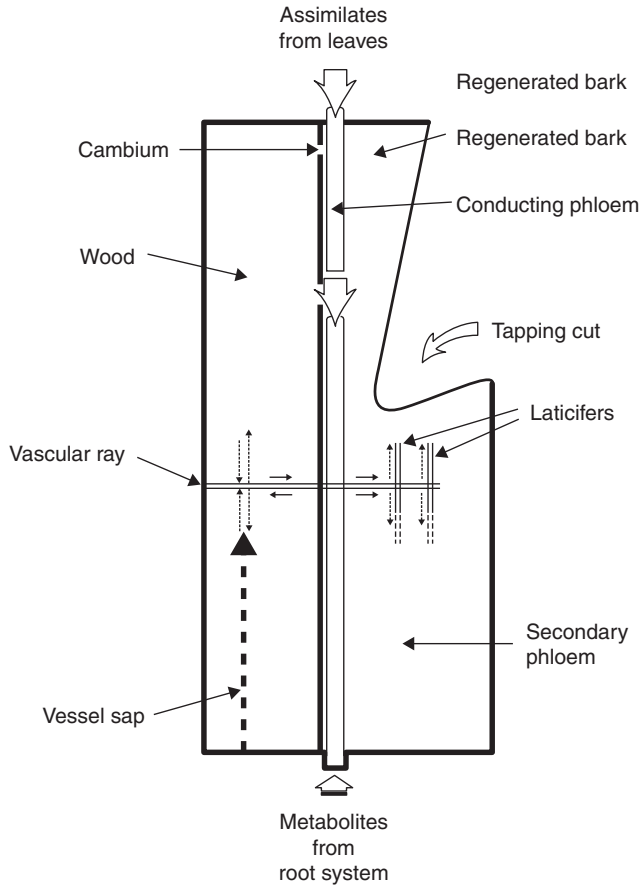


Fig. 4.6. Diagrammatic representation of a tapping panel.

tapping systems cause more drying of the bark tissue between tappings. A thicker bark shaving per tapping is required which experienced and skilled tappers adjust automatically. Annual bark consumption from different frequencies of tapping on a half-spiral cut will be: (i) 20–23 cm for alternate day tapping; (ii) 16–18 cm for third day tapping; and (iii) 14–16 cm for fourth day tapping. A higher yield can be obtained at dawn. This is believed to have a direct bearing on the turgidity of the tree with transpiration being at a minimum at this time of day under conditions of a high atmospheric pressure and high RH.

The tapping task for a tapper will depend on the tapping system, stand per hectare and topography of the land. In Malaysia, the normal task size with ½ S d/2 tapping is between 550 and 600 trees. Tapping is controlled wounding and it retards the growth of all trees, especially bud-grafted trees. This is clone dependent and such trees are likely to have unbalanced development and will become prone to wind damage. Hence, tapping systems must be tailored to the growth habit of cultivars after tapping. Longer cuts than half spiral tend to reduce the

rate of girth increment and hence long-cut systems are not preferred on young rubber trees. Two micro-tapping systems have been used in the planting industry, namely: (i) puncture tapping; and (ii) micro-X tapping. Both systems only work with stimulation and hence have their limitations. Puncture tapping has some attractions for bringing trees into early tapping (Abraham, 1981). Micro-X combines the use of puncture tapping and excision tapping (Ismail Hashim *et al.*, 1979).

4.6.3 Factors affecting tapping efficiency

Tapping is a skilled operation and hence quality of tapping varies from person to person. Usually the best tappers are given young trees to tap where tapping should be carried out with minimum wounding. Field supervision is a must to ensure that latex never spills over the panel and tapping is carried out to the proper depth to reduce yield loss. On flat land, a tapper may be able to tap 600 trees on panel B0-1 and on a steep hill slope only 500 trees. As mentioned earlier, tapping at dawn is most preferred since hydrostatic pressure of the tree is high (1.0–1.5 MPa) and diurnal variation in latex flow follows the vapour pressure deficit of the air. This implies the role of transpiration in turgor pressure (Rao *et al.*, 1990).

The girth of older trees is usually larger, and hence the tapping cuts are longer. Consequently, it requires more time to tap the older trees and a tapper who taps 600 trees on panel B0-1 may only be required to tap 575 trees on panel B0-2, 530 trees on panel BI-1 and so on. The length of the tapping cut is also determined by the tapping system. It is the owner of the estate who determines the task load of each tapper. For example, where tapping involves the use of a ladder, the tapper should be given only 65% of the number of trees that are given for low-level tapping. In control-led upward tapping (CUT), a tapper is given a slightly bigger task size than ladder tapping (Ismail Hashim *et al.*, 1981).

Two tapping knives are commonly used in the industry: (i) the Michie-Golledge; and (ii) the Jebong. A third type is the Gouge but this is meant mainly for CUT. While the Jebong is suitable for shaving off a thin layer of bark, the Gouge is used to push along the tapping cut to shave off the bark instead of being pulled along as with the Jebong. A modified Gouge with a long handle is widely used for CUT. Bidirectional knives are also available and are used for upward and downward tapping systems (Abraham, 1981). Spouts made of galvanized iron are fixed (without injuring the cambium) to the trees at the end of the tapping cut to enable the latex to flow from the tapping cut into the cup.

4.6.4 Yield stimulation

Stimulation of latex flow is principally an exogenous process to increase the yield above that normally obtained by tapping a rubber tree. The first known report on yield stimulation is that periodic scraping of bark led to an increase in yield (Kamerun, 1912). Stimulation is now an integral part of most exploitation

methods. The early history of stimulation has been reviewed, tracing the development to the commercial use of synthetic growth substances such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Abraham and Tayler, 1967). A wide range of substituted phenoxyacetic acids and substituted benzoic acids were screened for stimulant activity during the period 1956–1968. A number of experiments led to the conclusion that only 2,4,dichloro-5-fluoro-phenoxyacetic acid gave a comparable or better response than 2,4,5-T, but its use was not considered for economic reasons (Blackman, 1961; Abraham *et al.*, 1968). The results supported the continued use of 2,4-D and 2,4,5-T as a yield stimulant of *Hevea*.

The first use of a gas (ethylene oxide, which is toxic) was reported to increase latex flow (Taysum, 1961). Subsequently, acetylene was found to be a yield stimulant (Banchi and Poliniere, 1969). The yield stimulant action of acetylene and ethylene was later confirmed (d'Auzac and Ribaillier, 1969a, b) and all effective non-gaseous stimulants generate ethylene (Abraham *et al.*, 1968). Ethylene gas is believed to act more or less directly by inhibiting the plugging reaction of the trees (Abraham *et al.*, 1972). The success of (2-chloroethyl) phosphonic acid (ethephon) as a yield stimulant generated an intensive search for alternative ethylene-based stimulants. Though numerous chemicals were screened covering a wide spectrum, no alternative to ethephon could be named (Pakianathan, 1971). However, derivatives of ethephon, especially polysilylene phosphate derivatives, could constantly prolong the production of ethylene over time (Debouet *et al.*, 2003).

There are several methods of applying ethephon that are not labour demanding but consequently cheap, simple and very practical. These methods are described here briefly. A common method is applying the stimulant over the scraped bark below the tapping cut. This method entails demarcating a narrow band below the tapping cut, scraping off the outer corky tissue and then applying a thin layer of stimulant mixture over the scraped area. The ethephon on the applied portion is absorbed before the next application (Abraham *et al.*, 1976). In the second method, the stimulant is applied to the regenerating bark immediately above the cut, no scraping is done and the width of application varies with the frequency (Puddy and Warriar, 1961). In the third method, the stimulant is applied to the groove of the tapping cut by means of a paint brush after removal of the tree lace (the remnants of the latex flow from the cut down to the cup), on a non-tapping day at monthly intervals. This is as effective as the scraped bark method. The fourth method is the lace application, in which the stimulant is applied to the groove of the tapping cut without removal of the tree lace.

Gaseous stimulants are available using ethylene popularly known as HLE and RRIMFLOW (Guha *et al.*, 1992). In the case of HLE, a 1 mm puncture is made in the bark using a hypodermic needle and latex is extracted in a container with 8–10% ammonia solution, while RRIMFLOW is applied through a minicut (2.5 mm) with d/4 frequency. In both cases, a small portion of the bark is exposed to ethylene gas with the help of applicators. Labour productivity can be improved and yield maximized where there are a large number of trees (e.g. 900–1000) to be tapped by reducing tapping frequency from d/4 to d/6. Tapping at low intensity and frequency along with low dosage of stimulation using ethephon has

been suggested as an effective approach to increase productivity per tapper and thus reduce the cost of production (Zarin *et al.*, 1991; Thanh *et al.*, 1996).

The incidence of dryness in stimulated trees is generally higher than that in unstimulated trees and increases markedly in stimulated trees when the cut approaches the graft union (Sivakumaran *et al.*, 1981). Generally, trees on which stimulation was first introduced on virgin panels have a higher incidence of dryness than trees which were first stimulated on renewed panels. A previously tapped and stimulated panel has a greater chance of becoming dry.

Further, the extent of depression in girth increment is largely influenced by the age of trees and the intensity of tapping in stimulated trees. Thus, stimulation on virgin panels generally has a greater adverse effect on girth increment than stimulation on renewed panels when growth rate is lower and competition for assimilates is less. Generally, the depression in girth increases in proportion to the increase in the length of cut, with the most depression obtained in trees that are intensively tapped.

The effect of stimulation on bark thickness and number of latex rings varies according to cultivar. Thus, in a study of eight clones bark thickness of renewed bark was significantly increased in ethephon-stimulated trees of five clones relative to respective controls, while in three others the increase was not significant (Sivakumaran *et al.*, 1981). Similarly, for the number of latex vessels, there was an increase in six clones, while in two there was a marginal decrease (Ping, 1982).

In trees stimulated for 3 years with ethephon, both initial flow rate and turgor pressure were reduced in comparison with controls (Pakianathan, 1977). Also, a marked reduction in initial flow rates in long-term ethephon-treated trees was observed relative to unstimulated trees (Pakianathan *et al.*, 1982). Anatomical examination of bark taken from such low-pressure areas has shown there is an increase in stone cells in the soft bark and partial emptiness of latex vessels along with a reduction in sucrose levels (Tupy and Primot, 1976).

4.7 Tapping Panel Dryness (TPD)

TPD is a syndrome encountered in rubber plantations, characterized by spontaneous drying up of the tapping cut resulting in abnormally low yield or stoppage of latex production. The disease was reported for the first time in Brazil in 1887 in the Amazon forest and at the beginning of the 20th century in plantations in Asia (Rutgers and Dammerman, 1914). The symptoms range from partial dryness with no browning of the tapping cut, browning and thickening of the bark and cracking and deformation of the bark in some instances. The syndrome is characterized by: (i) the appearance of tylosoids and the coagulation of latex (Paranjothy and Ghandhimathi, 1976; de Fay and Hebant, 1980; de Fay, 1981); (ii) abnormal behaviour of the parenchyma cells adjoining the laticifers; and (iii) general increase in synthesis of polyphenols (Rands, 1921). A detailed review of the histological, histochemical and cytological study of the diseased bark was presented by de Fay and Jacob (1989).

Nandris *et al*. (2006) made a distinction between TPD and trunk phloem necrosis (TPN). While TPN almost invariably results in irreversible panel dryness,

TPD can be either reversible or irreversible. There are several reasons for which TPD or TPN can occur. Some are: (i) reduced water availability due to compaction of soils combined with disturbed sap flow (Nandris *et al*., 2006); (ii) involvement of impaired cyanogenesis in the necrotic process of bark tissue (Chrestin *et al*., 2004); (iii) occurrence of oxidative stress within the latex cells (Sookmark *et al*., 2006); and (iv) overstimulation (Sookmark *et al*., 2006). Further, involvement of a causative organism (Rands, 1921; Sharples, 1922; Keuchenius, 1924), existence of cortical necrosis (Peries and Brohier, 1965; Zheng Guanbiao *et al*., 1982) and rickettsia-like organisms were suspected to be responsible for TPD. However, there is no evidence so far for any of these contentions. High intensity of exploitation is known to promote the incidence of TPD in plantations; the proportion of dry trees increases with tapping intensity and particularly with tapping frequency (Chua, 1967; Bealing and Chua, 1972). Intensive exploitation is reported to result in: (i) excessive outflow of latex and consequent nutritional stress (Chua, 1967); (ii) inadequate organic resources (Chua, 1966; Tupy, 1984); and (iii) Cu and K deficiency (Compagnon *et al*., 1953).

The influences of climate and growth period were also believed to be the reasons for dryness (Harmsen, 1919; Compagnon *et al.*, 1953; Bealing and Chua, 1972). Unbalanced nutrition favouring the incidence of disease was reported by Pushpadas *et al.* (1975). Clonal sensitivity was also observed by many workers as a reason (Dijkman, 1951; Omokhafe, 2004). Although there is considerable evidence for the possible causes of dryness, the real reason and a cause of dryness that is accepted by all are yet to be confirmed.

The most common symptom of TPD/TPN is a phase of excessive and/or late dripping of latex and a simultaneous fall in the drc, followed by a sharp decline in the volume per tapping. The colloidal stability of the latex will also be reduced, resulting in particle damage, flocculation of rubber particles and early plugging of latex vessels (Chrestin 1985). A reduction in turgor pressure (Sethuraj *et al.*, 1977), change in latex flow pattern (Sethuraj, 1968) and a sharp increase in the BI (Eschbach *et al.*, 1983) can also occur. The starch reserve is not depleted (Chua, 1967) and the vascular rays function normally (de Fay, 1981).

Certain forms of bark dryness are transitory and do not display the characteristic symptoms of the formation of tylosoids or activation of the phenolic metabolism (de Fay and Jacob, 1989). Numerous traumas (e.g. mechanical trauma such as tapping, chemical trauma or pathological infection) cause the formation of ethylene and its influence in biochemical, anatomical and histological phenomena is proved (Liebermann, 1973). Overstimulation (dose and frequency) or over tapping can lead to excessive endogenous ethylene production and a deleterious effect on cellular systems (Chrestin, 1985). Deliberate overstimulation with Ethrel® can also result in imbalanced peroxidase activity and consequently the disorganization of membrane structures, thus leading to bark dryness. A reduction in sucrose, thiol and Mg contents and increase in redox potential are connected with a higher rate of bark dryness (Eschbach *et al*., 1986). Though tapping rest for varying periods can revive certain trees, in many cases reoccurrence of dryness is not uncommon.

Ecophysiological studies have shown that TPN-affected trees experience a significant water deficit with higher stomatal resistance and cytologically an

abnormal vascular connection between rootstock and scion. At the ultrastructural level, signs of degeneration have been observed in mitochondria – a typical feature of stress and ageing (Nandris *et al*., 2006). Construction of inner phloem cDNA suppression subtractive hybridization (SSH) libraries and bioinformatic analysis of more than 2000 ESTs sequenced from the SSH libraries (healthy versus TPN and TPN versus healthy) suggest there is differential gene expression (Kongsawadworakul *et al*., 2006). While investigating the biochemical and/or molecular markers related to the stress response and the role of oxidative stress in the onset of bark disorders, Sookmark *et al*. (2006) cloned and characterized three full-length cDNAs encoding Cu/Zn-superoxide dismutase (Cu/Zn-SOD), ascorbate peroxidase (APX) and glutathione peroxidase (GPX). While healthy trees showed a positive relationship between rubber yield and latex cytosolic GPX activity and gene expression, TPD trees showed exactly the opposite. And, in contrast, trees exhibiting TPN had both higher GPX activity and gene expression. So, although both TPD and TPN trees end up with a dry panel, they appear to differ in their origin (Sookmark *et al*., 2006). Much emphasis has been placed on the study of TPD/TPN, but a comprehensive picture of this syndrome is yet to emerge.

5 Genetics and Breeding

Breeding (cyclical recombination and selection) is oriented towards the improvement of latex yield and speed of growth. The technique of bud grafting is beneficial for it makes possible the vegetative multiplication of elite clones that are highly heterozygous genotypes, so allowing efficient use of the available genetic variability. However, there are drawbacks of which the main one is the long time needed for genotype evaluation, and also low female fertility which is an added constraint for recombination. The rubber industry in Asia and Africa was based on the Wickham population, but now the aim is to integrate new Amazonian wild populations with a view to enlarging the base of genetic variability.

5.1 Genetic Resources

5.1.1 *Hevea* as a species complex

The genus *Hevea* includes ten species, which are intercrossable (Clément-Demange *et al*., 2000). Schultes (1977a, b) and Wycherley (1992) refer readers to excellent reviews on the subject. The taxonomic considerations from 1874 to 1970 delineated the genus with several species on different occasions. Although the genus was considered to include 24 species in 1906, the species concept crystallized with nine species in 1970 (Schultes, 1977a, b). A tenth species, *Hevea camargoana* was added in 1971 (Schultes, 1987). *Hevea paludosa* has been identified in Brazil and is often considered as an 11th species (Pires, 1973; Gonçalves *et al*., 1990). Three botanists are considered to be the 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* (from the archive) 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' (Dijkman, 1951).

The ten species recognized today as belonging to the genus *Hevea* are: *H. brasiliensis*, *H. guianensis*, *H. benthamiana*, *H. pauciflora*, *H. spruceana*, *H. microphylla*, *H. rigidifolia*, *H. nitida*, *H. camporum* and *H. camargoana* (Webster and Paardekooper, 1989; Wycherley, 1992; Schultes, 1990). Seven species are found in the upper Rio Negro region, considered to be the centre of origin of the genus. *Hevea brasiliensis* is found in southern areas outside this centre, in the upper Rio Madeira, where five other species are represented. It has generally been assumed that the species are freely intercompatible (Baldwin, 1947; Seibert, 1947). Pires (1981) observed natural hybrids of *H. camargoana* × *H. brasiliensis*, and Gonçalves *et al*. (1982) analysed progeny derived from hand pollination from this type of crossing. Consequently, *Hevea* species might be considered as a species complex, due to the absence of a strict barrier to recombination between species. Many efforts have led to the identification of certain types which were formerly presented as other possible species. *Hevea paludosa* was identified in Brazil by Ule in 1905 and is often considered as an 11th species (Gonçalves *et al*., 1990; Priyadarshan and Gonçalves, 2003). An elaborate description of taxonomical and botanical aspects of *Hevea* has been reviewed by Schultes (1977a, b, 1987, 1990) and Wycherley (1992).

A summary of the salient features of different species of *Hevea* is presented in Table 5.1.

5.1.2 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, Colombia, French Guiana, Guyana, Peru, Suriname and Venezuela. All species except *H. microphylla* occur in Brazil, the centre of origin (Gonçalves *et al*., 1990). Four species have been found in Colombia and three occur in Venezuela. Two occur in Bolivia, French Guiana and Guyana. *Hevea guianensis* is the most widely adapted species (Fig. 5.1) (Pushparajah, 2001). Temperate-type rubber thrives up to 2500–3000 m in the Andes Mountains (Senyuan, 1990). These species of *Hevea* evolved in the Amazonian forests over 100,000 years ago (Clément-Demange *et al*., 2000). It is pertinent that species adaptation to a particular area is as per climatic and edaphic requirements. The centre 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* show only limited adaptation (Fig. 5.1). 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 noting that except for *H. benthamiana* (clones F 4512, F 4542) none of the other species has been utilized for the improvement of the rubber tree.

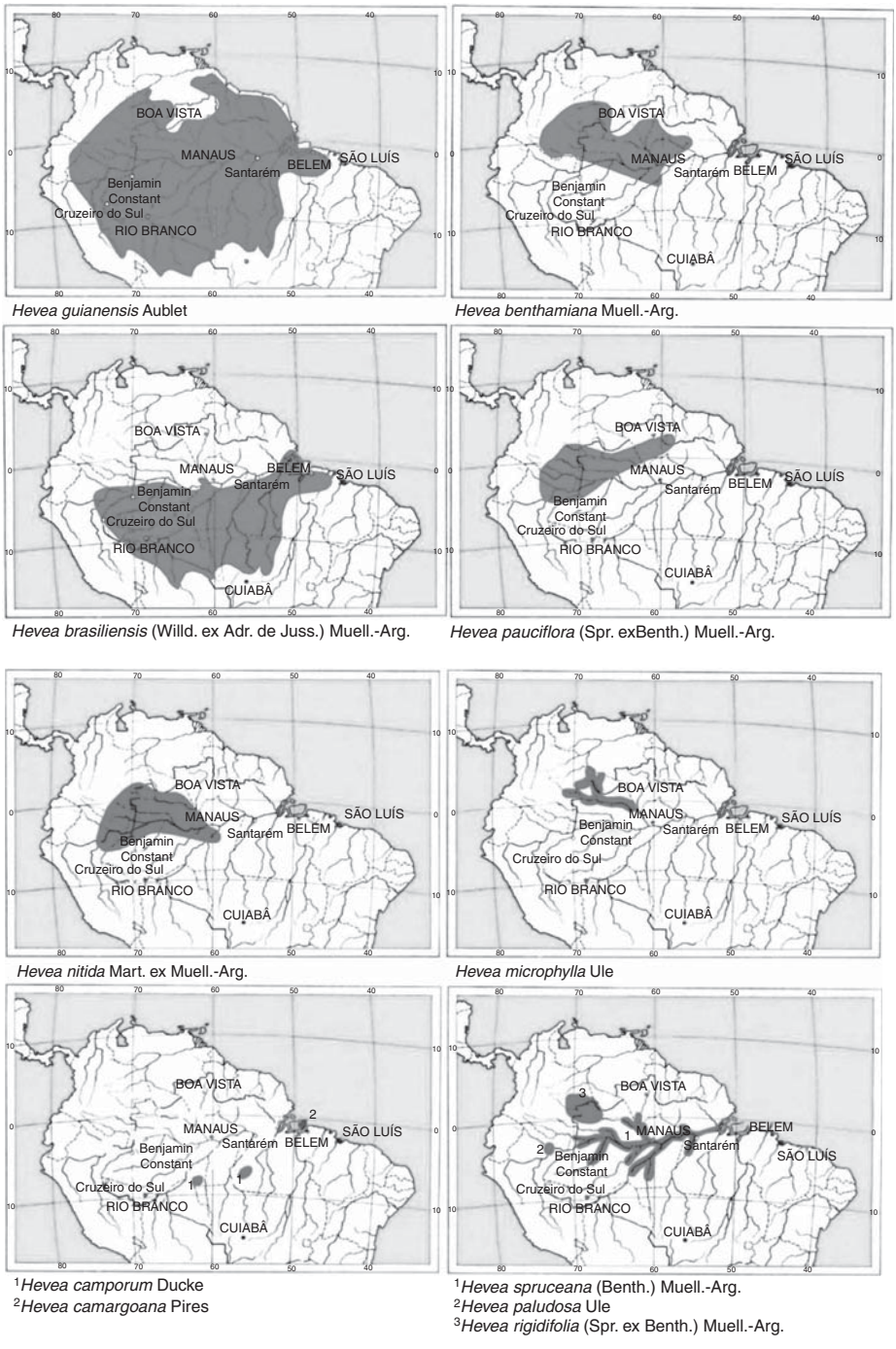
All *Hevea* species have $2n = 36$ chromosomes, with the exception of one triploid clone of *H. guianensis* ($2n = 54$) and the existence of one genotype of

Table 5.1. Allied species of the genus *Hevea* – occurrence and features (Source: Schultes, 1970, 1977b; Ministério da Indústria e Comércio, Brazil, 1971; Pires, 1973; Gonçalves *et al*., 1990; Wycherley, 1992).

Species	Occurrence	Notable features[a]
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. Adr. 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 hybridization with *H. brasiliensis*. From 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 Guiana, Peru, Colombia, Suriname, 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 regions of the geographic range of the genus	Complete defoliation of leaves. Small trees. They live on flooded area (igapós). Habitat: sandy or lateritic 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). Habitat: quartzitic soils
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 hillsides
H. rigidifolia (Spr. ex Benth.) Muell-Arg.	Among Negro river and its affluents, Uaupes and Içana rivers (Brazil, Colombia and Venezuela)	Retains old leaves even after inflorescences appear. Small tree from savannahs. Sometimes 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)	Retains old leaves until new leaves and inflorescences appear. Flowers reddish purple. Medium size tree. Habitat: muddy soils of islands
H. paludosa Ule[b]	Marshy areas of Iquitos, Peru	Small leaflets, narrow and thin in the fertile branches; up to 30 m in height. Habitat: marshy areas

[a] The wintering characteristics mentioned here have a bearing on the incidence of fungal diseases (e.g. *Oidium* infestation), especially secondary leaf fall since retention of older leaves may make the tree an '*Oidium* escape'. Dwarf types are desirable for wind fastness. All species are diploid ($2n = 36$) (Majumder, 1964), and are crossable among themselves (Clément-Demange *et al*., 2000).

[b] Pires (1973) considered 11 species including *H. paludosa* identified in Brazil.



Hevea guianensis Aublet

Hevea benthamiana Muell.-Arg.

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

Hevea pauciflora (Spr. exBenth.) Muell.-Arg.

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.

Fig. 5.1. Distribution of *Hevea* species in the Amazon valley (after Priyadarshan and Gonçalves, 2003).

H. pauciflora with $2n = 18$ (Baldwin, 1947; Majumder, 1964). Although *Hevea* behaves as a diploid, it is believed to be an amphidiploid ($2n = 36$; $x = 9$) that stabilized during the course of evolution. This contention is supported by the observation of tetravalents during meiosis (Raemer, 1935; Ong, 1976; Wycherley, 1976). *In situ* hybridization studies revealed two distinct 18S–25S rDNA loci and one 5S rDNA locus, suggesting a possible allotetraploid origin with the loss of 5S rDNA during the course of evolution (Leitch *et al*., 1998). But locus duplications are infrequent in the *Hevea* genome, and they could have occurred due to chromosomal modifications posterior to the polyploidization event (Seguin *et al*., 2003); consequently, the two unknown ancestral genomes of *Hevea* would have strongly diverged.

Low and Bonner (1985) characterized the *Hevea* nuclear genome as containing 48% of slowly annealing DNA (putative single copy) and 32% middle repetitive sequences with the remaining DNA being highly repetitive or palindromic. The size of the whole nuclear genome was first estimated as 6×10^8 base pairs. An estimation of the size, using flux cytometry, demonstrated 1.9×10^9 base pairs for *H. brasiliensis*, *H. benthamiana*, *H. guianensis*, *H. pauciflora* and *H. spruceana* (Seguin *et al*., 2003). The evolution of the cytoplasmic genome was slower, due to the lack of genetic recombination through meiosis. The estimated mean molecular size of chloroplast DNA (ctDNA) is 152 kb (Fong *et al.*, 1994). Differentiation of the genus into species appears to be linked with the evolution of the Amazonian forest over the last 100,000 years. Alternations of humid and semi-arid periods responsible for the forest extension or fragmentation resulted in the formation of forest islets. These are assumed to have become zones of protection and differentiation under local selection pressures.

5.1.3 New genetic resources

The 'Wickham' population developed in Asia, issued from the collection of seeds in Brazil by Wickham in 1876, has been the basis for rubber domestication and was reputed to have a narrow genetic base. This justified the organization of other collections and transfers of wild germ plasm from Amazonia to the main rubber-producing countries, mainly for *H. brasiliensis* but also for allied species. Moreover, the Ford Company and Firestone (companies owning rubber estates in Latin America) as well as Brazilian research contributed to the creation of a stock of selected 'Amazonian' and 'Wickham × Amazonian' germ plasm (F, FX, MDF, FDR, IAN, IAC clones).

From the details of the story of the first transfer of rubber seeds from Brazil to Asia (Dean, 1987; Baulkwill, 1989), it is difficult to evaluate how narrow the genetic base initially was for what has now become the 'Wickham' domesticated population. Much importance was conferred to a small number of 22 seedlings disseminated from Singapure to Malaysia after 1876; but a significant part of the Wickham seedlings which germinated in Kew Botanic Gardens was then sent to Ceylon (now Sri Lanka), raised and disseminated to different countries, especially India. However, it must be underlined that the original Wickham stock was collected in only one Brazilian site, Boïm, on the western banks of the Tapajoz

river, not far from Santarem. From then, directional selection applied to this population for more than one century and the limitation of the low fruit set in *Hevea* probably further contributed to reducing the extension of this genetic base. Genetic diversity can now be compared with that of the available wild Amazonian populations by use of molecular genetic markers.

Many other introductions from Brazil to Asia and also Africa were carried out between 1896 and 1974, including some species that differed from *H. brasiliensis* (Dijkman, 1951; Brookson, 1956; Baptist, 1961; Wycherley, 1968; Hallé and Combe, 1975; Nicolas, 1976; Ong *et al*., 1983; Ong and Tan, 1987; Tan, 1987). All collections were quantitatively rather limited, especially for non-*brasiliensis* species.

In 1981, the IRRDB organized an international collection in Brazil composed predominantly of seeds, but also of budwood and seedlings (Nicolas, 1981; Nouy, 1982; Tan, 1987; Simmonds, 1989). This collection was carried out over three states (Acre, Rondonia and Mato Grosso) from 60 different locations spread over 16 districts. It resulted in the provision of around 10,000 new accessions for breeding. Of this, 37.5% of the seeds were sent to Malaysia and 12.5% to Côte d'Ivoire. Half of the collections were maintained in Brazil. The accessions from the budwood collection were brought to Malaysia and Côte d'Ivoire after a quarantine period of 1 year on the island of Guadalupe (as a protection from SALB disease). After the establishment of two IRRDB germ plasm centres in Malaysia and Côte d'Ivoire, other IRRDB member countries were supplied with budwood from this material according to their request.

The field evaluation of this wild Amazonian germ plasm showed that the latex yield was as low as about 10% of GT 1, one of the most cultivated clones. Attempts to improve it through Wickham × Amazonian crosses resulted in recombinants that still had a low yield, ranging between 30% and 50% of the level of GT 1, probably due to the important genetic gap lying between the two populations. Conversely, a wide variability was found within these crosses for growth, enabling the selection of very vigorous Wickham × Amazonian clones. A clear difference in branching habit could be observed between accessions from Acre and Rondonia, which more often have tall trunks with poor branching located at a high height, and those from Mato Grosso, which display abundant branching at a low height. Obviously, this wild Amazonian germ plasm is bearing an important genetic burden in terms of unfavourable alleles. From the evaluation of IRRDB 1981 germ plasm in Côte d'Ivoire, a working population of 287 accessions was selected, taking into account genetic diversity but mainly based on yield; the average yield level of this population is estimated at 36% of the level of GT 1 (Nicolas *et al*., 1988; Clément-Demange *et al.*, 1998). Four genetic groups of this population could be the basis of pre-breeding work aimed at improving their yield level before testing them by crossing with the Wickham population (see Chapter 6).

In 1995, an expedition was launched by the Rubber Research Institute of Malaysia (RRIM) to collect rubber seeds from Brazil. From this collection, about 50,231 seedlings were planted in Malaysia, including allied species (RRIM, 1997; MRB, 1999). In order to enlarge the genetic variability of *Hevea*, some research was carried out on mutation breeding (Ong and Subramaniam, 1973;

Markose *et al*., 1977) and on polyploidization of the '2*n* = 36 *H. brasiliensis* species' (Mendes and Mendes, 1963; Shepherd, 1969; Zheng *et al*., 1980, 1981). An artificial triploid has been produced by crossing a diploid and a tetraploid (Saraswathyamma *et al*., 1988). Naturally occurring triploids have also been reported (Nazeer and Saraswathyamma, 1987). The existence of some putative genetically dwarf or semi-dwarf genotypes have been mentioned (Ong *et al.*, 1983); *H. camargoana* would have a dwarf growth habit (Gonçalves *et al.*, 1982). Some molecular genetic markers were associated with the dwarfing trait (Venkatachalam *et al*., 2004).

5.2 Early History of Rubber Breeding

Dijkman (1951), in a global presentation of rubber research in the first half of the 20th century, related how rubber breeding was pioneered in the East Indies (Indonesia). Seeds from the first Wickham trees planted in Asia after 1876 were used for the establishment of new plantations ('random unselected seedling populations'). Cramer analysed the variability of these seedlings for latex yield (Cramer, 1914, 1934). Almost at the same period in Malaysia, it was reported that 9.8% of a population of seedlings produced 28% of the total crop (Whitby, 1919). Such variation analyses led to the identification of better yielding trees and to the preferential use of their seeds for new plantings ('mother-tree seedlings') in an extensive process of mass selection. While vegetative multiplication by bud grafting was being developed by van Helten (1918) for multiplying the best seedlings as clones (Dijkman, 1951; Cramer, 1956), the method for producing recombinants (full-sib families) by hand pollination was also standardized. During this period, this method was seen as a way to produce elite seeds directly for commercial planting. Cramer and Dijkman were distinguishing the two methods by calling them: (i) vegetative selection; and (ii) generative selection or 'breeding'. However, generative selection could produce only limited quantities of seeds, while the quality of 'mother-tree seedlings' gradually improved as a result of the intensive directional selection of the mother trees. In 1916, the bud grafting method was good enough to be used commercially, but no clone had been selected, and the bud grafting technique had to overcome the many doubts of the planters. This sceptical attitude was motivated by varied practical and economic reasons. For example, the raffia strips which were used in bud grafting for binding the buds to the rootstock were not rainproof and were poorly effective as compared with the plastic strips that became available more than 30 years later. As a result, bud-grafting success at that time was relatively low. The competition between mother-tree seedlings and bud-grafted clones led to two tree types available to planters until the end of World War II when bud-grafted clones began to emerge as the most productive. These two ways were not only competitive but also complementary for state-owned research institutions and large companies (see Dijkman, 1951, p. 97: Flow chart of vegetative selection and breeding in *Hevea*). The selection of mother trees and the observations on their seedlings were important sources of information for the selection of new clones, and 'breeding', the generative way, became the basis for clone selection, the vegetative way. Also, the best clones were used

for establishing polyclonal seed gardens. All lines of breeding can be streamlined under three main headings: (i) evaluation of established clones for quick recommendations; (ii) recombination breeding and derivation of hybrids; and (iii) evaluation of polycross progenies like ortets (mother trees) and polyclonal seedlings (Priyadarshan and Clément-Demange, 2004). All these, either individually or in combination will lead to new clones. Details of these programmes will be considered here. However, while presenting these, a strict separation is not possible since these lines of work are mutually complementary.

5.3 Evaluation of Clones

When considering planting a new area that may be suboptimal for rubber growth the best plants to use are polyclonal seedlings to ensure maximum stand development, sacrificing yield since these areas are affected by stress conditions. However, evaluation of established clones gives quick information on what clones are suitable for a new area. Many planting recommendations made in several countries are based on such clone evaluations. Detailed accounts of the performance of clones in non-traditional rubber-growing areas of India, Brazil, Vietnam and China are available in Tables 5.2–5.5. A worldwide account is given in Table 5.6. A comparison of yield and other abilities of polyclonal seedlings and clones is given in Table 5.7. Varied performances were noticed among these clones in all those areas (see Chapter 8 for further details).

5.4 Recombination Breeding

Natural pollination was the basis of 'random seedling populations' and of 'mother-tree seedlings'. Natural pollination was exploited by creating polyclonal seed gardens with the best mixed clones, planted in isolated sites in order to protect them from outside pollen. They produce 'polyclonal seeds', such as the outstanding PBIG/GG seeds (Prang Besar Isolation Gardens/Gough Gardens) which were used for commercial plantings and proved to be tough competitors of the best clones, even after World War II (Simmonds, 1996a, b). Natural pollination in seed gardens can lead to a certain amount of selfed seedlings with potential inbreeding. This is why it was advised not to use seeds from monoclone plots ('monoclone seedlings'), where selfing could reach rather high rates. This inbreeding effect, noted by Sharp (1940, 1951) and discussed by Ross and Brookson (1966) and other authors (Tan, 1981), is suspected to be mainly due to the highly heterozygous nature of rubber, but its real importance has not actually been estimated. This 'generative' way of breeding still has some supporters. Arguments in its favour would be that: (i) planting seeds is easier for smallholders; (ii) seed gardens can provide seeds for growing improved rootstocks; and (iii) they are sources for creating 'rubber forest plantations' for timber (in both cases by use of vigorous parental clones). However, the limitations are: (i) the economic competitiveness of such synthetic cultivars cannot be guaranteed (unless intensive research is committed to this); (ii) a long time is required

Table 5.2. Yield and secondary attributes of 20 clones being evaluated in Tripura, India.

Clone	Stand (initial)	Girth (mature)	Projected yield (kg ha^{-1})[a]	Crop efficiency (g cm^{-1} of the tapping cut)	Wind damage	TPD	*Oidium* incidence
RRII 5	Average	Low[b]	1118[d]	0.85	Moderate	Low	Severe
RRII 105	Good	Moderate[b]	1297[d]	1.00	Moderate	Low	Severe
RRII 118	Good	High[b]	1389[d]	1.07	High	Mild	Moderate
RRII 203	Good	Moderate[b]	1647[d]	1.14	Low	Low	Mild
RRII 208	Good	Moderate[c]	1597[e]	0.93	High	Very mild	Severe
RRIM 600	Good	Moderate[b]	1499[d]	0.99	Low	Moderate	Severe
RRIM 605	Good	Moderate[b]	1074[d]	0.74	Moderate	Moderate	Moderate
RRIM 703	Average	Moderate[b]	1426[d]	1.21	Moderate	Low	Mild
RRIC 52	Average	Moderate[b]	992[d]	0.51	High	Low	Mild
RRIC 105	Average	High[b]	1093[d]	0.59	High	Low	Low
PB 5/51	Good	Low[b]	984[d]	0.74	Low	Mild	Very severe[f]
PB 86	Good	Low[b]	1083[d]	0.77	Moderate	Low	Moderate
PB 235	Good	High[b]	1858[d]	1.34	Moderate	Moderate	Severe
GT 1	Good	Moderate[b]	1077[d]	0.85	Low	Mild	Moderate
GI 1	Good	Low[b]	557[d]	0.44	Mild	Low	Severe
HARBEL 1	Average	Low	752[d]	0.58	Low	Low	Severe
PR 107	Good	Good[c]	878[e]	0.29	Very low	Mild	Very severe[f]
SCATC 88/13	Good	Good[c]	910[e]	0.67	Low	Moderate	Severe
SCATC 93/114	Good	Good[c]	822[e]	0.24	Medium	Very mild	Low
HIAKEN 1	Good	Good[c]	1011[e]	0.68	Medium	Mild	Moderate

[a] Projected yield = g per tree per tap × number of tappings × total stand (350).
[b] Over 13 years.
[c] Over 7 years.
[d] D panel.
[e] B panel.
[f] With secondary infection.

Table 5.3. Yield and yield depression over 9 years at São Paulo, Brazil.

Clone	Mean yield (g per tree per tap)[a]	Yield (g per tree per tap)		Yield depression during regime I (%)	Projected yield (kg ha^{-1})[b]
		Regime I (May–September)	Regime II (October–January)		
RRIM 600	54.81	42.65	66.97	36.30	1579
PB 235	64.69	47.24	82.14	42.49	1863
GT 1	52.06	43.24	60.88	28.98	1499
PR 255	47.65	34.10	61.20	44.28	1372
PR 261	41.75	37.20	46.30	19.65	1202
IAN 873	45.80	32.10	59.50	46.05	1319
FX 3864	60.51	52.91	68.10	22.31	1743
PB 252	53.40	46.80	60.00	22.00	1538
PB 330	41.59	35.10	48.08	27.00	1198
PB 217	74.55	70.00	79.09	11.49	2147
PR 107	40.65	28.40	52.90	46.31	1171

[a] Tapping systems: ½ S d/4 5d/7, with 2.5% ethephon stimulation.
[b] Projected yield = g per tree per tap × number of tappings (72) × total stand (400).

Table 5.4. Main characteristics of clones in suboptimal areas of Vietnam, Kontum Province (Highlands – 550 m above sea level).

Clone	Girth at opening (cm)	Yield over 10 years in kg ha^{-1} (g per tree per tap)	*Oidium* infestation	*Phytophthora* leaf fall	TPD
GT 1	Moderate	1191 (46.4)	Moderate	Moderate	Moderate
PB 235	High	1607 (59.8)	Severe	Low	Moderate
PB 255	Moderate	1174 (56.2)	Moderate	Moderate	High
PB 310	Moderate	1659 (52.8)	Low	Low	Moderate
PR 255	Low	1191 (49.8)	Moderate	–	Moderate
PR 261	Low	1197 (64.2)	Moderate	–	High
RRIC 110	High	1558 (66.3)	Low	Moderate	High
RRIM 600	Moderate	1177 (57.9)	Low	High	Moderate
VM 515	Moderate	1539 (63.4)	Moderate	High	High

between the setting of seed gardens and the availability of seeds; and (iii) the low seed productivity of seed gardens. On the other hand, the 'clone revolution' achieved through bud grafting of elite genotypes has been the main factor in the increase in rubber production, first in Malaysia, then in Thailand (with smallholders) and in other countries. This revolution is still to be achieved in Indonesia, where many smallholders still use seedlings.

Selection from seedling trees of commercial plantations gave 'primary clones' of unknown parental origin. A good example was provided by Gough (Prang Besar, Malaysia), who surveyed some million trees in the Kajang area of

Table 5.5. Yield[a] and secondary attributes of some clones in China.

Clone	Site	Stand	Girth	Mean yield (kg t^{-1} $year^{-1}$)	Yield (kg ha^{-1})	Years of tapping	Wind damage	Cold damage	*Oidium* incidence	TPD	Remarks
GT 1	Yunnan	Average	Moderate	2.87	1257.2	9	–	Low	Moderate	Moderate	Commercial trial
PR 107	Yunnan	Average	Moderate	3.15	1007.9	10	Very low	Moderate	Severe	Low	Commercial trial
RRIM 600	Hainan	Average	Moderate	5.12	1252.3	10	Moderate	Moderate	Moderate	Moderate	Commercial trial
GT 1	West Guang-dong	Average	Low	2.25	994.0	9	–	Low	Moderate	Moderate	Commercial trial
REYAN 93–114	West Guang-dong	Average	Low	2.14	980.3	9	–	Very low	Moderate	Low	Commercial trial
YUNYAN 77–2	Yunnan	Average	Moderate	4.61	1874.5	9	–	Low	Severe	Mild	Advanced trial[b]
REYAN 88-13[c]	Hainan	Average	Moderate	5.05	1700.0	8	Low	Moderate	Severe	Moderate	Advanced trial
REYAN 7-33-97[c]	Hainan	Average	Moderate	4.35	1910.0	9	Low	Low	Moderate	Moderate	Advanced trial
HAIKEN 1	Hainan	Average	Low	1.84	886.6	10	Very low	Moderate	Severe	High	Advanced trial

[a] Tapping system: the first 3 years: S/2·d/3, and without ethylene stimulation, about 75 tappings $year^{-1}$; after first 3 years of tapping: S/2·d/2, and without ethylene stimulation, about 110 tappings $year^{-1}$.

[b] An advanced trial means that the clone has been trialled for some time and is close to release for commercial production.

[c] REYAN is the new name for SCATC.

Table 5.6. Worldwide profile of prominent clones.

Clone[a]	Parentage	Yield (kg ha^{-1})[b]	Girth increment during tapping[c]	Resistance to[c]						
				Wind damage	Panel dryness	Pink disease	*Oidium*	*Colleto-trichum*	*Coryne-spora*	*Phyto-phthora*
RRII 105 [I]	Tjir 1 × Gl 1	2210	3	3	5	5	3	5	5	1
RRII 203[I]	PB 86 × Mil 3/2	1618	4	3	2	3	3	NA	3	3
RRII 208[I]	Mil 3/2 × AVROS 255	1587	3	3	3	NA	3	NA	NA	NA
RRIC 100 [M]	RRIC 52 × PB 83	1774	3	5	3	3	4	3	5	NA
RRIM 600[M]	Tjir 1 × PB 86	2199	4	4	4	1	3	3	1	1
RRIM 712[M]	RRIM 605 × RRIM 71	2264	2	5	4	3	3	1	3	3
RRIM 936[M]	GT 1 × PR 107	2146	3	4	3	4	3	4	4	2
RRIM 937[M]	PB 5/51 × RRIM 703	2483	2	5	3	4	3	3	5	3
RRIM 2015[M]	PB 5/51 × IAN 873	2760	4	NA	NA	NA	4	4	4	3
PB 217[M]	PB 5/51 × PB 6/9	1778	4	4	4	2	2	3	4	1
PB 235[M]	PB 5/51 × PB S/78	2485	3	2	2	3	2	2	4	3
PB 255[M]	PB 5/51 × PB 32/36	2283	3	4	2	2	2	2	4	2
PB 28/59[M]	Primary clone	2023	1	3	3	2	2	2	4	2
PR 255[M]	Tjir 1 × PR 107	2018	3	4	3–4	3	1	3	4	3
PR 261[M]	Tjir 1 × PR 107	1838	3	4	3–4	3	1–2	4	3	3
IRCA 111[CD]	PB 5/51 × RRIM 600	1446	5	3	3	NA	NA	NA	NA	NA
IRCA 230[CD]	PB 5/51 × GT 1	1807	5	3	3	NA	NA	NA	NA	NA
RRIT 163[I]	PB 5/51 × RRIM 501	2086	2	NA	NA	NA	3	NA	3	NA
HAIKEN 1[C]	Primary clone	1500	3	4	3	2	NA	NA	NA	NA
REYAN 8-333[Cd]	SCATC 88-13 × SCATC 217	2187	3	3	3	NA	3	NA	NA	NA
IAN 873[Be]	PB 86 × FA 1717	1920	4–5	3	4	NA	4	4	NA	NA

(*Continued*)

Table 5.6. *Continued*

Clone[a]	Parentage	Yield (kg ha^{-1})[b]	Girth increment during tapping[c]	Resistance to[c]						
				Wind damage	Panel dryness	Pink disease	*Oidium*	*Colleto-trichum*	*Coryne-spora*	*Phyto-phthora*
IAC 301[B]	RRIM 501 × AVROS 1511	2320	4	4	4	NA	4	4	NA	NA
IAC 40	RRIM 608 × AVROS 1279	2420	4	3	3	NA	2	3	NA	2
IAC 300	RRIM 605 × AVROS 353	1887	3	2	2	NA	3	2	NA	2
FX 3864	PB 86 × PB 38	1755	4	3	3	NA	2	2	NA	3
IAN 4493[B]	FX 441 × Tjir 1	1711	3	3	2	NA	2	2	NA	2
RRIV 4[VN]	RRIC 110 × PB 235	2103[10Y]	2	2	4	3–4	2–3	2	NA	4

[a] Under conditions of: B, Brazil; C, China; CD, Côte d'Ivoire; I, India; M, Malaysia; VN, Vietnam.
[b]Tapping system = S/2 d/2 6d/7 86%; number of tapping days year^{-1} = 158 ± 11 (with wide regional variation depending on weather); trees ha^{-1} = 327 ± 34. In Vietnam (south-east) the tapping notation followed was = S/2 d/3 6d/7. All entries are yield average over 6 years except for entry labelled '[10Y]', which is the average over 10 years.
[c] 1, Poor; 2, below average; 3, average; 4, good; 5, very good; NA, not available, since the disease is not prominent.
[d] REYAN is new name for SCATC.
[e] IAN 873 exhibits good tolerance to SALB.

Table 5.7. Comparison of polyclonal seedlings and multiclonal populations in Tripura 10 years after field planting (Source: Sasikumar *et al.*, 2001).

Attribute	Polyclonal seedlings	Multiclonal population
Wind damage (%)	19.1	25.6
Uprooting (%)	Nil	1.3
Panel dryness (%)	2.5	3.8
Powdery mildew (%)	65	90
Girth (cm)	68.7 (30.5–100.6)	63.1(38.5–89.5)
Mean yield (g per tree per tap)	21.1 (2.6–70.3)	21.4 (9.3–35.5)
Mean yield (g per tree per tap)		
Regime I (May–September)	16.1	11.2
Regime II (October–January)	26.1	31.5

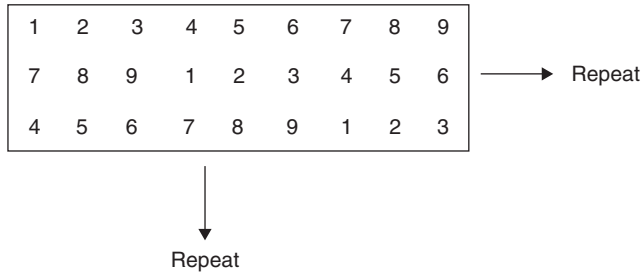


Fig. 5.2. Two-dimensional design for planting rubber plants of different genotypes (1–9) in order to generate polyclonal seeds (after Simmonds, 1986).

the Prang Besar Rubber Estate, from which he could select a dozen primary clones (Tan *et al*., 1996). Selected seedlings issued from polyclonal seed gardens were also used for selecting new clones. This possibility led to the establishment of other seed gardens not designed to produce commercial seed lots but specifically aimed at being a source of seedlings for selection. This process, based on natural pollination, has been called 'ortet selection' (the word 'ortet' is equivalent to 'mother tree'). A tree raised through bud grafting is a 'ramet' and not a 'clone'. However, since the term 'clone' is widely used in the rubber literature, this term will be used here. The concept of polyclonal seed gardens for the selection of clones is still considered interesting (Simmonds, 1986) (Fig. 5.2), notably for the improvement of the wild Amazonian populations and more generally for population improvement in rubber (including Wickham populations). Breeding orchards without any plan are also used (Fig. 5.3).

By 1930, the emphasis shifted to recombinant full-sib progenies from controlled hand pollination. Under this system, there was no selfing, and the crossing of related parents was avoided. The advantage of hand pollination was the possibility to cross two known parental clones carefully chosen for their high level of performance or their complementarities, and to trace back the clones to their

Fig. 5.3. A breeding orchard.

ancestors. With the development of biometry and quantitative genetics, this opened up new possibilities for evaluating the genetic worth of parents and progenies (for extensive reviews on breeding, see Tan, 1987; Priyadarshan and Clément-Demange, 2004; Priyadarshan *et al*., 2008).

5.5 Breeding Pattern

Latex yield and growth are polygenically or quantitatively controlled (Simmonds, 1989). It is very clear that growth rapidity is made of many different physiological processes occurring at successive stages of the development of trees. As a result, most genetic populations are normally distributed for growth measurements. In contrast, the usual distribution of genetic populations for latex yield, notably of full-sib families, although continuous, is strongly dissymmetric with most genotypes having low yields and few of them having high yields. This was initially mentioned by Maas (1934) and could be an indication of a more important role of some genes with rare favourable alleles related to some specific physiological processes such as: (i) the partition of assimilates; (ii) sucrose uptake by the laticifer cell; or (iii) regulation of ethylene metabolism.

Initially, the highest yielding clones were empirically intercrossed, on the assumption that additive genetic variance was predominant and that the best clones would be the best parents. This was confirmed, to some extent, by a first genetic analysis showing that vigour and yield in seedlings were strongly additive (Gilbert and Dodds, 1965, unpublished results cited by Wycherley, 1969; Gilbert *et al.*, 1973; Nga and Subramaniam, 1974; Tan and Subramaniam, 1975; Tan *et al.*, 1975; Tan, 1977, 1978a, b, 1979, 1981; Simmonds, 1979). There was no significant dominance effect, and each parent would have to be assessed for its

general combining ability (GCA), estimated from the evaluation of its progenies. For parents which had not undergone GCA assessment, their performance could be assumed as good, since their yield performance has been proved, with some possible exceptions (Simmonds, 1989). However, it seems that the assumption of a good relationship between GCAs and their performance as potential clones has not actually been checked. Moreover, a possible decline in additive genetic variance over successive phases of breeding must be considered (Tan, 1981). Clearly, genetic parameters in the Wickham population would have to be re-estimated with more recent sets of parents and associated progenies.

It must be acknowledged that accurate GCA estimations of the clones, and more generally genetic studies, are not easy to achieve routinely, due to the limitations imposed by hand pollination and by the low fruit set in most genotypes. Hand-pollination exercises usually end up with incomplete or partial diallel that will give only unbalanced data from the progenies. The yield data need to be analysed with special statistical tools like ASReml (a statistical software package) or with a program specially written in SAS (statistical analysis system) for the purpose. This calls for a special statistical model meant for an incomplete diallel. Hence, the implementation of factorial mating designs in rubber necessitates more than one hand-pollination campaign for accumulating the required full-sib families which must then be planted together into the same trial. In contrast, the simple comparison of full-sib families can routinely provide rough estimations of each parent's worth, by accurate estimations of paired crosses, thus allowing family selection.

Observation of heterosis was never demonstrated in rubber, although this word was sometimes inadequately applied in two ways: (i) in the case of clones yielding more than their two parents, which is very usual at the upper tail of progeny distribution; and (ii) when the mean of the progenies of one cross is higher than the mean of the two parents. When the two parents are similar, the progenies may even exhibit a higher level than the best parent. If the number of progenies is large enough, the mean of the progenies actually exhibits the real level of the sum of gene actions contained by the two parents, whereas the specific combinations of the two parents exhibit only a partial view. As heterozygotic genotypes, most of rubber clones probably express a good amount of the possible heterosis in the Wickham population. The plant material which would be necessary for comparing doubled haploid lines and their hybrids has never been available for assessing heterosis. In the Wickham population, there are no known complementarities between two types of parents resulting systematically in superior progenies. Considering the existence of metabolic typology of Wickham clones, hypothetic complementarity between the two opposed metabolic types could be tested. Most of the Wickham × Amazonian crosses express low rubber yields, still far from the Wickham level; but their mean vigour is equivalent to that of Wickham × Wickham crosses. They generate many clones with faster growth. Rather than heterosis, this is because such crosses exhibit a higher variability than that of Wickham families.

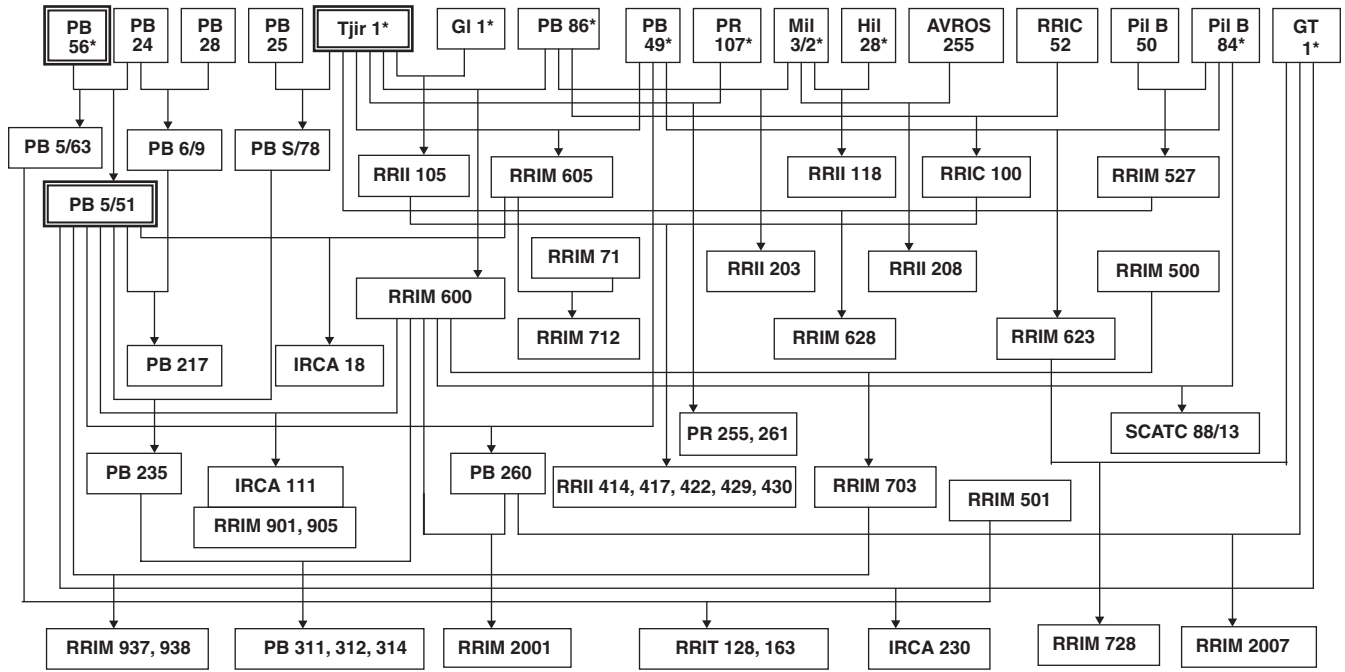
The breeding policy which was based on crossing 'the best with the best' generation-wise assortative mating (GAM) led to an intensive selection of clonal parents. The low female fertility of most of the clones often led to the preferential

choice of fertile clones as seed parents, which contributed to the suspected reduction of the genetic base of the Wickham population. Tracing back to the ancestors of many cultivated clones shows that these ancestors are few in number. The list of the parents of clones bred in Malaysia since 1927 (Tan, 1987) is made of 33 clones (PBIG, SR 1, PB 23, PB 186, PB 24, PB 25, PB 28/59, PB 28, PB 49, PB 56, PB 86, LCB 1320, PR 107, TK 14, AV 33, AV 49, AV 157, Tjir 1, WAR 4, GT 1, Lun N, Pil A44, Pil B16, Pil B50, Pil B58, Pil B84, Pil D61, Pil D65, RRIM 71, Ford 351, GL 1, BD 5 and BR 2) and most of the modern clones were bred from ten clones (Fig. 5.4 shows the parentage of outstanding clones). Some other clones were recently added, notably some including an Amazonian contribution (FX 25, IAN 873). Though this could seem well diversified enough, only four of these ancestors (PB 49, PB 86, Tjir 1 and Pil B84) played a major role; moreover, at intermediate level, the clones PB 5/51, RRIM 501, RRIM 600, RRIM 605 and RRIM 623 were extensively used. As a consequence, many recent clones are more or less related, and this discouraged intercrossing. Although the Wickham population was shown to be homogeneous (Seguin *et al*., 2003), one solution could be to split it into two subpopulations by separating two groups of ancestors (based on molecular studies), which could help in managing the genetic variability of this population. Another feature is that not more than three or four generations have been produced by hand pollination since the original primary clones of the 1920s, further reducing the importance of recombination.

In order to achieve a good diversification of the crosses, many different crosses are usually made, resulting in rather small numbers of progeny per family (from ten to 50 seeds per family) which is theoretically enough for a first comparison of the growth and yield levels of the families. The best families would then have to be made again in a large size (towards 200 or more seeds per family) in order to extract elite clones from them. However, few results have been published on the comparisons of the families and on the identification of the best ones. A suggested explanation is that, with the benefit of bud grafting, most efforts are targeted towards the early identification of elite individual genotypes, which globally led to neglecting family selection. As a matter of fact, Simmonds (1996a) advised in favour of family selection in rubber as well as other crops.

5.6 Selection

Before World War II, for commercial seedlings, planters were confronted with the high tree variability. In 1928, Ashplant raised a controversy with his proposal to carry out a very early selection on seedlings and Cramer (1938) proposed to 'grade' the young rubber plants at field level, or even at the nursery stage, with a view to eliminating the lowest yielding plants. This first early selection procedure was carried out with a special tapping tool, the 'Testatex' knife. Although aware of the limited effectiveness of such a simple test, Cramer suggested that this method could also be used for selecting mother trees and creating new clones. Evers (1955) suggested planting seedlings at a high density and practising early heavy selective thinning on the basis of growth or of test tapping. This idea was tested with a thinning based only on vigour, but the only effect of



AVROS = Algemene Verneiging Rubber planters Oostkust Sumatra, Indonesia; GI = Glenshiel, Malaysia; GT = Gondang Tapen, Indonesia; Hil = Hilcroft, Sri Lanka; IRCA = Institut de Recherches sur le Caoutchouc en Afrique, Côte d'Ivoire; Mil = Milakande, Sri Lanka; PB = Prang Besar, Malaysia; Pil = Pilmoor, Malaysia; PR = Profestation voor Rubber, Indonesia; 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; SCATC = South China Academy of Tropical Crops (now REYAN); Tjir = Tjirandji, Indonesia.

Fig. 5.4. Parentage of outstanding clones. Most of the modern clones were bred from ten clones (identified by an asterisk).

thinning was to reduce the heterogeneity of the seedling stand and reach uniformity similar to that of bud-grafted clones. After the beginning of tapping, yield was not improved by this method (Wycherley, 1969).

From the 1920s to the present day, clone selection has been carried out: (i) from seeds of the mother trees selected in commercial plantations; (ii) from natural pollination in polyclonal seed gardens (ortet selection); and (iii) increasingly from hand pollination. The best seedling trees could be bud grafted and multiplied as clones for further testing in different selection stages, with a gradually increasing number of trees per genotype and a decreasing number of genotypes.

The methodology of 'ortet selection', as developed by Prang Besar and the RRIM, has been described by Malaysian researchers (Ho *et al*., 1979). The main results of the programme initiated in 1972 by RRIM were presented by Tan *et al.* (1996). Shepherd (cited in Wycherley, 1969) drew attention to this alternative source of new clones, and noted that 15% of the clones selected by Prang Besar since 1945 were obtained in this way. The seeds issued from ortet selection did not have the genetic properties of full-sib progenies. Due to the imbalance between pollinators in natural pollination and the uncertain and variable amount of selfing, family selection appeared to be inappropriate for these seeds, and individual selection was applied intensively. In the Prang Besar breeding programme in Malaysia, the small-scale trial was called the 'preliminary proof trial', and the large-scale trial, where clonal seedling families derived from seed gardens were also tested, was called the 'further proof trial'. On the basis of this scheme, many options were suggested and tested. Two contrasting cases can be considered: (i) a long process with 5 years in seedling evaluation trials (SETs), 10 years in small-scale clone trials (SSCTs), and 15 years in large-scale clone trials (LSCTs) (with a total of 30 years); and (ii) a short process with 3 years in SETs, 5 years in SSCTs, and 12 years in LSCTs (with a total of 20 years). After this, block trials are to follow to ascertain the true potential under multilocations.

The last stage (LSCTs) is time consuming, since the dynamics of latex yield, TPD, incidence of diseases and wind endurance are to be studied. Some attempts to find early predictors of susceptibility to TPD or to wind damage still remain unsuccessful. LSCTs are trials carried out in the planters' field. As each LSCT comprises a small number of clones (from five to 25), these clones are compared as simple units, with no reference to their familial origin and their genetic relatedness. Usually, these trials are raised in a geographical network so that the clones can face various ecological conditions. Some multilocation monoclone blocks (clonal block trials; CBTs) can also be set up simultaneously, initiating a pre-recommendation process. Clone recommendations are mainly based on the data from LSCTs and/or CBTs. There is no real rule concerning the number of trials and the number of testing years before one clone can be recommended. In Malaysia, LSCTs have been replicated so as to be exposed to the varied environments of the country. Clones were recommended for experimental-scale planting when the LSCTs were set up, and some clones could be recommended for large-scale planting after 10 years of tapping on virgin bark in LSCTs (17 years from establishing LSCTs). Large estates are often willing to take some risks and devote a few hundred hectares to 'prospective' clones. It can be suggested that a clone can be recommended and planted on a moderate scale after it was seen

performing well during 7 tapping years in two different LSCTs. Assessments from data on clone trials indicate that yield is seen to become stabilized after 7 years (Chandrasekhar *et al*., 2007).

The breeder's work is more focused on the first two stages: (i) SETs with one seedling per genotype; and (ii) SSCTs with ten to 40 budded trees per genotype. Research is devoted to the possible early measurements, to the correlations between early and adult traits or the correlations between traits measured at the two successive stages, to create a balance between the two stages. In any selection, two types of error are unavoidable but must be minimized: (i) discarding good genotypes; and (ii) keeping bad genotypes (Simmonds, 1985). With two selection stages, the one stage is likely to discard the good genotypes taken from the preceding stage. Theoretically speaking, it is advisable to concentrate most of the selection intensity, for an index of the main traits, only on the one stage that appears to be the most efficient globally.

Until 1960 at the RRIM, all the genotypes derived from hand pollination were tested simultaneously as seedlings in the nurseries (SETs) and as budded clones in SSCTs. Then, with the increasing number of seeds produced, a first selection was applied to seedlings planted at a high density in the nursery (SET), based on their vigour during the first year, and a reduced number of genotypes was then tested in SSCT (Wycherley, 1969). Test tapping was applied to the nursery stage, using a system proposed by Hamaker (1914, cited in Morris and Mann, 1938). Significant correlations between yields of individual seedlings and of the derived budded clones had been found by Brookson (1959). However, Ross (1965) found significant correlations between the mean yields for the first 5 years of tapping of the same clones in LSCTs and SSCTs, but not with those of the original seedlings. According to Wycherley (1969), selection to LSCT would require 3–5 years tapping in SSCT, and the duration of the SSCT would be around 10 years. The proportion of selection from SSCT to LSCT was 2–10%. Another process, with greater selection in the nursery and a second selection stage in 'promotion trials', was also tried in Malaysia (Ong *et al.*, 1985). Such a system cuts out the SSCT stage, so reducing the selection period by about 10 years, but also reducing considerably the accuracy of selection.

At the Prang Besar Rubber Estate (in Malaysia), 2 years of test tapping were applied to the seedlings, and a selection made of the genotypes to be tested as clones in a preliminary proof trial with 15–20 trees per clone. After at least 2 years of test tapping in the preliminary trial, clones were chosen for assessment in further proof trials with each clone replicated in blocks over 0.4 ha per clone (Shepherd, 1969).

There has been some investigation of the possibility of using photosynthetic rate as a criterion in early selection for yield. Samsuddin *et al.* (1987a) made measurements in a hand-pollinated seedling population, derived from a breeding programme and grown in a nursery, but found no significant correlation between photosynthetic rate and nursery yield (early test tapping) or girth measurements. On the other hand, measurements of photosynthetic rate made on mature leaves of young bud-grafted plants of 23 clones grown in a controlled environment chamber were found to be significantly and positively correlated with the mean yields over 5 years of tapping of the same clones in a number of

large-scale field trials in Malaysia (Samsuddin *et al.*, 1987b). However, as the correlation coefficient was low (0.469) and only significant at $P = 0.05$, these workers concluded that further investigation was needed before it can be decided whether the determination of photosynthetic rates in growth chambers is likely to be useful as a criterion for early culling of low yielders in a breeding programme. Although the leaf area index (LAI) of most commercial clones in mature stands may be similar, it is quite possible that differences between clones in crown architecture and in the partition of assimilates between growth and latex production may preclude any direct relationship between the photosynthetic rate of single leaves and the yield of mature trees (see Chapter 3 for more details).

At the Institut de Recherches sur le Caoutchouc en Afrique (IRCA) programme in Côte d'Ivoire, SETs lasted 2 or 3 years and were planted at a high density of 2000 trees ha^{-1} (2000 genotypes unequally distributed among around 40 full-sib families). Girth increment from 1 to 2–3 years old was measured, and test tapping applied to the trees during 2–8 weeks. About 100 genotypes were bud grafted to raise plants for SSCTs. The duration of SSCTs was limited to 8 years, because as time progressed there was increasing competition between the genotypes set up in small plots. Additionally, selection in SSCTs was made with test tapping at 3 or 4 years old (for clones with a trunk girth bigger than 25 cm) for 6 months. A 3-year tapping period was observed for 5–8-year-old trees. Selection of some three to five clones was made from both these sets and LSCTs were raised immediately for further confirmation of their yielding potential (Odier, 1983).

A method of very early yield assessment in SETs, based on leaf morphology, proved to be ineffective (Amand, 1962). Assessing the density of stomata was proposed (Senanayake and Samaranayake, 1970). A fast and simple method was developed to predict yield potential through quantifying latex oozing out of leaflets or petiolules during the first months of the nursery stage (Zhou *et al.*, 1982). But, as simplification went too far, correlation between yields measured in SETs and in SSCTs fell below significant levels. Gnagne (1988) and Gnagne *et al.* (1990) studied the relationships between girth and latex yield measured on 2-year-old seedlings and on the corresponding 3-year-old bud-grafted clones in SSCTs but found no significant correlation for girth between SETs and SSCTs. However, significant correlation, although rather small, for latex yield between SETs and SSCTs ($r = 0.2$–0.3) was evident. Tan (1998) studied the relationships between selection in nursery and mature yield in SSCTs and concluded that nursery yield is the major selection criterion.

Although latex yield and girth are the main attributes, SETs and SSCTs demand selection for secondary attributes such as branching habit and resistance to leaf diseases. Physiological parameters were proposed as selection criteria, such as bursting index (BI), an indicator of susceptibility to early coagulation (Dintinger *et al.*, 1981), and photosynthetic rates (Samsuddin *et al.*, 1987b). Anatomical parameters, such as bark thickness, number of latex vessel rings, latex vessel density and plugging index (PI), were also investigated (Tan, 1998). PI, an indicator of the rate of coagulation after tapping, is negatively correlated with latex yield (Milford *et al.*, 1969). The number of latex vessel rings showed a consistent correlation with yield (Sanderson and Sutcliffe, 1929a, b; Frey-Wyssling,

1930; Wycherley, 1969; Huang *et al*., 1981), but counting the number of rings was difficult to carry out on young seedlings in the nursery. Considering vigour and yield in SSCTs, Wycherley (1969) suggested selecting clones that yielded more than predicted by the regression of yield on girth. Ho (1976) found that girth, number of latex vessels and PI accounted for 75% of the variation in yield between clones at the nursery phase, but only 40% at maturity. From the results of Hénon and Nicolas (1989), the thickness of the bark could not be considered a reliable attribute to predict yield, and it was assumed that the use of anatomical parameters as selection criteria is effective only if the genetic variation is large enough, which is not always the case in the selection of advanced Wickham clonal material (but it could assist in assessing the Amazonian and Wickham × Amazonian populations).

Correlations were found between the aforesaid physiological traits and latex yield, and the clonal nature of these traits was established (Eschbach *et al*., 1984). A method called 'latex diagnosis', based on four biochemical parameters of the latex (dry rubber content, sucrose ratio, inorganic phosphorus ratio and thiol groups ratio) was developed for optimizing the tapping systems and for monitoring commercial plots under tapping (Jacob *et al.*, 1987). A clonal metabolic typology of clones was also established (Jacob *et al.*, 1989; Gohet *et al*., 2003). The weak point of early tapping tests was that they tended to promote the genotypes having an easy flow with low viscosity. Latex diagnosis, applied to young bud-grafted 3–4-year-old trees which were tapped early with no ethephon stimulation in SSCTs, allowed the classification of the clones for the intensity of their metabolic activity and for the availability of sucrose in the latex (as a source of energy and of carbon for rubber biosynthesis). This method was used for selecting clones with active metabolism, fast and long latex flow, and high initial yield, as well as clones with lower initial yield but with a high ratio of sucrose indicating the capacity to provide an important response to stimulated yield.

The experimental design of SETs can be: (i) a total randomization of the seedling trees; (ii) a total randomization of replicated familial plots (with five to ten seedlings of the same full-sib family per plot); or (iii) a randomized block design with familial plots of five to ten seedlings distributed in three to four blocks. One characteristic of these trials is that the number of seedlings per family usually varies widely from ten to more than 100, and the trials are unbalanced. SSCTs are made of replicated clonal plots in randomized block or simple lattice designs. As each budded tree actually exhibits the performance of a stock–scion interaction, it is preferable to have a minimum of three trees per plot, so as to analyse average growth and yield data. The total number of trees per clone in an SSCT can vary from a minimum of six (two replications of three trees, a possible case for Amazonian germ plasm improvement) to about 40 (four replications of ten trees). A large number of different bud-grafted clones can be tested in SSCTs (100 or more) in incomplete block designs called α-designs to achieve a better control of the environment (Patterson and Williams, 1976).

Breeders have been aware of the limited effectiveness of selection in SETs, mainly due to the variation in environment applied to only one non-replicated tree per genotype (Evers, 1959; Ho *et al*., 1979). Unsatisfactory attempts were made to multiply the seedlings as cuttings. As each of the two cotyledons of a

rubber seed bears an axillary bud, a method was developed by Ramaer for splitting each seedling into two twin trees (Meyer, 1938; Dijkman, 1951) and, in this way, there were two replications of each genotype. But this method could never be developed routinely. As a matter of fact, selection in SETs must have a dual view: (i) genetic; and (ii) biometric. Usually, the families in one SET may not have the required genetic structure of a nested or factorial design, which would enable the estimation of the genetic values of each seedling by use of the equations based on the genetic relatedness between individuals, full sibs or half sibs (Falconer, 1961). From a biometric point of view, the estimation of genetic value of the genotypes in a SET faces some critical limitations (Gnagne *et al*., 1998). The genetic variance between different full-sib families can be estimated but with no replication of the genotypes. While family selection is possible in a SET, a combined family-individual (genotypic) selection could be done only with a rough estimation of the necessary parameters. In contrast, bud grafting for SSCTs allows many replications of each genotype, so enabling real genotypic selection.

Criticism has been raised against phenotypic selection in SETs with the hope of picking up exceptional trees. Although full-sib families have been created, the range of variation is limited depending on the recombinant combinations possible and/or available. Such selections produce very limited exceptional individuals. Hence, the method of family selection was advocated (Jayasekera and Hettiarachi, 1988; Simmonds, 1996a). Considering the limitations imposed on the very early selection in a SET and the principle of concentrating on selection of multiple traits in only one selection stage, Simmonds (1996a) proposed the following modifications: (i) carry out measurements during a maximum of 2–3 years and perform only a mild selection in a SET, by discarding the low-performing families as well as the low-performing genotypes within the selected families; (ii) set up an SSCT with the selected families represented by ten to 20 genotypes per family; (iii) concentrate the measurement efforts in SSCTs during the 8–10 years possible before competition between plots becomes too important; and (iv) analyse SSCT data as for a combined family-individual selection. When a routine tool for early selection is lacking, these proposals can only end up with the chances of losing more desirable high yielders.

There is a word of caution here: one cannot predict the potential of an 8-year-old tree when it is 1 or 2 years old. The capacity to attain increased yield develops with maturity and that depends on a number of physiological and morphological attributes coupled with the genetic nature of the seedling. Test tapping a seedling will never give an early indication of the true potential. There can be early yielders but there are late starters as well. To ascertain this, one has to wait till the tree attains tappable girth. Yet another vital point to be noted is that all the hybrids should be tapped and their potential assessed against the reference clone. A general error committed by breeders is that they test tap the hybrids that attain girth early and reject other hybrids. Such a selection can yield clones that attain girth early but need not always result in high-yielding clones. All the hybrids need to be assessed in due course. Taking into consideration all these constraints to successful early selection, Priyadarshan and Clément-Demange (2004) proposed a new scheme of deriving high-yielding clones. The proposal is: (i) the full sibs are tapped upon attainment of girth (50 cm) and evaluted for

yield against the reference clone; (ii) high yielders are selected, multiplied and are evaluated in a clonal nursery against the reference clone; and (iii) the final selections are multiplied and distributed for block level trials. Nearly 8 years can be saved in this way. A clone can be derived in 20 years. While undertaking this scheme, it is advisable to conduct more hand pollinations in the desired crosses (based on the analysis of progeny yield data through statistical tools like ASReml/ SAS) since the data are expected to be unbalanced. More full-sib families ensure greater chances of selecting desired genotypes. Figure 5.5 shows a comparison of breeding schemes by which clones of recommended genotypes can be derived, including a scheme that combines germplasm improvement with

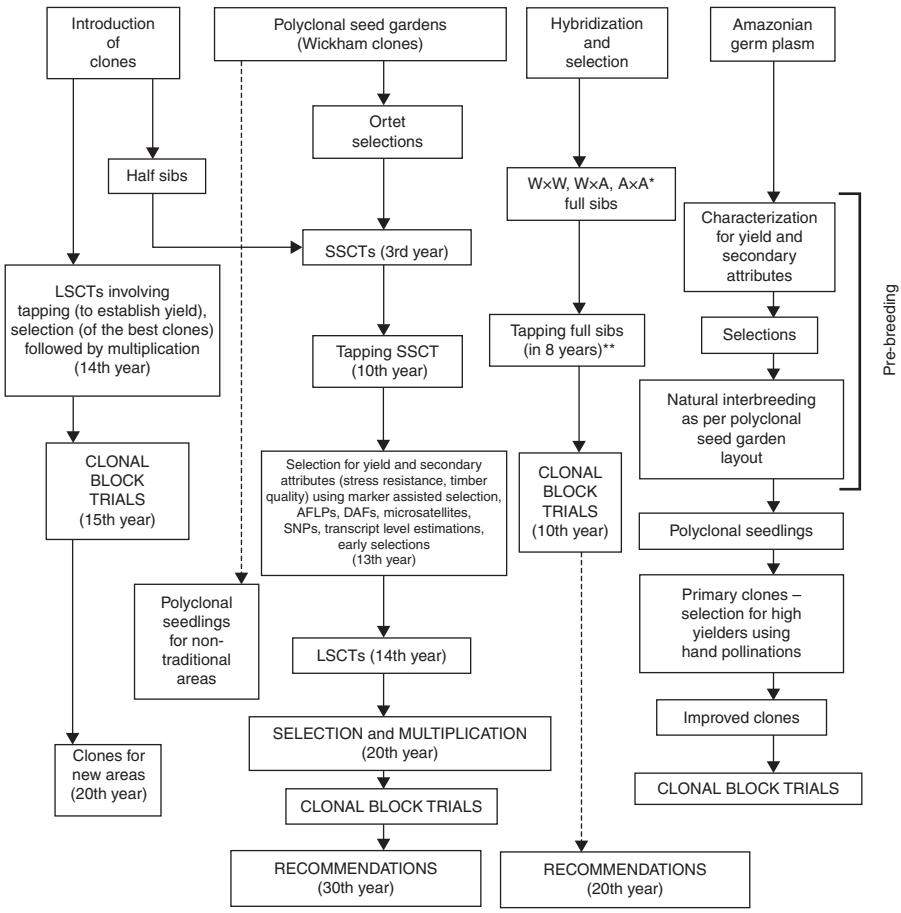


Fig. 5.5. Comparison of schemes for the derivation of clones. AFLPs, Amplified fragment length polymorphisms; DAFs, DNA amplification fingerprinting; LSCTs, large-scale clone trials; SNPs, single-nucleotide polymorphisms; SSCTs, small-scale clone trials; *, W = Wickham and A = Amazonian; **, full sibs will be cut back and kept as budwood points after yield evaluation (modified from Priyadarshan, 2003a).

mainstream clone selection. This scheme would supersede the others that takes nearly 35 years to derive a clone (Priyadarshan *et al*., 2008).

5.7 *Hevea* Clones

Rubber clones are denominated with a first part in letters (abbreviation of the origin) and a second part in numbers. Dijkman (1951) provides a list of denominations with their emblematic clones that were developed during the first half of the 20th century, such as AVROS (AV 49, AV 255, AV 352, AV 2037), Bodjong Datar (BD 5, BD 10), Djasinga (Djas 1), Glenshiel (Gl 1), Gondang Tapen (GT 1), Kali Djeroek (KD 1), Landb. Mij. 'Oud Djember' (LMOD 53), Lands Caoutch Bedrijf (LCB 1320), Pataroeman (Pat 190), Pilmoor (Pil D65), Prang Besar (PB 186), Proefstation voor Rubber (PR 107 = LCB 510), Tjirandji (Tjir 1, Tjir 16), Waringiana (War 4), etc. In addition a denomination was established to indicate precisely the different types of seeds or the genetic origin of clones issued from recombination: (i) 'illegitimate seedling families' (ill.) are issued from commercial plantings, with no known genetic origin; (ii) when only the mother parent of one clone is known (i.e. AV 163), the origin of the clone is denominated as AV163 ill.; and (iii) 'legitimate' full-sib seedling families issued from hand pollination are indicated by mentioning first the female (seed) and then the male (pollen) parent (i.e. PB 186 × Tjir 16).

The main denominations of rubber clones are as follows:

- AVROS;
- F and FX, clones from the collections and recombinations of the Ford Company in Brazil;
- IAC, Brazil, Instituto Agronomico do Campinas;
- IAN, Brazil, Instituto Agronomico do Norte;
- IRCA, Côte d'Ivoire, clones created by the Ivorian-French breeding programme in Côte d'Ivoire since 1974;
- IRRI, Indonesia, Indonesian Rubber Research Institute (clones IR, but also LCB, PR and BPM);
- MDF, Madre de Dios Firestone (clones collected in the Madre de Dios, Peru by Firestone);
- PB, Malaysia, Prang Besar Rubber Estate;
- RRIC, Sri Lanka, Rubber Research Institute of Ceylon;
- RRII, India, Rubber Research Institute of India;
- RRIM, Malaysia, Rubber Research Institute of Malaysia (now integrated within the Malaysian Rubber Board); with also OS (clones issued from ortet selection) and PC (clones issued from Promotion Clone trials);
- RRISL, Sri Lanka, Rubber Research Institute of Sri Lanka;
- RRIT, Thailand, Rubber Research Institute of Thailand;
- RRIV, Vietnam, Rubber Research Institute of Vietnam;
- SCATC, China, South China Academy of Tropical Crops (Hainan) – the code SCATC has now changed to REYAN; and
- YITC, China, Yunnan Institute of Tropical Crops (Yunnan).

Cramer (1914) was the first to select the three 'Cultuurtuin' clones (primary) Ct3, Ct9 and Ct88 from 33 seedlings derived from the Penang Wickham trees (imported seeds), which were established at Buitenzorg (Bogor) in 1883 (Dijkman, 1951). The most striking result from the mother-tree selection in Indonesia during the 1920s was the identification of GT 1 and PR 107, which are being cultivated even now. AVROS 49, recommended thereafter, was the standard clone in all the AVROS station experiments.

The ortet selection of Gough in Prang Besar (Malaysia) during the 1920s, based on a preselection of 618 seedlings from the Kajang area, produced important primary clones, among which is PB 86, one of the most important clones ever produced, as well as PB 23, PB 25 and PB 186 (Simmonds, 1996b). Sanderson and Sutcliffe (cited in Dijkman, 1951) obtained the primary clones Pil A44, Pil B16, Pil B84 (from the Pilmoor Estate in Malaysia) and Gl 1 with the same approach.

It is difficult to provide yield data for different clones which successively emerged in clone recommendations, because there has been no standard for characterizing the yield level of the clones over time in the successive experimentation programmes. Tan (1987) suggested using the mean annual yield over 10 tapping years with 550 kg ha^{-1} for unselected Wickham seedlings, 1175 kg ha^{-1} for PilB 84 (selected in the 1920s), 1425 kg ha^{-1} for RRIM 501 (1928–1931), 2000 kg ha^{-1} for RRIM 600 (1937–1941) and 2125 kg ha^{-1} for RRIM 712 (1947–1958). Using the same criterion, Simmonds (1989) indicates 1890 kg ha^{-1} for the mean of 20 clones recommended in Malaysia, and 1330 kg ha^{-1} for PBIG seedlings. As a matter of fact, successive evaluations of sets of clones lead to a regular evolution of the clone recommendations without any global comparison.

Clone recommendations in Malaysia have evolved over time and this indicates the diversity of clones that were prominent at different periods. From 1939 to 1988, 18 clones have been recommended in 'Class I', with the successive entries: Tjir 1, Tjir 16, PB 86, PilB 84, PB 25, Gl 1, RRIM 501, RRIM 513, PR 107, PB 5/51, RRIM 605, RRIM 623, GT 1, RRIM 600, PR 255, PR 261, PB 217 and RRIM 712. Most of these clones have also been used extensively as parents. For Indonesia, it is worth noting that PR 107 and GT 1, identified as mother trees in the 1920s and still cultivated now, got Class I status in Malaysia only in 1955 and 1967, respectively. PB 5/51 was withdrawn in 1977 because its yield was superseded by other clones. PR 107 was withdrawn in 1977 for low initial yield, although the mean yield over a long period is still among the best performances. GT 1 was withdrawn in 1992 due to its susceptibility to *Colletotrichum*, and because its yield appeared to be less competitive compared with that of other clones. In 1995, the recommendation system was deeply modified; however, the clones RRIM 600, PR 255, PR 261, PB 217 and RRIM 712 were maintained in the 'Group I' of the new system, and withdrawn only in 1998. PB 260 was elevated to 'Class I' in 1992, and is still in 'Group I', together with more recent clones of the RRIM 900 series and also PB 280, PB 350, PB 355, PB 359, PB 366 and PM 10. Clones of the RRIM 2000 series are in 'Group II'. The prominent role of RRIM 600, the most widely adaptable clone of the world, as well as GT 1, must be underlined. From 1986 to 1995, statistics of planting materials used in Malaysia show that the most important clones were PB 260, PB 217 and

PB 235. PB 260 and PB 217 are still very important in many producing countries. From a physiological point of view, they have opposite behaviours. PB 260 exhibits a very active metabolism and a fast initial yield, but low sucrose reserves in the latex, a poor response to stimulation, and a high susceptibility to dryness and brown bast (a physiological disease). In contrast, PB 217 is a slow starter but with a high level of sucrose reserves in the latex, a very good response to stimulation, a low susceptibility to dryness and brown bast, and a very high yield potential over a long tapping period. PB 235 is similar to PB 260 and it has a very fast growth and can be tapped at 4½ years in favourable conditions, whereas PB 260 can be tapped from 5 to 6 years, and GT 1 and RRIM 600 at 5½ years. PB 235 has a high initial yield but is prone to dryness and wind damage (PB 260 faces roughly the same problems). However, in non-traditional rubber-growing areas, these attributes are altered significantly with few or no symptoms of dryness (Priyadarshan, 2003a).

Other important clones that have emerged from the breeding programmes of other countries include: (i) BPM 24 and BPM 1 in Indonesia; (ii) RRII 105 in India; (iii) RRIC 100 in Sri Lanka; (iv) RRIV 2 and RRIV 4 in Vietnam; (v) HAIKEN 1 and SCATC 88/13 in China; and (vi) IRCA 18 and IRCA 230 in Côte d'Ivoire. Also there are many other promising clones which are still under multilocation trials. Historically, Malaysia played a major role in rubber breeding, following the initial work of Dutch researchers in Indonesia. One main conclusion that can be drawn is that the turnover of the clones is slow but regular, which illustrates the fact that many years are necessary for accumulating the necessary observations and gaining a stabilized knowledge of the rubber clones. This is in contrast to the rather limited information that is drawn from the initial selection in SSCTs.

6 Biotechnology and Molecular Biology

The attainment of yield plateau and prevalent intraclonal variations in yield of *Hevea* prompted researchers to tackle these problems through employing the modern tools of biotechnology. However, higher yield alone would not encourage cultivation of *Hevea*, since the species is sensitive to biotic and environmental attributes and physiological disorders. The long breeding cycle and the large size of the crop also make breeding time-consuming. Biotechnology applied to *Hevea* can be discussed under two headings: (i) *in vitro* culture; and (ii) molecular breeding. While *in vitro* culture deals mainly with regeneration and propagation, molecular breeding includes identification, characterization, introduction and expression of novel genes.

6.1 *In Vitro* Culture

Experimentation with *in vitro* culture of rubber commenced during the 1960s with Chua (1966) attempting to derive callus cultures from the plumule tissues of seedlings. The effects of osmotic concentration, carbohydrates and pH of the culture media were also studied. Later, the RRIM took the initiative of undertaking large-scale tissue-culture work through maintaining callus cultures from various explants (Paranjothy and Gandhimathi, 1976). It expanded to somatic embryogenesis and micropropagation through stem explants. While anther culture was employed to achieve pure lines first and exploitation of heterosis thereafter, micropropagation and somatic embryogeny were used to generate homogeneous populations. Although research on *in vitro* culture commenced nearly 45 years ago, even after rigorous experimentation, these areas are still in their infancy due to shortcomings towards commercial applicability. Expectations of better performance of these multiplication techniques are based on three considerations: (i) cloning the root system would generate new and more homogeneous rootstocks or monogenetic clones; (ii) selection of clonal roots would

improve the exploitation of existing genetic variability; and (iii) use of rejuvenated clonal plant material would potentially provide important agricultural attributes towards higher growth, latex yield and resistance to wind and dryness.

Carron *et al*. (1989, 1995a, b, 2001, 2005) has amply reviewed *in vitro* approaches applied to the rubber tree (including tissue culture, haplogenesis, microcutting, somatic embryogenesis, protoplast culture, germination of immature embryos and cultivation of laticiferous tissue). Microcuttings and somatic embryogenesis were studied in *Hevea* in order to achieve rapid clonal propagation as an alternative to the drawbacks of the use of cuttings and bud grafting techniques. Somatic embryogenesis as a means of regeneration opens up possibilities for transgenic technology. *In vitro* culture is made up of the application of many laboratory protocols involving hormones, nutrients, culture medium and of histo-cytological controls; details can be found in the works of Chen *et al*. (1982), Chen (1984), El Hadrami *et al*. (1991), Etienne *et al*. (1991, 1993, 1997a), Carron *et al*. (1992), Housti *et al*. (1992), Montoro *et al*. (1993), Veisseire *et al*. (1994a, b), Wang and Chen (1995), Seneviratne and Wijesekara (1996), Cailloux *et al*. (1996), Linossier *et al*. (1997), Wang *et al*. (1998), Sushamakumari *et al*. (2000a) and Kumari Jayasree *et al*. (2001).

Bouychou (1953), Chua (1966), Wilson and Street (1975), Paranjothy and Gandhimathi (1976) and Audley and Wilson (1978) were the first rubber researchers to develop calli and tissue culture derived from epicotyl, green stem or plumule tissues of young seedlings. The aim was to use calli to study the laticiferous system and the action of ethephon, but they encountered problems of ploidy instability. The RRIM took the initiative of maintaining callus cultures from various explants that later expanded to somatic embryogenesis and micropropagation through stem explants (Paranjothy and Gandhimathi, 1976).

6.1.1 Anther culture

The Rubber Research Institute of Ceylon (RRIC) was the first to carry out culture of anthers to raise haploid plants (Satchuthananthavale and Irugalbandara, 1972). However, the first plants from *Hevea* pollen were made available during 1977 at the Baoting Institute of Tropical Crops, Hainan, China (Chen *et al*., 1979). Since then, at least four laboratories in China took the lead in researching production of haploid plants *in vitro* (Carron *et al*., 1989). In addition, attempts were made to produce plants through gynogenesis (Guo *et al*., 1982; Yang and Fu, 1997).

Carron *et al.* (1989) enumerated three phases for the production of haploids from anther culture. In the first phase, production of callus and embryos takes nearly 50 days. Here, the media formulation is vital since the balance between callus development and initiation of embryos needs to be maintained (Chen, 1983). The modified MB (microbouturage) medium (Chen, 1984) is widely used with the addition of naphthalene acetic acid (NAA) and coconut water, which regulate development of microspores, and a judicial concentration of sources of N, K and sugar leads to the production of calli and embryos. The somatic callus then degenerates and the embryos develop from microspores. Subculture must

be carried out at this stage into differentiation medium in order to avoid degeneration of embryos (Chen *et al*., 1982). Maturity of embryos is the crucial factor in the second phase. The cultures need 2–3 months for the apical bud to develop. Coconut water at this stage will be substituted with gibberellic acid (GA_3) for better development of cotyledons. In the third phase, progressive increment of GA_3, gradual withdrawal of other growth regulators, addition of 5-bromouracil and reduction of sugar results in the development of plants from embryos.

Cytological investigations of callus, embryos and plantlets showed mixoploidy (Qin *et al*., 1979). However, when the plants develop *in vitro*, there is a progressive tendency towards diploidy (Carron *et al*., 1989). Above all, the developmental stage of the anther is vital for the right results. The anthers from male flowers that have a yellow corolla should not be selected, for the microspores will be in a binucleate stage. Such anthers will repress callus development and embryogenesis. Only uninucleate pollen is ideal for haplogenesis, which can be obtained from greenish-yellow flowers (Chen, 1984; Shije *et al*., 1990).

6.1.2 Somatic embryogenesis and meristem culture

Somatic embryogenesis research has been reviewed by Carron *et al*. (1995a, b, 2001). *Hevea* somatic embryogenesis was first developed in China and Malaysia, using the anther wall as the initial mother-tissue explant (Carron *et al*., 1989). Paranjothy (1974) obtained the first *Hevea* somatic embryos. Successful plantlet formation and acclimatization were achieved in Haiken 1, Haiken 2 and SCATC 88/13 (Wang *et al*., 1980). In 1985, the first plantlets from clones RRIM 600 and GL 1 were transferred to the soil in Malaysia. Research on somatic embryogenesis from immature inflorescences was also reported by Sushamakumari *et al*. (2000a). Chen *et al*. (1979) reported the production of somatic plants through embryogenesis issued from anther explants, and their use as explants for microcuttings, to generate a new type of 'self-rooting juvenile clone'.

At the RRII, high-frequency somatic embryogenesis and plant regeneration were achieved from immature anthers of Indian *Hevea* clones by Kumari Jayasree *et al*. (1999). Optimum callus induction was observed on modified Murashige and Skoog (MS) medium supplemented with 2.0 mg l^{-1} 2,4-D and 0.5 mg l^{-1} kinetin. The maximum number of somatic embryos was produced on medium supplemented with 0.7 mg l^{-1} kinetin and 0.2 mg l^{-1} NAA. Further development of the embryos into plantlets was achieved on a hormone-free medium and these were subsequently established in the field. Cytological analysis revealed that all the plantlets tested were diploid. Sushamakumari *et al*. (2000a) evaluated the independent effect of kinetin and benzylaminopurine (BA) in combination with NAA and GA_3 on normal *Hevea* somatic embryo induction. The promotive effect of GA_3 on normal *Hevea* somatic embryogenesis was assessed by Kumari Jayasree and Thulaseedharan (2001). Embryo germination in *Hevea* was affected at higher concentrations of GA_3 (Kumari Jayasree *et al*., 2001). The protocols developed for *Hevea* regeneration were published by various research groups.

At CIRAD, the inner integument of immature seeds was chosen as the maternal tissue explant (Carron and Enjalric, 1982) for developing somatic

embryogenesis through four successive phases: (i) callogenesis; (ii) differentiation of embryos; (iii) multiplication of embryos; and (iv) germination of embryos and development into plantlets. The first development of embryos was achieved with RRIM 623, PB 260 and some others. Procedures were developed for reducing callus browning due to culture stresses, and optimizing each component of the process. This involved histology and biochemistry, water balance between the explants and the culture medium, the exchanges of minerals, CO_2, ethylene and polyamine synthesis, growth regulator types and concentration, type of medium support and oxidative stress. The simultaneous presence of embryos from unicellular and multicellular origins was demonstrated. Following the primary somatic embryos issued from explants, subculturing of the primary calli was investigated in order to achieve friable and embryogenic secondary calli, to increase the multiplication process (proliferation). The reliable induction of friable calli with a high calcium supply has been analysed by Montoro *et al*. (1995). Suspension cultures issued from the disaggregation of friable calli were sustained for more than 1 year for clones PB 260, PR 107 and GT 1, with embryogenic rates from 6 to 12%, but plantlets were obtained only from PB 260 and PR 107. The dynamics of somatic embryo development was compared with that of zygotic embryos for development and maturation stages and for their efficiency to germinate into plantlets (Etienne *et al*., 1993). Two protocols were established for somatic embryogenesis from the inner integument of immature seed: (i) a short method, which could not be used for mass propagation, using the rapid but ephemeral formation of embryos on compact calli (150 days), with six phases (initiation of callogenesis, embryogenesis expression, pro-embryo development, maturation of somatic embryos, germination of somatic embryos and plantlet development); and (ii) a longer method which involves inducing and proliferating friable embryogenic calli, and then isolating microcalli from the suspension for regeneration through the 'short' process (Carron *et al*., 1995b).

Carron *et al*. (1995a) presented a qualitative and quantitative study comparing somatic embryogenesis in four clones (PB 260, PR 107, RRIM 600 and PB 235), starting from 600 explants for each clone, following the primary embryogenesis protocol (the 'short method'). The following percentages of plantlets were obtained: 77, 31, 17 and 2% for the four clones, respectively. PB 260 exhibited a high embryogenic efficiency (77%) but a corresponding low rate of conversion into plantlets. Eventually in 1992, 18 plantlets obtained from PB 260, 66 from PR 107 and two from RRIM 600 were acclimatized and transferred to a field trial at CNRA (Côte d'Ivoire) for comparison with the corresponding bud grafted clones. In the absence of an embryo maturation phase, survival during acclimatization was very low (see Fig. 6.1 for micropropagated plantlets).

Carron *et al*. (1995a) compared the micropropagation capacities of clones with explants issued either from mature plants or from theoretically rejuvenated somaplants (plants from somatic embryos). The micropropagation capacity of explants from somaplants was much greater than that of explants from mature trees. This suggests that plants from somaplants are completely rejuvenated and behave as seedlings.

Etienne *et al*. (1997b) standardized a pulsed-air temporary immersion system (the RITA® system) for enhancing embryo production, through culturing

Fig. 6.1. Micropropagated plants.

Fig. 6.2. (a) The RITA® system for rapid multiplication of somatic embryos; (b) development of a somatic embryo; (c) a plantlet with taproot; (d) a plantation in Côte d'Ivoire grown from *in vitro* cultured plants.

embryogenic calli under immersion in an autoclavable filtration unit. A high concentration of calcium in the culture medium stimulated the regeneration potential of embryogenic calli (Etienne *et al*., 1997a). Somatic embryo production was three to four times greater than that on a semi-solid medium, producing 400 embryos g^{-1} fresh weight with a smaller number of abnormal embryos (Fig. 6.2). Lardet *et al*. (1999) demonstrated that protein accumulation in zygotic embryos commenced from week 13, and starch accumulation from week 15, leading to

development and maturity. The smaller size of somatic embryos accumulating less starch and protein reserves leads to lower vigour and lower conversion rates and lower acclimatization success.

CIRAD is now conducting field trials in cooperation with various partners (Rubber Research Institute of Thailand, RRIT; CNRA, Côte d'Ivoire; and the Michelin Company in Nigeria) with the planting of 13,000 somaclonal plantlets, predominantly from clone PB 260 (Carron *et al*., 2001). Research is now focusing on the limiting factors of mass propagation, which include: (i) developing new friable embryogenic callus lines; (ii) selecting among the lines for improved quality; (iii) maintaining those callus lines through proliferation or cryopreservation; (iv) improving the regeneration of embryos into plantlets; and (v) mastering the acclimatization conditions. The quality of embryogenic lines is assessed with regard to the embryogenic capacity (production of somatic embryos), the ability for regeneration and the vigour of young somaplants at field level. An attempt based on the comparison of mRNAs from different lines (differential display) was carried out for identifying markers specific for embryogenic capacity (Charbit *et al*., 2001). The final acclimatization of *in vitro* plantlets is still a bottleneck for field evaluation. Apart from their use in mass propagation, somaplants are being considered as juvenile mother trees for developing juvenile bud grafted clones on classical rootstocks and for developing clonal rootstocks by micropropagation.

Meristem culture follows three phases: (i) primary culture; (ii) multiplication and rooting; and (iii) acclimatization. Selection of explants is crucial. Indeed, very juvenile stem pieces have exhibited a higher rate of successful aseptic cultures than older material. Culture treatments are rather rigorous due to higher anticipated infection by fungi and bacteria. A mixture of gentamycin, kanamycin, chlortetracycline, chloramphenicol, rifampicin and the fungicide benomyl is found to be ideal for disinfecting the explants. Primary culture involves soaking explants in a solution of growth regulators – indole butyric acid (IBA) and benzylaminopurine (BAP) – for 2–3 h. Bud grafting is initiated in MB medium (Carron *et al*., 1989) without growth regulators. Isolated buds are cultured in half-strengh Lepoivre medium with IBA and BAP. These buds are subcultured to form microshoots that will in turn be cultured as explants in the multiplication phase. Soaking the base of the root in an IBA-NAA mixture for 3–4 days induces roots. Rooted microcuttings can be transferred to soil in 4–5 weeks. A number of clones, such as RRII 105, PB 5/51, PB 235, IRCA 438, IRCA 440, IRCA 442, PR 107 and GT 1, have been multiplied through micropropagation (Carron *et al*., 1995a). However, the acclimatization of plants is crucial, with a balance between RH and temperature governing the establishment of plants in the soil (Leconte and Carron, 1988).

The commercial value of the aforesaid procedures needs to be debated. Although gross experimentation was conducted in the past for standardizing *in vitro* technologies, there had been many setbacks in commercializing these procedures (Carron *et al*., 1992; Thulaseedharan *et al*., 2000). A number of aspects inherent in the explant tissue are responsible for the delay in commercialization. These include: (i) the release of phenols; (ii) contamination by bacteria and fungi; (iii) their recalcitrant status; (iv) reduced axillary branches; (v) lack of sufficient juvenility; and above all (vi) increased sensitivity of *in vitro*-raised plantlets

towards environmental attributes. There are, however, remedial measures for these setbacks. Since the contamination of microorganisms is location-specific, newer chemicals are to be tried to raise aseptic cultures. Instead of treating the explants with antioxidants, the incorporation of them in the media decreased browning (Seneviratne and Wijesekara, 1996). The use of support systems like cellulose plugs in liquid media reduced synthesis of polyphenols, and embryogenesis activity could be maintained for more than 200 days (Housti *et al*., 1992). On the other hand, the growth regulators used to induce axillary branches and somatic embryogenesis are more or less the same throughout. Judicious combination of new growth regulators that have shown positive results in other tree species can be tried in rubber. Also, metabolism of ethylene and polyamines during callus development must be controlled by appropriate adjustment of growth regulators (Carron *et al*., 1992). More prominently, the water status of the embryogenic callus is a governing factor to enhance embryogenesis (Etienne *et al*., 1991). Further, Lardet *et al*. (1999) demonstrated that protein and starch accumulation commenced from the 13th and 15th week, respectively, and the development and maturity of zygotic embryos happen accordingly. Somatic embryos also display the same accumulation abilities. However, the smaller size of somatic embryos can accomplish a relatively small mass of starch and protein reserves. This is related to lower vigour and conversion rates. The vigour is directly related to acclimatization success. Hence, increasing the size of somatic embryos through nutrient supplies must deserve priority. Techniques like air layering to achieve rooted cuttings and progression of this conventional multiplication for three to four generations can produce juvenile plants. They can be used as source plants for explants with increased juvenility *in vitro*. Increased turgidity and increased absorption of N, P and K are prerequisites for embryo induction. Juvenility is yet another crucial factor, wherein the successful micropropagation of mature stem apices micrografted on to 3-week-old seedlings grown *in vitro* increased the success of micropropagation (Perrin *et al*., 1994). Such basic studies must be conducted in meristem culture also, in order to implement these technologies in a commercial way. If commercialized in the strict sense, these technologies can assist breeding programmes and enhance productivity significantly.

6.1.3 Protoplast culture and embryo rescue

Attempts towards protoplast culture and fusion were carried out using young immature leaves (Cailloux and Lleras, 1979; Wilson and Power, 1989), using discs of pith in the apical part of young green shoots or anther calli (Othman and Paranjothy, 1980). Subsequently, Cazaux and d'Auzac (1994) obtained microcalluses from embryogenic callus-derived protoplasts of *H. brasiliensis*, but without plant regeneration. Recently, Sushamakumari *et al*. (2000b) reported successful plant regeneration from embryogenic cell suspension-derived protoplasts of *Hevea*. Protoplasts were isolated from immature inflorescence-derived cell suspensions and produced microcolonies on 'KPR' medium (Kao and Michayluk, 1975). Protoplast-derived cell colonies proliferated, upon subculture

on MS-based regeneration medium, with 40% of the protoplast-derived calli developing somatic embryos. Subsequently, they germinated into plants on the same medium. Fusion of protoplasts was aimed at hybridizing different *Hevea* species for breeding resistance to SALB.

In vitro germination of mature and immature zygotic embryos issued from hand pollination has been considered as a way of improving the success rate of genetic recombination in rubber (Muzik, 1956; Paranjothy and Gandhimathi, 1976; Carron, 1981). Good results (90% success in germination) were achieved only for immature embryos that were at least 3–3.5 months old after fertilization. It also appeared that immature seeds of this age could be germinated *in vivo* under controlled conditions. However, this procedure, which is expensive, did not appear to guarantee increased efficiency, nor was it a means for rescuing rare progenies.

6.1.4 Direct gene transfer

Somatic embryogenesis in rubber is becoming standardized in different laboratories worldwide as an efficient system for plant regeneration from cells. At the same time efforts have been made to transform *Hevea* cells in order to increase genetic variation in a targeted way (a new form of mutagenesis) and complement plant breeding efforts with the possibility of modifying already selected high-performing clones with specific genes (addition or inactivation), while avoiding meiotic recombination. However, in the short term, genetic transformation is becoming a powerful tool for investigating how the rubber genome functions with the assistance of targeted mutations.

The first transgenic rubber trees were reported by Arokiaraj *et al*. (1994, 1996, 1998), who used the particle bombardment method and then the *Agrobacterium tumefaciens* system on anther-derived calli from clone GL 1, with a view to *in vivo* production of high-value recombinant proteins (Yeang *et al*., 1998). The first experiments were carried out with plasmid vectors harbouring the strong and non-specific cauliflower mosaic virus (CaMV) 35S promoter, and *gus*-encoding β-glucuronidase and *nptII* reporter genes encoding neomycin phosphotransferase. Plant regeneration rates are strongly affected by genetic modification and require improvement. However, fluorometric assays and ELISAs were performed to prove the expression of *gus* and *nptII* genes, respectively, in calli and embryoids (Arokiaraj *et al*., 1996). The expression of foreign proteins in *Hevea* latex was demonstrated by Arokiaraj *et al*. (1998). This transformation appeared to be stable even after three vegetative generations with no chimeras, indicating that a single transformed plant is sufficient to achieve a population through bud grafting. Lately, a gene for an antibody fragment against the coat protein of the bacterium *Streptocuccus sanguis* (Yeang *et al*., 2002), a gene coding for 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR; involved in rubber biosynthesis), and a gene for human serum albumin (Arokiaraj *et al*., 2002) have been expressed in rubber latex through genetic transformation. At the present time these transformation experiments are based on a limited number of regenerated trees.

Stable transgenic callus lines starting from integument-derived friable embryogenic calli of the clone PB 260 have been transformed by *A. tumefaciens* (Montoro *et al*., 2000, 2003), and more than 200 transformed plantlets, incorporating the *uidA* reporter gene, have been transferred to soil in a greenhouse (Pujade-Renaud *et al*., 2005). Recently, Jayasree *et al*. (2003) achieved *A. tumefaciens*-mediated genetic transformation and the regeneration of transgenic plants in *H. brasiliensis*. This was the first report of the production of transgenic plants with a superoxide dismutase gene (*HbSOD*) under the CaMV 35S promoter using an *A. tumefaciens*-mediated gene transfer system for *H. brasiliensis*. The morphology of the transgenic plants was similar to that of untransformed plants. Histochemical *gus* assay revealed the expression of the *uidA* gene in embryos as well as leaves of transgenic plants. The presence of the *uidA*, *nptII* and *HbSOD* genes in the *Hevea* genome was confirmed by PCR amplification and genomic Southern blot hybridization analyses. Subsequently, an efficient and reproducible protocol for *A. tumefaciens*-mediated genetic transformation and plant regeneration of *Hevea* with the gene coding for superoxide dismutase under the control of figwort mosaic virus (FMV) 34S promoter has been reported by Sobha *et al*. (2003). Further, the authors claimed that the transgenic plants are being grown in poly bags and they will be bud grafted for the evaluation of oxidative stress later.

Evidence on the functionality of introgressed genes, and identification of genes of interest for research investigation or for clonal improvement are issues relating to the field of genomics (discussed in the next section). To complement this process, research has been under way for identifying, cloning and characterizing specific promoters to be associated with genes of interest in a plasmid vector, in order to optimize the gene expression, especially at the level of the latex cells of the tapped rubber tree. Cloning of ethylene-inducible and/or laticifer-specific promoters from the rubber tree has been undertaken (Pujade-Renaud *et al*., 2000, 2001). Glutamine synthetase (*gs*) and hevein (*hv*) gene promoters were targeted, based on the fact that *gs* overexpression was observed in latex after ethylene treatment (Pujade-Renaud *et al*., 1997), and the hevein protein has been found only in laticifers (Broekaert *et al*., 1990). Genomic clones of genes were obtained and partially sequenced (*hv1*, *hv2* and *gs1*, *gs2*, *gs3*). Unfortunately, the promoter region of *gs1* was lacking. It was not possible to distinguish *gs2* from *gs3*, or *hv1* from *hv2*, as these genes were highly homologous, including in their non-coding regions. Gene expression analysis revealed that: (i) both *gs1* and *gs2*/*gs3* were responsive to ethylene in latex, with *gs1* apparently strictly induced and *gs2*/*gs3* overexpressed; (ii) *hv* gene expression in latex was very strong but not significantly responsive to ethylene; (iii) *gs1* and *gs2*/*gs3* were differentially expressed in tissues derived from *in vitro* culture at various stages of development; (iv) both *gs* and *hv* genes were highly expressed in undifferentiated tissue; and (v) *hv* gene expression increased with embryo development, according probably to the laticifer differentiation stage. Sub-cloning of *hv1*, *hv2*, *gs2* and *gs3* promoter regions in a vector for transformation, in fusion with the *gus* reporter gene, was undertaken in order to analyse the functionality of these promoters. As a preliminary result, the *gs3* promoter-*gus* construct was introduced into rubber tree callus tissue by particle gun bombardment.

Transient *gus* activity was detected, which demonstrated functionality of the isolated *gs3* promoter. As *gs* genes revealed differential expression during the embryogenesis process, isolated *gs* promoters combined with a fluorescence reporter gene could become, under non-destructive conditions, a potential marker of embryogenesis. As *hevein* belongs to a multigene family, different *hevein* precursor genes were cloned and compared by sequence alignment, revealing a divergence between two groups (*Hev1* and *Hev2*) in their promoter regions. One representative in each group was chosen (*Hev1.1* and *Hev2.1*) and promoter-*gus* constructs were introduced into rice callus tissues by *A. tumefaciens* for functional analysis in a heterologous host (Pujade-Renaud *et al*., 2004). The two promoters were found to be functional and, to some extent, inducible, but *Hev1.1* expression level was very low, adding to other observations (P. Arokiaraj, unpublished results; P. Montoro, unpublished results), suggesting that the range of tissues and organs expressing the *hevein* promoters may be larger and not restricted to latex cells. *Hev2.1* was activated by wounding in rice, confirming Northern blot expression profiles observed in rubber, and was also induced by pathogen infection (*Magnaporthe grisea*) in rice. Functional analysis of these promoters is now continuing in the rubber tree itself; however, the *Hev2.1 hevein* gene promoter is assumed to be able to drive efficient overexpression of genes transferred to the rubber tree at the level of latex cells. New molecular constructs have recently been prepared with promoter *Hev2.1* and genes *Cu/Zn-SOD* and *GCL* (codes for glutamyl cysteine ligase, involved in resistance to oxidative stress).

6.2 Molecular Breeding

Developments over the past two decades have led to a new phase of plant genetics – plant genomics. This is the application of a wide range of novel methods and technologies to the analysis of the structure of the genome and its interaction with cell metabolism for protein synthesis, and the use of this knowledge for better understanding the functioning of plants or improving them into new varieties. One spectacular result achieved at the beginning of the 2000s was the entire genomic DNA sequencing of *Arabidopsis* and *Oryza* (rice), the main dicotyledon and monocotyledon plant models used due to their small genomes. Some recent powerful technologies are: (i) automatic DNA sequencing, where one machine can read two million base pairs a day; (ii) microarrays and DNA chips where tens of thousands of genes can be scanned for activity levels at the same time; and (iii) automated genotyping machines that can assay tens of thousands of DNA diagnostic points a day. In fact, it will soon be possible to monitor whole genomes by use of DNA molecular genetic markers (MGMs) or analysis of gene expression on single chips. Two main fields must be distinguished: (i) MGMs, which are non-coding DNA fragments independent from the variation of the environment; and (ii) expressed genes.

Genomics technologies were taken up by various research groups working with *Hevea*, in order to increase knowledge and also to identify new targets for breeding and/or complementary genetic transformation, and assist rubber breeders in various strategies.

6.2.1 Non-expressed molecular genetic markers (MGMs)

In conventional plant breeding, many morphological traits were used as markers for analysing genetic traits and identifying cultivars, but specific genetic information on Mendelian traits has been rare in *Hevea*. In the 1980s, isozymes, the expressions of which are not modified by the environment, have been used in rubber as proteic genetic markers for: (i) cultivar identification; (ii) genetic diversity analysis; (iii) control of progenies originating from hand pollination; and (iv) reproductive biology (Chevallier, 1988; Leconte *et al*., 1994; Paiva *et al*., 1994). Analysis of isozymes was developed at CIRAD with a set of 13 polymorphic isozymic systems to formulate a diagnostic kit associated with a clonal identification database. This kit proved to be able to differentiate a large set of cultivated clones (Leconte *et al*., 1997). However, the analyses are to be carried out near the field sites due to the fragility of isozyme molecules exposed to normal temperature, or otherwise the samples need to be freeze-dried before transportation to the laboratory. Moreover, isozyme-based analyses are limited by the rather small number of marker loci available and a general lack of polymorphism for these loci.

MGMs are the ideal means for identifying genotypes and tracing the segregation and inheritance of alleles related to economically important characters. MGMs are powerful tools that could enhance the speed and effectiveness of rubber breeding as is already the case for maize and some other plants. General advantages of DNA markers include: (i) their ability to reveal the sites of variation in DNA segments among many individuals; (ii) their abundance and distribution over the whole genome; and (iii) their independence from the variations of the environment. There is a growing arsenal of MGMs that are currently used, notably in identifying quantitative trait loci (QTLs). The process of using such markers as selection criteria is called marker-assisted selection (MAS), the methodology of which is still at research level in rubber. There are several types of MGMs currently used: (i) restriction fragment length polymorphism (RFLP); (ii) random amplification of polymorphic DNA (RAPD); (iii) amplified fragment length polymorphism (AFLP); (iv) single sequence repeats (SSR) or sequence tagged microsatellite sites (STMS); (v) DNA amplification fingerprinting (DAF); (vi) microsatellite primed-PCR (MP-PCR); and (vii) single nucleotide polymorphism (SNP).

RFLP involves the use of restriction enzymes to cut chromosomal DNA at specific short restriction sites; polymorphism results from variations in length of the fragments due to duplications or deletions between the sites or mutations at the restriction sites. RFLP provided the basis for most early work but requires a relatively large amount of DNA and is rather expensive in a large screening programme.

RAPD utilizes low stringency PCR amplification with single primers of arbitrary sequence to generate strain-specific arrays of anonymous DNA fragments. The method requires tiny DNA samples and analyses a large number of polymorphic loci.

AFLP requires digestion of cellular DNA with a restriction enzyme, then using PCR and selective nucleotides in the primers to amplify specific fragments.

The method measures up to 100 polymorphic loci and requires a relatively small DNA sample for each test.

SSR analysis is based on DNA microsatellites, (short-repeat) sequences that are widely dispersed throughout the genome of eukaryotes, which are selectively amplified to detect variations in the number (and length) of simple sequence repeats. SSR analysis requires tiny DNA samples, and has a low cost per analysis.

SNPs are detected using PCR extension assays that efficiently pick up point mutations. The procedure requires little DNA per sample and costs little per sample once the method is established.

MGMs, independent from the environment such as isozymes, have been used in the same way to determine the degree of variability within plant populations. They allow direct access to the coding and non-coding regions of the genome, making their number potentially unlimited. Among different characteristics, they can be easily visualized and identified (such as by probes and Southern blots, PCR amplification and electrophoresis, radio-labelling, fluorescence). The more efficient MGMs generally assign one sole locus, and the diversity of their possible alleles determines the level of their polymorphism.

The first applications to rubber, on nuclear and cytoplasmic genomes, were made with RFLPs. Then, when a PCR technique was developed with random primers, RAPDs were used, as well as AFLPs, which combine restriction enzymes and PCR. Microsatellites (SSRs), of PCR developed from specifically designed primers, appeared very powerful due to their high polymorphism (between 15 and 20 alleles per marker). In comparison with isozymes or mRNA, the high stability of DNA makes it possible to send leaf samples from any remote plantation site to a laboratory by normal mail for analysis.

Although RFLPs are powerful tools for studying genetic diversity and mapping, this technology is not preferred now since it is labour-intensive and requires large DNA samples. Its marker index value (expressed as the number of polymorphic products per sample) is also low with only 0.10 compared with PCR-based marker systems like RAPDs (0.23), SSRs (0.60) and AFLPs (6.08) (Low *et al*., 1996). Ever since isozymes were utilized for clonal identification (Chevallier, 1988), tools like minisatellites (Besse *et al*., 1993a), RFLPs (Besse *et al*., 1993b, 1994), mitochondrial and chloroplastic RFLPs (Luo *et al*., 1995), RAPDs and DAFs (Low *et al*., 1996; Varghese *et al*., 1998; Venkatachalam *et al*., 2001, 2002), AFLPs (Lespinasse *et al*., 2000a) and SSRs (Besse *et al*., 1993a; Atan *et al*., 1996; Low *et al*., 1996) were developed and used in detection, increasing the number of molecular markers in *H. brasiliensis*. Polymorphism in microsatellites was detected also in *H. pauciflora*, *H. guianensis*, *H. camargoana* and *H. benthamiana* (Low *et al*., 1996) and cross-species amplification was also done in these species (Souza *et al*., 2009). Microsatellite-enriched libraries were produced and led to the identification of large numbers of microsatellite markers (Atan *et al*., 1996; Seguin *et al*., 2003; Saha *et al*., 2005); sequences of 472 of them are currently accessible on the EMBL/Genbank databases. CIRAD has also developed a database for rubber clonal identification by use of ten microsatellite markers; microsatellite markers from rubber pathogens can also be used for distinguishing the genetic differences between the races, as has been done with 11 markers for *Microcyclus* (Le Guen *et al*., 2004).

Although considerable progress has been made to increase the yield in *Hevea* clones in the recent past, satisfactory resistance to biotic and abiotic stress has not been achieved because of limited genetic resources within the *Hevea* gene pool. Wind damage is one of the serious problems in rubber-growing countries; each year there is a considerable loss of rubber trees due to wind damage in rubber plantations. The incorporation of the dwarf character into high-yielding *Hevea* clones would be useful for generating a high-yielding tree with a desirable architecture (dwarf stature) (Venkatachalam *et al*., 2004). Information on the genetic and molecular basis of the dwarf character in this species could provide insights on the development of high-yielding dwarf clones that would eventually lead to decreasing the negative consequences of wind damage (abiotic stress) and high-density planting but might have negative effects on rubber wood productivity. The identification of molecular markers for the dwarf character would be important for isolating true-to-type high-yielding dwarf hybrid lines in the early stage of plant breeding programmes. Venkatachalam *et al*. (2004) identified a dwarf genome-specific RAPD marker in the rubber tree. The primer OPB-12 generated a 1.4 kb DNA marker from both natural and controlled F_1 hybrid progenies (dwarf stature) derived from a cross between a dwarf parent and a normal cultivated clone as well as from the dwarf parent; it was absent in the other parent (RRII 118). To validate this DNA marker, 22 F_1 hybrids (13 with a dwarf stature and nine with a normal stature) were analysed; the dwarf genome-specific 1.4 kb RAPD marker was present in all dwarf-stature hybrids and absent in all normal-stature hybrids. This DNA marker was cloned and characterized. DNA marker locus specificity was further confirmed by Southern blot hybridization.

6.2.2 Molecular genetic diversity

In comparison with isozymes, fingerprinting through RFLP minisatellite probes appeared to be more powerful (but also more expensive). Identification of 73 Wickham clones was carried out with 13 probes associated with the restriction enzyme *Eco*RI (Besse *et al*., 1993b). RFLPs and DAFs were also used for identification of progenies with two common parents (Low *et al*., 1996). Besse *et al*. (1994) assessed the genetic diversity of 92 Amazonian and 73 Wickham clones based on RFLP profiles by using homologous probes from a CIRAD *Hevea* bank, and demonstrated a genetic enrichment brought by the Amazonian population to *Hevea* germ plasm. They also studied the ribosomal DNA variations (Besse *et al*., 1993a) (Fig. 6.3). One clone from Rondonia, RO/C/8/9, showed eight specific restriction fragments and a unique *malate dehydrogenase* (*MDH*) allele, indicating its interspecific origin (Besse *et al*., 1994). Application of microsatellites to genetic diversity analysis confirmed that wild accessions are more polymorphic than cultivated Wickham clones. Some accessions such as RO/OP/4 20/16 and RO/A/7 25/133 seem unique since they do not fall under any cluster due to their high level of specific alleles (Lekawipat *et al.*, 2003a, b). Such diversity analyses were extended to larger samples and combined with the information coming from isozymic studies.

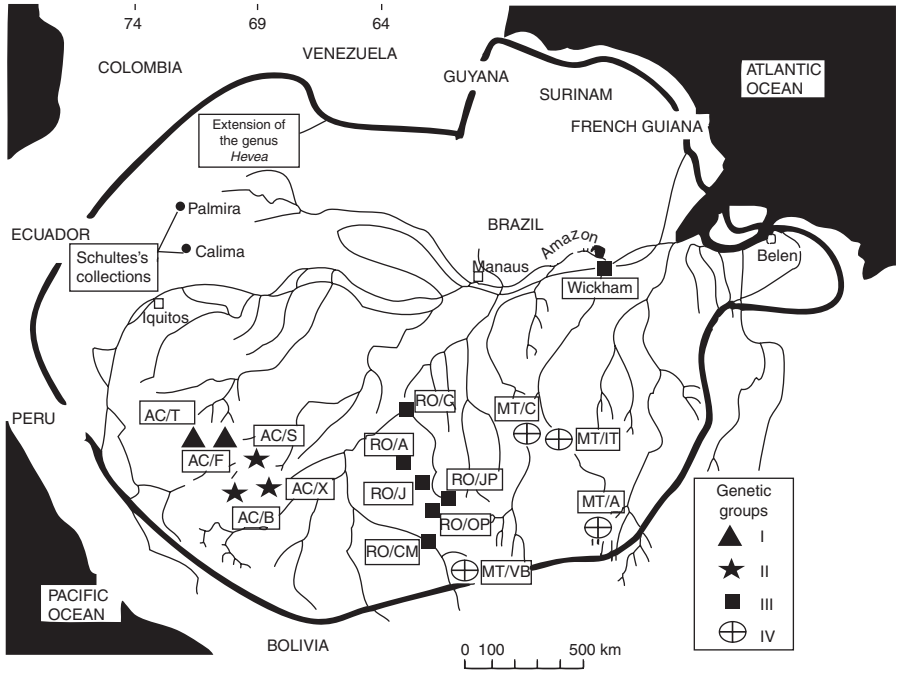


Fig. 6.3. Geographical origin of *Hevea* clones analysed with isozymes or RFLP markers for genetic diversity assessment (after Besse *et al.*, 1994).

Using RAPD analysis, Varghese *et al*. (1997) evaluated 24 cultivated *Hevea* clones to estimate genetic distances. More recently, Venkatachalam *et al*. (2002) described the genetic relationships for 37 *Hevea* clones using RAPD analysis. This molecular information concurs with the reported high morphological variability in *Hevea*. The clones are classified into seven major groups based on DNA markers. The phenogram showed that RRII 105 (India) and RRIM 600 (Malaysia) were nearly identical. They are also closely related to the PB clones (Malaysia) and Chinese clones. It is interesting to note that most of the Malaysian clones have been put in two clusters. Three clones belonging to Thailand have been put in the same cluster. The two clusters formed by the Indian clones were distinct with one group (RRII 201, RRII 204, RRII 205, RRII 207 and RRII 209) clustering with one Malaysian clone.

Venkatachalam *et al*. (2002) used the RAPD profiles to construct dendrograms and clones originating from different countries were clearly distinguished. This analysis clearly distinguished all the 37 *Hevea* clones from each other based on the polymorphic bands. The clones RRIM 501 and PB 25 were grouped together as they were separated from the remaining clones. The clones PB 312 and PB 314 were grouped together with a maximum similarity of 0.152, followed by PB 260, PB 280 and PB 217, which showed a similarity of 0.403. Clones PB 312 and PB 314, which come from the same cross (RRIM 600 × PB 235), appeared to be genetically very close. In this dendrogram based on RAPD

markers, 69% of the bands observed were polymorphic between the 37 *Hevea* clones. This seems to be relatively high when compared with the reports of other RAPD studies in *Hevea* (Varghese *et al*., 1997). RAPD analysis therefore reflects genetic differences and the geographical origin of the *Hevea* clones. The Indian RRII 203 clone is very dissimilar to the Malaysian PB 255 clone (similarity index: 0.692). The next most dissimilar clones are PB 25 and RRII 203 with a 0.672 similarity index. From the dendrogram it is intriguing to note that several primary clones developed in different countries, such as Tjir 1, Gl 1, PB 86, Mil 3/2, AVROS 255 and BD 10, are closely clustered. In most cases, clones with common ancestors, such as RRII 105, RRIM 600, PB 311, PB 312, PB 314, KRS 128, KRS 163, PB 217, PB 255, PB 260, RRII 204, RRII 209, were observed to cluster together. Further, Le Guen *et al*. (2009) studied microsatellite markers from 307 individuals of the International Rubber Research and Development Board (IRRDB) collection and claimed the diversity followed a hydrographic network.

Mitochondrial DNA (mtDNA) was also analysed in over 395 genotypes using heterologous probes from broad bean (Luo and Boutry, 1995; Luo *et al*., 1995). A high mtDNA polymorphism was found in Amazonian accessions; in contrast, the diversity of mtDNA in the Wickham population is almost nil as only GT 1, a male-sterile clone, exhibited a different type from that of 49 other Wickham clones analysed. The mtDNA appears to be a valuable tool for studies on classification and phylogeny in plants, as it results more from DNA rearrangements rather than nucleotide substitutions. Sequencing of a highly polymorphic mtDNA fragment from 23 genotypes showed real potential for phylogenetic analysis in *Hevea* (Luo and Boutry, 1995). Chloroplast DNA (cpDNA) analysis was carried out in over 217 accessions representing 126 mitochondrial genotypes; only two cpDNA RFLP profiles were found, so showing a much lower polymorphism and indicating the high level of conservation of this chloroplast genome (Luo *et al*., 1995).

As a synthesis of these diversity studies, good relationships were found between the results coming from the different genetic markers. Even if the contribution of isozymes is important by itself, molecular markers provided important clarifications for the distinction of different groups. There would be no barrier to migration of *Hevea* genes within the Amazonian basin. However, the wideness of the area and the limited dispersion of *Hevea* seeds allowed the preservation of the current structure, which is assumed to have initially resulted from the fragmentation of the Amazonian forest during the Pleistocene period, according to the refuge theory presented by Haffer (1982). Moreover, the genetic structure of *Hevea* germplasm clearly follows the geographic structure of the hydrographic network of the Amazonian forest, which confirms the role of rivers and inundated zones in the transport of seeds and dissemination of the species (Luo *et al*., 1995; Seguin *et al*., 1996a, b). The mtDNA of the Wickham population has less variation since their female progenitors are restricted to a very small set of primary clones. Cytoplasmic donors for most of the improved clones are either PB 56 or Tjir 1 (see Chapter 5). Obviously, this is the reason for the mtDNA profile showing only two clusters (Priyadarshan and Gonçalves, 2003) (Fig. 6.4). A possible explanation for greater polymorphism in mtDNA of wild accessions is that many might have evolved through interspecific hybridization.

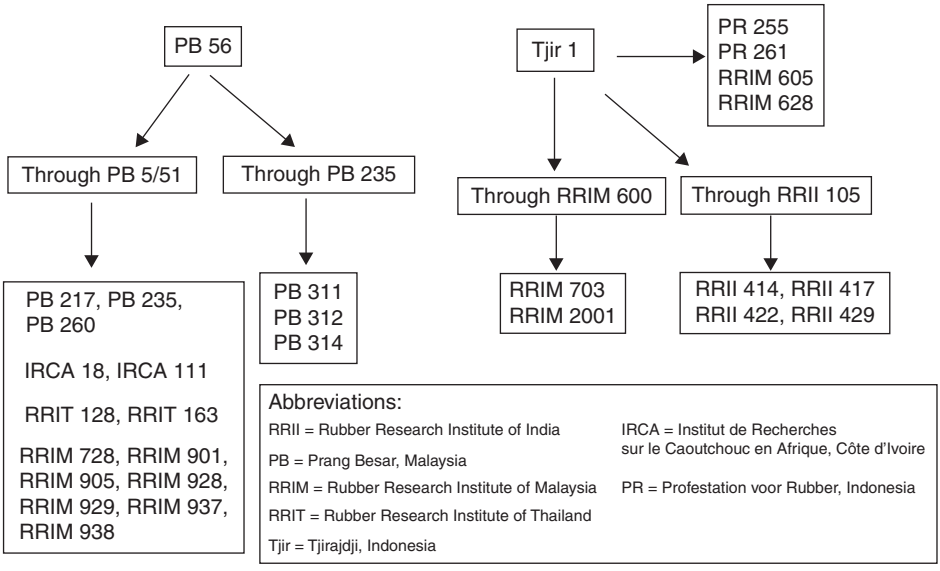


Fig. 6.4. Transmission of cytoplasm of PB 56 and Tjir 1 in the derivation of modern clones.

Seguin *et al*. (2003) proposed a general organization of *H. brasiliensis* germ plasm into six genetic groups arising from different areas of the Amazonian basin:

- **Group 1** made up of germ plasm from the two districts AC/T (Tarauaca) and AC/F (Feijo) in the western part of Acre, and with the Calima component of the Schultes collection;
- **Group 2** made up of germ plasm from the three districts AC/B (Brasileia), AC/S (Sena Madureira) and AC/X (Xapuri) in the eastern part of Acre;
- **Group 3** made up of germ plasm from six districts of Rondonia (RO/A (Ariquemenes), RO/C (Calama), RO/CM (Costa Marques), RO/J (Jaru), RO/JP (Jiparana), RO/OP (Ouro Preto)), the district MT/VB (Vila Bella) of Mato Grosso, and accessions MDF (Madre de Dios Firestone) from the Firestone collection in Peru;
- **Group 4** made up of germ plasm from three districts MT/A (Aracatuba), MT/C (Juruena) and MT/IT (Itauba) of Mato Grosso, and the district RO/PB (Pimenta Bueno) of Rondonia;
- **Group 5** made up of germ plasm from the Palmira component of the Schultes collection; and
- **Group 6** made up of germ plasm of the domesticated Wickham population.

Even if no prediction can be made about the progenies of crosses between these groups, they can be used as a basis for managing the genetic variability in the long term and organizing the recombination process.

Methodological research has been carried out in order to select genotypes that will make up a reduced-size collection of the Amazonian germ plasm, representative of the predominant part of the total variability of this germ plasm, according to the concept of a 'core collection' (Hamon *et al*., 1998). The germ plasm

characterization and diversity analysis studies coordinated by CIRAD were funded by the European Union from 1985 to 1997.

6.2.3 Paternity identification

Pre-breeding of the Amazonian genetic groups has been considered to be carried out by recurrent selection based on recombination through seed gardens and natural pollination, and intensive selection. For methodological purposes, one seed garden made up of 50 Amazonian genotypes and the GT 1 clone, planted at CNRA (Côte d'Ivoire), was subjected to the analysis of gene flux and paternity identification with isozymes and microsatellites (Blanc *et al*., 2001; Lidah, 2005). Paternity identification with microsatellites was carried out using the Cervus software (Marshall *et al*., 1998). A high level of confidence was found for paternity identification carried out using eight microsatellite markers. The distribution of the contribution of the different genotypes to pollination was found to be highly unequal, with four genotypes accounting for 40%, 14 genotypes accounting for 80%, and 25 genotypes accounting for 95% of the total fertilization of the seed garden. The variation of selfing rate was assessed among the genotypes with an average of 5%, and no selfing was found on GT 1, as expected for a male-sterile clone. The isolation of the seed garden was confirmed since no allele other than those belonging to the parental population was found. The efficiency in paternity identification which is made possible by microsatellites suggests that a new method of selection may be possible by which the best trees are selected from seedlings resulting from natural pollination and their paternity is identified *a posteriori*.

6.2.4 Genetic mapping

The availability of numerous MGMs led to the development, in most animal and plant species, of genetic linkage mapping based on the analysis of the percentage of crossing over between the loci of two markers during meiosis (a genetic and not a physical distance), and the ranking of the different loci on different chromosomes of one species. Due to the heterozygous nature of rubber clones, the construction of genetic linkage maps in *Hevea* requires specific methodology. Unlike annual crops, a cross between two heterozygous parents in *Hevea* can yield information about up to four alleles, which are segregated further. The first comprehensive genetic linkage map of *H. brasiliensis* has been built recently, mainly by the use of RFLP markers but also AFLPs, microsatellites and isozymes (Lespinasse *et al*., 2000a). This was accomplished through a double pseudo-test cross as per the methodology of Grattapaglia and Sederoff (1994) and a map was constituted separately for each parent. Further, homologous markers segregating in both parents were ascertained and a consensus map prepared. The parents used were PB 260 (PB 5/51 × PB 49) and RO38 (F4542 × AVROS 363). F4542 is a clone of the species *H. benthamiana*. The F_1 synthetic map of 717 markers was distributed in 18 linkage groups corresponding to the 18 chromosomes. These comprised 301 RFLP, 388 AFLP, 18 microsatellite and ten

g10
0 gHbCIR113
4.8 rDNA.L2
10.1 gHbCIR347
21.1 EM17/4
30.5 EM57/17
31.1 EM61/3
35.3 EM 34/12
35.3 EM 54/2
36.3 EM 48/7
37.2 EM36/6
38.6 EM34/4
39.8 EM38/1
42.4 EM48/13
43.6 gHbCIR626
45.8 EM23/6
48.9 EM29/4
51.2 gHbCIR168
53.7 EM2/8
58.3 EM36/9
62.3 M338
65.6 gHbCIR426
67.4 EM16/8
69 EM54/5
71.2 gHbCIR664
71.6 gHbCIR570
73.7 EM9/8
78.1 gHbCIR377
78.6 ICD
80 gHbCIR403.L2
80.2 GS
88.6 gHbCIR480
90.8 EM3/4
94 gHbCIR135/476
95.7 gHbCIR66
97 gHbCIR372.L2
97.8 EM36/7
99.1 gHbCIR36
99.4 gHbCIR46
100.1 gHbCIR364.L2
102.1 EM3/0'
107.2 EM24/10
109.4 EM23/5
111.5 EM23/7
112.5 gHbCIR158
112.5 gHbCIR509
112.7 gHbCIR625.L2
113.1 gHbCIR163
113.3 gHbCIR541
114 gHbCIR627
116 gHbCIR85.L1
118.5 EM33/11
122.9 gHbCIR687
123.3 EM24/13
126.6 gHbCIR82
131.6 gHbCIR457
135.9 EM3/1
g11
0 MnSOD
2.4 EM31/3
4.5 M379
8.4 EM57/15
14.4 EM34/8
23.6 EM2/7
25 EM36/12
26.8 EM23/9
31.2 gHbCIR295.PB
51.4 EM3/6
51.7 EM12/2
53.2 EM2/5
54.1 EM32/5
55.5 EM13/7
55.8 EM3/25
56.1 EM32/3
56.6 EM16/10
56.8 EM33/18
57.6 EM16/1
62.2 gHbCIR507
63.8 gHbCIR636
65.2 gHbCIR339.L1
66.6 EM3/24
69.7 gHbCIR295.RO
73.7 EM57/9
86.3 gHbCIR247
90.5 gHbCIR688
97.1 gHbCIR215
103 EM2/10
104.4 EM34/2
110 EM17/6
112.4 EM13/1
115.7 M613
116.6 RGAnbs13.L1
119.1 RGAnbs22
120.3 RGAnbs10/36/p9
120.6 RGAnbs29/33/p1
123 RGAnbs2
130.4 EM17/3
133.9 EM13/4
g12
0 gHbCIR387
4.4 EM15/9
9.6 gHbCIR995.L2
13.7 gHbCIR181
17.7 gHbCIR560.L2
21.3 gHbCIR690
22.4 LAP
23.7 gHbCIR29
25.7 gHbCIR513
29.5 EM45/4
30.5 EM63/14
30.9 EM15/3
32 gHbCIR80/317
33.1 EM21/4
35.5 gHbCIR81
38.4 EM40/5
38.8 EM15/2
39.5 EM33/14
41.7 gHbCIR415
41.8 EM16/12
42.6 ADH
44.9 gHbCIR145
48.6 EM13/10
48.6 EM14/2
50 EM58/3
51.3 EM48/4
52.6 gHbCIR213
64.3 EM57/13
66.3 EM61/10
68.4 EM22/8
68.5 EM24/8
70.5 gHbCIR164
75.3 EM2/2
76 EM40/8
76.3 EM32/7
g13
0 EM2/12
6.7 gHbCIR671.L2
10.6 RecHevein.L2
19.6 EM9/10
28.8 EM3/12
35.7 EM58/11
45.2 gHbCIR402
46.7 EM21/1
50 EM34/11
51.1 EM36/13
51.6 EM14/10
52.4 EM2/9
52.8 gHbCIR506.L2
53.2 EM36/14
59.3 EM48/11
60.3 EM29/3
60.9 EM32/2
61.6 EM9/15
62 EM58/4
62.3 EM7/5
62.5 EM17/1
62.8 gHbCIR31
62.8 EM16/5
66.7 EM38/7
67.3 EM2/12
70.5 gHbCIR333
71.5 gHbCIR287
73.9 gHbCIR360
74.3 EM54/4
74.9 gHbCIR521
75.1 gHbCIR303
76.2 EM38/5
84.5 EM41/1
90.4 EM30/2
100.6 EM58/10
101.4 EM57/16
102.5 gHbCIR670.PB
104.1 EM48/1
106 EM34/6
110.6 gHbCIR110.L2
116.9 EM38/4
118.3 gHbCIR670.RO
g14
0 gHbCIR6
8.9 EM32/8
10.5 gHbCIR685
10.9 EM33/3
16.7 gHbCIR365.L2
18.6 gHbCIR425
21.6 gHbCIR261
24 gHbCIR682.L2
31.6 gHbCIR631
38.9 gHbCIR330
45.6 EM31/1
48.8 EM23/8
49.1 gHbCIR412/493
52.8 gHbCIR602
64 M124
70.2 EM3/15'
75 EM63/7
76.3 EM16/16
78.1 EM21/7
79.6 EM16/11
79.7 EM9/3
81.1 EM23/10
82.9 EM47/4
83.7 EM22/15
87.5 EM57/1
91.2 EM22/9
91.9 gHbCIR20.L1
93 EM48/12
96.3 gHbCIR290
104.9 gHbCIR409
109.5 gHbCIR644
g15
0 M382
4.4 gHbCIR436
7.1 M574
9.8 RGAnbs7
13.4 RGAnbs14.L1
14.7 RGAnbs14.L2
15.9 RGAnbs14.L3
16.6 RGAnbs13.L2
17.8 EM2/6
20 RGAnbs20
21.3 RGAnbs14.L4
26.8 gHbCIR998
28.2 gHbCIR441
29.3 gHbCIR139
33 EM33/15
35.8 EM12/4
37.3 EM15/6
37.5 EM13/8
39.8 EM12/1
48.1 EM24/6
48.8 EM24/15
51.8 gHbCIR406
52 gHbCIR265.L2
54.6 EM14/4
55.1 gHbCIRE171
63.1 gHbCIR494
64.5 EM22/11
72.8 EST
77.1 EM24/7
78.5 EM24/14
80 EM22/14
82.5 gHbCIR79
85.4 gHbCIR542
87.9 gHbCIR 167
94.5 EM7/9
106.5 EM21/3
114.1 gHbCIR45
127.8 EM3/17
g16
0 EM57/5
5.2 gHbCIR161.L2
10.6 EM24/5
17.5 EM21/9
19.1 EM54/1
21.3 EM22/7
23.9 gHbCIR689.L2
31.5 gHbCIR523.L2
40.4 EM3/11'''
51.8 EM2/14
68.6 gHbCIR130
69.2 gHbCIR191
71.7 EM17/7
72.1 EM24/4
73.8 EM3/13
74 EM14/13
75.1 EM15/11
87.5 gHbCIR302
93.1 gHbCIR647.L2
110.3 gHbCIR373.L2
116.8 gHbCIR588
131.1 gHbCIR421
138.8 EM3/3
g17
0 EM15/10
8.9 gHbCIR585
18 EM22/6
29.5 EM16/17
31.6 EM17/9
33.2 EM45/8
36.1 gHbCIR420
38.5 gHbCIR586
46 gHbCIR339.L2
47.7 EM32/1
51.6 EM7/4
54.3 M340
57.3 gHbCIR366
60.3 EM48/10
61.1 EM61/1
61.3 gHbCIR635
67.2 gHbCIR526
68.1 EM57/11
68.3 gHbCIR617
70.6 EM40/2
70.7 EM63/12
71.8 EM23/11
72.9 EM9/11
74.7 EM47/3
77.6 EM36/4
91.8 gHbCIR357
g18
0 EM22/13
6.4 gHbCIR41/47
8.8 gHbCIR266
12.4 gHbCIR227
24.8 PGD
29 EM3/21
30.1 EM22/16
32.5 gHbCIR618
34.1 GS2
38.7 EM16/14
42.3 gHbCIR112
43 gHbCIR443
43.3 gHbCIR259
44.4 gHbCIR101
47.6 gHbCIR20.L2
61.8 EM16/19
65.7 EM9/1
81.5 gHbCIR423
84.8 EM21/12
98.1 EM21/13
98.9 gHbCIR533
125.3 EM16/6
126.8 EM13/3
127.7 EM23/2
135.1 EM3/23
136.3 EM21/14
137.6 gHbCIR597
138.6 EM57/19
141.1 EM2/3
143.2 EM7/11
145.1 EM41/8

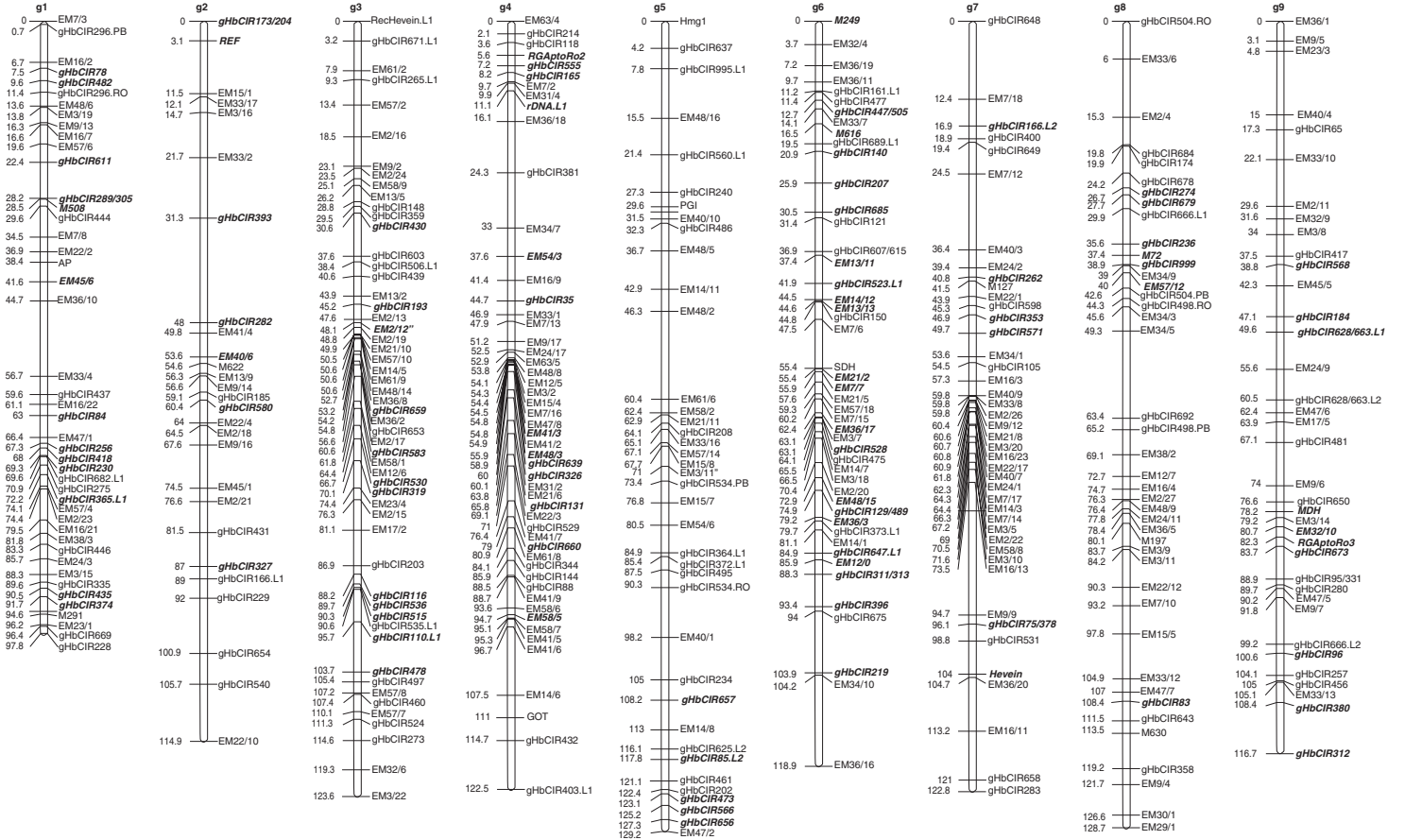


Fig. 6.5. F_1 synthetic map of 717 markers distributed in 18 linkage groups. This map encompasses 301 RFLP, 388 AFLP, 18 microsatellite and ten isozyme markers (Lespinasse *et al.*, 2000a). With kind permission from Springer Science and Business Media.

isozyme markers. The genetic length of the 18 chromosomes was fairly homogeneous with an average map length per chromosome of 120 cM. Many AFLP markers were seen in clusters, which were attributed to regions of reduced recombination frequency. Although the RFLP markers were well distributed all over the 18 linkage groups, these were insufficient to saturate the map. The AFLPs and a few microsatellites together contributed to saturating the map. A partially non-random arrangement of duplicate loci observed in the RFLP profiles indicate that they have homology descending from a common ancestor (Lespinasse *et al*., 2000a) (Fig. 6.5). The origin of such duplications is still unknown and *H. brasiliensis* continues to behave as a diploid.

Genetic linkage maps associated with phenotyping studies (field evaluation of the genotypes) can generate phenotypic comparisons between a huge number of classes of alleles and lead to the identification of QTLs. The research developed on the cross PB 260 × RO38 was targeted at understanding the genetic determinism of the resistance of this cross to SALB, first with manual infection at the laboratory level (Lespinasse *et al*., 2000b). Eight QTLs, with one predominant on linkage group g13, were identified for resistance in the RO38 map through the Kruskel-Wallis marker-by-marker test and the interval mapping method (Lander and Botstein, 1989; Oojen van *et al*., 1992). The F_1 consensus map confirmed the results obtained in the parental maps. It was further rationalized that the resistance alleles of RO38 were 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 found available in RO38, with specificity of resistance to different strains. Field evaluation against the pool of *Microcyclus* strains available in French Guiana was carried out under real infestation conditions, and it confirmed the presence of the predominant QTL in g13 previously found under controlled infestation (Le Guen *et al*., 2003). Then it was shown that this major QTL was no more efficient against two widely virulent and highly aggressive strains; and, for one of them, another QTL located on the linkage group g12 was able to reduce the aggressiveness. This genetic mapping and QTL approach is currently being continued with other crosses in order to analyse the genetic determinism of different sources of SALB resistance.

Research for identifying and cloning the real gene(s) responsible for this QTL in linkage group g13 is undertaken at CIRAD in the framework of the building of a bacterial artificial chromosome (BAC) bank and of a physical map of the rubber tree based on the clone RO38, which inherited the resistance trait from F4542. Among other applications, this will make possible the search for the DNA fragments bearing the QTL g13 and the development of the 'chromosome walking' technique towards genes associated with QTL g13 on these fragments. This physical map with a high density of MGMs (fine mapping) will also allow assessment of the stability of linkage between the neighbouring genetic markers.

More recently, genetic mapping and field assessment in Thailand were carried out on the cross RRIM 600 × PB 217 in order to study the genetic bases of different factors related to latex production. Genetic mapping was developed with a set of 247 microsatellite markers complemented by 198 AFLPs (Prapan *et al*., 2004). Genetic mapping is also developed at the Malaysian Rubber Board on crosses RRIM 937 × RRIM 600 and PB 5/51 × IAN 873 by use of RAPD and

AFLP markers, and at the Indonesian Rubber Research Institute targeting the analysis of resistance to *Corynespora* on the cross AVROS 2037 (resistant) × PPN 2444 (susceptible).

6.2.5 Expressed genes in *Hevea*

Lekawipat (2004) performed a genetic diversity analysis of *H. brasiliensis* germ plasm over 66 Amazonian and 40 Wickham accessions, by use of non-expressed MGMs (12 microsatellites) and also 17 markers of expressed genes (single-strand conformation polymorphism (SSCP), based on PCR and the secondary conformation structure of single-strand DNA on non-denaturing acrylamide gel, aimed at mutation detection in expressed genes). It was found that microsatellites could detect higher polymorphism than gene-specific primers of SSCP in rubber accessions, although markers of expressed genes can be assumed to be more related with some putative breeding objectives. SSCP markers could not differentiate the Wickham and the Mato Grosso accessions.

By the use of reverse genetics from mRNA to cDNA libraries (RT-PCR), the fields of functional genomics and molecular physiology are being developed in rubber by different teams, predominantly working on latex cells, on such themes as:

- rubber biosynthesis;
- latex-cell functioning;
- the latex coagulation process;
- ethylene biosynthesis and metabolism;
- oxidative stress;
- tapping cut dryness and brown bast (the reversible or irreversible forms of TPD);
- allergenic proteins in the latex;
- heterologous genes to be expressed in the latex;
- drought tolerance;
- leaf fungus diseases;
- cyanogenesis metabolism or defence proteins;
- photosynthesis; and
- on an increasing number of other proteins.

Latex as a cytoplasm can be efficiently used as a model for research on molecular cell biology. But also in other tissues of the rubber tree the complexity of cell functioning and developmental biology is addressed in the framework of varied topics. In the field of reproductive biology, rubber flower and inflorescence development has been characterized: one important gene regulating flower induction and development (*leafy*/*floricaula*) was cloned and its expression was analysed and localized by *in situ* hybridization (Dornelas and Rodriguez, 2005). In the field of post-germination changes in rubber seeds, proteomics (2D-Page and mass spectrometry methods) were implemented for examining the changes in protein expression from the mature seed to the germinated seed (Wong and Abubakar, 2005). The suppression subtractive hybridization (SSH) technique is currently widely implemented between different pairs of mRNA samples for the production of molecular resources

by RT-PCR in the form of subtracted cDNA libraries; microarrays or sequencing and comparison with entries from the databases will then assist in searching the functions of these expressed genes (candidate gene approach) (see Sathik *et al*., 2009).

Expressed sequence tags (ESTs, or small and partial 5′-end sequences of expressed genes) related to various metabolic aspects are being collected to create EST banks that broadly represent the genes expressed in one tissue, such as latex cells, and this assists in the study of gene function and regulation. Entries of these banks are compared with public databases of already known genes for identifying the putative functions of the corresponding genes. These EST banks will also create the way for macroarray- or microarray-based studies of *Hevea* gene expression. The 'Latex Lambda Triplex' EST-cDNA library (Ko *et al*., 2003) published in the EMBL/GenBank databases (858 entries) showed that about 16% of the database matched ESTs encoding rubber biosynthesis-related proteins. Rubber biosynthesis-related genes appeared to be mostly expressed, followed by defence-related genes and other protein-related genes (Han *et al*., 2000). Published DNA sequences of the latex allergens were matched against these ESTs, so indirectly providing a ranking of the allergens depending on their concentration in the latex; more than 1000 ESTs matched with the sequences of *REF* (gene for rubber elongation factor, or *Hev.b.1*) and *SRPP* (gene for small rubber particle protein, or *Hev.b.3*).

Genes responsible for the synthesis of rubber transferase, the key enzyme for polymerization of polyisoprene (natural rubber), appeared to be among the most abundantly expressed genes in the latex. Hevein, a chitin-binding protein, one of the defence proteins that play a crucial role in the protection of wound sites from fungal attack, is also involved in the coagulation process; it belongs to a multigene family, and the specificity of its expression in the latex is under investigation (Broekaert *et al*., 1990; Pujade-Renaud *et al*., 2004). Nearly 12.6% of the proteins available in the latex are defence related (Han *et al*., 2000). Among 200 distinct polypeptides (Posch *et al*., 1997), mainly three rubber synthesis-related genes are expressed in the latex: (i) *REF* (Dennis and Light, 1989; Goyvaerts *et al*., 1991); (ii) *HMGR* (Chye *et al*., 1992); and (iii) *SRPP* (Oh *et al*., 1999). The most abundantly expressed gene is *REF* (6.1%) and then *SRPP* (3.7%) (Han *et al*., 2000). References and partial- or full-length sequences of these cloned genes can be found in the EMBL/GenBank databases.

Unlike photosynthetic genes, transcripts involved in rubber biosynthesis are 20–100 times greater in laticifers than in leaves (Kush *et al*., 1990). On the other hand, transcripts for chloroplastic and cytoplasmic forms of glutamine synthetase are restricted to leaves and laticifers, respectively (Kush *et al*., 1990), indicating thereby that the cytoplasmic form of glutamine synthetase plays a decisive role in amino acid metabolism of laticifers. The transcript levels of hydrolytic enzymes, namely polygalacturonase and cellulase, might be taken as indicators for a better development of the laticifers. Genes expressed in the latex of *Hevea* can be divided into three groups based on the proteins they encode: (i) defence-related proteins such as hevein, chitinase, β-1,3-glucanase and HEVER; (ii) rubber biosynthesis-related proteins such as rubber elongation factor (REF), HMGR and 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMGS), *cis*-prenyltransferase (CIS), geranyl-geranyl diphosphate (GGPP) synthase, small rubber particle

protein (SRPP) and isopentenyl diphosphate (IPP) isomerase; and (iii) latex allergen proteins such as Hev.b.3, Hev.b.4, Hev.b.5 and Hev.b.7. The biological functions of the allergenic proteins are largely unknown (Oh *et al*., 1999).

Rubber biosynthesis

Rubber biosynthesis in *Hevea* laticifer cells has become a major field of research applied to the expression and regulation of genes, with a view to possibly opening the way to genetic manipulation of the isoprenoid biosynthesis pathway enzymes. Rubber molecules (1,4 *cis*-polyisoprene) are formed from polymerization of molecules with five carbons, IPP, and aggregated as rubber particles packaged within a membrane which protects them from oxidation, in latex vessels. The general metabolic pathway of rubber biosynthesis is as follows. Sucrose from photosynthesis is actively transported into laticiferous cells through the plasmalemmic membrane, and is then hydrolysed into glucose and fructose by invertase. These sugars are then converted into acetyl-coenzyme A (acetyl-CoA) through glycolysis. Three molecules of acetyl-CoA are condensed into mevalonic acid and then IPP. Polymerization of thousands of molecules of IPP leads to dimethylallyl diphosphate (DMAPP) and GGPP, with the action of the enzyme rubber transferase associated with REF, a molecule fixed on the rubber particles' membranes.

Although natural rubber is synthesized and made almost entirely of isoprene units derived from IPP, an allylic disphosphate is also required as the priming co-substrate to initiate the subsequent extensive prenyl chain elongation process for the formation of rubber macromolecules. Both the HMGS and the HMGR have been shown to be involved in the early steps of rubber biosynthesis by forming 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) using HMGS. The HMGS catalyses the condensation of acetyl-CoA and acetoacetyl-CoA to form HMG-CoA (Suwanmanee *et al*., 2002, 2004; Sirinupong *et al*., 2005). In plants, HMG-CoA is reduced by HMGR to mevalonate (MVA) and is subsequently converted into IPP. Chye *et al*. (1992) reported that there are three genes encoding HMGR in *Hevea*, namely *hmg1*, *hmg2* and *hmg3*. The *hmg1* gene is likely to be involved in rubber biosynthesis, whereas the *hmg3* is possibly involved in isoprenoid biosynthesis of another nature. Gene expression analysis by type of tissue indicated that MVA-pathway genes were highly expressed in latex, as compared with other types of tissue, and that HMGS and HMGR exist in multiple copies and have different expression patterns (Sando *et al.*, 2008).

The first step in rubber biosynthesis is the isomerization of IPP to DMAPP by IPP isomerase. The successive head-to-tail condensation reactions of the five-carbon intermediates, catalysed by the enzyme rubber transferase, have been assumed to yield rubber. Oh *et al*. (2000) have isolated and characterized a cDNA clone encoding IPP isomerase from *Hevea* and showed in an *in vitro* rubber assay that the recombinant IPP isomerase was required for rubber biosynthesis. In order to examine possible participation of GGPP synthase in the enzymatic prenyl chain elongation in natural rubber biosynthesis, Takaya *et al*. (2003) studied the GGPP synthase gene from *Hevea*. Based on their investigation, GGPP synthase would catalyse the condensation of IPP with allylic diphosphates to give GGPP. Therefore, GGPP is one of the key precursors in the biosynthesis of biologically significant isoprenoid compounds. Natural rubber is thought to be

made almost entirely of *cis*-isoprene units derived from IPP, and the enzyme responsible for polymerization is believed to have characteristics similar to the *cis*-prenyl diphosphate synthases. The gene responsible for the *cis*-1,4-polymerization of isoprene units has been isolated and characterized in *Hevea* recently by Asawatreratanakul *et al*. (2003). It was suggested that rubber biosynthesis in *Hevea* is mediated by the association of a soluble *trans*-prenyltransferase with the REF, a 14.6 kDa protein, tightly bound to the rubber particles in the laticifers (Dennis and Light, 1989). Farnesyl diphosphate (FPP) is a key intermediate in the biosynthesis of at least 20,000 isoprenoids. FPP is also the allylic diphosphate initiator of rubber biosynthesis in plants. FPP is synthesized by the enzyme farnesyl diphosphate synthase (FPS), which has been cloned and characterized from *Hevea* by Adiwilaga and Kush (1996). FPP formed by FPS is assumed to be the initiating substrate for rubber formation. The REF protein was isolated and studied extensively and the results indicate that the FPS and REF complex was responsible for the *cis*-1,4-polyprenol condensations observed in isolated rubber particles (Light and Dennis, 1989). The *REF* gene was isolated and the involvement of REF on rubber formation was analysed by Attanyaka *et al*. (1991) and Goyvaerts *et al*. (1991). Oh *et al*. (1999) reported a novel *Hevea* cDNA sequence associated with SRPP, and the sequence analysis revealed that this protein is highly homologous to the REF. It is possible that the rubber biosynthesis pathway is coordinately regulated by these enzymes. However, the precise mechanism for the biosynthesis of rubber molecules has not yet been established. Moreover, the exact site of the formation of new rubber molecules still remains unknown.

Molecular biology of tapping panel dryness (TPD)

The two forms of TPD (reversible simple 'tapping cut dryness' related to overexploitation of the laticifer tissue, and the quite irreversible 'brown bast' related to the development of necrosis within the bark of tapped trees) are currently addressed at the level of gene expression and protein synthesis. Dian *et al.* (1995) showed that: (i) the latex from trees displaying tapping cut dryness exhibited five proteins specific to the cytosolic compartment of the latex cells and these were related to the disease; (ii) major changes consisted of a dramatic increase of a 14.5 kDa protein and a 26 kDa protein in diseased plants; and (iii) the 26 kDa protein was linked to the coagulation process. Then Sookmark *et al*. (2002) observed that the two main polypeptides (here called P15 and P22) were found to accumulate in the cytosol of the TPD-affected trees; P15 and P22 were identified as *REF* (*Hev.b.1*) and *SRPP* (*Hev.b.3*), respectively. Specific molecular probes were designed for a detailed characterization of *REF* and *SRPP* gene expression and RFLP mapping. This allowed the demonstration that *REF* and *SRPP* display very similar expression profiles. They are highly overexpressed by the tapping-induced metabolic activation, although not by wounding per se, or ethylene or abscisic acid. In addition to this similarity in gene expression, they were found to share one common locus in the genome. Eventually, no significant difference in *REF* and *SRPP* gene expression was observed between healthy and TPD trees, indicating that their TPD-related accumulation in the cytosol was not transcriptionally regulated. Western blot analysis demonstrated that osmotic lysis of the sedimentable organelles (lutoids) *in vitro* caused the release of *REF* and

SRPP from the rubber particle membrane into the cytosol. A mechanism of cellular delocalization as a consequence of the lutoid instability was proposed to explain *REF* and *SRPP* accumulation in the cytosol of TPD trees (Sookmark *et al*., 2002).

In recent years, studies aimed to identify genes associated with TPD have also been carried out. Chen *et al*. (2003) reported that the expression of *HbMyb1* was likely to be associated with TPD syndrome. At RRII, the TPD research was focused on identification of TPD-responsive genes by SSH technology applied to mRNA isolation from latex (Venkatachalam *et al*., 2005). The goal of this study was to identify genes whose mRNA levels are differentially expressed in the rubber tree during TPD development. To identify the genes involved in this process, two SSH cDNA libraries were constructed. For the forward subtracted cDNA library, healthy RNA was used as the tester and TPD RNA served as the driver, whereas TPD RNA was the tester and healthy RNA was the driver for the reverse subtracted cDNA library. A total of 1079 putatively positive clones were screened from these two libraries; 352 of these clones were positive by differential screening with forward and reverse subtracted probes and were selected for sequencing analysis. The putative functions of clones were predicted by BLASTX/BLASTN analysis. Among these, 64 were genes whose function had been previously identified while the remaining clones were genes with either unknown protein function or insignificant similarity to other protein/DNA/EST sequences in existing databases. Differentially expressed genes selected by subtractions were classified into 12 broad categories according to their putative functions generated by BLAST analysis. The possible links between the identified regulated genes and TPD syndrome were considered by dot-blot analysis, and compared where two unique genes were strongly downregulated under the TPD condition. Two genes, *Myb transcription factor* and *translationally controlled tumour-induced protein* (*TCTP*), that were unigenes to the forward substracted cDNA library (upregulated), were selected for expression analysis. The expression of two selected gene transcripts was examined by Northern blot analysis using plant tissues of both healthy and TPD trees. Results from Northern analysis confirmed that the expression of these two genes was downregulated in TPD trees. This was the first study reporting a set of suppressed genes in tapping cut dryness-affected trees by the SSH technique. Some other known genes identified in this study might provide new insights into TPD development in the rubber tree (Venkatachalam *et al*., 2005). Similar research based on SSH is currently being developed on brown bast (Kongsawadworakul *et al*., 2005).

Apart from genetic engineering, studies on laticifer-specific gene expression could have important implications for selection and breeding. The use of mRNA transcript levels as molecular markers for early selection could be considered (Kush *et al*., 1990). It is also felt that extensive studies on the expression of genes and the regulation systems in different fields may open new paths for rubber breeding. Functional genomics in rubber will develop faster and faster, taking advantage of research developed on other species (through comparison with the information of public databases), and by focusing on specific areas of interest in order to gain a good understanding of the functioning of the network of interacting genes and regulating factors.

7 Soil Tillage, Crop Establishment and Nutrition

Nearly all the early plantations were established on land cleared from virgin forest, and the trees thrived on the high inherent fertility from the organic matter reserves built up over a long period. Overall growth was generally considered satisfactory, even with complete weeding, and fertilizers were not used. However, in experiments with nitrogen (N) and phosphorus (P) fertilizers, better growth was obtained on almost all inland soils but not on alluvial soils (RRIM, 1939). Similarly, response to potassium (K) fertilizer depended on the soil type. On coastal and heavier inland soils, added K often depressed growth, especially in the absence of fertilizer N, but, on sandier soils, growth was increased. On most soils there was some response to fertilizer, especially if a combination of N, P and K fertilizers was used, and this became recommended for general use on rubber, once a native forest tree, in Malaysia (Bolton, 1964).

High-yielding clones grown on sandier soils showed magnesium (Mg) deficiency in the leaves (Bolle-Jones, 1956; Bolton and Shorrocks, 1961). The fertilizer recommendations of the RRIM (1958) were therefore modified to include this element. A mixture of sulfate of ammonia (N), rock phosphate (P) and potassium chloride (K), with or without Mg, was recommended for all inland soils, while fertilizer was not considered necessary on alluvial coastal soils. Therefore on the basis of terrain alone, which affects drainage and runoff, two broad groups of soils suitable for rubber were differentiated (inland and alluvial coastal soils), and fertilizers are managed accordingly. Under the high rainfall and highly acidic conditions of inland soils, various types of phosphatic rocks are widely used, which slowly release available P to plants over time. Rock phosphates remain a cheap source of P for both rubber trees and leguminous cover crops. They have a high residual value so that they benefit the crop for a long time (Middleton and Pushparajah, 1966).

Legume cover crops have long been an important part of soil management for rubber. Initially they were used to provide effective cover against soil erosion in newly planted or replanted rubber on steep terrain, and later to supply

additional N. Over the years, field experiments with various types of cover crops and fertilizers have shown that the amount of fertilizer, especially N, required by young rubber trees can be reduced after the third or fourth year from planting if a good legume cover is maintained (Coulter, 1972; Broughton, 1977). However, regular top-dressing with rock phosphate during the first few years is required to establish and maintain the legume cover (Watson, 1966). With good management of the cover crop and fertilizer, trees can be brought into production at only 4 or 5 years, reducing the immature, unproductive period by 1 or 2 years. Cover crops also protect the soil, and at the same time maintain or even increase its overall fertility.

Improved planting materials require substantial amounts of applied nutrients if they are to give their maximum economic potential yield. Forest clearance reduces the overall soil fertility in a humid tropical climate and this is overcome by replacing it with a tree crop ecosystem. Both the forest and the tree crop are stable and sustainable systems, but the latter gives a better economic return and is more demanding in terms of nutrient supply. On appropriate terrain, both protect the soil against dehydration, destruction of the soil structure and erosion, and add organic matter to the surface soil. The nutrient cycle, which maintains the sustainability of the forest ecosystem before clearance, is temporarily broken during the early phase of tree-crop establishment. Providing immediate soil cover by means of an appropriate fast-growing legume cover crop protects the soil and helps to build up a pool of nutrients in the topsoil, at the same time restoring soil fertility temporarily lost after forest clearance. Cover crops of this kind take advantage of one of the assets of the tropical climate – the warm, moist conditions favour the rapid re-establishment of vegetation. This generally takes longer in a temperate environment with its shorter growing season.

The use of perennial crops tolerant of acid soils and aluminium toxicity, together with applications of rock phosphate to both the legume cover crop and the rubber crop, is another technique being widely used to overcome the chemical constraints, particularly the lack of P, found in highly weathered soils in upland regions. Low-cost rock phosphate is widely used, taking advantage of the soil's acidic nature, which results in the slow release of P to tree and cover crops. Removal of nutrients through the crop is less in rubber than in the forest ecosystem, where large quantities of nutrients get locked up in the biomass of the trees and are lost permanently from the soil system with the removal of timber at the time of replanting. Therefore, to ensure optimum growth and yield and to protect sustainability of the system, maintenance of soil fertility through regular application of fertilizers is very important.

The four major components of soil are: (i) inorganic or mineral materials; (ii) organic matter; (iii) water; and (iv) air. The solid mineral particles comprise about 45% of the total soil volume. The organic matter is 5% and the balance is made up of pore spaces occupied by air and water. The proportion of air and water in soil is subject to rapid and wide fluctuations. The inorganic portion of soil, quite variable in size and composition, is composed of small rock fragments and mineral particles of various kinds and sizes. Rock fragments are comparatively larger in size. Some stone and gravel pieces may be as large as small rock fragments. Sands are somewhat smaller in size (0.02–2.0 mm diameter) and can

be seen easily with the naked eye. The sand particles do not stick together and feel gritty when rubbed between the fingers. Still smaller in size are the silt particles (0.002–0.02 mm), which are powdery when dry and even when wet are not sticky. The smallest mineral particles are the clays (< 0.002 mm), which form a sticky mass when wet and aggregate when dry. The smallest clay particles have colloidal properties and can be seen only with the aid of an electron microscope. Clay acts as a storehouse for both water and nutrients.

Soils for rubber cultivation should have a minimum depth of 1 m without any intervening hard pan or impenetrable layer. The water table should also be well below 1 m so that at least 1 m of soil with good aeration, essential for root penetration, is available. A good soil depth helps the plant to tide over the drought season more efficiently as moisture stored in the lower depths will become available to the roots during a drought. The yield of rubber is found to be very much reduced in shallow soils as compared with soils having adequate depth (Dijkman, 1951). Moreover, shallow soil or soil with a high water table does not ensure proper anchorage of the tree, which therefore becomes susceptible to wind damage by uprooting.

Well-drained soils are essential for optimum growth and yield of rubber plants. In marshy areas, owing to poor physical properties and waterlogged conditions, growth of rubber is always found to be very poor. Soil drainage is affected by: (i) topography; (ii) intensity and duration of rainfall; (iii) soil physical characteristics; and (iv) the condition of ground cover. In rubber plantations on level land, drainage can be facilitated by providing open drains. In hilly areas, the ravines and depressions which form natural waterways can be utilized with the adoption of proper methods for reducing stream velocity. Drainage of excess water considerably improves the physical structure of waterlogged soils and ensures better aeration of soil. Taproots penetrate deep into the soil while lateral roots spread into more shallow strata. Drainage ensures better anchorage because of the formation of a well-developed root system. Absorption of water and nutrients depends on the proper aeration of soil, which in turn is improved by good drainage. Drainage also increases the activity of beneficial soil microflora.

7.1 Chemical Properties

The important chemical properties of soils are: (i) soil reaction (pH); (ii) organic matter content; and (iii) fertility status. The optimal pH of soil for rubber cultivation lies in the range of 4.0–6.5 but the crop can tolerate a pH range of 3.8–8.0. Young seedlings tend to be more sensitive to low pH than mature trees. A soil pH above 8.0 causes growth retardation. Most of the soils in the humid tropics are acidic in reaction. The organic matter content and fertility status can be improved by proper management of the soil, which includes growing a leguminous cover crop, application of manures and fertilizers and adoption of soil and water conservation measures.

Soil contains an accumulation of partially disintegrated and decomposed plant and animal residues and other organic compounds synthesized by the soil microbes as decay occurs. Organic matter after decomposition attains a

more-or-less stable form called humus, which is dark coloured, amorphous and colloidal in nature. Humus is very similar to clay in physico-chemical properties. It carries negative electric charges on its surface, which attract and hold ions like K, calcium (Ca) and Mg. Humus acts as a cementing material and assists greatly in granulating clay particles to form stable crumbs. It facilitates movement of water in the soil and improves aeration.

The organic matter content of well-drained soils varies from 1.0 to 6.0% and the level requires maintenance by regular application of plant and animal residues to the soil. The capacity of the soil to retain moisture and nutrients is also increased by the addition of organic matter. Every soil has certain ability to hold cations on its negatively charged sites. The total of all such sites (of both mineral and organic constituents) in a soil is known as its cation exchange capacity (CEC). It is expressed as C mol kg^{-1} (previously in milliequivalents (mEq) 100 g^{-1} of soil). The CEC can vary from < 5.0 to 40.0 C mol kg^{-1} depending upon: (i) the amount and type of clay; (ii) the organic matter content; and (iii) the pH. Soil fertility indicates the inherent capacity of a soil to supply plant nutrients. The nutrient needs of the rubber tree are less than those of other plantation crops such as coffee, cocoa or oil palm. Rubber, therefore, can be grown on soils poor in nutrient content but with good physical properties. Any deficiency in the fertility can be supplemented by proper manuring and good agromanagement practices.

There are at least 16 elements which are essential for the growth and development of all plants. The essential elements are carbon (C), hydrogen (H), oxygen (O), N, P, K, Ca, Mg, sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), molybdenum (Mo) and chlorine (Cl). The nutrients which are required in large amounts are called primary, major or macronutrients and those required in small amounts are called secondary elements, micronutrients, minor elements or trace elements. Primary elements are N, P and K, which are mainly supplied through fertilizers, and secondary elements are S, Ca and Mg. In addition to these essential elements, sodium (Na), silicon (Si) and cobalt (Co) have been identified as beneficial elements. These beneficial elements, even though not essential, are found to stimulate growth in certain plant species, under certain conditions.

Plants obtain C, H and O_2 from air and water and the rest of the nutrients from soils, fertilizers and manures. N from the air is fixed by leguminous plants through the symbiotic association with bacteria that colonize their root nodules. C and O_2 from the air enter the leaves as carbon dioxide (CO_2) and a major portion of H is obtained from water.

7.2 Planting Density

Planting density of rubber is determined by considering the growth requirement of mature rubber trees. At the agronomic optimum, plants should capture and utilize growth resources in the most efficient manner providing the highest yield per unit area of land. Among the four main growth resources (i.e. CO_2, light, nutrients and water) CO_2 is highly dispersive in the atmosphere, providing equal opportunity for each plant to access. Availability of nutrients for plant growth

could be manipulated by adding the required quantity of fertilizer. If rubber is grown in wet areas, competition for soil water would be minimal but this could be a matter of concern in dry areas. Therefore, in general, light appears to be the most limiting factor and results in plants competing with one another. Competition between rubber trees begins at the fourth year after planting when the sparse canopies of individual trees intermingle forming a closed canopy (Rodrigo *et al*., 1995; Obouayeba *et al*., 2005). Irrespective of the planting density, trees have more or less equal opportunity to capture light and other resources in the immature phase, particularly before the fourth year of growth. Therefore, a higher planting density has an advantage of greater dry matter production per unit area during this early stage of growth. This has a carry-over effect in that the tree density with the highest growth or biomass production per unit area is above the density that captures maximum resources at a particular point of time. For instance, the LAI at maturity was found to be the same across a wide range of planting densities (i.e. between 500 and 800 trees ha^{-1}). However, latex yield and total timber volume per hectare were high in high planting densities despite the decrease in those values on a plant basis (Silva, 2007). Similar status with respect to latex yields and light interception were also recorded earlier (Rodrigo *et al*., 1995).

Although clonal propagation is practised to maintain homogeneity, heterogeneous rootstocks together with initial vigour in planting materials and spatial variability of the edaphic and aerial environment create some level of heterogeneity in rubber plantations. This is advantageous for more vigorous plants under competitive conditions. Therefore, substandard plants have less opportunity to become tappable in areas of high-density planting, resulting in a greater percentage of runts being found under high compared with low densities. Although the absolute number of trees being tapped increases with the increase in planting density, the percentage of trees being tapped in higher densities is less than that in low densities (Silva, 2007). The highest density tested so far recorded was 1067 trees ha^{-1} (Tiong *et al*., 1994) and most experiments recorded to date show a decline in yield per tree but an increase in yield per hectare with the increase in planting density (Tiong *et al*., 1994; Rodrigo *et al*., 1995; Siagian, 2000; Obouayeba *et al*., 2005). However, the rate of yield increase per hectare eventually declines so that the optimum density was found to be in the range of 500–700 trees ha^{-1}.

Planting geometry is also an important factor in the determination of the optimum planting density. For convenience in crop management, rubber trees are grown in alleys/clusters and the distance between trees within the alley/cluster is less than that between alleys/clusters. Therefore, competition between trees could be separated into two categories: (i) the competition *within* alleys/clusters; and (ii) the competition *between* the alleys/clusters. If the space between trees is equal in all directions (e.g. square planting), then there would be no such distinction of competition as the magnitude of the above two types of competition would be the same. This would be ideal for tree growth; however, there are some practical limitations when rubber is grown on sloped lands. Soil conservation practices would be rather expensive with a great number of terraces, and the walking distance for tapping and latex collection increases, making harvesting

more difficult. Also, canopy closure is quicker than in avenue planting systems and there is little space available for cultivating any other crop with rubber (Rodrigo *et al*., 2004). Rodrigo *et al*. (2004) carried out an experiment looking at light distribution at ground level under different spatial arrangements of planting at a density of 500 trees ha^{-1}. They demonstrated that, when the gap between alleys/clusters is increased at a particular planting density, the distance within the alley/cluster decreases. With that, the competition among trees within the alley/cluster increases and the competition between alleys/clusters decreases. However, the former is compensated by the latter within a certain limit beyond which plant growth will be affected. Therefore, spatial arrangements complying with the grower's requirements are to be selected within these limits. The planting density of rubber adopted in different countries varies from 400 to 550 trees ha^{-1} with different spatial arrangements. In Sri Lanka, a planting density of 500 trees ha^{-1} is generally recommended with three types of spatial arrangements: (i) 4.2 m × 4.8 m; (ii) 3.6 m × 5.4 m; and (iii) 2.4 m × 8.1 m. The first one is advantageous on rocky lands where row arrangements are difficult. The second system is generally used on flat lands while the third one is used for short-term intercropping with other crops or to reduce terraces on sloped lands (Tillekeratne and Nugawela, 2001).

7.3 Resource Capture in Intercropping Systems

During the initial stage of growth, rubber plants behave as discrete units with no canopy closure. This leads to temporal waste of resources, particularly light, resulting in excessive weed growth; hence additional inter-cultivation activities (e.g. weeding) are required that have no direct financial benefits. Intercropping with short-duration crops provides an opportunity to utilize those resources, enabling farmers to earn extra income during this period in the rubber crop in which there is no financial return. A wide range of crops with different spatial arrangements and combinations has been tested on immature rubber lands. In such instances, land-use efficiency has increased with increase in overall growth and yield. This has clearly been associated with increased radiation use in intercrops: for instance, overall radiation use during the first 2 years of growth in rubber/banana intercropping was over threefold when compared with the values recorded for sole-cropped rubber (Rodrigo *et al*., 2001). Except with grasses, crop combinations have shown no negative effects on the growth of rubber; on the contrary, in most cases intercropping has resulted in improved growth of rubber plants (Rodrigo *et al*., 1997, 2001; Pathiratna and Perera, 2002). Among the indices for crop growth assessments in intercrops, the crop performance ratio (CPR) and the land equivalent ratio (LER) are suitable to evaluate the component crops separately and the cropping systems as a whole, respectively. The CPR assesses the productivity of the component crop with respect to its productivity in the sole crop and the proportional density in the intercrop (Azam-Ali *et al*., 1990); in contrast, the LER is calculated from the sum of the ratio of the yield of the component crops in the intercrop relative to their sole crops showing the relative land area required as sole crops to produce the same yields achieved in the intercrop (Reddy and Willey, 1981; Willey, 1985). Values above unity for

CPR and LER indicate the intercropping advantages of the particular component crop and the whole system, respectively. As an example, the CPR value of intercropped rubber with banana was 1.48 while the LER of the whole rubber/banana intercrop was recorded as 2.6 (Rodrigo *et al*., 1997).

It is well known that the light response of photosynthesis is curvilinear at the leaf level approaching light saturation above 1000 mol m^{-2} s^{-1} in rubber (Nugawela, 1989; Senevirathna *et al*., 2003). Nevertheless, in view of the fact that the majority of leaves in a crop canopy are shaded to different degrees, canopy photosynthesis operates at an almost constant photosynthetic efficiency for intercepted radiation. This results in a linear relationship between dry matter production and intercepted radiation, with the slope of the relationship providing a measure of radiation-use efficiency (Monteith, 1994). During the early stage of growth, leaves of rubber plants are almost fully exposed to the incident light, hence operating at or above the light saturation of photosynthesis, particularly during the mid-hours of the day. Excessive radiation loads are stressful to the photosynthetic apparatus in that some level of photo-inhibition, related to the downregulation of photosynthesis, has been observed (Senevirathna *et al*., 2003). Moreover, around midday, a reduced rate of instantaneous photosynthesis associated with low water status of leaves and increased stomatal resistance (r_s) has also been observed (Rodrigo, 1997). Partial shading in intercropping alleviates the radiation stress on rubber plants, resulting in improved productivity. For instance, growth of rubber in rubber/banana intercropping showed higher growth than that in sole-cropped rubber (Rodrigo *et al*., 1997) and this has been attributed to improved radiation-use efficiency (Rodrigo *et al*., 2001). This phenomenon is very common when rubber is grown under suboptimal conditions (e.g. in dry regions). Although dry conditions affect the growth of banana, rubber plants in the rubber/banana intercrop in drier regions of Sri Lanka have shown higher growth than that in the sole crop even under smallholding conditions where no fertilizer was applied to banana (Rodrigo *et al*., 2003). Moreover, alleviation of radiation stress on photosynthesis around midday and associated improved growth have been reported in rubber grown with sugar cane under smallholding conditions in a dry region of Sri Lanka (Rodrigo *et al*., 2000).

In addition to the benefits associated with light use, heterogeneity of the root system in intercrops results in improved resource capture in the edaphic environment. For instance, total water consumption increased in rubber/banana intercropping (Rodrigo *et al*., 2005a) and increased dry matter per unit area in the same experiment (Rodrigo *et al*., 1997) provided evidence for greater nutrient uptake in intercrops over the sole crops. In intercropping systems, deep-rooted tree crops bring the nutrients from the lower soil horizons to the surface through leaf fall and make these available to the shallow-rooted companion crops (Noordwijk *et al*., 1996). In addition, the close proximity of roots at different depths tends to reduce nutrient leaching.

With the understanding of the benefits of intercropping, attempts have been made to incorporate perennial crops with rubber. As observed with short-term and semi-perennial crops, shade-tolerant perennial crops such as cocoa and coffee have been planted successfully with the standard density of rubber recommended for sole crops. However, light penetration through the mature

rubber canopy is not sufficient for sun-loving crops, demanding new spatial arrangements for such intercropping systems (e.g. rubber/tea, rubber/cinnamon). Two approaches have been tried for improved light penetration: (i) reduced planting density; and (ii) close planting of a few rows and then a wide gap for intercropping. The former reduces the intraspecific competition of rubber, allowing a safe margin for the interspecific competition. In the latter, intraspecific competition within the closely planted rows (clusters) increases while that between clusters decreases, providing sufficient resources for other crops to grow. In Sri Lanka, rubber/tea intercropping is practised with a reduced planting density of rubber (i.e. *c.* 75% of the sole crop density) with the spacing of 2.4 m × 12 m (Rodrigo, 2001). Being a tree crop that is taller than most other crops, rubber dominates even in such systems and no sign of a negative effect of tea on rubber was observed (Iqbal *et al*., 2005). Instead, rubber showed an improved performance over the sole crop, which could be a combined effect of low tree density and increased access to nutrients given to the tea. Placement of root barriers between the rubber and tea crops in this system had no clear positive effect on tea growth unless the rubber canopy was pruned to improve the light penetration (Iqbal *et al*., 2005). Obviously, the shorter the crop the weaker it is in crop competition, as access to light is limited by the taller crop. Moreover, the reduced planting density (up to 75%) has enabled the tea crop to produce a good harvest but for only up to about 7 years, highlighting the need for improved spatial arrangements. Planting rubber in paired rows without reducing the density has given improved light penetration. However, the gap between paired rows appeared not to be sufficient for long-term intercropping and also the spacing among rubber plants within the paired row did not allow rubber plants to grow adequately (Rodrigo *et al*., 2004). Therefore, a combination of the reduction in the planting density and the use of paired rows for improved light penetration is under investigation for long-term intercropping. The gaps used between paired rows were either 15 or 18 m while plants were placed within the paired rows at either 3 m × 3 m or 3.5 m × 3.5 m spacing (Seneviratne, 2005). Growth performance of rubber trees planted in cluster planting systems (i.e. three to four plants within a closely planted cluster) was comparable with that obtained with traditional ways of planting at the standard planting density (Rodrigo *et al*., 2004). Nevertheless, there have been no proper records for such systems at reduced planting densities targeting long-term intercropping. Selective pruning of the rubber canopy would also be another option to improve light penetration and experiments are to be set up to investigate its influence on the overall productivity and the cost-effectiveness.

In addition to the effect on photosynthesis in the understorey, improved light penetration through the rubber canopy in wide-row intercropping systems appears to be beneficial in cooler climates. Rubber trees grown in the cooler climates of China are subject to freeze damage in the collar region of the tree and light penetration through the wide inter-row gap between the rubber rows in rubber/tea intercropping has led to a temperature increase in the rubber tree trunk in winter with a minimum incidence of such damage (Feng *et al*., 1982). It is also expected that heterogeneous canopies in intercrops would conserve the heat inside the system through added resistance for heat transfer.

8 Constraints – Environmental and Biological

The great Amazonian basin is the ideal environment for growing rubber. This area, between the equator and 15°S is one of flat land with altitudes not exceeding 200 m with a wet equatorial climate (Strahler, 1969). The climate is characterized by a mean monthly temperature of 25–28°C and abundant rainfall of more than 2000 mm $year^{-1}$. The attributes ideal for rubber cultivation are: (i) 2000–4000 mm rainfall distributed over 100–150 rainy days $year^{-1}$ (Watson, 1989); (ii) mean annual temperature around 28 ± 2°C with a diurnal variation of about 7°C (Barry and Chorley, 1976); and (iii) sunshine hours of about 2000 h $year^{-1}$ at the rate of 6 h day^{-1} in all months (Ong *et al*., 1998). In a study using a hydrothermal index, Rao *et al*. (1993) rationalized Senai of Malaysia (1°36′N; 103°39′E) to be the most suitable for rubber cultivation and production.

The increased global demand for rubber, as with the extension in cultivation of other agricultural crops, has prompted countries outside what has been the traditional rubber-growing zone to focus their attention on the cultivation of rubber (Pushparajah, 1983, 2001). This tendency has meant that rubber is often grown in suboptimal soil and environmental conditions.

8.1 Non-traditional Environments and Geoclimatic Stresses

Specific areas of China, Thailand, Vietnam, India, Côte d'Ivoire and the southern plateau of Brazil fall into non-traditional rubber-growing zones that experience one or more stress situations, namely: (i) drought; (ii) low temperature; (iii) high altitude; (iv) diseases; and (v) strong winds. The mean annual temperature decreases with a move away from the equator with more prominent winter conditions during November–January. The north-eastern states of India and China, lying between 18° and 24°N, are regions well recognized as being inhospitable for the crop, as they exhibit stress situations such as low temperatures and typhoons (Zongdao and Yanqing, 1992). It may also be worthwhile noting that the

rubber-growing areas of China and Tripura (north-east India) fall in the same latitude range, although climatic conditions in pockets of China vary since China's tropical and subtropical regions are undulating and diversified (Priyadarshan, 2003a; Priyadarshan and Gonçalves, 2003). In Brazil, there are four main climatic zones: (i) the Amazonian basin; (ii) the Brazilian plateau; (iii) the coastlands within the tropics; and (iv) the southern states. Rubber is found in all these areas except the southern states. The southern Brazilian plateau (450–500 m above MSL), especially the area around São Paulo, is an area where growers are experimenting with rubber cultivation. This move to areas seasonally affected by dry and cold conditions is mostly motivated in Brazil in order to escape from the climatic conditions congenial to South American Leaf blight (SALB). These areas, apart from the high altitude, offer high rainfall that often exceeds the basic requirements of rubber.

A geoclimatic comparison of various environments including India, China, Brazil, Côte d'Ivoire, Indonesia, Vietnam and Thailand reveals a spectrum of climatic conditions over which rubber is grown (Table 8.1). Climatic conditions in these countries are also variable (Table 8.2). In India, marginal areas delineated as non-traditional rubber-growing zones (spread over the states of Maharashtra, Orissa, Tripura, Assam, West Bengal, Meghalaya and Mizoram) pose a multitude of hazards, namely: (i) moisture stress; (ii) low temperature; (iii) wind; (iv) high altitude; (v) disease epidemics; and (vi) altered physical soil properties.

Nevertheless, even if breeding can be efficiently involved, adaptation of the rubber crop to non-traditional environments will not overcome the constraints on growth and latex yield compared with traditional rubber-growing zones. So the success of the crop in non-traditional areas becomes an issue related to socio-economic conditions (rubber price, cost of labour, availability of land). Moreover, in the short term, adaptation to new areas needs more ecophysiological research with a better evaluation of existing clones in various environments rather than the creation of new clones, which can be considered only as part of a long-term perspective.

8.2 *Hevea* Grown Under Marginal Conditions

Climatic factors play a pivotal role in the establishment and development of any crop. Ambient temperature, rainfall, wind speed, vapour pressure deficit and the number of hours of sunshine are some of the factors that govern these (Table 8.3). Different genotypes need to be evaluated under various environments in order to give an idea of which genotype(s) would be suitable for planting in a particular environment.

Clones that attain the required girth (50 cm) early are preferred since yield can be retrieved from them relatively quickly, especially in a new environment. Accordingly, girth increment in the immature phase becomes a crucial attribute in *Hevea*. In a comparison of girth increment of clone RRIM 600 between traditional and non-traditional rubber-growing areas, Sethuraj *et al*. (1989) reported that the trees were 4.3 cm smaller in girth in the north-eastern region of India compared with the girth of trees in the traditional rubber-growing belt. While RRII 105 is counted as one of the most suitable clones for the traditional growing

Table 8.1. Spectrum of climatic features of rubber-growing countries (Source: Priyadarshan and Gonçalves, 2003).

Country	General climatic features
Malaysia	Tropical, annual south-west (April–October) and north-east (October–February) monsoons
Thailand	Tropical; rainy, warm, cloudy south-west monsoon (mid-May–September); dry, cool north-east monsoon (November–mid-March); southern isthmus always hot and humid. North and north-east areas are non-traditional for rubber
India	Tropical monsoon type with winter (November–January), summer (March–May), south-west monsoon season (June–September) and post-monsoon or north-east monsoon season (October–December). Most of the rainfall brought by south-west monsoon. Because of the geographical diversity of India, regional climatic conditions in the extreme north, east and west vary from the general conditions given here. Specific areas of west, east and north-east are non-traditional for rubber
Sri Lanka	Tropical monsoon; north-east monsoon (December–March); south-west monsoon (June–October)
Indonesia	Tropical climate all year round. 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 subarctic in the north, with great climatic differences resulting from the monsoon, the expanse of the land mass and the considerable differences in altitude. Typhoons occur in south-east 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–mid-September) and warm, dry season (mid-October–mid-March). Diverse range of latitude, altitude and weather patterns produces enormous climatic variation. North Vietnam like China has two basic seasons: (i) a cold humid winter from November to April; and (ii) a warm, wet summer for the remainder of the year. South Vietnam is relatively warm. Central highlands and the coastal regions are non-traditional areas for rubber
Côte d'Ivoire	Tropical along coast, semi-arid in far north; three seasons: (i) warm and dry (November–March); (ii) hot and dry (March–May); and (iii) hot and wet (June–October). Low-rainfall areas in north (less than 1300 mm $year^{-1}$) are non-traditional experimental zone for growing rubber
Nigeria	Varies – equatorial in south, tropical in centre and arid in north. Two principal wind currents affect Nigeria: (i) the harmattan (dry, dusty wind) from the north-east is hot and dry and carries reddish dust from the desert and causes high temperatures during the day and cool nights; (ii) the south-west 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, tropical and subtropical highlands. Annual average temperature in the Amazon region is 22–26°C. Brazil is in the south of the equator, so seasonal changes are the opposite to those north of the equator. Plateau of São Paulo is non-traditional area for rubber

Table 8.2. Climatic conditions of traditional and non-traditional rubber-growing areas (Source: IWMI, 2010).

Attributes	Bogor (Indonesia)[a]	Pindorama (São Paulo, Brazil)[b]	Kourou (French Guiana)[b]	Odienne (Côte d'Ivoire)[b]	Nong Khai (Thailand)[b]	Hainan (China)[b]	Agartala (Tripura, India)[b]	Senai (Malaysia)[ac]	Dak Lak (Vietnam)[b]
Temperature (°C) (mean)	27.4	22.9	26.3	25.6	26.8	22.6	25.4	26.9	21.5
Daily temperature range (°C)	9.1	11.8	7.8	12.7	10.2	7.8	9.9	7.2	7.9
Relative humidity (%)	79	67	81.5	67	74	79.9	76.8	82.3	75.7
Sunshine (% h)	61	55.1	49.9	59.2	58.1	46.8	50.8	47.8	48.8
Wind run (m s^{-1})	2.4	1.6	1.35	1.3	1.2	2.7	1.38	2.1	2.5
Rainfall (mm $year^{-1}$)	1791.5	1117.6	2573.53	1297.9	1455.96	1431.29	1960.1	2282.2	1669.31
Number of rainy days	159	117	193	119	128	151	93	182	163
Moisture availability index[d]	0.78	0.49	1.4	0.67	0.7	0.6	1.1	1.2	0.8
Penman ETo (mm day^{-1})[e]	4.4	3.87	3.78	4.3	3.97	3.48	3.39	3.9	3.57
Latitude	5°9′S	20°25′S	5°7′N	9°30′N	17°51′N	19°2′N	23°49′N	1°36′N	14°55′N
Longitude	106°58′E	49°59′W	52°56′W	7°34′W	102°44′E	109°30′E	91°16′E	103°39′E	108°10′E
Altitude (m)	16	505	48	451	164	671	31	13	655

[a] Traditional rubber-growing area.
[b] Non-traditional rubber-growing area.
[c] Senai (Malaysia) is considered as the area offering the optimum environment for growing rubber.
[d] The moisture availability index is a measure of the soil moisture, and ranges from 0 (dry) to 1.0 (saturated). The weekly moisture availability index values are calculated from the weekly precipitation and evaporation values in conjunction with the soil type and maximum soil-water availability values.
[e] The Penman ETo is a measure of evapotranspiration (ETo).

Table 8.3. Climatic factors influencing growth and yield of rubber.

Attribute	Manifestations	Reference
Ambient temperature (°C)		
< 0	Severe cold damage	Jiang (1984)
< 5	Cold damage	Zongdao and Xueqin (1983)
10	Mitosis occurs but photosynthesis discontinues	Zongdao and Xueqin (1983)
18	Plant cells divide normally just for survival (crucial temperature for tissue differentiation)	Zongdao and Xueqin (1983)
< 18	Yield decreases with late dripping of latex from the tree	Zongdao and Xueqin (1983)
18–24	Optimum for latex flow	Shuochang and Yagang (1990)
22–28	Favourable for latex flow	Shangphu (1986), Jiang (1988)
27–30	Optimum range for photosynthesis	Shangphu (1986), Shamshuddin (1988)
34–40	Respiration exceeds photosynthesis; retardation of growth and scorching of young leaves	Lee and Tan (1979), Chandrashekar *et al*. (1990), Ong *et al*. (1998)
Mean annual temperature range 20–28°C	Optimum for growth and latex production	Shamshuddin (1988)
Diurnal variation (7–10°C)	Optimum	Jiang (1988), Rao and Vijayakumar (1992)
Mean monthly temperature 20°C	Negligible growth	Pushparajah (1983)
Rainfall		
1300–1500 mm $year^{-1}$	Optimum for growth and production	Pakianathan *et al*. (1989)
1800–2000 mm $year^{-1}$	Optimum for growth and production	Liyanage *et al*. (1984)
9–11 mm day^{-1}	Optimum	Pushparajah (1983)
Rainy days 100–125 days $year^{-1}$ (at 125 mm $month^{-1}$)	Optimum	Ong *et al*. (1998)
Water requirement 3–5 mm day^{-1}	Optimum	Haridas (1985), Monteny *et al*. (1985)
Wind (m s^{-1})		
1.0	Favourable	Zongdao and Xueqin (1983)
1.0–1.9	No evident hindrance	Oldeman and Frere (1982)
2.0–2.9	Growth and latex flow become retarded	Yee *et al*. (1969)
> 3.0	Severe inhibition of growth and latex flow	Zongdao and Xueqin (1983)
8–13.8	Leaf laceration	Zongdao and Yanqing (1992)

(*Continued*)

Table 8.3. *Continued*

Attribute	Manifestations	Reference
17.2	Branch breaks, trunk snaps	Zongdao and Yanqing (1992)
24.5	Uprooting	Zongdao and Yanqing (1992)
Sunshine		
2000 h year^{-1}	Optimum	Ong *et al.* (1998)
Ambient vapour pressure deficit (mb)		
> 12	Decrease in latex flow	Paardekooper and Sookmark (1969)
28	Initiation of stomatal closure	Rao *et al.* (1990)
35	Stomata close	Rao *et al.* (1990)

areas, PB 235, RRIM 600, RRII 208 and the Chinese clone Haiken 1 are seen to be adaptable in the north-east region of India (Mondal *et al*., 1999; Priyadarshan *et al*., 2000). In a study with seven clones and five hybrids, Meenattoor *et al*. (2000) established that the hybrid clone 82/29 (RRII 429) attains higher girth in non-traditional environments. Girth increment is at a minimum during the winter months (Meenattoor *et al*., 1991; Priyadarshan *et al*., 1998a), which is over 20% of the total annual girth increment (Vinod *et al*., 1996b). These preliminary evaluations indicate that clones which perform well under traditional areas do not behave similarly under non-traditional environments.

In the water-limiting environment of the Konkan region of India, shrinkage of tree stems has been observed during the moisture deficit period (March–June). Although there is a lot of cloud and reduced hours of sunshine during the monsoon period (July–August), girth increment indicated that trees received adequate photosynthetically active radiation (Chandrashekar *et al*., 1998). Also, full irrigation during the dry period resulted in maximum growth that is 50% less than the growth observed in the preceding monsoon period (Mohanakrishna *et al*., 1991), presuming that *Hevea* prefers low vapour pressure deficits for growth. While the Konkan region experiences active girth increment between July and September, in north-east India May–August is the period for better growth (Chandrashekar *et al*., 1998; Priyadarshan *et al*., 1998a). Both regions require 8–9 years for the trees to attain maturity. In a comparative study involving 15 clones of Indian, Malaysian, Sri Lankan and Indonesian origin, RRII 208, RRIC 52, RRII 6, RRIC 100 and RRIC 102 were seen to exhibit better growth in the Konkan region of India. Low temperature affects north-east India, but despite this RRII 208 performs well in addition to PB 235, RRIM 600, RRII 118 and SCATC 93/114. Evidently, these clones were developed under hydrographic environments specific to each location. However, RRII 105, a potential clone for traditional growing regions, was not judged as drought/low-temperature tolerant, hence is not adapted to these conditions (Chandrashekar *et al*., 1998; Meenattoor *et al*., 1991). In a comparative stability analysis of girth in Tripura, Haiken 1, PR 107 and SCATC 93/114 were seen to be more stable over other clones.

However, RRII 208 followed by Haiken 1 and SCATC 93/114 made the best contribution towards girth increment. Clones with higher stability showed better wind endurance (Priyadarshan *et al*., 1998a).

In an analysis of clones of diverse geographic origin (GT 1, AVROS 2037, RRIM 600, PB 217 and PB 235) grown at different locations in Côte d'Ivoire, Dea *et al*. (1997) demonstrated that growth is influenced by the availability and extent of rainfall. Rainfall in these areas varied from 1090 to 1600 mm $year^{-1}$ with 4–6 dry months. Trees took 7–8 years to attain maturity. A similar exercise was carried out in Vietnam, where non-traditional growing areas imposed an immaturity period of 1½–2 years more compared with traditional zones (Tuy *et al*., 1998). The immaturity period also increased with altitude. GT 1, RRIC 110, RRIC 121, PB 235 and VM 515 had higher girth increment than other clones.

Although genotype–environment (GE) interaction studies had been undertaken at several sites earlier (Jayasekara *et al*., 1984), the environment had not been split into climatic and edaphic factors. In studies with seven clones of Indonesian (GT 1, PR 261, PR 255), Malaysian (RRIM 701, PB 235, RRIM 600) and Brazilian (IAN 873) origin, Gonçalves *et al.* (1998a) split the climatic and edaphic factors affecting the interactions. This was done by exercising clone–site interactions (at four test locations) through calculation of estimated heritability and genetic gains. The study showed that PB 235, IAN 873 and RRIM 600 had greater values than the other clones in different sites. In yet another study with half-sib progenies of 22 Asian clones evaluated in three test sites, genotype–site interactions were significant for rubber production and girth increment (Costa *et al*., 2000). However, these studies never rationalized clones suitable for a specific location. The aforesaid discussion amply proves that growth trends of clones are location specific and clones exhibiting better growth need to be developed for a specific environment.

8.2.1 Abiotic stress factors

Abiotic stress factors that particularly affect rubber include low temperatures, high vapour pressure and wind.

In China, two types of cold damage (chilling injury) have been identified: (i) radiative; and (ii) advective (Zongdao and Xueqin, 1983). In the radiative type, the night temperature falls sharply to 5°C, whereas the day temperature ranges between 15 and 20°C or above; in the advective type, the daily mean temperature remains below 8–10°C, with a daily minimum of 5°C. In both these types, under extreme circumstances, complete death of the plant is the ultimate outcome. A similar situation prevails in the north-eastern states of India. Reports from China point out that, while clones GT 1 and Haiken 1 can withstand temperatures as low as 0°C for a short time span, SCATC 93-114 can endure temperature as low as –1°C. The cold-wave conditions in Tripura state (north-east India) can be conveniently classified as relating to the radiative type. Chinese clones such as Haiken 1, SCATC 88-13 and SCATC 93-114 are being evaluated in Tripura. The initial yield pattern shows Haiken 1 to be a high yielder among the Chinese clones compared with RRIM 600, which is used as a local check.

Though SCATC 93-114 is proclaimed as a clone that can endure the cold under Chinese conditions, it never shows considerable yield potential under the conditions of Tripura, at least during the initial stages on the B0-1 panel (Priyadarshan *et al*., 1998a, b). Interestingly enough, as the temperature decreases, yield increases, but, when it goes below 18°C, yield also goes down (Fig. 8.1). China has also developed the clone Zhanshi 86, which is more cold tolerant than SCATC 93-114; this clone derives from seedlings out of a random cross between SCATC 93-114 and Wuxing I_3 (Senyuan, 1990). Further, clones like Zhanshi 306-15 (RRIM 600 × Guangxi 6-68) give around 10 kg of dry rubber per tree. But these contentions are to be proved at the block level (a tapping block of 300 trees). IAN 873, a SALB-resistant high-yielding clone developed in Brazil shows resistance to cold weather in China (Senyuan, 1990).

High vapour pressure during the summer months can be a problem for growing rubber trees in parts of India, for example the Konkan region. This is discussed in detail in the next section '8.2.2 Rubber-growing regions of India, Thailand and Vietnam'.

Wind is yet another abiotic stress influencing the establishment and growth of rubber. One impact is a contribution to the drying effect of drought conditions, especially with regimes of long-lasting steady winds such as occurs during the dry season in the highlands of Vietnam. It is argued that wind speeds of 2.0–2.9 m s^{-1} retard rubber growth and latex flow, and that speeds of 3.0 m s^{-1} and above

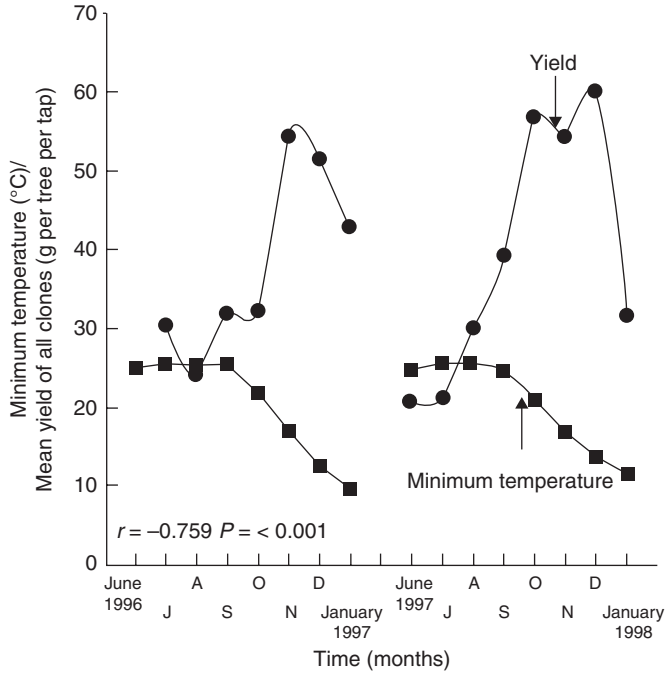


Fig. 8.1. Yield (●) in relation to minimum temperature (■) over 2 years (June1996–January 1997 and June 1997–January 1998).

severely inhibit normal growth (Table 8.3). Wind speeds of force 10 or over on the Beaufort scale (> 24.5 m s^{-1}) play havoc, resulting in breaks in branches and trunks and uprooting of trees; such conditions are prevalent in China during June–October (Watson, 1989). Studies in China revealed that clones PR 107 and Haiken 1 can endure high winds, and PB 5/51 can do so in Tripura (Priyadarshan, 2003a, b). Establishment of shelter belts, consisting of fast-growing and wind-resistant species, is one remedial measure being followed in China (Zongdao and Xueqin, 1983). However, proof is needed as to the effectiveness of shelter belts as well as taking into account the economic cost of their implementation and the land they occupy. Alternatively, adoption of judicial pruning of branches and induction of branches at a lower height can reduce wind damage from 25.3 to 13.7% (Zongdao and Xueqin, 1983; Bernardes *et al*., 1995). In Côte d'Ivoire, rubber plantations often experience wind damage due to storms occurring at the onset of the rainy season (April–May). Although hypotheses have been proposed about the relationship between architecture and wind susceptibility of different clones, no clear factual validation is available (Combe and du Plessix, 1974; Hofmann, 1981; Costes *et al.*, 1995). The quality of rubber wood in relation to wind resistance has also been investigated (Ahoba, 1985). Tapping heavily limits growth rate, to some extent in height but mainly in girth increment, in comparison with non-tapped trees (Clément-Demange *et al.*, 1995). Following these observations, a large-scale design comparing the initiation of tapping in two levels of girth standard (50 and 65 cm) was conducted in Côte d'Ivoire. Although the larger size of the trunk improved the stiffness of the tapped trees, waiting until the girth attained 65 cm before tapping delayed the productive period. A mechanical experiment consisting of artificially bending and breaking trees of different clones also confirmed the prominence of the relationship between the trunk section and the stiffness of the trees, irrespective of the clone (Fourcaud *et al.*, 1999).

8.2.2 Rubber-growing regions of India, Thailand and Vietnam

There are five climatic zones in India: (i) tropical rain; (ii) tropical wet and dry; (iii) subtropical wet; (iv) arid; and (v) desert (Fig. 8.2). Of these, the first three are identified as suitable for rubber cultivation. Several locations within these zones are counted as non-traditional growing regions due to latitude and altitude changes. North-east India (23–25°N and 90–95°E) offers a suitable non-traditional environment for rubber. Rubber is a prominent species in the states of Tripura, Assam, Meghalaya, Mizoram and Arunachal Pradesh. The constraints in these states are: (i) a low temperature period during November–January; (ii) complete defoliation during the period February–March; (iii) brief moisture stress during March; (iv) tropical storms during the monsoon (June–August); and (v) *Oidium* infestation during refoliation. Tripura (22°56′ and 24°32′N and 91°10′ and 92°21′E) is a representative environment of these states. The climate is subtropical with moderate temperatures (summer: 17.9–36.6°C; winter: 7.17–28.9°C) and high atmospheric humidity.

The areas between 15 and 20°N of western and eastern India also have been identified as non-traditional zones for rubber cultivation. For instance, the

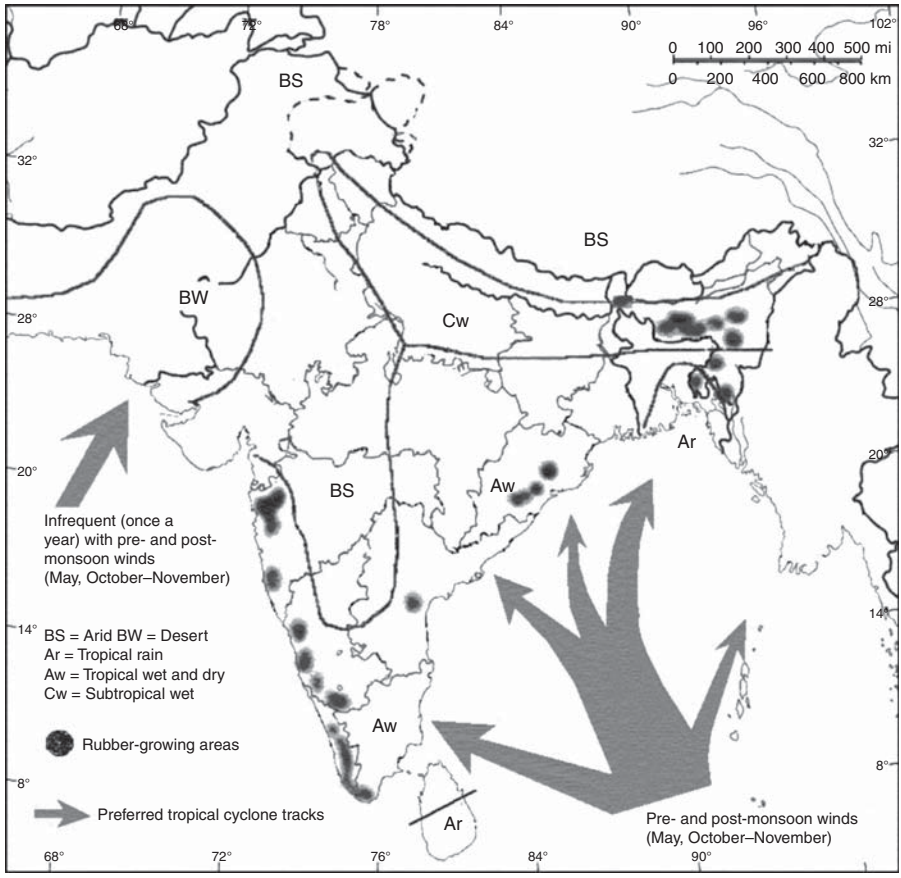


Fig. 8.2. Rubber-growing areas in India in relation to climatic zones and cyclone tracks.

Konkan region of western India experiences long dry periods, high temperatures, low atmospheric humidity and zero rainfall between September and May. Daytime temperatures range from 38 to 41°C during the summer months with occasional days getting as hot as 47°C. Although its rainfall is 2430 mm year^{-1}, the distribution of that rainfall is uneven (Devakumar *et al*., 1998). High solar radiation coupled with high temperature and low relative humidity (RH) results in high vapour pressure deficit between the leaf and the surrounding atmosphere, and this subsequently increases the evapotranspirative demand of the atmosphere. Thus, rubber trees in this region are subjected to prolonged periods of both soil and atmospheric drought stress. Irrigated plants showed 32% increment in leaf area index (LAI) leading to 52% more shoot biomass per tree (Devakumar *et al*., 1998). Water deficit in the dry period is 1070 mm whereas in traditional areas it is 350 mm (Jacob *et al*., 1999). Reduction in the girth of trees (0.2–0.5 mm) was observed during the summer months (Chandrashekar *et al*., 1996). Towards the end of summer, the moisture level falls below the permanent wilting

point (17.5%). The atmosphere during the summer results in high vapour pressure deficit between the leaves. The high intensities of sunlight, more than is required to saturate photosynthesis, can aggravate the harmful effects on *Hevea* leaves (Devakumar *et al*., 1998). Almost an analogous situation prevails in the eastern part of India also.

Similarly, the non-traditional rubber-growing areas of Thailand (13–18°N), namely Chachoengsao (east), Nong Khai and Chiang Mai provinces (in the north-east), experience stress factors including: (i) a marked dry season for 6 months; (ii) a severe moisture deficit (with temperatures between 14 and 39°C); and (iii) a minimum temperature of 5°C during January (Saengruksowong *et al*., 1983). Rainfall (1110–1550 mm) is mainly confined to June–September (Watson, 1989).

The rubber areas of Vietnam are scattered between 12 and 21°N (Fig. 8.3). The research and development of rubber in non-traditional areas are streamlined depending on altitude and are: (i) highlands of 450–600 m above MSL; (ii) highlands of 600–700 m above MSL; and (iii) coastal regions. The south-east area is the traditional region for rubber cultivation where nearly 300,000 ha are under rubber. While the terrain in the south-east region is relatively flat, the coastal regions are < 550 m. The highlands and coastal regions that are non-traditional growing areas experience low temperatures (5.5°C), regular strong winds, rainfall lasting for several days, less sunshine and a higher number of misty days (Hoa *et al*., 1998; Tuy *et al*., 1998). The soils in the highlands are predominantly ferrallitic and belong to a major family of red or yellowish-red soils. They are clayey, deep and based on basalt (Eschbach *et al*., 1998). Ever since rubber was introduced in 1897, Vietnam has taken steps to extend the area of rubber cultivation to 500,000 ha including expansion into marginal areas. Rubber is the second most important crop for Vietnam (Chapman, 2000).

8.2.3 Rubber-growing regions of China

China has been divided into six climatic zones: (i) tropical wet and dry; (ii) subtropical wet; (iii) subtropical summer rain; (iv) arid; (v) desert; and (vi) temperate continental (Fig. 8.4). Growers are experimenting with rubber cultivation in the first three of these zones. The rubber-growing areas of China fall within 18–24°N and 97–121°E, spread over five provinces of south China namely, Hainan, Guangdong, Fujian, Yunnan and Guangxi. These areas are tropical and subtropical with a monsoonal climate. Pronounced monsoon and dry seasons prevail from May to November and December to April, respectively. As discussed in section 8.2.1 'Abiotic stress factors', cold damage can be a particular problem in non-traditional growing areas of China.

8.2.4 Conditions in West Africa

Countries in West Africa (Côte d'Ivoire, Liberia, Ghana, Nigeria, Guinea and Sierra Leone) are suitable for rubber cultivation. Rainfall is confined to April–October

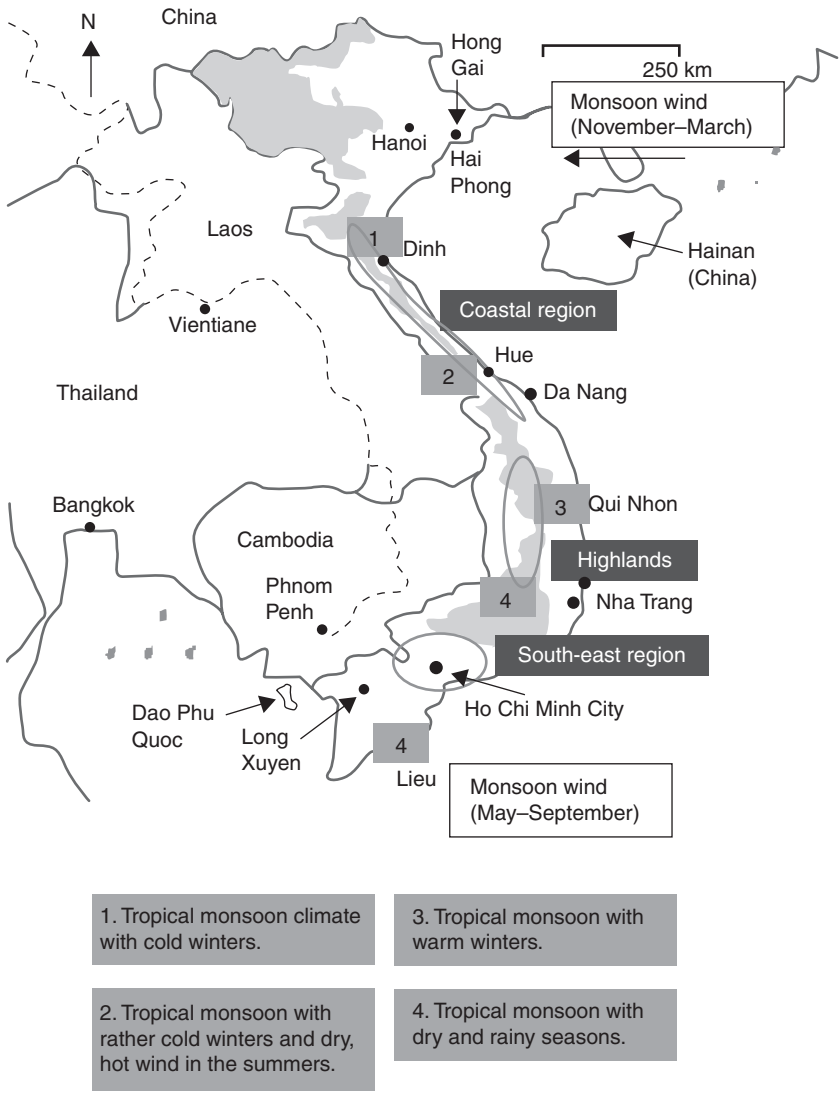


Fig. 8.3. Rubber-growing areas and climatic zones of Vietnam.

as the south-west monsoon winds over the Gulf of Guinea result in high rainfall in the coastal region and diminish steadily northwards (Edington, 1991). The presence of Mount Cameroon acts as a barrier for rain-bearing winds, resulting in the second highest rainfall in the world (1000 cm). These areas also experience average annual temperature of 25°C with a very small diurnal temperature range. During November to April the northern parts of the rubber-growing countries experience a dry wind, which originates in the Sahara desert, popularly known as the harmattan. Cameroon experiences tornadoes during the

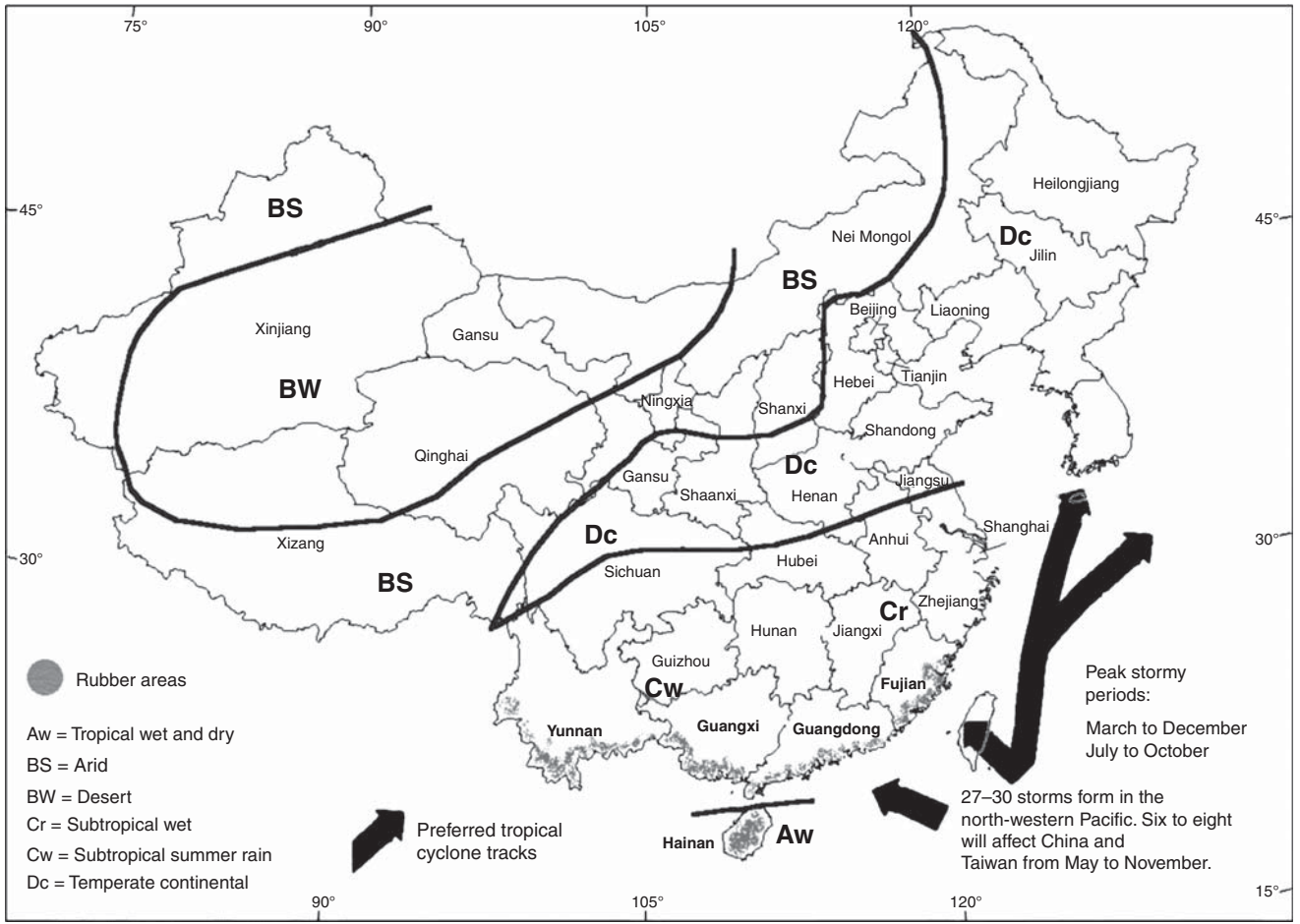


Fig. 8.4. Rubber-growing areas and climatic zones of China.

rainy season. The soils are derived from sedimentary rocks, which have been weathered, leached, eroded and deposited. They are naturally deep and poorly supplied with nutrients. But soils of west Cameroon are more fertile and have a tendency to fix nutrients. The coastal areas are densely forested and suitable for rubber.

Côte d'Ivoire, a prominent rubber producer, is located between latitudes 5 and 6°N and at longitudes 3 and 8°W. Although the areas fall under the tropical belt, water is the limiting factor due to low rainfall. Considering the 1500 mm isobar, and a dry season not exceeding 5 months (with a monthly rainfall below 100 mm), 20% of the area is suitable for rubber cultivation (Dea *et al*., 1997). Areas towards the north are identified as marginal, where rainfall is below 1300 mm. Even under moderate conditions, in spite of favourable rainfall and a short dry season, areas having gravel elements in the soil profile impose 20–30% weak growth in rubber (Dea *et al*., 1997).

8.2.5 Situation in South America

Brazil has four main climatic zones: (i) tropical rain; (ii) tropical wet and dry; (iii) subtropical rain; and (iv) arid (Fig. 8.5). Although the former two are suitable for rubber, the southern plateau of São Paulo (20–24°S; 44–52°W) with a tropical wet and dry climate is the main production area, due to the absence of an epidemic of SALB. The most important production region is the north-western region, where the climate is tropical with a summer rainy season from October to March and a cold dry winter from June to August with temperatures reaching 15–20°C. The yearly total rainfall ranges from 1000 to 1400 mm. The ideal altitude for rubber is 350–900 m above MSL. The undulating flat areas have deep and well-drained podzolic and latossolic soils, both with autotrophic and dystrophic types. A few plantations are located on volcanic red soils of high fertility. The low duration of leaf wetness and relatively low temperature in the winter reduce the epidemics of SALB (Gonçalves *et al*., 1999).

8.3 Phenology Under Different Geoclimates

Hevea exhibits significant changes in phenology under different geoclimates. The shift in cultivation towards north and south of the equator induces ample phenological changes. A comparison is made here in relation to Tripura (north-east India) and São Paulo (south Brazil). Rubber in Tripura experiences wintering during December–January and reflushing commences by February, followed by flowering (Priyadarshan *et al.*, 2001). The yielding pattern of clones in Tripura shows a clear delineation between low- and high-yielding regimes (Priyadarshan *et al.*, 1998a). There are a multitude of factors that induce a low-yielding period, namely: (i) low temperature; (ii) utilization of carbohydrate reserves for refoliation, flowering and fruit development during February–April; (v) low moisture period during February–March; and (vi) powdery mildew (*Oidium heveae*

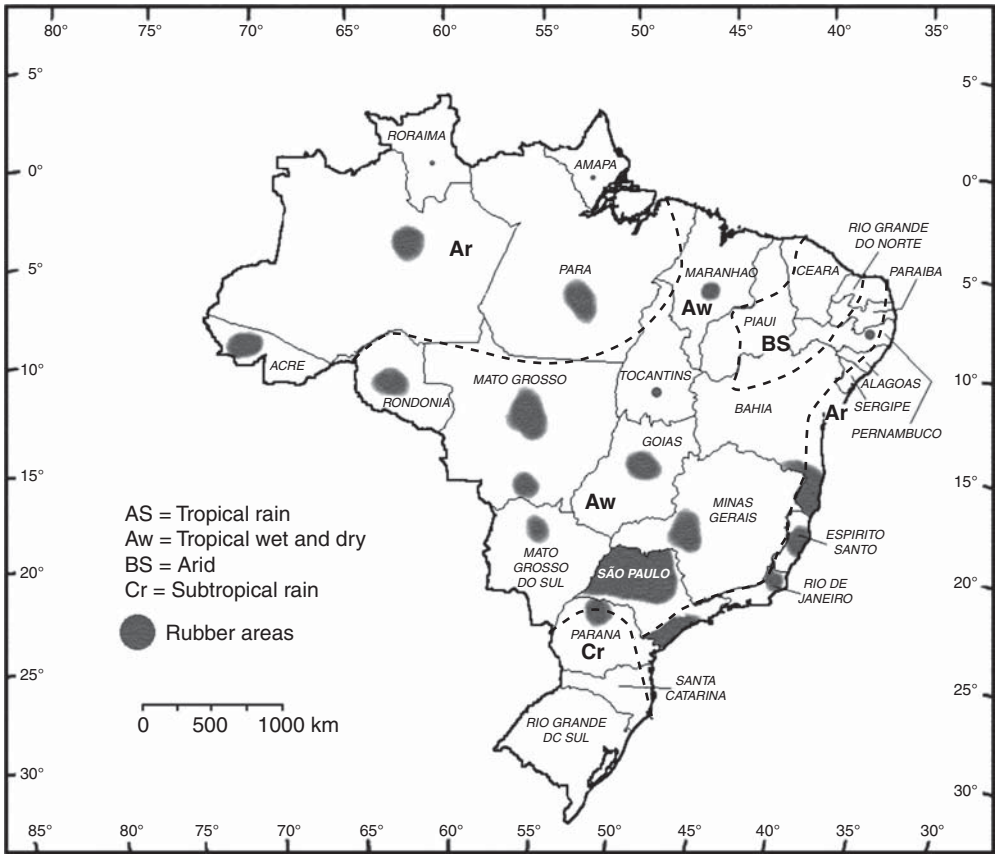


Fig. 8.5. Rubber-growing areas and climatic zones of Brazil.

Stein.) infestation. These factors together impose an ensuing low-yielding period during May–September (Priyadarshan *et al.*, 2000). A photoperiodic stimulus, operating because a slight change in the proportion of light to dark in the day length is sufficient to trigger flowering, has been very well established in several tropical tree species (Baker *et al.*, 1983). However, the fall in temperature during October–November stimulates yield while the mean temperature is 28°C, making the atmosphere most ideal for latex flow and production. The minimum temperature experienced in the early morning is 15–18°C, and after 10 a.m. the temperature shoots to 27–28°C. While the former is suited for latex flow, the latter is ideal for latex regeneration through accumulation (Ong *et al.*, 1998). The plateau region of São Paulo state, an escape area for SALB, has been referred to as the most important rubber-growing region of Brazil (Costa *et al.*, 2000). While Tripura lies at 22–24°N, São Paulo is at 20–22°S (400–500 m above MSL), making these non-traditional rubber-growing areas. Unlike Tripura, trees are exploited throughout the year in São Paulo. Reflushing, flowering and seed fall are experienced once a year in São Paulo (Ortolani *et al.*, 1998).

Plants react to different environments through phenological changes. For instance, defoliation is a phenomenon to circumvent moisture and low temperature stresses through minimizing transpiration so as to ensure reproduction, seed dispersal and perpetuation of generations. Flowering and fruit formation utilize large amounts of carbohydrate reserves. Hence, flowering and fruit formation precede a low-yielding phase in rubber both in Tripura and in São Paulo. The environmental conditions inducing defoliation, flowering and low- and high-yielding periods are analogous. The peak yielding period in São Paulo is January–May, followed by winter and defoliation, while in Tripura January–May is the low-yielding period. Hence the latitudinal changes south and north of the equator induce phenological changes in *Hevea* that are the opposite of one another. It is noteworthy that São Paulo is a disease-free zone for SALB. Similarly, Tripura experiences only *O. heveae.* But the traditional growing regions experience the incidence of at least two other diseases (caused by *Corynespora* and *Phytophthora*). It may be pertinent to believe that low temperatures and a dry spell extend an antagonistic effect over the environment to reduce disease incidence in a particular zone. Moreover, the non-existence of alternate hosts is another reason for the disease-free atmosphere. Since the peak yielding periods south and north of equator occur at the opposite time of year it is evident that minimum temperature and rainfall in a region are the major factors influencing yield and phenology of rubber. As the length of the dry period increases, the mean temperature decreases as one moves away from the equator. Minimum temperatures in the range of 15–20°C influence the yield north and south of the equator. The effect of rainfall assumes significance only after 2 months in increasing yield. The differential phenology in *Hevea* on both sides of the equator might be advantageous towards shortening the testing cycle in which new clones/genotypes are tested for suitability for production. For instance, on the island of Sumatra (Indonesia) there are two conspicuous flowering seasons, since the equator passes through its centre (Priyadarshan *et al*., 2001). This needs to be exploited fully by the breeders. The annual cycle of solar radiation intensity is shown to correspond closely with the flowering near the equator and in the subtropics. While in temperate regions the incoming solar radiation (insolation) is dependent on both day length and radiation intensity, the insolation at the equator is entirely due to radiation intensity. High solar radiation and bright sunshine induce synchronous anthesis around spring and autumn equinoxes at the equator (Yeang, 2007).

8.4 GE Interactions and Specific Adaptation

It is imperative that there are low and high yielding periods in non-traditional growing areas. This is evident from the analysis of yielding trends in Tripura (India), Vietnam and São Paulo (Figs 8.6–8.8). Under the hydrothermal situations of Tripura, in a study involving 15 clones of different geographical origin, all clones showed an increment in yield towards the onset of the cold season (i.e. during October–November). It is implicit that the cold weather (18–20°C) is very favourable for latex flow by pushing the coagulation time to a later period of the

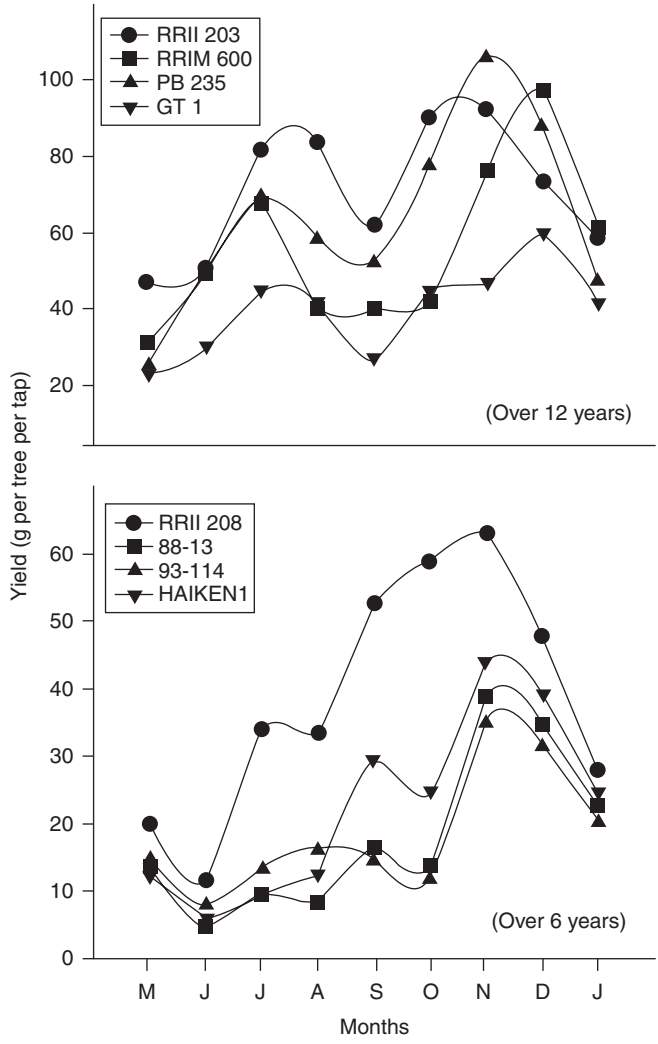


Fig. 8.6. Dry rubber yield of eight clones over months May–January in Tripura, India (mean values of data collected over 12 years, top graph, and 6 years, bottom graph).

day, and the onset of the cold season renders a stimulatory effect to maximize yield and the trend continues until the temperature falls below 15°C during January. The clones are classified under two categories: (i) those showing a slow escalation in yield from April onwards, reaching the maximum during November, and receding sharply during December and January; and (ii) those with a low-yield regime during April–October, and with the peak yield during November and December (high-yield regime), then receding during January. In São Paulo (southern hemisphere), the low- and high-yield regimes occur at the opposite

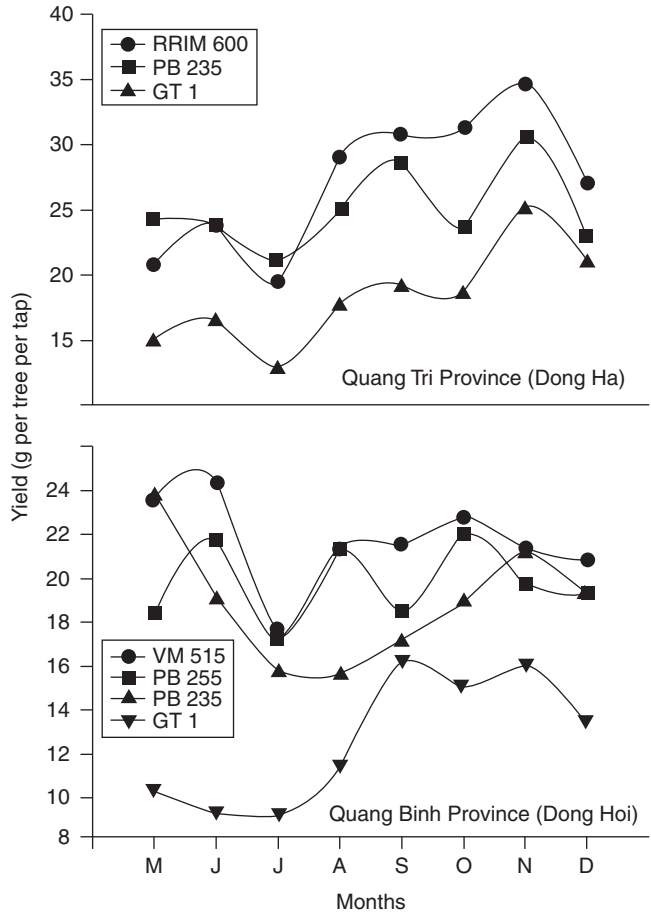


Fig. 8.7. Variation in yield over months May–December in coastal region of Vietnam.

time of year to that of Tipura. While clone PB 235 comes under the first category (i.e. showing a slow escalation in yield from July onwards, reaching the maximum during November and receding sharply during December) all the other clones come under the second. The trend shows that the first category is appreciable since the clones give considerable yield during regime I (May–September compared with regime II, October–January), which ensures better returns to the planter. The rationale is that fall in temperature along with reduced evaporation and low wind speeds prevail upon the microenvironment to influence yield stimulation during October–December (Priyadarshan, 2003b). PB 235 seems to own an adaptive mechanism, whereby it yields higher when the ambient temperature ranges between 22 and 28°C. When all the other clones continue with a higher yield in combination with descending temperature, the yield of PB 235 drops during January when the ambient temperature gets below 18°C. Studies conducted with RRII 208 endorse the same trend (Priyadarshan and Nair, 2002).

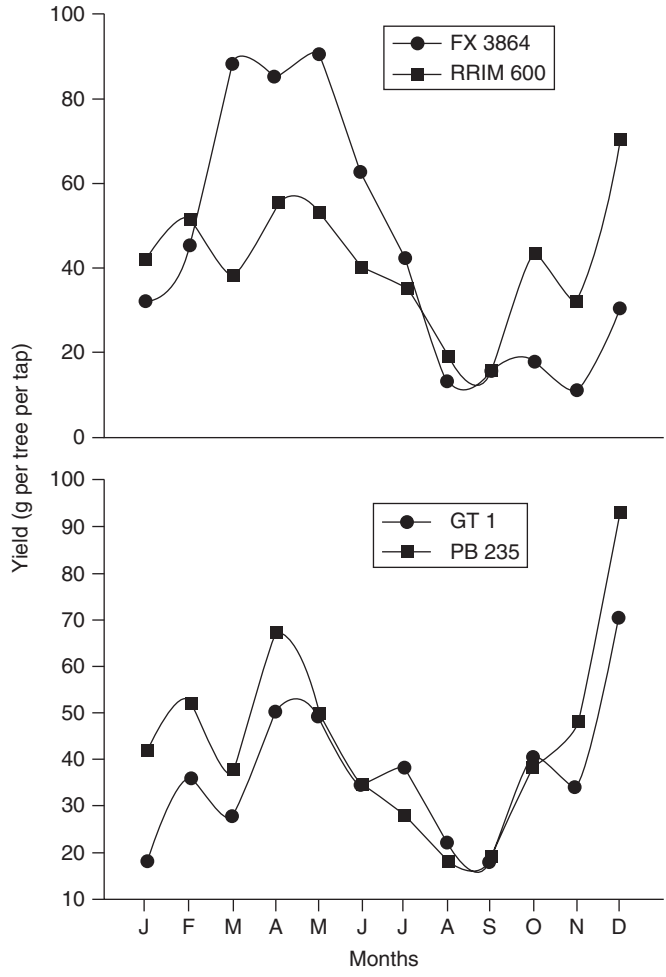


Fig. 8.8. Yield of four clones over months January–December in São Paulo, Brazil.

Evidently, the existence of genetic homeostasis and its subsequent expression in the changed environment might be the reason for the near uniform yield in these clones. In São Paulo, RRIM 526 showed higher yield during regime I in comparison with RRIM 600, RRIM 614 and AVROS 1328 (P. de S. Gonçalves, Instituto Agronômico de Campinas, São Paulo, personal communication). These observations clearly indicate that the clone (or clones) selected for planting should be one that is a consistent yielder (Priyadarshan *et al*., 2000).

The weather variables and environmental index were used as covariates while analysing the yield data to determine the variable that contributed to heterogeneity in the GE interactions. The test of heterogeneity for the environmental index showed high significance, so indicating that the high stability values of a few clones (s^2 i) over the years were due to the linear effect of the environment

(Priyadarshan, 2003b). However, under Malaysian conditions, Tan (1995) accounted for GE interactions with a non-linear effect of wind damage and disease. In fact, these hazards play a prominent role in differentiating the adaptation of clones to one or another location. Grouping of clones with a high mean and a low coefficient of variation is proved to be dependable in selecting better performers in a new environment (Tan, 1995; Priyadarshan *et al*., 2002). GE interactions were also significant for rubber production and girth increment under the conditions of São Paulo (Gonçalves *et al*., 1998a; Costa *et al*., 2000). This need for identifying specific adaptations of clones to the diversity of rubber planting tracts might lead to emphasizing the importance of ecophysiological research that can provide functional and predictive models for characterizing those adaptations.

8.5 Biotic Stresses

Diseases, especially SALB (caused by *Microcyclus ulei*), which is singularly devastating, are yet another stress factor limiting the yield of *Hevea.* It is noteworthy that, unlike other clonal species, *Hevea* is not affected by viral diseases (Simmonds, 1989). Other diseases of economic importance are the *Gloeosporium* leaf disease (*Colletotrichum gloeosporioides*), powdery mildew (*O. heveae*) and *Phytophthora* leaf fall (*Phytophthora* spp.). Clonal specificity is evident towards resistance to these diseases (Wycherley, 1969). A study with *Gloeosporium* showed that clones from Malaysia and Indonesia are fairly resistant while clones from Sri Lanka and China are less resistant. But clones from South America are seen to be highly resistant, indicating that 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 susceptible genotypes are rejected in the first instance and the survivors that are at least moderately resistant are selected for.

8.5.1 South American leaf blight (SALB)

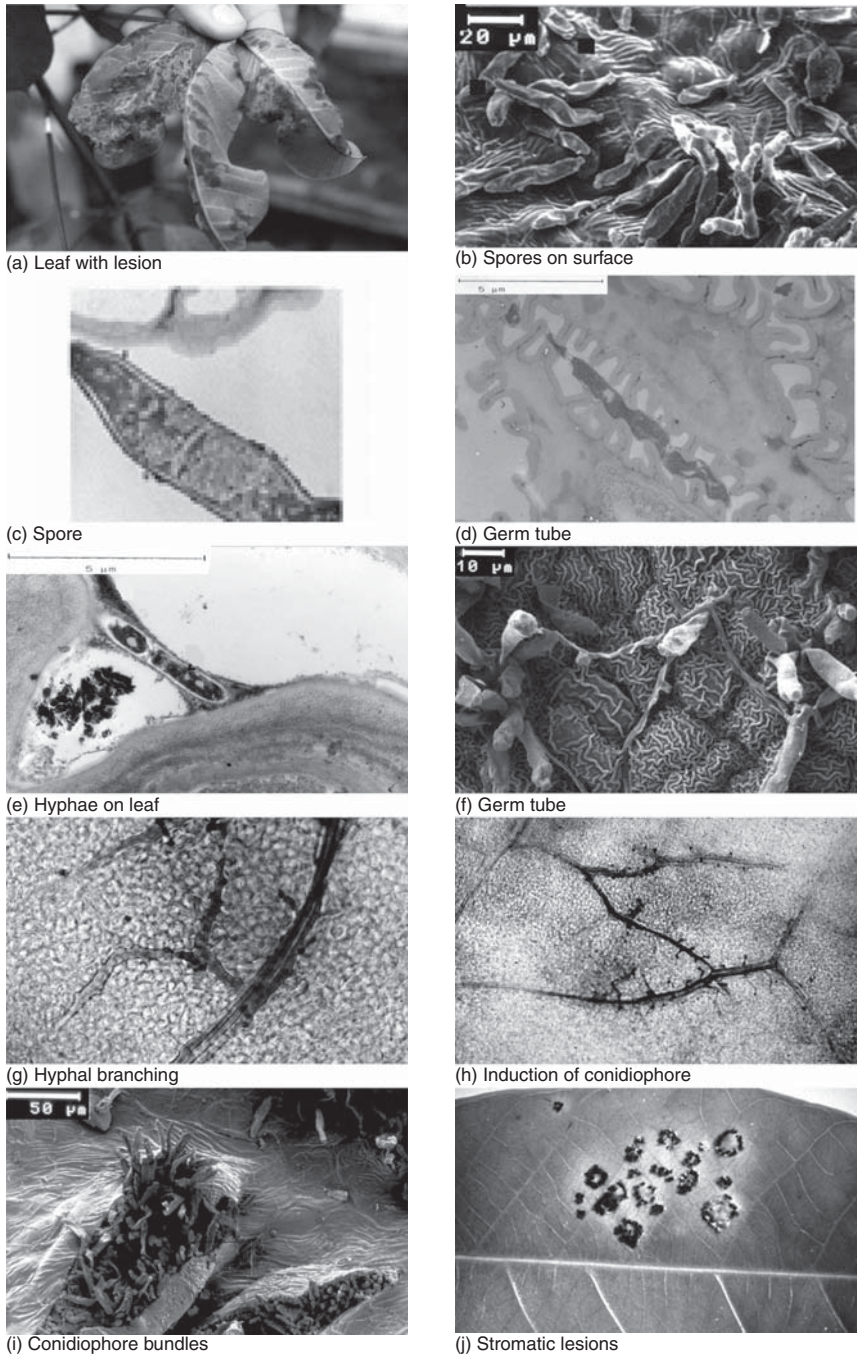
SALB has played and still plays a major role in the history and geographic distribution of the rubber industry, as on the one hand it prevents Latin America from developing rubber cropping in all the otherwise favourable climatic conditions, and on the other hand it represents a permanent major threat to the crop in Asia and Africa (Dean, 1987; Davies, 1997). Some amount of breeding work, mainly based on backcross technique, has been undertaken in the past to incorporate resistance to this disease in high-yielding clones. However, the efforts were in vain due to the unknown polygenic nature of the attributes, high variability of the pathogen and multiple interactions between fungus strains and rubber clones (Rivano, 1997a, b). Simmonds (1990, 1991) argues that the pathotype-specific resistance (vertical resistance) has resulted in catastrophic failures. Horizontal resistance should be more effective and durable (Rivano *et al.*, 1989; Simmonds, 1990). Resistance sources appear to be absent in the high-yielding Wickham population, but rather frequent within the Amazonian germ plasm. However, the

wild population is yet to be improved for yield. With these views, efforts have been reoriented towards the analysis of partial resistance components (Junqueira *et al*., 1990). Recently, the genetic determinism of the resistance source of *H. benthamiana* (F 4542), widely used in many former backcross programmes, has been characterized by a genetic map (Lespinasse *et al*., 2000b). A CIRAD-Michelin common research and breeding programme is currently being carried out in Brazil for reducing the incidence of SALB in rubber cropping.

SALB is present in Bolivia, Brazil, Peru, Ecuador, Colombia, Venezuela, Guyana, Surinam, French Guiana, Trinidad, Panama, Costa Rica, Nicaragua, EI Salvador, Honduras, Guatemala, Haiti and Mexico (Holliday, 1970; Compagnon, 1986) and has caused the abandonment of ambitious programmes of extensive rubber cultivation in the South American humid tropics.

Conidia and ascospores cause infection and both are equally important in completing the disease cycle (Langford, 1945; Chee, 1976, 1977). Rain plays an important role in the spread of leaf blight. It is believed that rain is the most effective disseminator of large masses of spores and wind is the chief means of dispersal. Lieberei (2006) observed that conidia survived for 2 weeks under normal laboratory conditions. However, the longevity of conidia decreases as RH increases. Under high humidity, conidia survive for 3 weeks and at 100% RH they are killed within 1 week. It seems probable that leaf blight could be spread by conidia carried on plants, plant parts or man himself. Outbreaks of leaf blight occur when the daily temperature is below 22°C for longer than 13 h, RH over 92% for a period longer than 10 h and rainfall above 1 mm day^{-1} for the previous 7 days (Holliday, 1969; Chee, 1976). The fungus can affect petioles, green stems, inflorescences and fruits. But the most obvious infection is on young leaves on the abaxial surface of 4–9-day-old, expanding tender leaves. They appear as greyish-black lesions covered with olive-green powdery sporulating masses (Lieberei, 2007) (Fig. 8.9). On the young infected leaves, lamina distortion, growth arrest, crinkling and shrivelling of leaflets, blackening, drying and abscission are the common symptoms. The secondary stage develops on the adaxial surface of the leaves as they harden. In Trinidad, the conidia have maximum dispersal in June and July and the peak ascospore concentration occurred from August to November during the wet season (Chee, 1976). In a mature stand of rubber, a fresh disease cycle probably starts when ascospores are released from leaves which fall due to wintering and also from infected leaves remaining on the trees. As infection builds up on the newly emerging flushes, conidia take over the spread during the wet season to complete the disease cycle.

After penetration of the leaf surface, the hyphae colonize the underlying tissue by intercellular growth. They often enter the tissue layers adjacent to the leaf vascular bundles and spread rapidly along the veins into the leaves (Fig. 8.9). In this biotrophic phase, compatible combinations do not show cell death. However, in resistant clones, the cells in direct contact with the penetration hyphae collapse. Hashim *et al*. (1978) ascribed this to a hypersensitive reaction and to preformed resistance factors (Blasquez and Owen, 1957; Figari, 1965), and proof was also given for induced defence compounds such as scopoletin (Tan and Low, 1975; Giesemann *et al*., 1986; García *et al*., 1995). This early detection process of the fungal presence in the attacked tissue of resistant plants leading to



(a) Leaf with lesion
(b) Spores on surface
(c) Spore
(d) Germ tube
(e) Hyphae on leaf
(f) Germ tube
(g) Hyphal branching
(h) Induction of conidiophore
(i) Conidiophore bundles
(j) Stromatic lesions

Fig. 8.9. Habit and habitat of *Microcyclus ulei* causal organism of South American Leaf Blight (SALB).

a hypersensitive response is a typical defensive reaction (Breton *et al*., 1997). This reaction is regarded as an indicator for complete or vertical resistance, but this concept is applicable only to mature leaves of *H. brasiliensis* and occurs with most genotypes of the previously uninvestigated host species *H. pauciflora* (Junqueira *et al*., 1988). At the biochemical level of host reactions, a hypersensitive response is often associated with well-described defence reactions such as: (i) formation of reactive oxygen-type compounds (García *et al*., 1999); (ii) deposition of autofluorescent compounds in the cell wall (Mevenkamp, 1992); (iii) synthesis of callose; (iv) occurrence of scopoletin as a phytoalexin (Giesemann *et al*., 1986; García *et al*., 1995b); and (v) finally cell death in a restricted area surrounding the penetrating hyphae. Detailed and quantified descriptions have been given by García *et al.* (1999). Rubber tree leaves are formed in a flush growth pattern. Directly after bud burst, the leaves are thin, have a high respiration rate, no net photosynthesis (Lieberei *et al.*, 1996) and are devoid of any resistance against the virulent isolates of *M. ulei*. In the course of maturation, rubber tree leaves change from susceptible to completely resistant (Chee, 1980). This maturation requires 12–20 days after bud burst and the maturation time is genotype dependent. Based on morphological criteria seen from bud burst until hardening of leaflets or leaves, Dijkman (1951) placed the developing leaves in four groups designated A–D (Fig. 8.10).

The causal fungus *Microcyclus ulei* (P. Henn.) von Arx & E. Muller (*Dothidella ulei* P. Henn.) is specific to *Hevea* species only. The pathogen has been recorded in four species: (i) *H. brasiliensis*; (ii) *H. benthamiana*; (iii) *H. guianensis*; and (iv) *H. spruceana.* SALB infection results in repeated defoliation, dieback of the shoots and even death of the mature trees (Holliday, 1970). An examination of the morphology and an updated taxonomic description of this species have appeared elsewhere (Chee and Holliday, 1986). In the South American plantations

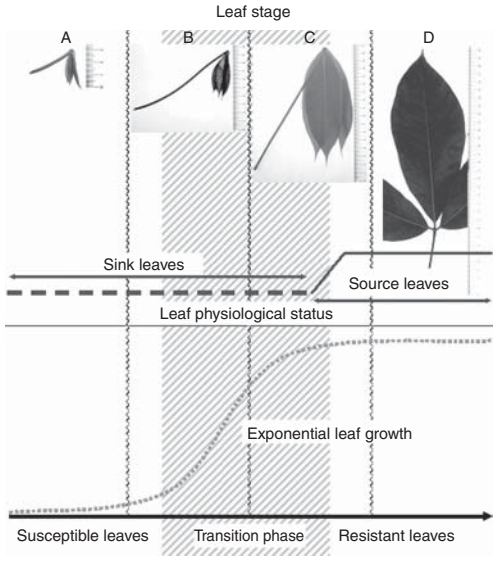


Fig. 8.10. Susceptibility to SALB: relationship between exponential leaf growth and the transition phase from completely susceptible leaf stage (A) to completely resistant leaf stage (D), and the short physiological step from sink to source leaves. This attempts to visualize the different developmental processes that turn a susceptible leaf into one that is completely age resistant. The hatched area shows the resistance factors that interact in various ways during the transition phase.

it reduced the yield by over 90%. More than 90% of the world's natural rubber requirement is being met by production from the Far East (Holliday, 1970). All the planted African and Asian rubber is extremely susceptible and the climatic conditions present in the rubber-growing areas of Asian and African countries are comparable to those of the American tropics. Hence introduction of SALB into these regions could destroy the existing plantations. This has prompted rubber-growing countries to implement quarantine regulations (Edathil, 1986).

Eleven physiological races (plus an avirulent one) of the pathogen have already been detected. Many clones which were reported to be tolerant/resistant to *M. ulei* have succumbed later with the appearance of more virulent strains. Six species in which natural infection has not been reported include *H. camporum*, *H. microphylla*, *H. nitida*, *H. paudflora*, *H. camargoana* and *H. rigidifolia.* The clones belonging to these species are being used in Brazil for crown bud grafting on high-yielding susceptible clones. Some resistant clones which are being used for crown bud grafting are PA 31, IAN 717, IAN 6486, IAN 7388, IAN 7657, FX 25, FX 614 and FX 636 (Holliday, 1970).

In case of an accidental entry of this disease, despite the phytosanitary measures, immediate adoption of eradication procedures should receive top priority. First, two to three rounds of spraying (aerial application) with protectant chemicals such as mancozeb, benomyl or thiophanate methyl are given and then the entire area is defoliated using n-butyI-2,4,5-T, folex, cacodylic acid or ethephon, so that the trees remain leafless for about 2 months (Abdul Aziz, 1976; Lim and Hashim, 1977).

Several plant protection operations are being carried out for controlling this disease. Aerial spraying (eight to ten rounds) is carried out using 300 g benomyl, 200 g thiophanate methyl or 2 kg mancozeb in 30 l water ha^{-1} at intervals of 7–10 days. For fogging 200 g thiophanate methyl or 1 kg mancozeb are being used in 6–8 l of agricultural spray oil ha^{-1} at intervals of 4–7 days (Martins and Silva, 1979; Chee and Wastie, 1980). Systemic fungicides like chlorothalonil (Daconil), triforine (Saprol) and triadimefon (Bayleton) have been found to be promising in small-scale trials (Chee and Wastie, 1980; Santos *et al.*, 1984).

Biological control of *M. ulei* using *Hansfordia pulvinata*, a hyperparasite which grows well on conidial lesions, has been attempted (Lieberei *et al.*, 1989). It was reported by Feldman (1990) that mycorrhizal fungi can cause an increase of resistance of the rubber tree to *M. ulei.* The generation period of spores was increased and the sporulation of the pathogenic fungus was decreased. The diameter of lesions was also decreased.

According to detailed studies by Seguin *et al.* (1996a, b) and Lespinasse *et al*. (2000b), *H. brasiliensis* has a diploid genomic organization with rare duplicated loci. These studies led to identification of 18 basic linkage groups in a rubber genome of 2150 cM total map length. Using the 195 progenies of the population derived from the cross PB 260 × FX 3899 and their response to six isolated strains of *M. ulei*, eight QTL with respect to resistance were identified on seven independent linkage groups. Le Guen *et al.* (2003) and Lespinasse *et al.* (2000b), on the basis of their molecular data, prepared the mapping of genes conferring field resistance to SALB. For the first time, it was shown that factors both for partial resistance and for complete resistance were quantitatively

expressed in the progeny and could be correlated with five loci. The molecular approach to this plant–pathogen combination has greatly enhanced the possibilities of proceeding with marker-assisted breeding and selection in a perennial tropical plant.

8.5.2 Abnormal leaf fall

Abnormal leaf fall is the most destructive disease in India and occurs during the south-west monsoon months of June, July and August. It infects pods, leaves and tender shoots causing heavy defoliation and dieback of tender twigs. The first report of this disease from India was in 1910 from estates near Palapilly, in the Trichur district of Kerala state (McRae, 1918). In due course, the disease spread to all other rubber-growing districts. Later, the disease was reported from Sri Lanka and Burma (Petch, 1921). Subsequently pod rot and leaf fall were also reported from Cambodia, Vietnam, Liberia, Ghana, Nigeria, Cameroon, Congo, Brazil, Peru, Nicaragua, Costa Rica and Venezuela. In Malaysia, a serious outbreak of this disease was noticed during 1966 (Chee *et al.*, 1967; Chee, 1969). Pod rot and leaf fall due to *Phytophthora* attack have been reported from Thailand also (Chee and Greenwood, 1968). Although this disease occurs in several countries, severe incidence necessitating adoption of control measures every year is observed only in south India.

Rainfall is the most important predisposing factor for the initiation and spread of the disease. In the traditional rubber-cultivated areas in India, a continuous spell of 250–350 mm rain for 7–10 days without intermittent hot sunshine, with minimum and maximum temperatures within the range of 22–25°C and 26–30°C, respectively, and RH above 90% are most suited for the outbreak of the disease. Under such conditions of low temperature and very high atmospheric humidity, the disease spreads rapidly and assumes epidemic proportions. Under normal monsoon conditions, the disease starts by the middle of June and reaches the peak by the middle of July. However, when the monsoon is late, very heavy incidence is noticed from the middle of July to the middle of August.

Different species of *Phytophthora* are reported to cause pod rot, bark rot, patch canker and leaf fall diseases of rubber in various countries. In India, four species of *Phytophthora* have been isolated from infected specimens: (i) *Phytophthora paimivora* (Butler) Butler; (ii) *Phytophthora meadii* McRae; (iii) *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterhouse; and (iv) *Phytophthora botryosa* Cheewere (Thankamma *et al.*, 1968; Edathil and George, 1976, 1980). However, the species most common in the traditional rubber-growing areas is *P. meadii.*

Hyphae of the fungus are found to ramify inside the tissues of the infected portions intercellularly or intracellularly. Sporangia are found emerging externally through the stomata. The shape and size of the sporangia vary according to the species. During favourable climatic conditions, the pathogen resorts to the production of profuse asexual sporangia, which aid in quick dispersal and rapid spread of the disease. The sporangia liberate binucleate, biciliate, reniform zoospores which swim in the available water and, on contacting the green tissues, produce germ tubes, thus establishing a fresh infection on the host. Sporangia

may also germinate directly, producing germ tubes which also cause fresh infection. The pathogen gains entry into the host tissue through stomata (Thankamma *et al.*, 1975). In general, all high-yielding clones and clonal seedlings are susceptible to abnormal leaf fall disease under Indian conditions. Clones like PB 86, PB 235, PB 260, PB 311, PB 28/59, RRIM 600, RRIM 628, RRIM 703, RRII 5, PR 255, PR 261 and Tjir 1 are observed to be susceptible to the disease.

Prophylactic spraying of rubber plants with 0.75% Bordeaux mixture is the popular method used to control the disease (Ashplant, 1928). Later experiments revealed that 1% Bordeaux mixture was more effective for the control of this disease and this is being adopted extensively by rubber planters. It was noticed that addition of 0.5% zinc sulfate to 0.5% Bordeaux mixture could give adequate protection to the clones RRIM 600 and RRII 105 and reduced the cost of spraying by about 35% when compared with spraying with 0.75 or 1% Bordeaux mixture (Idicula *et al.*, 1994). As an alternative to Bordeaux mixture, copper oxychloride dispersed in agricultural spray oil sprayed through low volume applicators proved effective for the control of this disease.

8.5.3 Powdery mildew

Powdery mildew disease was first reported in Indonesia (Arens, 1918). Subsequently, it was reported from Uganda (Small, 1924), Sri Lanka (Stoughton-Harris, 1925) and Malaysia (Sharples, 1926). In India, the disease was reported in 1938 (Mitra and Mehta, 1938). Since then, the disease has been reported from almost all rubber-growing countries. The disease affects the immature leaves of rubber when the trees refoliate after annual wintering, and it causes leaf fall. Tender leaves at the brown or light-green stage are highly susceptible. The presence of dull cool weather with intermittent light showers during refoliation predisposes the plants to severe disease attack. Prevalence of mist, dew and cloudy days with 75–80% RH are favourable for disease development. Early wintering clones usually escape from the disease because the climatic conditions during their refoliation period are not favourable for the disease development. Late wintering clones are usually severely affected. Dry weather conditions during the wintering period encourage early and rapid wintering and consequent escape from the disease. In India, the disease is severe in Kanyakumari, Idukki and Wynad districts of south India and in the north-eastern states.

The optimum temperature for germination, infection and sporulation ranges from 25 to 30°C (Liyanage *et al.*, 1985). The fungus is disseminated by airborne conidia. The peak sporulation is around noon. *Oidium heveae*, an obligate parasite, is responsible for the disease. The fungus produces superficial, branched, hyaline, septate hyphae. The hyphae are anchored on the host tissue with haustoria which help in deriving nutrients. The fungus has simple erect conidiophores which bear elliptical or barrel-shaped vacuolated conidia with round ends. The sexual stage has not yet been reported.

Leaf fall due to powdery mildew adversely affects the growth and yield of rubber trees. Wastie and Mainstone (1968) have reported a crop loss of 8.1% in the clone PB 5/51 over a period of 9 months, in Malaysia. Increased bark renewal

and girth increment of trees protected against powdery mildew compared with unprotected trees were also observed. Tan and John (1985) have reported 6.3–10.3% yield increase by controlling powdery mildew disease. In India, it was observed that, in clone PB 86, 8–12% more disease in unprotected plots when compared with protected plots resulted in 21–32% crop loss. Similarly, 8–18% more disease in unprotected RRIM 600 caused 14–29% crop loss. The disease caused reduction in yield throughout the year (Jacob *et al.*, 1992). Disease resistance has been reported only in the low-yielding clone LCB 870 from Sri Lanka. In India, clones PB 86, GT 1, Gl 1, PR 107, RRIM 703, RRII 208 and PB 310 show some tolerance. The clones Tjir 1, PB 5/51, RRIM 605, RRII 105, RRII 118, RRII 300, PR 261, PB 21.7, PB 235, PB 280 and PB 311 are susceptible.

Dusting with sulfur gives effective control of powdery mildew disease. Spraying wettable sulfur is preferred only in the nurseries and young rubber plantations as repeated spraying in mature areas is expensive and impracticable. Sulfur dust, having a minimum of 70% sulphur, is generally used for dusting. The dust should be dry, free flowing and should pass through a 325-mesh sieve (particle size 40 μm). Dusting is done at the rate of 11–13 kg ha^{-1} at an interval of 7–10 days. Three to six rounds of dusting are usually required. The first round of dusting is done when 10% of the trees start refoliation. A Micron duster is employed for this purpose. The duster should be carried along every fourth row of trees at a speed of 3–4 km h^{-1}. With one duster, nearly 10–12 ha can be covered in a day. Sulfur dusting should preferably be done early in the morning so that the dew on the leaves helps the dust to stick. The still air in the morning hours also helps to raise the dust to reach the canopy. An integrated approach using tridemorph and sulfur in dust form was found to be more effective (Edathil *et al.*, 1992). Carbendazim (Bavistin) 1.5% dust has also proved to be effective and could be used in integration with sulfur (Jacob *et al.*, 1996).

8.5.4 *Corynespora* leaf disease

Corynespora cassiicola (Berk. & Curt.) Wei. causes leaf spot and leaf fall disease. First reported in India from seedling nurseries (Ramakrishnan and Pillay, 1961), it was then reported from Malaysia (Newsam, 1963), Nigeria (Awodern, 1969), Indonesia (Soepena and Sinulingga, 1996), Sri Lanka (Liyanage *et al.*, 1986) and Thailand (Kajornchaiakul, 1987). The disease has now been found in almost all rubber-growing regions (Chee, 1988). Severe leaf fall was reported from Malaysia (Tan, 1990) and Indonesia (Soepena and Sinulingga, 1996). The disease appears in mature plantations during the refoliation period, infecting young leaves. The environmental factors favouring disease development are: (i) high humidity; (ii) a temperature of 28–30°C; (iii) humid air; and (iv) cloudy weather (Situmorang *et al.*, 1996). The conidia of the fungus, produced abundantly on infected leaves, are carried by wind and cause rapid spread of the disease. The spore release increases steadily from morning and reaches the peak by noon and thereafter falls to very low levels (Chee, 1988). The spore load in air has been negatively correlated with rainfall (Radziah *et al.*, 1996). The conidia remain viable for about a month. Although the host range of *Corynespora* is wide

(Liyanage *et al.*, 1986), cross-infectivity is doubtful (Chee, 1988). In the severe form of the disease, a characteristic browning and blackening of veins gives a 'fishbone'- or 'railway track'-like appearance (Fig. 8.11). Even a single leaf spot can cause defoliation. Severe infection on the midrib causes leaf blight. When leaf petioles are infected, greyish-black lesions are formed, causing defoliation without any symptoms on the lamina. Repeated defoliation and refoliation lead to shoot dieback.

RRIC 103, RRIC 104, RRIM 600, RRIM 725, Tjir I, RRIC 110, RRIC 133, RRIM 600, GT 1, PB 5/51, PB 217, PB 235, PB 260, PR 107, RRIM 901, RRIM 905 and Tjir 1 are susceptible to *Corynespora* leaf disease (Tan, 1990; Jayasinghe and Silva, 1996). AVROS 2037, BPM 24 and RRIC 100 are reported as tolerant from Indonesia (Azwer *et al.*, 1993). Studies conducted in France indicated PB 260 to be highly susceptible and GT 1 to be tolerant (Breton *et al.*, 1997).

Several fungicides have been recommended for the control of *Corynespora* leaf disease. Spraying of benomyl, mancozeb, captan or propineb is recommended for affected nursery plants (Hashim, 1994; Jayasinghe and Silva, 1996). Four to five rounds of spraying with mancozeb (Dithane M45 1.5–3 kg ha^{-1}) are

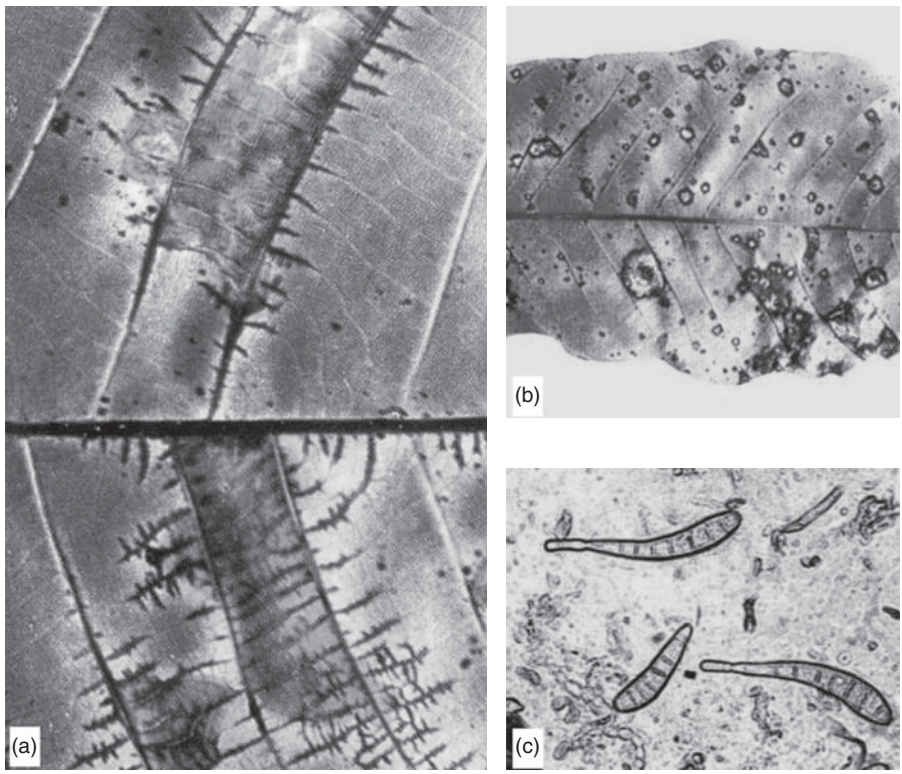


Fig. 8.11. *Corynespora* leaf disease. (a) Browning or blackening of leaf veins gives a 'fishbone' or 'railway track' appearance; (b) lesions over the leaf; and (c) *Corynespora* spores.

recommended for *Corynespora* control in Indonesia (Soepena and Sinulingga, 1996).

8.5.5 Shoot rot

The initial symptom of this disease is noticed on the terminal portions, especially on the purple-coloured leaflets. Within 24–48 h, the leaflets become dark-coloured and the rotting extends up to the petiole. In a short time, infection spreads to other leaflets also. Subsequently, infection spreads to the stem and progresses from the apex downwards. The affected portions of the stem are initially dark brown but later turn black and shrunken. The rotting of the shoot may extend from 15 to 75 cm in length. The diseased portion dries up and later new branches arise from below the infected portion. Clones that are susceptible to abnormal leaf fall are also susceptible to shoot rot.

The disease could be controlled by prophylactic spraying with copper fungicide for mature and immature plants in the field. Repeated spraying with 1% Bordeaux mixture or 0.5% Bordeaux mixture + 0.5% zinc sulfate at an interval of 7–10 days is required to protect the young plants in the nursery and field during the monsoon period (Idicula *et al*., 1992). Phosphorous acid at 0.16% and metalaxyl-M at 0.2% are also effective in checking the disease (Idicula *et al*., 1998).

8.5.6 *Gloeosporium* leaf disease

Even though this disease is not a serious problem in mature rubber, it has been observed throughout the rubber-growing regions. Although it is confined to seedling and budwood nurseries, immature plants are also being seriously affected. The disease is generally noticed during April–May, before the onset of the south-west monsoon and in August, September and October or whenever wet weather is prevailing. High humidity is a prerequisite for the formation of the sporocarp. Free water is necessary for optimum germination of the fungus. Germination of spores occurs in a few hours at 100% humidity; this takes a longer time at lower levels of humidity (Wastie, 1972).

Tender leaves produced soon after bud burst are more susceptible to infection. Under extensive damage, leaves become distorted, turn black, shrivel and fall off, leaving the petioles on the stem. The infection usually starts at the tip of the leaf and spreads towards the base. If the leaf gets infected at a later stage, it either becomes highly spotted or may be partially damaged along the tip and margin. As the leaf ages, the margins of the leaf spots become thick and raised above the surface as conical projections, this being the most important diagnostic feature of the disease. The pathogen is the fungus *Gloeosporium alborubrum* Retch. The hyphae of the fungus penetrate the tissues of the affected part. Intraepidermal or subepidermal stromata are formed on the infected region.

A severe incidence in immature plants in the field may lead to heavy defoliation and shoot dieback, resulting in girth retardation and extension of the

immaturity period. In Indonesia, the persistence of this disease over a long period resulted in up to 50% loss of yield and a delay in maturity of up to 3 years (Basuki, 1992). After 3 years of continuous artificial defoliation to control secondary leaf fall, a yield increase exceeding 30% was achieved in Malaysia (Radziah and Hashim, 1990). PB 217, PB 260 and RRIM 600 are clones that have some tolerance.

Copper oxychloride spraying carried out as a prophylactic measure in April/May, keeps this disease under check. Mancozeb (0.2%), carbendazim (0.05%), bitertanol (0.025%) and Bordeaux mixture (1%) were found to be effective in controlling the disease in young rubber plantations (Joseph *et al.*, 1994). Mechanical fogging of captafol-in-oil (0.6 kg ha^{-1}) three times at weekly intervals during refoliation gave good control of the disease in Malaysia (Tan and John, 1985). In Malaysia, artificial defoliation by aerial spraying of several chemical defoliants mixed in water is practised (Radziah and Hashim, 1990). In Cameroon, ethephon (3 l ha^{-1}) induced defoliation and early refoliation helps in avoiding secondary leaf fall (Senechal and Gohel, 1988).

9 Ancillary Income Generation

Hevea honey and wood are the two supplementary income-generating sources that can raise significant income from rubber plantations. A brief description is given here of the potential of these income sources in rubber plantations.

9.1 *Hevea* Honey

Neither male nor female flowers secrete nectar, but much is secreted by the extra-floral nectaries (on young leaf petioles and fleshy scales of young shoots) (Parkin, 1900). Over the years, honeybee rearing has seen major vicissitudes due to bees being unavailable. In India, there was a rehabilitation measure through the introduction of *Apis mellifera* with a reported average yield of 60 kg per hive year^{-1} compared with 19.46 kg per hive year^{-1} for the popular Indian honeybee, *Apis cerana indica* (Haridasan *et al*., 1987). The average number of *A. cerana indica* hives is 15–20 hives ha^{-1} and the results of a recent survey showed an average yield of 12.1 kg per hive year^{-1} for the Indian honeybee (Chandy *et al*., 1998). Therefore, the mature rubber plantations in India have the potential to produce 67,886 t year^{-1} of rubber honey.

The honey flow period of rubber plants ranges from January to March during which honeybees collect large quantities of nectar from the extra-floral nectaries. Lack of honey flow in rubber during the prolonged dearth period from April to December necessitates supplying an alternate bee flora for the off-season for rubber-plantation-based apiaries (Nehru *et al*., 1990). According to the Bureau of Indian Standards' specifications, rubber honey is medium grade (Grade A) with an average moisture content of 22%. The important properties of rubber honey are given in Table 9.1. Apart from honey, other principal hive products which also have industrial uses are: (i) pollen (bee-bread); (ii) propolis; (iii) beeswax; and (iv) bee venom. The major consumers of honey in the domestic market are the ayurvedic and allopathic pharmaceutical industry, bakeries and the

Table 9.1. Properties of rubber honey (Source: George *et al*., 2000).

Properties	Range	Average
Viscosity (in mPa s) at 27°C	550–3800	1358
Specific gravity at 27°C	1.40–1.34	1.38
Moisture (%)	21.5–25.5	22.0
Reducing sugars (%)	69.08–74.8	72.80
Levulose (%)	34.88–40.70	37.14
Dextrose (%)	33.57–37.97	35.98
Non-reducing sugars (%)	0.78–3.14	1.71
Acidity (%)	0.06–0.20	0.13
Ash (%)	0.09–0.39	0.22
Protein (%)	0.05–0.25	0.14
Yeast (million cells g^{-1})	103.9–158.0	139.39

confectionery, dairy and tobacco-manufacturing industries. Under the current rates, with 15 hives ha^{-1}, a production potential of 182 kg ha^{-1} $year^{-1}$can be realized. With a farm gate price of US$2 kg^{-1} for unprocessed honey, the estimated net income is around US$100 ha^{-1} $year^{-1}$.

9.2 *Hevea* Wood

Rubber wood, which can be transformed into furniture by sawing, but also into plywood, particle board, medium density fibreboard (MDF) and fuel wood after felling the old rubber plots, has become a second product of rubber cropping. It may represent about 15% of the total income of farmers, and it generated a profitable industry mainly in Malaysia and Thailand, but also in India, Vietnam, Indonesia and Cambodia. It has become a new challenge for breeders, and this was first addressed by the RRIM (Othman *et al*., 1995). Many clones of the RRIM 2000 series are considered latex-timber clones. The price of rubber-wood timber at field level, which was initially very low, has now reached the average level of US$5000 ha^{-1} in Malaysia and Thailand. The average ligneous biomass at felling of the aerial parts of the trees (excluding roots) is roughly around 180 m^3 ha^{-1}, from which 90 m^3 can be sawn, providing the industry with around 20–50 m^3 of sawn wood. Allied species and Amazonian germ plasm accessions have great potential as a source of rubber wood (Table 9.2).

The emergence of rubber wood as a basic renewable source (Fig. 9.1) is an outcome of the sustained research and development efforts since the 1970s through standardization of preservative treatment and drying procedures. Rubber wood is now extensively used in furniture manufacture, structural applications and interior decoration. The estimated world market size of rubber-wood-based furniture and other items is around US$1500 million and the export earnings of Malaysia alone were US$655 million in 1995 (Malaysian Timber Industry Board, personal communication, 1996). Malaysia and Thailand are the leading countries in terms of commercial production, consumption and export.

Table 9.2. Estimated wood volume from potential clones, accessions of Brazilian Amazonian and allied species (source: Arshad *et al.*, 1995).

Clone	Parentage	Age (year)	Volume (m^3 per tree)		
			Clear bole	Canopy wood	Total wood
RRIM 910	PB 5/51 × RRIM 623	22	0.76	0.57	1.33
RRIM 912	PB 5/51 × RRIM 623	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 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

Fig. 9.1. Processed *Hevea* wood.

Hevea rubber timber is whitish-yellow when freshly cut and turns pale cream after drying. The air-dry specific gravity is 0.557 with an average weight of about 515 kg m^{-3} at 12% moisture content (Sekhar, 1989). The growth rings are absent or ill-defined (Silva, 1970) and the growth-ring-like structure displayed in the cross-sectional view of the timber is merely false rings which are formed by the distribution pattern of tension wood fibres (Reghu *et al*., 1989). The sapwood is not differentiated from heartwood due to lack of deposition of pigmented extraneous materials that usually occur during heartwood formation in other hardwood timber species. Although reserve metabolites in the form of soluble sugar, starch, etc. are abundant in rubber wood, conversion of these materials into heartwood substances through the long-term ageing process and necrobiosis of storage cells does not take place, mainly due to the fast-growing nature of rubber trees. Hence heartwood formation is virtually absent in rubber trees and the storage tissue is always filled with soluble sugar and starch which in turn is easily attacked by fungi, insects, borers, beetles, termites, etc. (Kadir and Sudin, 1989). Early wood and late wood differentiation is not possible in rubber due to the long and continuous cambial activity associated with the fast-growing tendency. Also, cambial activity involves production of latex vessels, which is a constant process during the growth of a tree.

Rubber wood is composed of fibres, vessel elements (pores), axial parenchyma and rays in different proportions similar to those of other hardwood species. The fibres are lignified or partially lignified and are 1.1–1.5 mm in length (Bhat *et al*., 1984) and about 22 μm in thickness (Silva, 1970) and the vessels are small to moderately large with one to four pores mm^{-2}. The structure and distribution pattern of pores enhance the chemical impregnation capacity of rubber wood during preservative treatments. The lumen of the pores is usually filled with balloon-like parenchymatous outgrowths called tyloses, which are a characteristic feature of rubber wood. The nature and extent of tylosis formation and their impact on preservative impregnation are still obscure. Tension wood formation is considered as a natural abnormality which creates various problems (Isenberg, 1963; Harlow, 1970). Tension wood is characterized by its unlignified gelatinous fibres. Sharma and Kukreti (1981) observed 15–65% tension wood fibres that create a variety of drying, woodworking and finishing problems (Ipe *et al*., 1987). Rubber wood is lignocellulosic and its density is not uniform throughout (Midon, 1994).

9.2.1 Processing

Processing of rubber wood consists of impregnation with preservative and drying. The basic objective of preservative treatment is to protect rubber wood from biodeterioration caused by various biological agents. There are short-term and long-term methods of protection. For temporary protection, a dip treatment with a number of insecticides and fungicides is carried out. For long-term protection, the wood preservatives are allowed to penetrate deep into the timber for complete preservative penetration through either a dip diffusion process or a pressure impregnation process.

The deep diffusion or boron diffusion process is done only on freshly sawn timber which has more than 50% moisture content. Here, freshly sawn timber is immersed in a mixture of boric acid and borax in water (Gnanaharan, 1982; Gnanaharan and Mathew, 1982; Tam and Singh, 1987; Gnanaharan and Dhamodaran, 1993) and this gives a dry salt retention of 5 kg m^{-3} and 12 mm penetration (Gnanaharan, 1996). To circumvent fungal attack, sodium pentachlorophenate (NaPeP) at 0.5–1.0% is seen to be effective (Gnanaharan, 1983; Jose *et al*., 1989).

Pressure treatment is more popular. There are two types: (i) the vacuum pressure method (Bethel process); and (ii) the oscillating pressure method (OPM). In the former, the wood is impregnated with preservatives by creating a vacuum and using pressure. Partially dried timber ensures maximum penetration and retention of preservatives. The preservatives used are copper-chrome-arsenic (copper sulfate, potassium or sodium dichromate and arsenic pentoxide), copper-chrome-boron (copper sulfate, sodium or potassium dichromate and boron), boric acid and borax (Hong and Liew, 1989; Gnanaharan and Dhamodaran, 1993). The OPM is more complex requiring automated plant equipment for introducing ten to 15 cycles of vacuum and pressure for 10 min in a total time of 2 h. This is popular in Malaysia (Dahlan *et al*., 1994).

9.2.2 Production and consumption

Of late, selection for timber has become a very important objective. An estimation from RRIM shows that 1 ha of rubber plantation can yield around 190 m^3 of rubber wood, and 2.7 million m^3 of *Hevea* wood would be available from Malaysia (Arshad *et al*., 1995). Approximately 741 million m^3 of wood must be available from 8,927,000 ha worldwide. The demand is expected to increase fast, and the RRIM has been making earnest efforts in generating latex-timber clones (Othman *et al*., 1995). Lately, there has been some interest generated among scientists to evolve rubber as a factory producing useful chemicals, especially life-saving drugs (Yeang *et al*., 2002). In future, new requirements linked with environmental concerns such as reforestation or carbon sequestration might appear, but the implications for breeding are not clear for now (see Chapter 10). As far as rubber wood is concerned, the processing technique needs to be standardized following the environment of the country in question, since the infestation of pests differs with the environment.

10 *Hevea* and Clean Development Management

Accumulation of greenhouse gases (GHGs) in the upper atmosphere is leading to changes in climate, particularly in temperature. The average global surface temperature increased by 0.6 ± 0.2°C over the 20th century and is projected to rise by 0.3–2.5°C in the next 50 years and 1.4–5.8°C in the next century. Global warming changes the earth's atmospheric circulation, which leads to altered patterns of precipitation and also ushers in extreme climate events. Although the economic and ecological consequences of global warming will vary by region, in the tropics it may threaten production of crops and may even become a major cause of species extinction. CO_2 emanating from fossil fuel combustion, cement production and land use change makes a large contribution to the GHGs, which prevent radiation from being reflected into space and cause warming of the atmosphere (Kelkar, 2006). In the last 150 years, and more strikingly in the past couple of decades, the rise in temperature has exceeded the natural variation recorded until then (Fig. 10.1). Significant evidence has been accumulated to show the direct relationship between accumulation of GHGs and rising mean temperatures (Jacob, 2005). The concentration of CO_2 in the atmosphere was rising at the rate of approximately 1.6 ppm $year^{-1}$ during the 1980s and 1990s (Jacob, 2006). If this atmospheric CO_2 could be fixed by being absorbed by terrestrial ecosystems, it may reduce the alarming trend towards global warming. Although all these arguments for climate change are the cause of great concern for developed and developing countries alike, there is a counter argument that challenges this view and says there are real holes in the climate science that supports climate change (Schiermeier, 2010).

Under the Kyoto Protocol of the United Nations Framework Convention on Climate Change (UNFCCC), signatory countries must decrease emissions of CO_2 to the atmosphere, or increase rates of removal and storage. The Protocol's Clean Development Mechanism (CDM) allows a country that emits C above agreed-upon limits to purchase C offsets from an entity that uses biological means to absorb or reduce GHG emissions. The CDM is currently offered for

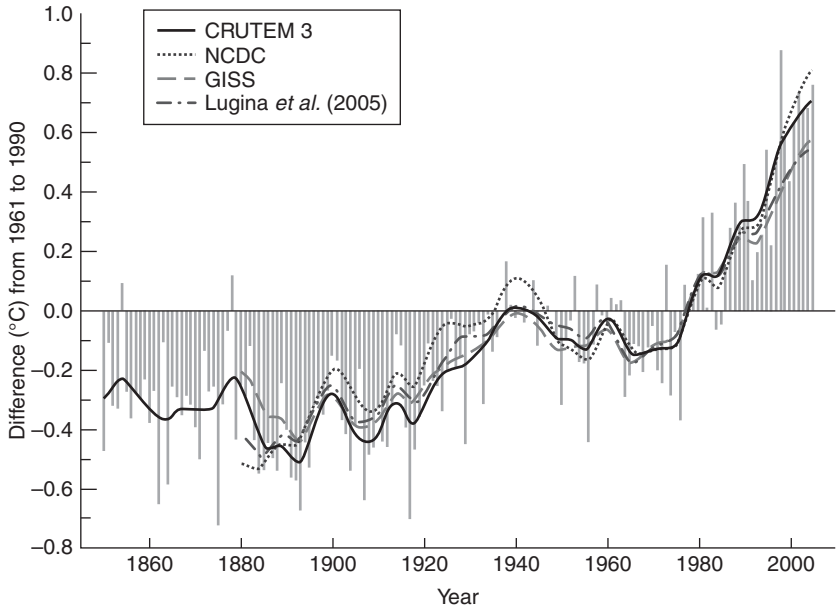


Fig. 10.1. Annual anomalies of global land–surface air temperature (°C), 1850–2005, relative to the 1961–1990 mean for CRUTEM 3 updated from Brohan *et al.* (2006). The curve from CRUTEM 3 (a gridded dataset of global historical land surface temperature anomalies) is compared with those from the National Climatic Data Center (NCDC; Smith and Reynolds, 2005), Goddard Institute for Space Studies (GISS; Hansen *et al.*, 2001) and Lugina *et al.* (2005) (adapted from Kelkar, 2006).

afforestation and reforestation projects, and is expected to be extended to C sequestration in agricultural soils. Markets for soil and plant C sequestration are also developing outside the protocol in addition to those promoted by CDM. The interest in C sequestration and trading as mechanisms for both environmental protection and poverty alleviation in developing countries has increased considerably in the last decade. The CDM could result in enhanced income and conservation of natural resources in the developing world. Two types of payments are anticipated, namely for C capture and C storage. Moreover, technological practices that are known to slow down soil C oxidation and increase C fixation are also being adopted in rubber plantations. Such strategies include improved soil and water conservation practices like leguminous cover cropping, application of organic manure, mulching, intercropping, etc., which are known to have increased enrichment of soil organic C by about 30–50%.

A mature rubber plantation would qualify as 'forest' as per the CDM definition (Kurian, 2006). The mean annual leaf litter fall for a mature *Hevea* rubber ecosystem which included falling branches, twigs and fruits was calculated to be around 3.7–7.7 t ha^{-1}. The total biomass accumulated in a tree over 33 years is 1.8 t, which amounts to 596 t ha^{-1}. The total amount of C sequestered in 1 ha of

rubber plantation made up of tree biomass, latex produced and the contribution from leguminous cover crops amounts to 680 t. The possible credit revenue entitlement per hectare at the end of 33 years at the rate of US$12 t^{-1} is about US$8160. However, C trading will not be able to function without a government's firm backing. Since technical and institutional conditions are not yet in place to make C sequestration a successful business venture, it is more practical for the resource-limited rubber industry to pursue C sequestration initially as a 'long-term pilot project' in partnership with global C trading ventures.

In a study with 21-year-old rubber trees, Jacob (2006) estimated 66.47 t of C $acre^{-1}$ being sequestered. More than 77% of the C pool was in the aboveground biomass and more than 22.6% was from below ground. Timber constituted the single largest C pool (35.7%). The maximum rate of C sequestration occurred during the seventh and eighth years and thereafter it decreased a little but remained constant until the 18th year. After that, it was estimated to be well below 3.17 t C $acre^{-1}$ $year^{-1}$. The CO_2 equivalent of the total C sequestered during 21 years would be 243.7 t $acre^{-1}$ or 11.6 certified emission reductions (CERs) $year^{-1}$. On the other hand, the net C assimilation rates through leaf photosynthesis can be in the range of 7.01–9.1 μmol CO_2 m^{-2} s^{-1} (Jacob, 2006). Cultivation of rubber trees on non-forested land could act as a C sink by sequestering C in biomass and indirectly in soils. International political and economic interests, following the Kyoto Protocol, require estimates of this C sequestration. Wauters *et al*. (2008) assessed the C stock in rubber in two contrasting climatic areas: (i) the western region in Ghana (2–14 years old); and (ii) Mato Grosso in Brazil (14–25 years old). Trees with a range of stand ages and clone types were felled and partitioned into log, live lignified branches, dead branches, non-lignified fine branches, leaves, taproot and lateral roots. Allometric relationships (log-transformed power functions) based on trunk circumference at a height of 170 cm (C_{170}) were used to predict the tree foliage, above ground, below ground and total C content (kg C $tree^{-1}$) (r^2 = 0.86–0.99). Predicted tree C stock for 14-year-old stands was higher in Ghana (76.3 t C ha^{-1}) than in Mato Grosso (41.7 t C ha^{-1}), which was partially explained by a difference in tree height growth. In the framework of the Kyoto Protocol, these results could be useful when drafting a Project Design Document (PDD) for the Afforestation and Reforestation (AR-) CDM. Although the aim of these two studies is complementary, the results are significantly different, probably because of the methodologies used. UNFCCC needs to acknowledge the methodology used for calculating the CERs before embarking on the process of C credit transfers.

Glossary

Adaptation: The process by which individuals (or parts of individuals), populations or species change form or function in such a way to better survive under given environmental conditions.

Amphidiploid: A polyploid formed from the union of two separate chromosome sets and their subsequent doubling. An organism produced by hybridization of two species followed by chromosome doubling. An allotetraploid that appears to be a normal diploid.

Avirulent: Inability of a pathogen to produce a disease on its host.

Backcross: A cross of a hybrid to either of its parents. In genetics this is a cross of a heterozygote to a homozygous recessive. (See also test cross)

Backcross breeding: A system of breeding whereby recurrent backcrosses are made to one of the parents of a hybrid, accompanied by selection for a specific character or characters.

Biotype: A group of individuals with the same genotype. Biotypes may be homozygous or heterozygous.

Bivalent: A pair of homologous chromosomes united in the first meiotic division.

Breeder seed: Seed produced by the agency sponsoring a variety and used to produce foundation seed.

Breeding: The art and science of changing plants or animals genetically.

Bulk breeding: The growing of genetically diverse populations of self-pollinated crops in a bulk plot with or without mass selection, followed by single-plant selection.

Certified seed: Seed used for commercial crop production produced from foundation, registered or certified seed under regulation of a legally constituted agency.

Character: An attribute of an organism resulting from the interaction of a gene or genes with the environment.

Clone: A group of organisms descended by mitosis from a common ancestor.

Combining ability: General, average performance of a strain in a series of crosses.

Crossing over: The exchange of corresponding segments between chromatids of homologous chromosomes during meiotic prophase. Its genetic consequence is the recombination of linked genes.

Diallel cross, complete: The crossing in all possible combinations of a series of genotypes.

Dihybrid: Heterozygous with respect to two genes.
Dioecious: Plants in which staminate and pistillate flowers occur on different individuals.
Diploid: An organism with two chromosomes of each kind.
Disease: A departure from normal metabolism and a reduction of its normal potential for growth, reproduction and yield.
Dominance: Intra-allelic interaction such that one allele manifests itself more or less, when heterozygous, than its alternative allele.
Donor parent: The parent from which one or a few genes are transferred to the recurrent parent in backcross breeding.
Double cross: A cross between two F_1 hybrids.
Emasculation: Removal of the anthers from a flower.
Epiphytotic: An unarrested spread of a plant disease.
Expressivity: The degree of manifestation of a genetic character.
F_1: The first generation of a cross.
F_2: The second filial generation obtained by self-fertilization or crossing F_1 individuals.
F_3: Progeny obtained by self-fertilization of F_2 individuals.
Factor: Same as gene.
Facultative: Parasites which can grow and live in environments other than living host tissue.
Family: A group of individuals directly related by descent from a common ancestor.
Fertility: Ability to produce viable offspring.
Fertilization: Fusion of the nuclei of male and female gametes.
Foundation seed: Seed stock produced from breeder seed under the direct control of an agricultural experiment station. Foundation seed is the source of certified seed, either directly or through registered seed.
Gamete: Cell of meiotic origin specialized for fertilization.
Gene: The unit of inheritance. Genes are located at fixed loci in chromosomes and can exist in a series of alternative forms called alleles.
Gene frequency: The proportion in which alternative alleles of a gene occur in a population.
Gene interaction: Modification of gene action by a non-allelic gene or genes.
Genome: A set of chromosomes corresponding to the haploid set of a species.
Genotype: The entire genetic constitution of an organism.
Germ plasm: The sum total of the hereditary materials in a species.
Haploid: A cell or organism with the gametic chromosome number (n).
Heritability: The proportion of observed variability which is due to heredity, the remainder being due to environmental causes. More strictly, the proportion of observed variability due to the additive effects of genes.
Heterosis: Hybrid vigour such that an F_1 hybrid falls outside the range of the parents with respect to some character or characters. Usually applied to size, rate of growth, yield.
Heterozygous: Having different alleles at one or more corresponding loci (opposite of homozygous).
Homology of chromosomes: Applied to whole chromosomes or parts of chromosomes which synapse or pair in meiotic prophase.
Host resistance: The result of genetic manipulation of the host which renders it less susceptible to pathogens that would or do attack the host.
Hybrid: The product of a cross between genetically unlike parents.
I_1, I_2, I_3...: Symbols that are used to designate first, second, third, etc. inbred generations.
Inbred line: A line produced by continued inbreeding, accompanied by selection.

Inbreeding: The mating of individuals more closely related than individuals mating at random.

Independence: The relationship between variables when the variation of each is uninfluenced by that of others, that is, correlation of zero.

Isogenic lines: Two or more lines differing from each other genetically at one locus only. Distinguished from clones, homozygous lines, identical twins, etc., which are identical at all loci.

Isolation: The separation of one group from another so that the mating between or among groups is prevented.

Line breeding: A system of breeding in which a number of genotypes, which have been progeny tested in retrospect to some character or group of characters, are composited to form a variety.

Linkage: Association of characters in inheritance due to location of genes in proximity on the same chromosome.

Linkage map: Map of position of genes in chromosomes determined by recombination relationships.

Linkage value: Recombination fraction expressing the proportion of crossovers versus parental types in a progeny. The recombination fraction can vary from zero to one-half.

Locus: The position occupied by a gene in a chromosome.

M_1, M_2, M_3...: Symbols used to designate first, second, third, etc. generations after treatment with a mutagenic agent.

Male sterility: Absence or non-function of pollen in plants.

Mass-pedigree method: A system of breeding in which a population is propagated in mass until conditions favourable for selection to occur, after which pedigree selection is practised.

Mass selection: A form of a selection in which individual plants are selected and the next generation is propagated from the aggregate of their seeds.

Mating system: Any number of schemes by which individuals are assorted in pairs leading to sexual reproduction. Mating systems include: (i) *Random* – assortment of pairs is by chance; (ii) *Genetic assortative mating* – mating together of individuals more closely related than individuals mating at random; (iii) *Genetic disassortative mating* – mating together of individuals less closely related than individuals mating at random; (iv) *Phenotypic assortative mating* – mating individuals more alike in appearance than the average; and (v) *Phenotypic disassortative mating* – mating of individuals less alike in appearance than individuals mating at random.

Meiosis: A double mitosis occurring in sexual reproduction which results in production of gametes with haploid (*n*) chromosome number.

Mitosis: The process by which the nucleus is divided into two daughter nuclei with equivalent chromosome complements, usually accompanied by division of the cell containing the nucleus.

Modifying genes: Genes that affect the expression of a non-allelic gene or genes.

Monoecious: Staminate and pistillate flowers present separately on the same plant.

Mutation: A sudden heritable variation in a gene or in a chromosome structure.

Obligate: Parasite that cannot multiply in nature without a host. (See also facultative.)

Oliogenic resistance: Resistance determined by one or a few genes whose effects are readily detectable.

Outcross: A cross, usually natural, to a plant of different genotype.

Parameter: A numerical quantity which specifies a population in respect to some characteristic.

Parasite: Lives in or on another organism and obtains nutrients from it.

Parthenogenesis: Development of an organism from a sex cell in respect to some characteristic.

Pathogen: A parasite which produces disease in its host.

Pedigree: A record of the ancestry of an individual, family or strain.

Pedigree breeding: A system of breeding in which individual plants are selected in the segregating generations from a cross on the basis of their desirability judged individually and on the basis of a pedigree record.

Phenotype: Appearance of an individual as contrasted with its genetic make-up or genotype. This term is also used to designate a group of individuals with similar appearance but not necessarily identical genotypes.

Phytolexins: Substances produced by host plants in response to injury, physiological stimuli, infectious agents or their products that accumulate to levels which inhibit the growth of microorganisms. Some include toxic substances produced to repel insects and nematodes.

Polycross: Open pollination of a group of genotypes (generally selected), in isolation from other compatible genotypes, in such a way as to promote random mating.

Polygenic: Determined by several genes whose effects are readily detectable.

Populations: In genetics, a community of individuals which share a common gene pool. In statistics, a hypothetical and infinitely large series of potential observations among which observations may actually constitute a sample.

Progeny test: A test of the value of a genotype based on the performance of its offspring produced in some definite system of mating.

Protandry: Maturation of anthers before pistils.

Protogyny: Maturation of pistils before anthers.

Pure line: A strain homozygous at all loci, ordinarily obtained by successive inbreeding.

Qualitative character: A character in which variation is discontinuous.

Quantitative character: A character in which variation is continuous so that classification into discrete categories is not possible.

Random: Arrived at by chance without discrimination.

Randomization: Process of making assignments at random.

Recessive: The member of an allelic pair which is not expressed when the other (dominant) member occupies the homologous chromosome.

Reciprocal crosses: Crosses in which the sources of the male and female gametes are reversed.

Recombination: Formation of new combinations of genes as a result of segregation in crosses between genetically different parents. Also the rearrangement of linked genes due to crossing over.

Recurrent parent: The parent to which successive backcrosses are made in backcross breeding.

Recurrent selection: A method of breeding designed to concentrate favourable genes scattered among a number of individuals by selecting, each generation, among the progeny produced by matings of the selected individuals (or their selfed progeny) of the previous generation.

Registered seed: The progeny of foundation seed normally grown to produce certified seed.

Resistance: The restriction of development of a pathogenic agent or parasite.

Rogue: A variant from the standard type of a variety or strain. *Roguing* is the removal of undesirable individuals to purify a stock.

S_1, S_2, S_3...: Symbols for designating first, second, third, etc. selfed generations from an ancestral plant (S_0).

Segregation: Separation of paternal from maternal chromosomes at meiosis and consequent separation of genes leading to the possibility of recombination in the offspring.

Selection: In genetics, discrimination among individuals in the number of offspring contributed to the next generation. In statistics, selection is discrimination in sampling leading to bias. It is opposed to randomness.

Self-fertilization: Fusion of male and female gametes from the same individual.

Self-incompatibility: Genetically controlled physiological hindrance to self-fruitfulness.

Single cross: A cross between two genotypes, usually two inbred lines, in plant breeding.

Species: The unit of taxonomic classification into which genera are subdivided.

Strain: A group of similar individuals within a variety.

Synthetic variety: A variety produced by crossing a number of genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination.

Test cross: A test cross involves breeding the individual in question with another individual that expresses a recessive version of the same trait.

Tetraploid: An organism with four basic (*x*) sets of chromosomes.

Top cross: A cross between a selection, line, clone, etc. and a common pollen parent, which may be a variety, inbred line, single cross, etc. The common pollen parent is called the top cross or tester parent.

Variation: The occurrence of differences among individuals due to differences in their genetic composition and/or the environment in which they were raised.

Variety: A subdivision of a species. A group of individuals within a species which are distinct in form or function from other similar arrays of individuals.

Virulence: Capacity of a pathogen to incite a disease.

***x*:** Basic number of chromosomes in a polyploid series.

X_1, X_2, X_3...: Symbols denoting first, second, third, etc. generations from irradiated ancestral plants (X_0).

Zygote: Cell formed by the union of two gametes and the individual developing from this cell.

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