Chapter 13
Breeding *Hevea* Rubber

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13.1 Introduction

Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell.-Arg., the prime source of commercial rubber, is a deciduous perennial tree of the family Euphorbiaceae (Fig. 13.1). The predominant constituent of rubber derived from *Hevea* is cis-1,4 polyisoprene $(C_5H_8)_n$ where n may range from 150 to 20,00,000 (Pushparajah 2001). The invention of vulcanization by Goodyear in 1839 adjudged rubber as a prime raw material that was otherwise unknown to mankind for over 450 years, since Christopher Columbus gave the first description of rubber in the fifteenth century (Priyadarshan and Clément-Demange 2004). It staked almost 40% of the export revenue of Brazil till 1940 (Dean 1987). However, Brazil and adjoining countries of Latin America share only 2% of the production due to the infestation of South American Leaf Blight (SALB-*Microcyclus ulei* (P. Henn. von Arx.). The Southeast Asian countries enjoy dominance in rubber production and trade by contributing more than 90% of the 7.97 million tons of rubber produced worldwide in 2003 (Sekhar 2004). Thailand with 2.3 million tons is at the helm followed by Indonesia, India, Malaysia, China, Vietnam, Côte d'Ivoire, Liberia, Sri-Lanka, Brazil, Philippines, Cameroon, Nigeria, Cambodia, Guatemala, Myanmar, Ghana, D.R. of Congo, Gabon and Papua New Guinea.

13.2 Commercial Importance

Rubber is the strategic raw material for more than 40,000 products, including 400 medical devices (Mooibroek and Cornish 2000). Primarily due to its molecular structure and high molecular weight (> 1 million daltons) it has resilience, elasticity, abrasion resistance and impact resistance that cannot easily be obtained by artificial polymers. Search for alternative sources of natural rubber resulted in

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Fig. 13.1 A view of rubber plantation

experiments on industrial exploitation of guayule (*Parthenium argentatum* Gray) as a source of high quality latex. Although economic considerations may prevent commercial exploitation of new rubber-producing microorganisms, transgenic yeasts and bacteria may yield intermediate or alternative (poly-) isoprenes suitable for specific applications.

13.3 Botanical Aspects

Rubber is synthesized in over 7500 plant species, confined to 300 genera of seven families, namely, Euphorbiaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Moraceae, Papaveraceae and Sapotaceae (Cornish et al. 1993). The genus *Hevea* includes 10 species (Table 13.1) (Webster and Paardekooper 1989; Wycherley 1992). A few species are inter-crossable (Clément-Demange et al. 2000). Consequently, the *Hevea* species can be considered as a species complex. Since an elaborate description of taxonomical and botanical aspects of *Hevea* is out of scope of this article, readers may refer other sources (Schultes 1977a, 1987; Wycherley 1992; Priyadarshan 2003a; Priyadarshan and Gonçalves 2003; Priyadarshan and Clément-Demange 2004) for narrations on the subject. The natural habitats of *Hevea* species are Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam and Venezuela (Webster and Paardekooper 1989). All species are diploids having $2n = 36$ chromosomes ($x = 9$), with the exception of one triploid clone of *H. guianensis* ($2n = 54$) and the existence of one genotype of *H. pauciflora* with 18 chromosomes (Baldwin 1947). However, *Hevea brasiliensis* behaves as amphidiploid (Ramaer 1935; Ong 1975; Wycherley 1976; Priyadarshan and Gonçalves 2003). All species were probably evolved in Amazonian forests over 100,000 years (Clément-Demange et al. 2000).

Table 13.1 Occurrence and features of *Hevea* species

Species	Notable features[1]
H. benthamiana Muell.-Arg.	Complete seasonal defoliation Medium size tree. Habitat: swamp forests
H. brasiliensis (Willd. ex. Adr. Juss.) Muell.-Arg.	Complete defoliation. Medium to large tree size. Habitat: well drained soils
H. camargoana Pires	Possibility of natural hybridization with *H. brasiliensis*. 2–25 m tree height Habitat: seasonally flooded swamps
H. camporum Ducke	Retains old leaves until new leaves appear. Maximum 2 m tall. Habitat: dry savannahs
H. guianensis Aublet	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	Complete defoliation. Small trees. They live on flooded area (igapós). Habitat: sandy soils
H. nitida *Mart. ex Muell.-Arg*.	Inflorescences appear when leaves are mature. Small to medium size trees (2 m).
H. pauciflora (Spr. ex Benth.) Muell.-Arg.	Retains old leaves until new leaves and inflorescences appear. No wintering. Small to big size trees. Habitat well drained soils, rocky hill sides.
H. rigidifolia (Spr. ex Benth.) Muell.-Arg.	Retains old leaves even after inflorescences appear. Small tree from savannahs. Sometime tall, with small crown on the top. Habitat: well drained soils
H. spruceana (Benth.) Muell.-Arg.	Retain old leaves until new leaves and inflorescences appear. Flowers reddish purple. Medium size tree Habitat: muddy soils of islands
H. paludosa Ule[2]	*Small leaflets, narrow and thin in the fertile branches; Habitat: marshy areas*

After Wycherley (1992), Schultes (1977a), Gonçalves et al. (1990), Pires (1973), Pires et al. (2002) Brazil (1971).

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

[2] Pires (1973), Pires et al. (2002) considered 11 species including *H. paludosa*; Brazil (1971) considers 11 species.

(Modified from Priyadarshan and Gonçalves 2003).

13.4 Historical Aspects

Fusée Aublet was the first to give a botanical description of the genus *Hevea* in 1775. Five distinguished men played pivotal role for rubber domestication, namely,Clement Markham (of British India Office), Joseph Hooker (Director of Kew Botanic Gardens), Henry Wickham (Naturalist), Henry Ridley (Director of Singapore Botanic Gardens) and R. M. Cross (Kew Gardner). Kew Botanic Gardens played the crucial role for rubber procurements and distribution. As per directions of Markham, Wickham collected 70,000 seeds from Rio Tapajoz region of Upper Amazon (Boim district) and transported them to Kew Botanic Gardens during June 1876 (Wycherley 1968; Schultes 1977b; Baulkwill 1989). Of the 2,700 seeds germinated, 1,911 were sent to Botanical Gardens, Ceylon during 1876, where 90% of them survived. Later, during September 1877, 100 *Hevea* plants specified as 'Cross material' were sent to Ceylon. However, 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 1,000 tappable trees found by Ridley during 1888. An admixture of Cross' and Wickham's materials might have occurred, as the '22 seedlings' were unspecified (Baulkwill 1989). Seedlings from Wickham's collection of Ceylon were also distributed worldwide. But somehow, rubber trees covering millions of hectares in Southeast 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 into East Asia, Thomas (2001) drew the conclusion that the modern clones have invariably originated from the 1,911 seedlings sent to Ceylon in 1876. Hence, the contention that the modern clones were derived from '22 seedlings' is debatable. Moreover, if the modern clones are derived from 1,911 seedlings, then the argument that they originated from a 'narrow genetic base', as believed even now, needs to be reviewed.

P. J. S. Cramer conducted experiments on variations observed among 33 seedlings introduced from Malaysia in 1883 from which the first clones of 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 China, Thailand, India, Sri Lanka and Vietnam and rubber became an integral part of the economy of Southeast Asia toward latter half of the 20th century. Around 1950, bud grafted clones proved to be overwhelmingly popular because of higher productivity.

Progress in yield improvement in *Hevea* resulted in a gradual increment, from 650 kg/ha in unselected seedlings during 1920s to 1,600 kg/ha in best clones during 1950s. The yielding potential was further enhanced to 2,500 kg/ha in PB, RRIM (Malaysian), RRII (Indian), RRIC (Sri Lankan), IRCA (Côte d'Ivoire), BPM (Thai), NIG (Nigeria), IAC (Brazilian) and RRIV (Vietnamese) clones during 1990s. During these 70 years of rigorous breeding and selection, notable clones like RRIM 501, RRIM 600, RRIM 712, PB 217, PB 235, PB 260, RRII 105, RRIC 100, IRCA

18, IRCA 230, IRCA 331, BPM 24, IAC 35 and IAC 40 were derived (Tan 1987; Simmonds 1989; Clément-Demange et al. 2000; Priyadarshan 2003a; Priyadarshan et al. 2005; Omokhafe and Nasiru 2005). Primary clones selected during the aforesaid period (PB 56, Tjir 1, Pil B84, Pil D65, Gl 1, PB 6/9 and PB 86) became parents of improved clones. It must also be acknowledged that primary clones like GT 1 and PR 107 are still widely used although their identification traces back to the 1920s.

13.5 Propagation Systems

Rubber is currently planted in the form of grafted trees, at a density of about 450 trees per hectare. It experiences an immature phase varying from 5 to 9 years, depending on climate, soil conditions and management. Propagation through grafting enables the multiplication of elite genotypes as clones. The high level of homogeneity in bud-grafted trees should exhibit intra-clonal variation in yield to a minimum, barring factors such as (a) soil heterogeneity, (b) difference in juvenility of buds and (c) variable seedling rootstocks. On the contrary, such clonal populations exhibit significant variations. In an experiment with RRII 105, total volume of latex and dry rubber yield ranged between 5.0 to 325.0 ml and 1.8 to 144.0 g, respectively (Chandrashekar et al. 1997). The differences exhibited are significant and refutable for a homogeneous population. Due to the lack of an efficient cloning technique, the root system directly affects soil–plant relationships, such as water and mineral uptake, water stress resistance and resistance to wind uprooting (Ahmad 2001). Moreover, efficient breeding for growth of budded clones and the increasing use of fast growing clones may have generated an imbalance between stock and scion, so emphasizing the 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, yield improvement and adaptation to various environments. Rubber trees can be propagated as seedlings also, for the polyclonal seedlings are desired plant materials for non-traditional areas (Sasikumar et al. 2001).

13.6 Laticifer System

Hevea has articulated laticifers issued from the anastomose of latex cells in newly formed parts of the tree, forming a paracirculatory ramified structure (syncytium). This laticifer system is notably developed in the soft bark of the trunk from which latex can be extracted by tapping (Figs. 13.2 and 13.3). The laticifers successively generated by the cambium are organized in cylindrical rings that are not interconnected. There are no plasmodesmata between the latex vessels, or between them and their surroundings (de Faÿ and Jacob 1989); there are also no associated companion cells in contrast with sieve tubes. Connections exist between the laticifer systems of the stock and the scion, evidenced by the transport of latex (Bonner

Fig. 13.2 Tapping Hevea rubber in Amazonian forests

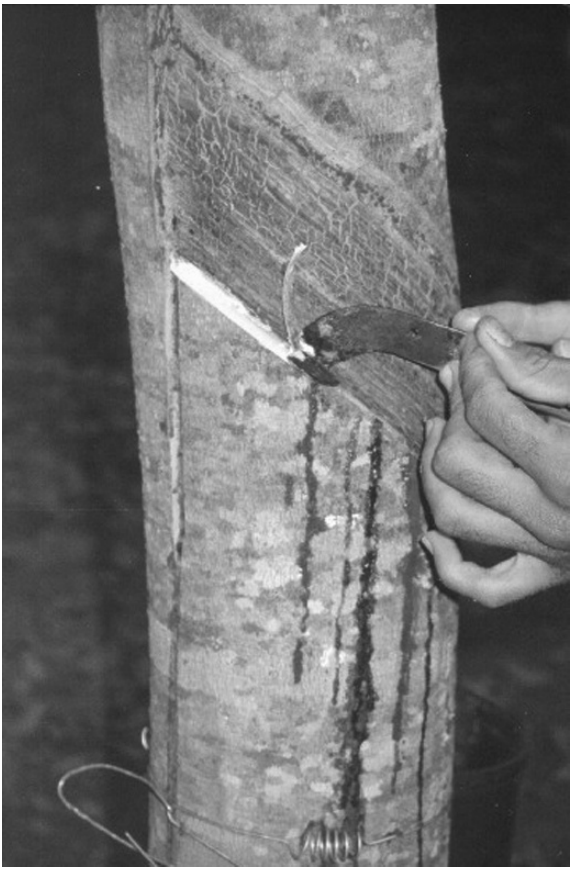


Fig. 13.3 Refined commercial tapping of bud grafted tree

and Galston 1947). The latex is a cytoplasm that contains predominantly rubber particles, as well as lysosomal microvacuoles known as lutoids; it also contains double-membrane organelles rich in carotenoids, which look similar to plastids but their role has not yet been fully elucidated (Paardekooper 1989). On tapping, nuclei and mitochondria remain adhered to the plasmalemma, and consequently, cannot be found in the latex, which makes possible latex regeneration after tapping. Rubber particles, made of clustered polymer chains, are surrounded by a phospholipoglycoprotein monomembrane with outside negative charge that ensures the colloidal stability of the latex before coagulation and protects the rubber chains against oxidative degradation.

Coagulation at the level of the tapping cut is an important limiting factor of rubber yield as it stops latex flow. Lutoids with coagulating factors, such as hydrolytic enzymes, other proteins and many ions with positive charge, cause the rupture of their membrane and play a key role in this process. High osmotic gradient near the cut also is an adding factor that leads to the plugging of laticifers (Southorn and Edwin 1968). Plugging index, a ratio between initial and final flow of latex, shows differences between clones (Milford et al. 1969) and can be used as a selection variable. Turgor pressure in the laticifers, as high as 10–14 atmospheres before sunrise, is vital for the flow of latex. Water plays a key role in latex flow after tapping and all the ecophysiological factors that affect water balance and water flux in the tree influence latex flow and coagulation.

Regeneration of the latex and rubber between two tappings is related to the cellular metabolism of the laticifer system and with the ecophysiological functioning of the tree. The full regeneration of the latex after one tapping was estimated to be around 72 h (Serres et al. 1994). Assimilation (photosynthesis), transport of sugars, and allocation to the different competing sinks play a key role in the regeneration of the latex. One of the most striking features of tapping is that it generates a direct competition for carbohydrate assimilates between latex regeneration and the whole growth of the tree. This can easily be observed by the strong reduction in growth increments in trunk growth and in tree height, a few weeks after the beginning of tapping. Faster growth resumes when tapping is stopped. This partition of assimilates has been theoretically modelled by Simmonds (1982), based on some ideas of Templeton (1969). As such, assimilate partitioning has become vital especially when rubber wood is getting increasing importance (Fig. 13.4).

The intensity and the duration of latex flow and latex regeneration determine latex yield. Ethephon stimulation delays coagulation and prolongs latex flow in laticifers. This is a clonal response and is a genetic component of yield potential, which can be subjected to selection and breeding. A review (d'Auzac et al. 1997) took stock of the physiological functioning of the laticifer system and its response to stimulation, and presented the concept of the physiological typology of the clones, which is based on the metabolic activity of the laticiferous cells, the provision of these cells with sucrose and their protection from oxidative stress. Based on this concept,a biochemical diagnosis (the "latex diagnosis") was developed from the measurements of the dry rubber content, sucrose ratio, inorganic phosphorus ratio and thiol ratio in the latex (Jacob et al. 1995; d'Auzac et al. 1997). Gohet et al. (1996) fine-tuned this

Fig. 13.4 Rubber wood ready for chemical impregnation

methodology. Further, it was envisaged that latex diagnosis could be used to detect physiological stresses (Lacote et al. 2004).

The current best clones, with annual yield little more than 2,500 kg of latex per hectare appear still far below the yield summit (4,000 kg) estimated by Templeton (1969). This has provided encouragement to rubber breeders to continue their efforts in genetic improvement.

13.7 Breeding Objectives

Improving dry rubber yield is the exclusive objective of *Hevea* breeding. Growth of the trunk during immature phase, yield per tree over a specific period, stability of the stand per unit area and resistance to stresses (tapping panel dryness, wind damage, varied diseases, low temperature, higher altitude and moisture deficit) are some of the factors that govern productivity levels. Latex yield and growth are not correlated, obviously due to differential partitioning of assimilates. Breeding and selection are exclusively applied to scion and the choice of rootstock is very limited. The possibility of cloning the whole plant *in vitro* would allow breeding to be applied to the root system for resistance to root diseases, for better adaptation to specific soils and for anchorage. This leads to the concept of 'compound tree' with three different genetic components, namely roots, trunk and canopy, each selected for its own requirements (Simmonds 1985, 1989). High yielding trunks with canopies resistant to SALB have been experimented by the way of crown budding, but it failed commercially.

Adaptation and yielding potential of clones to specific environments are optimized through multi-location trials and localized experimentation. Characterization of the architecture of the trees in connection with wind risk and phenology is assessed in relation to susceptibility to leaf diseases (*Colletotrichum gloeosporioides* Penz. Sacc., *Microcyclus ulei*) are vital (Priyadarshan et al. 2001). Studies on adaptation of clones to new environments, especially to sub-optimal or marginal

areas, are gaining momentum (Priyadarshan 2003a,b). In all these aspects, diversification of clones allows large plantations to mitigate risks. Among those clones, the more stable ones are identified for recommendation to small holders, since small holders represent a predominant share. A selection focused on fast growing trees with effective competence toward weed growth, canopy adapted to multi-cropping, clones adapted to uneven and intensive tapping systems and climatic variations needs to be exercised.

Derivation of clones for timber has emerged as a recent objective. An estimation from RRIM shows that a hectare 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). Also, there is some interest generated among the scientists to evolve rubber as a factor producing useful chemicals, especially life saving drugs (Yeang et al. 2002). Possibilities of using rubber trees for reforestation or carbon sequestration may come up in future, which breeders may have to take up with required priority.

13.8 Genetic Resources and Variability

Hevea brasiliensis is believed to be an amphidiploid ($2n = 4x = 36$) that got stabilized during the course of evolution. This contention is amply supported by the observance of tetravalents during meiosis (Ong 1975). However, for practical purposes, *Hevea* is considered as a diploid genus ($2n = 2x = 36$). 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). Hence, as long as a potential ancestor with $2n = 18$ is not known, rubber tree would be considered as an amphidiploid (Priyadarshan and Gonçalves 2003). Locus duplications are infrequent in *Hevea* genome, and they could have occurred due to chromosomal modifications posterior to the polyploidization event (Seguin et al. 2003). Consequently, the two ancestral genomes of *Hevea* would have strongly diverged. Only a comprehensive molecular analysis along this objective will reveal the details of origin.

Allied species of *Hevea* make up a gene pool for breeding purposes, especially for the identification and introduction of genes of resistance to leaf diseases (Priyadarshan and Gonçalves 2003). Within *Hevea brasiliensis*, a clear distinction needs to be made between 'Wickham' population and the 'Amazonian' population. While the Wickham population was domesticated and bred for more than a century, Amazonian populations are still under evaluation and despite poor yield, they display a fairly high resistance to leaf diseases, such as *Microcyclus* or *Corynespora cassiicola* Berk et. Curt. Wei. (Clément-Demange et al. 2000).

During 1951–1952, 1,614 seedlings of five *Hevea* species (*H. brasiliensis, H. guianensis, H. benthamiana, H. spruceana* and *H. pauciflora*) were introduced in Malaysia (Tan 1987). Brookson (1956) has given an account of these introductions. In Sri Lanka, 11 clones of *H. brasiliensis* and *H. benthamiana*, and 105 hybrid materials were imported during 1957–1959, through triangular collaboration of USDA, Instituto Agronomico do Norte (IAN), (Brazil), and Liberia. Many of these clones

were later given to Malaysia (Tan 1987). During 1981, due to initiative taken by the International Rubber Research and Development Board (IRRDB), 63,768 seeds, 1,413 m of bud wood from 194 high yielding trees, and 1,160 seedlings were collected from Brazilian Amazonia (Gonçalves 1981; Tan 1987; Simmonds 1989). This collection was carried out over three states, namely, Acre, Rondonia, and Mato Grosso, from 60 different locations spread to 16 districts. 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 budwood collection were brought to Malaysia and Côte d'Ivoire after quarantine against SALB. After the establishment of two IRRDB Germplasm Centers in Malaysia and in Côte d'Ivoire, other IRRDB member countries were supplied with material according to their request.

Attempts to improve the yield of wild accessions through Wickham × Amazonian crosses resulted in recombinants with low yield, ranging between 30 and 50% of the level of GT1, probably due to the important genetic gap lying between the two populations. Conversely, a wide variability was found within these crosses for growth, with probable heterotic effects enabling the selection of very vigorous Wickham × Amazonian clones. It is quite evident that the Wickham population, though originally meager in number, was subjected to natural pre-breeding. This must have occurred in two ways, one through indirect selection of ortets exhibiting adaptation to specific hydrothermal environment and the other by evaluation of useful recombinants. 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 great heights and those from Mato Grosso that display trees with abundant branching at low heights (Clément-Demange et al. 1998). Furthermore, during 1995 an expedition was launched by RRIM to collect rubber seeds from Brazil. From this collection, about 50,231 seedlings were planted in Malaysia, including allied species (RRIM Annual Report 1997; MRB Annual report 1999).

13.9 Breeding Methodologies and Achievements

Breeding methodologies utilized for maximizing genetic gain are based on breeding objectives with the specific aim of providing farmers with high yielding clones. Such methodologies are backed by the theory of quantitative genetics, which derives clones well adapted to a given environment. Elements of breeding methodologies are available with major contributions of Dijkman (1951), Shepherd (1969), Wycherley (1969), Tan (1987), Simmonds (1989), Clément-Demange et al. (2000); Priyadarshan (2003a) and Priyadarshan and Clément-Demange (2004). These ideas are discussed here with a separate section on biotechnology.

13.9.1 Primary Clones

The first clones released out of seedlings were those of Cramer's *Cultuurtuin* (Ct3, Ct9, Ct88) selected from 33 seedlings planted in Penang through Java in Indonesia

(Dijkman 1951). Mixed planting of these clones gave yield over 1,700 kg/ha, against unselected seedlings (496 kg/ha) (Tan et al. 1996). During 1924, Major Gough selected 618 seedlings from a population of about 1 million in Kajang district of Malaysia that yielded prominent primary clones like Pil A44, Pil B84, Pil B16, PB 23, PB 25, PB 86, PB 186 and Gl 1 (Tan et al. 1996). By 1930, it was understood that the primary clones had reached a plateau of yield (Tan 1987). Hence, the emphasis shifted from primary clones to recombinants issued from controlled pollination (Fig. 13.5). Simultaneously, polyclonal seed gardens were organized with improved clones for raising polyclonal seedlings for ensuring supplementary planting materials. Thus, the best seedlings came from Prang Besar Isolated Gardens (PBIG), Gough Gardens (GG), and Prang Besar Further Proof Trials (Tan et al. 1996). By 1970, polyclonal seedling areas extended to 7,700 hectares. Both yield and secondary attributes were given deserving importance while selecting clones based on 65% and 35% scores for yield and secondary attributes respectively (Ho et al. 1979; Tan et al. 1996). The procedure involved field selection in the estates, nursery selection applied to seedlings, small-scale selection with 16 plants per genotype, and large scale testing with 128 plants per genotype.

Polyclonal seed gardens involving clones with high general combining ability (GCA) ensures panmictic conditions for deriving seedlings with high genetic divergence. Selection for both vigor and high yield can be exercised in such seedlings (Simmonds 1986). After popularization of clones in 1950s, the potentiality of extending rubber to marginal areas was realized. This seems to be an appreciable option since results on the yield of polyclonal seedlings from non-traditional areas like Tripura (northeast India) and Konkan (western India) are encouraging (Sasikumar et al. 2001; Chandrashekar et al. 2002). There is a contention that yield and girth variation can be largely accounted for by additive genetic variance (Gilbert et al. 1973; Nga and Subramaniam 1974; Tan 1981). As per general genetic principles, selection based on genotypic values as reflected by GCA would be more

Fig. 13.5 A view of recombinants (hybrids) of Hevea rubber

reliable and desirable. GCA could be estimated by the evaluation of seedling progenies, in order to select the best parent clones. DNA fingerprinting can contribute significantly to assess molecular diversity of parents and their progenies. Optimum number of parents is crucial while constituting seed gardens and Simmonds (1986) suggested that a lay out involving nine clones with all hetero neighbors as the best.

The extent of selfing due to lack of self-incompatibility may reduce the vigor of first generation population (SYN_1), since there is no evidence of self-incompatibility. Since inbreeding reduces zygotic ability to germinate, the presumption is that only cross-pollinated seeds will survive (Simmonds 1986). Till recently, such SYN_1 progenies were considered as Class I planting material in Malaysia and must be of better use in non-traditional/marginal areas. However, factors like agronomic performance of such synthetic seedlings, the long time taken to attain seed production and supply of seeds in tune with demand need to be evaluated before utilizing this methodology. In contrast, seed gardens can be viewed as a recombination tool for addressing the improvement of wild Amazonian populations, where the ability to flower and set seed make the major criteria to ensure maximum genetic combinations (Fig. 13.6). With this view, 50 Amazonian parents were analysed in Côte d'Ivoire using microsatellite markers (Blanc et al. 2001). Most of the paternal contribution to the progenies was due to a restricted number of male parents with substantial flowering, hence were very far from a panmictic status. It is implicit that each seed garden need to be evaluated with DNA fingerprinting.

13.9.2 Derivation and Evaluation of Recombinants

Recombination breeding starts with production of full-sib families, followed by Seedling Evaluation Trial (SET), Small Scale Clonal Trial (SSCT) and Large Scale Clonal Trial (LSCT) with selection practiced at every level. This process is cyclical, with the best clones becoming candidates for recombination in the next cycle. Yield improvement from 500 kg/ha in primary clones to 2,500 kg/ha in the current clones could be attained through recombination breeding and selection in RRIM and Prang Besar (Malaysia). RRIC 100 series released in Sri Lanka during 1970s is yet another example. Much of the hybridisation work in Malaysia, Indonesia, India, Côte d'Ivoire, Brazil, Thailand and Vietnam further strengthened the array of hybrid clones with differential genetic set-up, obviously due to selection pressure applied under varied conditions (Table 13.2).

At least 16 primary clones are considered prime progenitors of many modern clones (Fig. 13.7). However, many valuable recombinants must have been lost during the course of assortative mating of primary and hybrid clones followed by subsequent directional selection for yield under varied geo-climates (Priyadarshan 2003a). The crossing of 'the best with the best' (GAM, Generation-wise Assortative Mating), with strong emphasis on selection for precocious yield within Wickham material (Wycherley 1976) has been practiced in all these recombination breeding

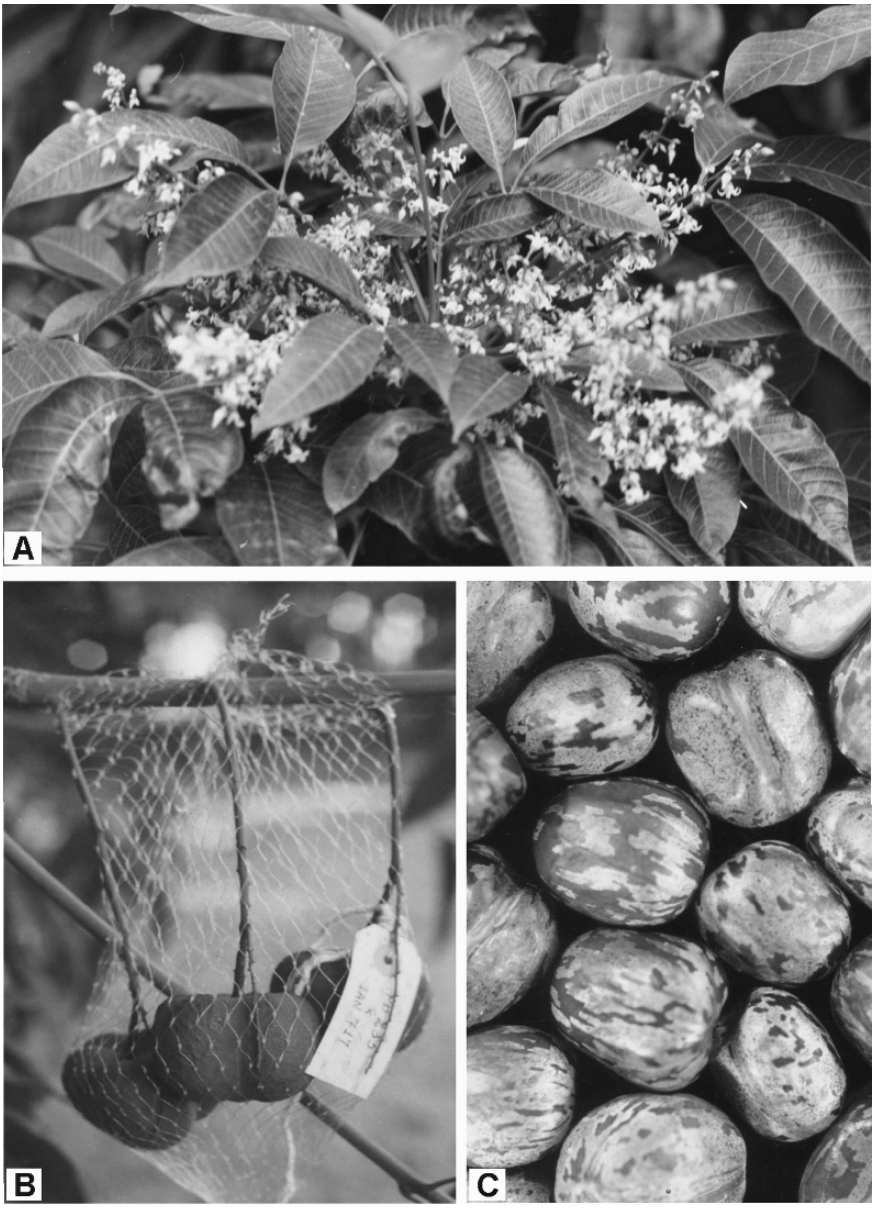


Fig. 13.6 (**A**) Flowers; (**B**) Bagged fruits borne from hand pollinated flowers; and (**C**) seeds

programs. Breeding for disease resistance has to take account of specific aspects related with host × pathogen interactions. But this exercise has to go a long way before it achieves clones combining resistance and higher yield. Interestingly, in the clones developed in Nigeria, the cross involving one primary and a secondary clone ended with high yielders, which are under evaluation (Table 13.3).

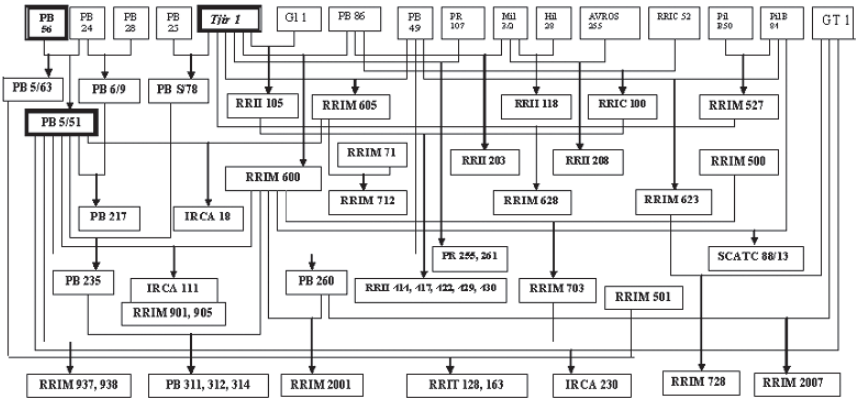
Table 13.2 Profile of prominent clones

Clone	Parentage	Yield (kg/ha)	Girth increment during tapping	Resistance to						
				Wind damage	Panel dryness	Pink Disease	Oidium	Colletotrichum	Corynespora	Phytophthora
RRII 105[I]	Tjir 1 × Gl 1	2,210	3	3	5	5	3	5	5	1
RRII 203[I]	PB 86 × Mil 3/2	1,618	4	3	2	3	3	NA	3	3
RRII 208[I]	Mil 3/2 × AVROS 255	1,587	3	3	3	NA	3	NA	NA	NA
RRIC 100[M]	RRIC 52 × PB 83	1,774	3	5	3	3	4	3	5	NA
RRIM 600[M]	Tjir 1 × PB 86	2,199	4	4	4	1	3	3	1	1
RRIM 623[M]	PB 49 × PB 84	1,622	4	2–3	3	2–3	1–2	3–4	4	1
RRIM 712[M]	RRIM 605 × RRIM 71	2,264	2	5	4	3	3	1	3	3
RRIM 936[M]	GT 1 × PR 107	2,146	3	4	3	4	3	4	4	2
RRIM 937[M]	PB 5/51 × RRIM 703	2,483	2	5	3	4	3	3	5	3
RRIM 2015[M]	PB 5/51 × IAN 873	2,760	4	NA	NA	NA	4	4	4	3
PB 217[M]	PB 5/51 × PB 6/9	1,778	4	4	4	2	2	3	4	1
PB 235[M]	PB 5/51 × PB S/78	2,485	3	2	2	3	2	2	4	3
PB 255[M]	PB 5/51 × PB 32/36	2,283	3	4	2	2	2	2	4	2
PB 28/59[M]	Primary clone	2,023	1	3	3	2	2	2	4	2
PR 255[M]	Tjir 1 × PR 107	2,018	3	4	3–4	3	1	3	4	3
PR 261[M]	Tjir 1 × PR 107	1,838	3	4	3–4	3	1–2	4	3	3
IRCA 111[CD]	PB 5/51 × RRIM 600	1,446	5	3	3	NA	NA	NA	NA	NA
IRCA 230[CD]	PB 5/51 × GT 1	1,807	5	3	3	NA	NA	NA	NA	NA
RRIT 163[I]	PB 5/51 × RRIM 501	2,086	2	NA	NA	NA	3	NA	3	NA
HAIKEN 1[C]	Primary clone	1,500	3	4	3	2	NA	NA	NA	NA

Table 13.2 (continued)

Clone	Parentage	Yield (kg/ha)	Girth increment during tapping	Resistance to						
				Wind damage	Panel dryness	Pink Disease	Oidium	Colletotrichum	Corynespora	Phytophthora
REYAN 8–333[C]	SCATC 88–13 × SCATC 217	2,187	3	3	3	NA	3	NA	NA	NA
BPM 24[M]	GT 1 × AVROS 1734	1,394	2	3	3	3	3	2	3–4	4
IAN 873[B]	PB 86 × FA 1717	1,920	4–5	3	4	NA	4	4	NA	NA
IAC 301[B]	RRIM 501 × AVROS 1511	2,320	4	4	4	NA	4	4	NA	NA
IAC 300[B]	RRIM 605 × AVROS 353	887	3	2	2	NA	3	2	NA	2
Fx 3864[B]	PB 86 × PB 38	1,755	4	3	3	NA	2	2	NA	3
IAN 4493[B]	EX 441 × Tjir 1	1,711	3	3	2	NA	2	2	NA	2
IAC 303[B]	RRIM 505 × AVROS 1511	2, 190[6Y]	3	3	2	NA	2	2	NA	2
PB 260[VN]	PB 5/51 × PB 49	1, 691[10 Y]	4	3–4	3–4	3	4–5	4	NA	3–4
RRIC 121[VN]	PB 28/59 × IAN 873	1, 654[10 Y]	4–5	4	4	4	2	3–4	NA	4
GT 1[VN]	Primary clone	1, 459[10 Y]	3	5	5	3	2–3	3	NA	3–4
RRIV 4[VN]	RRIC 110 × PB 235	2, 103[10 Y]	2	2	4	3–4	2–3	2	NA	4

1 = poor; 2 = below average; 3 = average; 4 = good; 5 = very good. NA = Not available, since the disease is not prominent.
Under conditions of M = Malaysia; I = India; C = China; CD = Cote d'Ivoire; B = Brazil.
Tapping system = s/2 d/2 6d/7 86%; No. of tapping days per year = 158 ± 11 (with wide regional variation depending on weather); Trees per hectare = 327 ± 34.
IAN 873 exhibits good tolerance to SALB.
Tapping under Vietnamese (south east) conditions =S/2 d/3 6d/7.
6 Y=average over 6 years; 10 Y=average over ten years.
REYAN is new name for SCATC.



Gl = Glenshiel, Malaysia; GT =Gondang Tapen, Indonesia;
IRCA = Institute de Recherches sur le Caoutchouc en Afrique, (Côte d'Ivoire);
Mil = Milakande, Sri Lanka; RRIC = Rubber Research Institute of Ceylon (currently Sri Lanka);
RRII = Rubber Research Institute of India; RRIM = Rubber Research Institute of Malaysia;
RRIT = Rubber Research Institute of Thailand; Tjir = Tjirandji, Indonesia; PB = Prang Besar, Malaysia;

Fig. 13.7 Parentage of outstanding clones

13.9.3 Genetic Resource Management

Yield in rubber analyzed according to different types of mating designs was shown to have a large additive genetic variance. Heritability and general combining abilities for yield and growth have been investigated at the RRIM and are high, thus justifying GAM (Gilbert et al. 1973; Nga and Subramaniam 1974; Tan 1977, 1978, 1981; Simmonds 1989). Importance of low female fertility of many parents emerges here as a limiting factor for producing every full-sib progeny. The need for selecting highly heterozygous clones and reducing the risk of narrowing the genetic base are the two prime attributes that need attention (Simmonds 1989). One option is to involve Amazonian germplasm in breeding programs. Although such crosses appear as the best way to introgress the new germplasm into breeding populations, most of the Amazonian genotypes bear a large part of alleles unfavorable for yield (genetic burden in heterozygous plants). Pre-breeding appears necessary within the Amazonian groups before using them as progenitors in crossing with Wickham (Baudouin et al. 1997; Priyadarshan and Clément-Demange 2004). Since a detailed evaluation of whole *Hevea* germplasm is quite impossible, a working population of 287 accessions was extracted at the IRRDB African Germplasm Centre (Clément-Demange et al. 1998). It was proposed to combine the use of field experiments and molecular markers (microsatellites) for extracting a clonal population of reduced size (core collection) containing maximised genetic variability (Hamon et al. 1998; Brown 1989; Clément-Demange et al. 2000).

Table 13.3 Twenty four clones of *Hevea brasiliensis* developed in Rubber Research Institute of Nigeria (RRIN)

	Class 1 clones*				Class 2 clones**			
	National Code	RRIN Code	Parentage Mean Yield		National Code	RRIN Code	Percentage	Mean Yield
1.	NIG 800	RRIN C 76	RRIM 501 × Har 1	2679	NIG 901	RRIN C 289	PB 5/51 × PR 107	3528
2.	NIG 801	RRIN C 83	RRIM 600 × PR 107	2229	NIG 902	RRIN C 292	PB 5/51 × PR 107	3351
3.	NIG 802	RRIN C 114	RRIM 501 × RRIM 628	2014	NIG 903	RRIN C 291	PB 5/51 × PR 107	3237
4.	NIG 803	RRIN C 48	RRIM 600 × PR 107	2765	NIG 904	RRIN C 367	PB 5/51 × RIM 600	3233
5.	NIG 804	RRIN C 1	RRIM 600 × Tjir 1	3207	NIG 905	RRIN C 227	RRIM 501 × RRIM 628	3152
6.	NIG 805	RRIN C 15	RRIM 628 × RRIM 501	1944	NIG 906	RRIN C 380	PB 5/51 × RRIM 600	3069
7.	NIG 806	RRIN C 163	RRIM 501 × RRIM 628	2723	NIG 907	RRIN C 321	RRIM 501 × Har 1	3043
8.	NIG 807	RRIN C 145	RRIM 501 × RRIM 628	2699	NIG 908	RRIN C 366	PB 5/51 × RRIM 600	3033
9.	NIG 808	RRIN C 143	RRIM 501 × RRIM 628	2411	NIG 909	RRIN C 369	PB 5/51 × RRIM 600	3009
10.	NIG 809	RRIN C 150	RRIM 501 × 628	2388	NIG 910	RRIN C 368	PB 5/51 × RRIM 600	3000
11.	NIG 810	RRIN C 159	RRIM 501 × RRIM 628	2383				
12.	NIG 811	RRIN C 154	RRIM 501 × RRIM 628	2334				
13.	NIG 812	RRIN C 162	RRIM 501 × RRIM 628	2312				
14	NIG 813	RRIN C 202	RRIM 600 × PR 107	2090				

Source: Omokhafe, and Nasiru, I. (2005) Genetic improvement of *Hevea brasiliensis* in Nigeria. International Natural Rubber Conference, Cochin, India, pp. 13–17.

*: Tested in multilocation trials.

**: Undergoing multilocation trials.

13.9.4 Selections

Breeding cycle in rubber extends to 20–30 years between pollination and yield assessment, distributed over three selection stages. This justifies standardization of early selection methods to optimise and shorten the cycle as much as possible. One component of early selection is identification of traits at young age that have correlated response with yield at maturity and the other is combined management of different selection stages to improve the accuracy of estimation of genetic value. Several parameters, namely, girth, height, bark thickness, latex vessel number, latex vessel and sieve tube diameters, and rubber hydrocarbon in bark and petiole were inconsistent in having relations with yield both at seedling and mature stages (Gunnery 1935). Also, parameters like quantity of latex oozing out of leaflets or petiolules, plugging index, photosynthetic rate, number of stomata (Senanayake and Samaranayake 1970; Ho 1976; Zhou et al. 1982; Samsuddin et al. 1987) were studied but only plugging index and latex vessel number showed consistent and significant correlations with yield (Huang et al. 1981). Hénon and Nicolas (1989) showed that thickness of the bark cannot be considered as a reliable attribute to predict yield but the number of latex vessel rings can help to differentiate poor yielders in both Amazonian and Wickham populations. Recently, Sreelatha et al. (2004) demonstrated ATP concentration in latex as a possible indicator of high yield. However, this relationship needs to be confirmed in high yielding clones under varied environments, due to differential yielding pattern of clones under different environments. The first stage of selection is applied to full-sib seedlings (SET, Seedling Evaluation Trial) and information from this stage is used for selecting new clones to be evaluated as grafted trees in Small Scale Clone Trial (SSCT) (Fernando and de Silva 1971). Analysis of different procedures for assessing yield on young seedlings confirmed the use of only mild selection at the first nursery stage (Gnagne et al. 1998; Tan 1978).

For combining SET and SSCT, Gnagne et al. (1998) studied the relationships between the two stages of early selection. A combined family × individual selection was proposed in the form of a linear combination of family value and individual values. At nursery stage, with only one seedling tree per genotype, it will almost be impossible to directly assess the environment effect, so limiting the predictive efficiency of this first stage. With this view, early selection does not aim for priority to shorten the cycle but to improve the selection efficiency. Alternately, molecular genetic markers are considered independent of the environment and using them as predictors can contribute to improve the accuracy of genetic value assessment according to the concept of marker-assisted selection or MAS (Lynch and Walsh 1998). But this technique needs further refinement. The third stage of selection is Large Scale Clonal Trial (LSCT), involving evaluation of individual genotypes in sets and on rather large plots over a long period at different locations. Further, Priyadarshan and Clément-Demange (2004) proposed an alternate method, where the seedlings raised at a moderate spacing will be evaluated at maturity and then cut back for bud wood multiplication. The high yielders will be evaluated directly

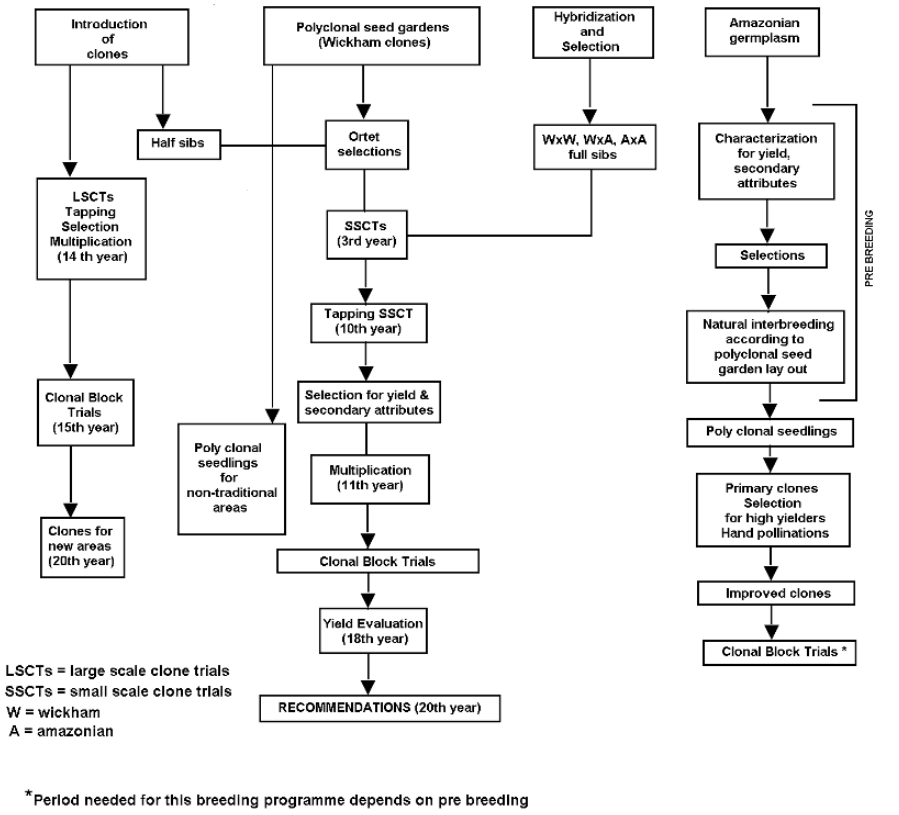


Fig. 13.8 Scheme for breeding clones

as LSCT before they are recommended for a specific location. This can reduce the experimental period from 34 to 20 years (Fig. 13.8).

13.9.5 Breeding Against Stresses

The increased global demand for rubber prompted the countries outside the hitherto traditional zone to focus their attention on the cultivation of rubber (Pushparajah 1983). Rubber was also extended to sub-optimal environments of the countries coming under the traditional belt. This is mainly due to three reasons, namely, increasing demand for rubber, crop diversification under traditional areas and efforts to upgrade the living standards of the people under the so-called sub-optimal environments (Priyadarshan 2003a). Specific areas of China, Thailand, Vietnam, India, Côte d'Ivoire and southern plateau of Brazil fall under sub-optimal environment (non-traditional areas) that experiences one or more stress situations, namely, drought,

low temperature, high altitude, diseases and strong winds. Latitudinal range will be more than 10 °N or S of the Equator (Table 13.4). On the other hand, the traditional rubber growing tracts extend up to 10 °N and S of equator, and offer environmental conditions ideal for rubber cropping. They are: (a) 2,000–4,000 mm rainfall distributed over 100–150 days per annum (Watson 1989); (b) mean annual temperature around 28 ± 2 °C with a diurnal variation of about 7 °C (Barry and Chorley 1976) and (c) sunshine hours of about 2,000 h per year at the rate of 6 h per day in all months (Ong et al. 1998). In a study with hydrothermal index, Rao et al. (1993) rationalized Senai of Malaysia (1° 36′N; 103° 39′E) to be the most suitable area for rubber cultivation and production.

Latitudinal increase will imply fall in mean annual temperature and more prominent winter conditions during November – January. North-eastern states of India, the highlands and coastal areas of Vietnam and south China that lie between 18° and 24 °N are regions well recognized as inhospitable for the crop, exhibiting stress situations like low temperatures and typhoons (Zongdao and Yanqing 1992; Priyadarshan and Gonçalves 2003). It may also be worthwhile to note that rubber areas of China and Tripura fall under the same latitude range, though climatic conditions in vivid pockets of China shall vary because its tropical and sub-tropical regions are undulating and diversified (Priyadarshan 2003a). Southern plateau of Brazil, especially São Paulo (20–22 °S; 450–500 m MSL) is a prominent rubber area. This move to grow rubber seasonally affected by dry and cold conditions is mostly motivated to escape from the climatic conditions congenial to SALB. These areas, apart from high altitude, offer high rainfall that often exceeds the basic requirements. North Côte d'Ivoire is also being experimented with rubber, where warm climatic conditions prevail (Dea et al. 1997). A geo-climatic comparison of various environments with Tripura, China, Brazil, Côte d'Ivoire, Indonesia, Vietnam and Thailand would amply reveal a spectrum of climatic conditions over which rubber is grown. In India, marginal areas delineated as non-traditional zones, spread over to the states of Maharashtra, Orissa, Tripura, Assam, West Bengal, Meghalaya and Mizoram, pose a multitude of hazards, namely, moisture stress, low temperature, wind, high altitude and disease epidemics, apart from altered soil physical properties (Priyadarshan 2003a). Adaptation of existing clones to non-traditional environments with clone specific/area specific tapping schedules and fertilizer inputs are of prime importance to achieve latex yields compared to favorable zones.

13.9.6 Stress Factors and Yield

Low temperature, wind, diseases, climatic changes due to higher altitude and latitude are the stress factors influencing rubber culture. In China, two types of cold damages (chilling injury) have been identified, namely, radiative and advective (Zongdao and Xuequin 1983). In radiative type, the night temperature falls sharply to 5 °C, whereas the day temperature ranges between 15 and 20 °C or above; in advective type, the daily mean temperature remains below 8–10 °C, with a daily

Table 13.4 Geo-climatic feature non-traditional rubber areas of India, Vietnam, China and Brazil

Attributes	India (Agartala)	Vietnam (Pleiku-Highlands)	Vietnam (Dong Hai-Coastal)	China (Hainan Island)	China (Xishuangbanna, Yunnan)	Brazil (Pindorama-Sao Paulo)
Temperature						
Annual mean	30.5	21.8	24.6	23–25	20.9–21.7	22.68
Coldest month mean	17.5	13.3	16.0	16.2–28.3	15.2–15.7	19.5
Extreme minimum	3.8	5.7	7.7	1.4–5.1	1.3–3.7	−2.1
Annual precipitation	1818.0	2272	2159	960–2400	1200–1535	1390
No. of days with rain	129	154	135	95–200	165–193	116
Penman ET_o (mm/day)	3.39	3.1	3.3	–	–	–
Relative Humidity (%)	80–85	80	83	79–86	83–86	64.1–80.3
Wind speeds (m/s)	1.38	2.8	2.8	1.2–4.5	0.5–0.8	1.5
Maximum wind speed (m/s)	35	28	40	80	24	10
Sunshine (h)	2500–2600	2377	1750	1747–2662	1787.8–2152.9	2376
Latitude	22°56′ and 27°32′N	13°59′N	17°28′N	18°10′ and 20°10′N	21°08′ and 22°34′N	21°13 S
Longitude	91°10′ and 92°21′E	108°E	106°37′E	108°35′ and 111°03′E	99°57′ and 101°51′N	48°56′E
Altitude (m)	30	778	7	5.5–328.5	100–1180	562
Soil type	Laterite/Sandy loamy	Ferrasols on basalt	Ferrasols on schists	Latosol/latosolic red soils	Latosol/Latosoic red soils	Red yellow podzolic/ medium texture
Geomorphology	Hillock/low lying areas	Relatively flat	Hills/Flat coastal areas	Hills/Flat coastal areas	Hills/valleys	Hillocks/high altitude ranges

minimum of 5 °C. In both types, under extreme circumstances, complete death of the plant is the ultimate outcome. An analogous atmosphere prevails in northeastern states of India also. Reports from China point out that while clones GT 1 and Haiken 1 can withstand temperatures as low as 0 °C for a short 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 like Haiken 1, SCATC 88-13 and SCATC 93-114 are being evaluated in Tripura. The yielding pattern shows Haiken 1 to be a high yielder among Chinese clones, as compared with RRIM 600, which is used as a local check. Though SCATC 93-114 is known for its cold endurance, it never shows considerable yield potential under the conditions of Tripura, at least during the initial stages on B0-1 panel (Priyadarshan et al. 1998a,b). China has also developed Zhanshi 86, a clone borne out of a random cross between SCATC 93-114 and Wuxing I_3 is cold enduring than SCATC 93-114 (Senyuan 1990). Further, clones like Zhanshi 306-15 (RRIM 600× Guangxi 6–68) give around 10 kg of dry rubber per tree. But these conditions will be tested at the block level. IAN 873, a SALB resistant high yielding clone developed in Brazil shows resistance to cold weather in China (Senyuan 1990) (Tables 13.5 and 13.6).

In India, areas between 15–20 °N of western and eastern side have been identified as non-traditional zones. For instance, the Konkan region of western India experiences long dry periods, high temperatures, low atmospheric humidity and zero rainfall between September and May with daytime temperatures ranging at 38–41 °C during summer months (with a maximum of 47 °C). Though it gets rainfall of 2,430 mm, the distribution is uneven (Devakumar et al. 1998). The atmosphere during summer results in high vapor pressure deficit. Almost an analogous situation prevails in the eastern part of India.

Wind is yet another abiotic stress influencing establishment and growth of rubber. Contributing to the drying effect of drought conditions, it induces regimes of long-lasting steady winds during the dry season in highlands of Vietnam. Wind speeds of 2.0–2.9 m/sec retard rubber growth and latex flow, and 3.0 m/sec or above severely inhibit normal growth. Wind over Beaufort force 10 (more than 24.5 m/sec) play havoc with branch breaks, trunk snaps and uprooting of trees, mainly prevalent in China, during June to October (Watson 1989). Studies in China revealed that clones PR 107 and Haiken 1 can be wind enduring, and PB 5/51 is wind enduring in Tripura (Priyadarshan et al. 1998a). Establishment of shelterbelts, consisting of fast growing and wind resistant species, is one remedial measure being followed in China (Zongdao and Xuequin 1983). But this exercise needs proof, taking into account their effects on total stand as well as the economy of their implementation and land occupation. Alternatively, adoption of judicial pruning of branches and induction of branches at lower height can reduce wind damage from 25.3 to 13.7% (Zongdao and Xuequin 1983). In Côte d'Ivoire, rubber plantations often experience wind damage due to storms occurring at the onset of the rainy season (April–May) (Clément-Demange et al. 1995). In coastal areas and high lands of Vietnam and Sao Paulo state of Brazil also, clones perform differently owing to greater GE interactions (Tables 13.7 and 13.8).

Table 13.5 Yield and secondary attributes of 20 clones being evaluated in Tripura

Clone	Stand (initial)	Girth (mature)	Yield (projected) kg/ha	Crop efficiency*	Wind damage	TPD	Oidium Incidence
RRII 5	Average	Low[1]	1,618 #	0.85	Moderate	Low	S
RRII 105	Good	Moderate[1]	1,635 #	1.0	Moderate	Low	S
RRII 118	Good	High[1]	1,484 #	1.07	High	Mild	MT
RRII 203	Good	Moderate[1]	2,021 #	1.14	Low	Low	T
RRII 208	Good	Moderate[2]	1,534 @	0.93	High	Very mild	MT
RRIM 600	Good	Moderate[1]	1,817 #	0.99	Low	Moderate	T
RRIM 605	Good	Moderate[1]	1,341 #	0.74	Moderate	Moderate	MT
RRIM 703	Average	Moderate[1]	1,741 #	1.21	Moderate	Low	T
RRIC 52	Average	Moderate[1]	1,013 #	0.51	High	Low	T
RRIC 105	Average	High[1]	1,164 #	0.59	High	Low	MT
PB 5/51	Good	Low[1]	963 #	0.74	Low	Mild	HS[$]
PB 86	Good	Low[1]	1,136 #	0.77	Moderate	Low	T
PB 235	Good	High[1]	2,248 #	1.34	Moderate	Moderate	HS
GT 1	Good	Moderate[1]	1,374 #	0.85	Low	Mild	MT
Gl 1	Good	Low[1]	644 #	0.44	Mild	Low	HS
HARBEL 1	Average	Low	739 #	0.58	Low	Low	HS
PR 107	Good	Good[2]	669 @	0.29	Very low	Mild	HS[$]
SCATC 88/13	Good	Good[2]	1414 @	0.67	Low	Moderate	MT
SCATC 93/114	Good	Good[2]	848 @	0.24	Medium	Very mild	MT
HAIKEN 1	Good	Good[2]	1,276 @	0.68	Medium	Mild	MT

[1]Over 13 years; [2]over nine years; * g/cm of the tapping cut; [$] with secondary infection; # BO II panel; @ BO I panel; Projected yield = g/tree/tap x no of tappings x total stand (350). S = susceptible, MT = moderately tolerant, T = tolerant, HS – highly susceptible.

Table 13.6 Yield and secondary attributes of some clones in China

Clone	Site	Girth	Yield kg/ha	Years of tapping	Wind damage	Cold damage	oidium Incidence	TPD	Stand
GT1	Yunnan	Moderate	1,257.2	9	–	Low	Moderate	Moderate	Commercial
RRIM600	Yunnan	Moderate	1,190.3	10	Moderate	Moderate	Moderate	Moderate	Commercial
PR107	Yunnan	Moderate	1,007.9	10	Very low	Moderate	Severe	Low	Commercial
GT1	West Guangdong	Low	994	9	–	Low	Moderate	Moderate	Commercial
93–114	West Guangdong	Low	980.3	9	–	Very low	Moderate	Low	Commercial
YUNYAN 77-2	Yunnan	Moderate	1,874.5	9	–	Low	Severe	Mild	Advanced trial
REYAN 88-13*	Hainan	Moderate	1,700	8	Moderate	Moderate	Severe	Moderate	Advanced trial
REYAN 7-33-97	Hainan	Moderate	1,910	9	Low	Low	Moderate	Moderate	Advanced trial
REYAN 8-333	Hainan	Moderate	2,187	7	Moderate	Low	Moderate	Moderate	Advanced trial
DAFENG95	Hainan	Moderate	1,509.6	8	Low	Low	Moderate	Low	Advanced trial
WENCHANG11	Hainan	Low	1,953.5	10	Very low	Moderate	Low	Moderate	Advanced trial
HAIKEN 1	Hainan	Low	886.6	10	Very low	Moderate	Severe	High	Advanced trial

Tapping systems: the first three tapping years: $s/2 \cdot d/3$, and without Ethylene stimulation, about 75 tapping days per year after first three years of tapping: $s/2 \cdot d/2$, and without Ethylene stimulation, about 110 tapping days per year; *erstwhile SCATC

Table 13.7 Main characteristics of clones under marginal areas of Vietnam Kontum Province (Highlands – 550 m a.s.l. grey soil)

Clone	Girth at opening	Girth (mature)	Yield over ten years in kg/ha (g/tree/tap)	Oidium infestation	Phytophthora leaf fall	TPD
GT 1	Moderate	Moderate	1,191 (46.4)	Moderate	Moderate	Moderate
PB 235	High	Moderate	1,607 (59.8)	Severe	Low	Moderate
PB 255	Moderate	Moderate	1,174 (56.2)	Moderate	Moderate	High
PB 310	Moderate	Moderate	1,659 (52.8)	Low	Low	Moderate
PR 255	Low	Moderate	1,191 (49.8)	Moderate	–	Moderate
PR 261	Low	Moderate	1,197 (64.2)	Mmoderate	–	High
RRIC 110	Hhigh	Moderate	1,558 (66.3)	Low	Moderate	High
RRIM 600	Moderate	Moderate	1,177 (57.9)	Low	High	Moderate
VM 515	Moderate	Moderate	1,539 (63.4)	Moderate	Hhigh	High

TPD = tapping panel dryness

Daklak Province (Highlands- 700 m a.s.l, basaltic soil)

Clone	Girth at opening	Girth (mature)	Yield over seven years (kg/ha)	Oidium infestation	Phytophthora leaf fall	TPD
GT 1	Moderate	Moderate	1,005	Moderate	Moderate	Moderate
PB 235	Moderate	Moderate	998	Severe	Low	Moderate
PB 310	Moderate	Moderate	1,065	Low	Low	Moderate
PR 107	Low	Moderate	669	Severe	–	Moderate
RRIC 110	High	Moderate	1,422	Moderate	Moderate	Moderate
RRIM 600	Moderate	Moderate	1,153	Low	High	Moderate
RRIM 712	Moderate	Moderate	1,170	Moderate	Moderate	Moderate
VM 515	Moderate	Moderate	1,056	Moderate	High	High
RRIV 1	Moderate	Moderate	1,236	Low	Moderate	Low

Quang Tri Province (Coastal region- 50 m a.s.l., basaltic soil)

Clone	Girth at opening	Girth (mature)	Yield over five years (kg/ha)	Oidium infestation	Phytophthora leaf fall	TPD
gt 1	Moderate	Moderate	966	Low	–	–
pb 235	High	High	1,368	Low	–	–
pb 310	High	High	1,005	Low	–	–
rrim 600	Moderate	Moderate	1,355	Low	–	–
lh 82/92	High	Moderate	1,281	Low	–	–

RRIV 1, LH 82/92 = clone bred by RRIV (after Priyadarshan et al. 2005).

13.9.7 Diseases

Despite having all favourable climatic conditions, South American Leaf Blight (SALB) prevents Latin America from developing rubber plantations and it represents a permanent major threat to rubber in Asia and Africa (Chee 1976; Dean 1987; Davies 1997). Breeding work mainly based on backcross technique was undertaken to incorporate resistance in high yielding clones. However, the efforts were in vain due to unknown polygenic nature of the attributes, high variability of the pathogen and multiple interactions between strains and clones (Rivano1997a,b). Simmonds

Table 13.8 Yield and secondary attributes of 20 clones being evaluated in the plateau region of São Paulo State

Clone	Stand (initial)	Girth[1] (mature)	Yield[1] (projected) kg/ha[5]	Crop efficiency[2]	Wind damage	TPD	Oidium incidence
RRIM 600	Good	Moderate	2,100[3]	0.83	Low	Moderate	Low
PB 235	Good	High	1,834[3]	1.10	Moderate	Mild	Severe
GT 1	Good	High	1,679[3]	0.95	Moderate	Moderate	Moderate
PR 255	Good	Moderate	1,700[4]	0.93	Moderate	Moderate	Mild
PR 261	Average	Moderate	1,973[4]	1.21	Moderate	Low	Mild
IAN 873	Good	Moderate	1,890[3]	1.02	Moderate	Low	Moderate
Fx 3864	Good	High	1,755[3]	1.07	Moderate	Low	Moderate
PB 330	Good	Moderate	1,980[3]	0.99	Low	Mild	Moderate
PB 217	Average	Low	2,100[3]	0.68	Moderate	Mild	Mild
PR 107	Good	Moderate	1,870[3]	0.55	Low	Mild	Moderate
IAC 35	Good	Moderate	2,100[3]	0.82	Low	Moderate	Low
IAC 40	Good	High	2,400[3]	1.20	Moderate	Moderate	Low
IAC 56	Average	Moderate	1,900[3]	0.95	Moderate	Moderate	Low
IAC 300	Good	High	2,200[3]	0.82	Moderate	Moderate	Moderate
IAC 301	Good	Moderate	2,100[3]	0.65	Low	Moderate	Low
IAC 302	Good	Moderate	1,800[3]	0.80	Moderate	Low	Moderate
IAC 303	Good	Moderate	5,191[3]	0.82	Low	Low	Low
IAN 4493	Average	High	1,711[3]	0.93	Moderate	Moderate	Low
RO 45	Average	High	1,500[3]	0.70	Moderate	Low	Low
IAN 3156	Low Average	Moderate	2,500[3]	0.60	Moderate	Moderate	Moderate

[1] Over seven years.
[2] g/cm of the tapping cut.
[3] Tapping system 1/2S d/3 6d/7 (with ethephon stimulation 2.5%).
[4] Tapping system 1/2S d/2 6d/7.
[5] Prospected yield = g/tree/tap × number of tapping × total stand (400) (after Priyadarshan et al., 2005).

(1990, 1991) argues that the pathotype-specific resistance (vertical resistance-VR) has resulted in catastrophic failures. Horizontal resistance (HR) should be more effective and durable (Rivano et al. 1989; Simmonds 1990). Amazonian germplasm with resistant sources is yet to be improved for yield. With these views, efforts have been reoriented toward 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).

Resistance to other diseases was studied in some detail. Clones with an early refoliation like AVROS 2037, RRIC 100, RRIM 600, or PB 260, can develop their new leaves before the onset of the rainy season, and so are able to escape incidence of *Colletotrichum gloeosporioides*. In contrast, the widely planted clone GT 1 with late defoliation has been seriously affected in many areas of Malaysia, Indonesia (Kalimantan) and Central Africa. This consequence of early defoliation on the resistance of some leaf diseases of rubber was successfully used for the development and implementation of artificial early defoliation by Ethephon® aerial spray in Cameroon and Gabon for escaping from *Colletotrichum* (Sénéchal 1986). *Corynespora* Leaf Fall Disease (CLFD) has become a major threat for rubber cropping in South-East Asia and West Africa. An escape strategy related with early defoliating clones or by the way of artificial defoliation is not operative. It was demonstrated that the fungus is acting through the emission of a toxin (*cassiicoline*) in the leaves (Onesirosan et al. 1975). Studies conducted under controlled conditions have not put evidence of a significant interaction between clones and strains, but GT 1 is known to be tolerant and PB 260 and RRIC 100 to be highly susceptible (Breton et al. 2000). *Oidium heveae* seems to be favored by rather cold conditions prevalent toward the onset of refoliation (Rajalakshmy et al. 1997). In a comparative study with clones of various geographic origins, SCATC 93–114, Haiken 1 and RRIM 703 were adjudged as resistant to *Oidium* in the traditional areas of India (Rajalakshmy et al. 1997; Alice et al. 2000). While studying sensitivity relationships between clones, Alice et al. (2001) confirmed these results and marked SCATC 93–114, RRIM 703, Haiken 1, RRII 208, RRII 5 and PB 310 as stable sources of resistance over the years. Molecular markers for resistance to *Oidium* are to be developed to augment breeding programs with a cautious approach since the cost effectiveness of this technique is yet to be proved.

13.9.8 Breeding for SALB Resistance

Microcyclus was first identified by K.Ule on wild rubber trees in 1900. Endemic to the Amazonian forests where *Hevea* trees are dispersed in small groves, SALB proved to become epidemic in monospecific plantations, and still stands as an impediment to the extension of rubber plantations in Latin America. Significant efforts to develop rubber plantations in Brazil were initiated by the Ford Motor Company in 1928 with the creation and management of Fordlandia Estate, with

Wickham clones from Asia. However, the severity of SALB attacks led Ford Company to initiate rubber breeding programs since 1937. This breeding programme, initiated by H.Weir and then conducted by C.H.T. Townsend Jr., was first devoted to the collection of resistant sources of Amazonia (like upper Amazon, Acre, Peru and Bolivia, whereas the accessions from the Tapajos river banks proved to be highly susceptible). A crossing program between these sources and Wickham clones was carried out for combining resistance to SALB and higher yield (creation of clones FX). One of the most widely used resistant parents was F4542 from the *Hevea benthamiana* while accessions from other species, notably *Hevea pauciflora* and *Hevea spruceana*, were also assessed. Very low productivity and disease tolerance of the seedlings from Amazonian origin was observed, and the very high susceptibility of the Wickham genetic stock was confirmed. Such efforts were also carried out by Goodyear in Costa Rica (Turrialba station), and by the IAN (Instituto Agronomico do Norte) in Belem (Brazil) since 1940. Since the beginning of 1960s, the new concept of resistance consisting of two forms, vertical and horizontal, was elaborated by Van der Planck (1968). Robinson (1971) described the host-pathogen relationship as a system that breeding should aim to stabilize. A genetic model of the host-pathogen interaction in the case of vertical resistance was proposed (Flor 1955). Polygenic, horizontal resistance is assumed to be expressed in a continuous distribution of the resistance level among the genetic material (quantitative resistance). Horizontal resistance, if only partial, could reduce the capacity of new fungus races to emerge within the pathogenic population. But this general concept had to be confronted with the specificity of the *Hevea/Microcyclus* relationships. Considering rubber and *Microcyclus*, RRIM developed a research program in Trinidad, and Chee (1976) demonstrated that most of the vertical resistances to *Microcyclus* were expressed in the form of a classical hypersensitive reaction. To identify the different existing virulence factors expressed by the host–pathogen interactions (with any race bearing one or many virulence factors), successive studies demonstrated an increasing number of races and progressively evidenced the wide genetic and pathogenic variability of these races (Holliday 1970; Chee 1976; Darmono and Chee 1985; Hashim and Almeida 1987; Junqueira et al. 1988, 1990; Rivano et al. 1989; Rivano 1997a). Virulence was defined in rubber as the compatibility of one *Microcyclus* race with one clone, whereas aggressiveness was defined as the severity of the infection produced by a virulent race on a susceptible clone. With these facts and these new concepts, breeders had to begin their task anew, in a more complex framework.

Breeding the rubber tree for a sustainable, horizontal, polygenic, race-nonspecific resistance to *Microcyclus* appears as mandatory for counteracting the dynamics of new virulent strains. Even if total resistance was unattainable, good tolerance would be appreciable. This effort requires the gathering of a wide genetic variability for resistance, and the use of different identified components of resistance. Some of these components are (1) incubation period (from infection to the first symptoms), (2) infection latency period (from infection to the first sporulation of conidia), (3) duration of the leaf susceptibility period, (4) number of lesions per

leaf area unit, (5) average diameter of the lesions, (6) conidia sporulation intensity (Rivano et al. 1989), and (7) stromata emergence period. Earliness of wintering can contribute partly to avoid the disease development at the resumption of the rainy season. In order to identify horizontal resistance non-specific of any race, it is necessary to check the resistance or tolerance of the clones at field level in different sites (with different sets of races), and/or in the laboratory by controlled inoculation with different types of races, or with polyvirulent races. Controlled inoculation in the laboratory can be applied to leaf discs only for testing virulence in a 'clone-race' couple, for assessing other factors related with the aggressivity of the virulent races, the use of plants in pots is necessary.

These general ideas and concepts on the nature of the resistance have been developed for rubber by many authors (Simmonds 1982; Wastie 1986; Rivano et al. 1989; Simmonds 1990). From these studies, there is no evidence that there could be a strong intrinsic negative genetic relationship between rubber yield and resistance to the disease. Probably, genetic sources of resistance lie in some wild populations of the *Hevea* germplasm, which in the absence of any previous selection, bears the genetic burden of many alleles unfavorable to rubber yield. One possible way could be to improve these Amazonian populations for yield with no introgression from the Wickham population, in order to maintain a high level of resistance. Although requiring a lot of time, the improvement of resistant Amazonian populations for yield might help to select economically viable clones after only one cycle of crossing with Wickham clones or with the best yielding Wickham × Amazonian tolerant clones. Although a long process, this strategy can gain support from genetic determinism of the existing resistance sources with the possible help of molecular genetics.

Since 1992, Centre de Cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD), Michelin and Brazilian universities (Sao Paulo, Viçosa, Santa Cruz), commenced concerted efforts *Microcyclus* challenge. The CMB project ('CIRAD-Michelin-Brazil') were conducted at the field sites of Michelin in Bahia (formerly a Firestone estate) and Mato Grosso and in French Guyana (Garcia et al. 2002a, b). The Mato Grosso estate, located in an 'escape' area (Wickham clones can be cultivated there), is used for conservation of the collections, hand pollination and preliminary testing. The diversity of virulences was proved to be much higher in the Bahia estate than in the French Guyana. Consequently, Bahia estate became the main field experimentation site. The germplasm collected by Firestone and conserved at Bahia estate, together with the Amazonian accessions of 1981 IRRDB collection, were re-evaluated for resistance. The accessions from states of Acre and Rondonia (western) were resistant than those from the Mato Grosso Eastern (western) (Le Guen et al. 2000). Although yielding low, Amazonian clones such as MDF180AS have been identified as expressing horizontal resistance. Eight clones were isolated to be resistant to SALB in the experiments conducted at Bahia (Garcia et al. 2004). The first major resistance gene ever identified and localized on a genetic map is of a minor disease, *Phyllachora huberi* (Le Guen et al. 2000).

13.9.9 Tapping Panel Dryness (TPD)

TPD is a physiological anomaly resulting in the cessation of latex flow and reduction of the tapping stands. Attention was first drawn at the beginning of the twentieth century on seedlings (Rutgers and Dammerman 1914), a phenomenon called 'Brown Bast disease' that progressed with browning of the inner part of the bark, its necrosis and malformation (de Faÿ 1981; de Faÿ and Jacob 1989, Eschbach et al. 1989). There is to be a range of dryness, starting from without any sign of browning to complete dryness (Fig. 13.9). Some researchers assume that there is a progressive evolution from tapping cut dryness to brown bast, whereas others think that they are two distinct diseases differing in their origin. It was found that tapping cut dryness was reversible, depending on tapping intensity and with seasonal variations, whereas brown bast is quite irreversible and leads to the loss of tapped trees (Jacob et al. 1994).

Reversible over-exploitation-induced tapping cut dryness is observed when the exploitation intensity exceeds the physiological capability of the tree to regenerate the latex. Here, the latex cell metabolism is severely disturbed (Bealing and Chua 1972). Before the occurrence of the first visible symptoms (partial drying of tapping cut), sucrose and dry matter are seen to decrease and inorganic phosphorus to increase in the latex (Pakianathan et al. 1982, Sivakumaran et al. 1984) with impaired rubber synthesis (Krishnakumar et al. 2001a). Membrane destabilization leading to bursting of the lutoids and consecutive in situ latex coagulation has been proposed to be associated with the occurrence of an uncompensated oxidative stress within the latex cells (Chrestin et al. 1984, Chrestin 1989). If the surrounding tissues do not seem to be much affected (absence of necrotic symptoms), they show some alteration of their biochemical composition (Yusof et al. 1995, Krishnakumar et al. 1999), with accumulation of proline (Wickremasinghe et al. 1987), decrease in cytokinin (Krishnakumar et al. 1997) and increased bark respiration (Krishnakumar et al. 2001b).

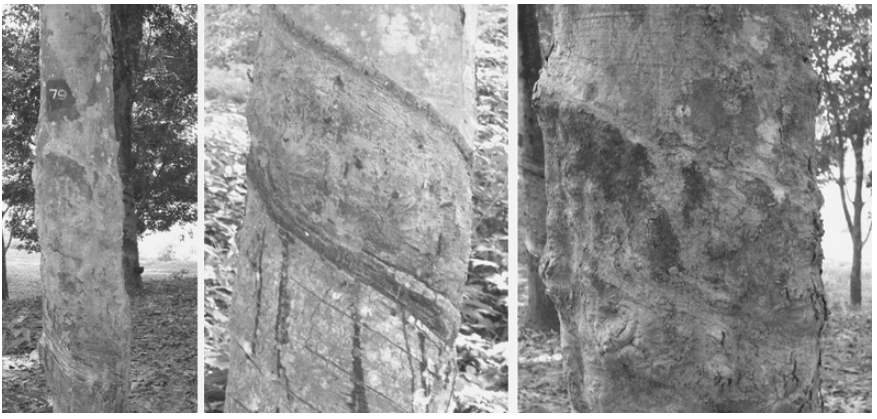


Fig. 13.9 Tapping panel dryness – various forms (*See* Color Insert)

Brown bast appears as the major visible problem as it contributes to the irreversible reduction of the tapped stand. Initial browning develops in the inner bark, seldom from stock-scion union (Jacob et al. 1994) and then necrosis appears on the outer part and spreads through the whole tapping panel. Since the syndrome spreads to neighboring trees, the possibility of a pathogen involvement has been investigated (Nandris et al. 1991a,b). A viroid has been recently claimed to be associated with this bark disease (Ramachandran et al. 2000). However, an alternative hypothesis that brown bast (as well as tapping cut dryness) is a physiological syndrome resulting from abiotic stress appears evident (Jacob et al. 1994; Faridah et al. 1996; Pellegrin et al. 2004). Though many hypotheses like localized soil characteristics in heterogeneous plots (Nandris et al. 2004), impaired cyanide metabolism, differences in the rates of cyanogenic compounds of leaves (Chrestin et al. 2004) have been put forward, none of these explain comprehensively the reason for panel dryness. In addition, terms like *tapping panel dryness, brown bast* and *bark necrosis* are to be classified and well defined wholly or separately to investigate the causes.

In the months just following opening, tapping cut dryness can be detected first but such cases can mask the development of real brown bast. In contrast, after two or three tapping years, regular census of trees affected by brown bast makes it possible to evaluate the development of disease. But clonal susceptibility to dryness seems to be related to the metabolic typology of clones. Clones with intensive metabolic activity and low sucrose reserves being less responsive to stimulation are more susceptible to dryness. Application of latex diagnosis to small scale clone trials (early stage of selection) could indicate dryness. The variation in clonal susceptibility is important and the ranking of clones seems to be roughly the same for the two syndromes, indicating thereby the possible occurrence of common causal factors. Studies on defense proteins suggested that a network of proteins (like chitinase and β-1,3-glucanase) are involved that may function as a biochemical barrier for invading harmful materials, thus becoming a governing factor for the onset of tapping panel dryness (Hao et al. 2004).

13.9.10 G × E Interactions

Yielding trends in Tripura (India), Vietnam and São Paulo (Brazil) that there are low and high yielding periods (Fig. 13.10). Under the hydrothermal situations of Tripura, in a study involving 15 clones of vivid geographical origin, almost all clones show an increment in yield toward the onset of cold season, that is, during October to November. Onset of cold season renders a stimulatory effect to maximize yield and the trend continues till the temperature falls below 15 °C during January. The clones are classified under two categories (a) one showing a slow escalation in yield from April onward, reaching the maximum during November, and receding sharply during December and January and (b) the other with a low yield regime during April to October, and with the peak yield during November and December (high yield regime), then receding during January. While PB 235 comes under the first category,

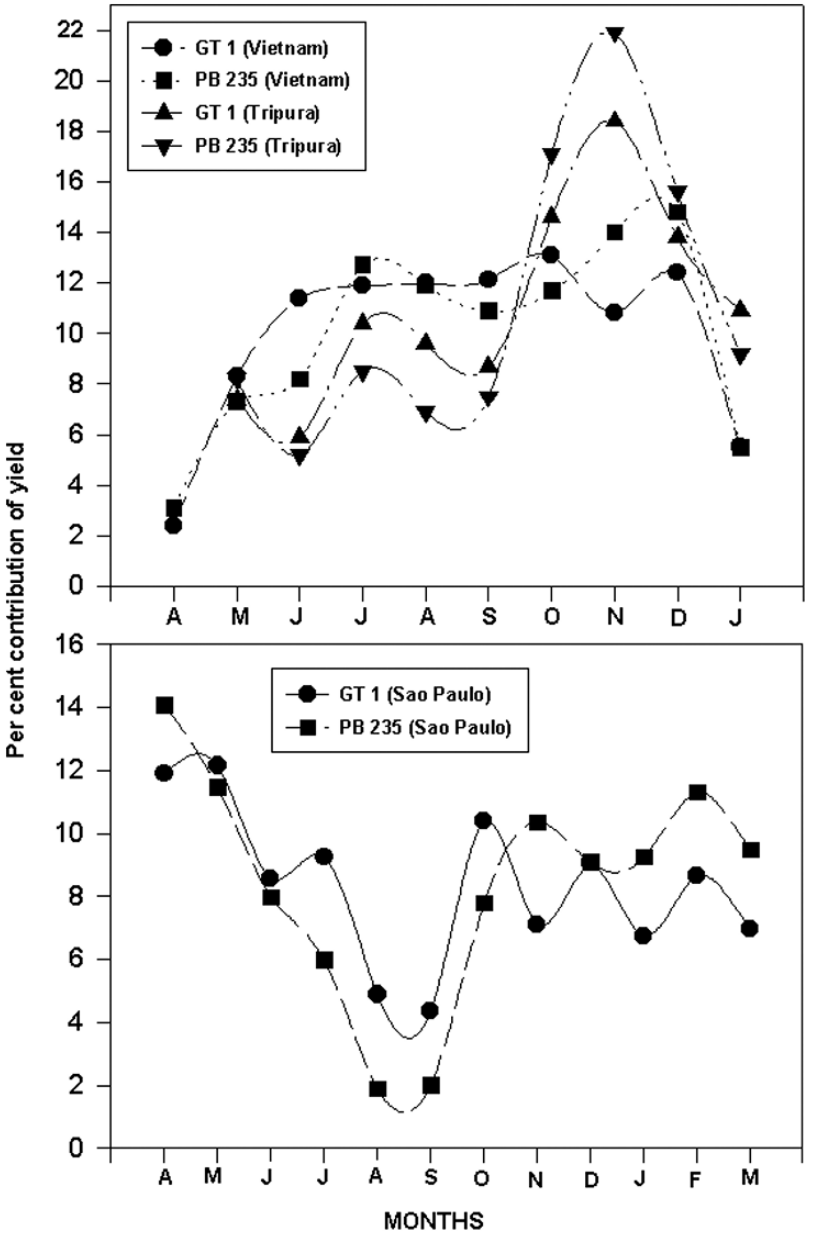


Fig. 13.10 Contribution toward yield in GT1 and PB 235 over months in Vietnam (highlands), India (Tripura) and Brazil (Sao Paulo)

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, 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 micro-environment to influence yield stimulation during October to December (Priyadarshan et al. 2000). The test of heterogeneity for environmental index showed high significance, so indicating that the high stability values of few clones (s^2i) over the years were due to linear effect of the climatic attributes (Priyadarshan 2003b). In São Paulo, RRIM 526 showed higher yield during low regime in comparison to RRIM 600 (Gonçalves, IAC, São Paulo, personal communication). These observations clearly rationalize the selection to be in favor of consistent yielder (Priyadarshan et al. 2000).

Under Malaysian conditions, Tan (1995) accounted 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 different locations. Grouping of clones with high mean and 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. 1998; Costa et al. 2000). In an investigation with seven clones (GT 1, PR 255, PR 261, IAN 873, RRIM 701, PB 235 and RRIM 600), over five environments, IAN 873 was adjudged as the most stable clone over years and locations (Gonçalves et al. 2003). Though GT 1 and IAN 873 were stable for girth and yield respectively, the change in rank of genotypes across the environments suggests that a breeding strategy of selecting specifically adapted clones in a mega environment will ensure the required productivity. Planters will also perceive yield stability as the most important socioeconomic aim to minimize crop failure, especially in sub-optimal environments.

13.10 Applications of In Vitro Culture

Long breeding cycle and larger size of the crop make the breeding process time consuming. Attainment of yield plateau prompted researchers to employ biotechnology tools to induce, increase and exploit new genetic variation. Biotechnology applied to *Hevea* can be discussed under two categories, namely, in vitro culture and molecular genetics. In vitro culture deals with regeneration and propagation, and molecular genetics involves identification, characterization, introduction and expression of novel genes.

Chua (1966) attempting derivation of callus from plumule tissues of seedlings was the first to attempt in vitro culture of rubber in 1960s. Further, the Rubber Research Institute of Malaysia 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). While anther culture was employed to achieve pure lines followed by exploitation of heterosis, micropropagation and somatic embryogeny were worked out to have homogeneous

populations. Though research on in vitro culture commenced nearly 38 years back, even after rigorous experimentations, due to shortcomings toward commercial applicability, these areas are still under experimentation.

13.10.1 Anther Culture

Plants from *Hevea* pollen were initially 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). Carron et al. (1989) enumerated three phases for the production of haploids from anther culture, namely, production of embryos, maturity of embryos and plant regeneration. Embryo production from callus takes nearly 50 days. The balance between callus development and initiation of embryos need to be maintained though use of MB medium with the judicious addition of NAA, coconut water, nitrogen, potassium and sugar for the production of calli and embryos (Chen 1984). The somatic callus then degenerates and the embryos develop from microspores. Sub-culture must be carried out at this stage into differentiation medium in order to ensure maturity of embryos (Chen et al. 1982). 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. For plant regeneration, progressive increment of GA3, gradual withdrawal of other growth regulators, addition of 5-Bromouracil and reduction of sugar shall result 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 toward diploidy (Carron et al. 1989).

13.10.2 Somatic Embryogenesis and Meristem Culture

First plants from somatic embryogeny were obtained simultaneously in China and Malaysia from anther wall (Carron et al. 1989). Later, inner integument that represents mother tissue was used to produce somatic embryos by CIRAD in France (Carron and Enjalric 1982). The successive phases are callogenesis, differentiation, multiplication and plant regeneration. The judicious combination of 2,4-D, IAA and benzylaminopurine (BAP) and an increase in sucrose concentration promotes callogenesis under dark. Cultures are then taken to light with a changed macro-element composition to increase tissue proliferation (Carron et al. 1989). The differentiation medium is enriched with naphthoxyacetic acid (NOA) and BAP with low sucrose concentration. It takes 5–6 months in this medium for the embryos to develop. Carron et al. (1989) claim that nearly 3,000 globular or lanceolate embryoids could be achieved in 4 months. For plant regeneration, addition of indolebutyric acid (IBA) is crucial for promoting root and cotyledon formation. Successful plantlet formation

and acclimatization have been achieved in Haiken 1, Haiken 2 and SCATC 88/13 (Wang et al. 1980). Anther wall requires 2,4-D, NAA and kinetin (KN) for both callogenesis and embryo induction. BAP and zeatin are essential in addition to NAA and 2,4-D. GA_3 is found to increase the number of embryoids. BAP and GA_3 together with lower sucrose level are shown to improve plant regeneration (Sushamakumari et al. 2000). Carron et al. (1995a,b) gave a detailed account of the procedure and media formulation for somatic embryogenesis in *Hevea*.

Significant genotype-medium interactions are experienced in aforesaid procedures (El Hadrami et al. 1991; Montoro et al. 1993). Tissue-medium interactions are also very prominent. This is evident in integument culture, where a different additive of 234 mM sucrose, 9 mM BAP and 2,4-D were needed for embryogenesis. Abscisic acid (ABA) was essential for embryo development (Etienne et al. 1993; Veisseire et al. 1994a,b). Plant regeneration takes 25 days. Low germination percentage and plant conversion are seen as setbacks in this procedure, since the mechanism involved in this technology is poorly understood (Cailloux et al. 1996; Linossier et al. 1997). For instance, initiation and germination of embryos are seen to progress at higher temperature of 24–27 °C (Wang and Chen 1995; Wang et al. 1998). Polyethylene Glycol (PEG) and high $CaCl_2$ are seen to stimulate embryo production (Etienne et al. 1997a; Linossier et al. 1997). Thus, the clone-tissue-media interactions prevail in this technology that necessitates extensive basic studies. More recently, Etienne et al. (1997b) standardized a pulsed-air temporary immersion system for enhancing embryo production, through culturing embryogenic callus under immersion in an autoclavable filtration unit RITA™. Somatic embryo production was three to four times greater than those on a semi-solid medium, to the tune of 400 embryos per gram fresh weight with lesser number of abnormal embryos. Rubber Research Institute of Thailand (Bangkok) and CNRA (Côte d'Ivoire) planted 13,000 embryo-derived plants for field trials (Carron et al. 1995b). Clones PR 107 and PB 260 were highly regenerative. This is a leap toward regeneration of *Hevea* in vitro, since higher regeneration should be ensured to have homogeneous populations and rapid gene transfer system in *Hevea*.

Juvenile stem pieces are desirable for meristem culture that follows three phases, namely, primary culture, multiplication with rooting and acclimatization. Pretreatments with a mixture of Gentamycin, Kanamycin, Chlortetracycline, Chloramphenicol, Rifampicin and the fungicide Benomyl make the explants aseptic. Primary culture involves soaking explants in a solution of growth regulators (IBA and BAP) for 2–3 h. Budding is initiated in MB medium (Carron et al. 1989) without growth regulators. Isolated buds are cultured in half-strength of Lepoivre medium with IBA and BAP. These buds are sub-cultured to form micro-shoots that will in turn be cultured as explants in multiplication phase. Soaking base of the root in IBA-NAA mixture for 3–4 days induces roots. Rooted micro-cuttings can be transferred to soil in 4–5 weeks time. A number of clones like 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 very crucial with a balance between relative humidity and temperature governing the establishment of plants in the soil.

Although gross experimentations were conducted for standardizing in vitro technologies, there had been many setbacks in commercializing these procedures (Carron et al. 1992). A number of aspects inherent in the explant tissue, namely, release of phenols, contamination of bacteria and fungi, recalcitrant status, reduced axillary branches, lack of sufficient juvenility, and above all, increased sensitivity of in vitro raised plantlets toward environmental attributes are responsible for the delay in commercialization. There are, however, remedial measures for these setbacks. Since the contamination of micro-organisms is location specific, newer chemicals are to be tried to raise aseptic cultures. Instead of treating the explants with anti-oxidants, the incorporation of the antioxidant 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, water status of 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 13th to 15th week, respectively, leading to development and maturity of zygotic embryos. However, the smaller size of somatic embryos that can accomplish relatively small mass of starch and protein reserves can lead to lower vigor and conversion rates where vigor is directly related to acclimatization success. Hence, increasing the size of somatic embryos through nutrient supplies deserve priority. To increase juvenility, air layering and progression to three to four generations can be exercised and explants from such source plants shall be used. If commercialized in the strict sense, these technologies can assist breeding programs and enhance productivity significantly.

13.11 Molecular Genetics and Breeding

Due to long generation time and larger size of the crop, new tools could be developed in order to manage germplasm variability and assist breeders in their recombination strategies. Molecular markers were developed that can be classified into three categories, namely, first generation (RFLPs, RAPDs and modifications), second generation mainly based on targeted PCR techniques with Simple Sequence Repeats (SSRs) or microsatellites, Amplified Fragment Length Polymorphism (AFLPs) and their modifications, and third generation markers like Expressed Sequence Tags (ESTs) and Single Nucleotide Polymorphism (SNPs) (Rudd et al. 2005). Though RFLPs are powerful for studying the genetic diversity and mapping, the technology is not preferred now since it is labor intensive, requires large DNA samples, and often involves radioisotopes. Its marker index value is also low (expressed as the

number of polymorphic products per sample) with only 0.10 compared to 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; Yeang et al. 1998), tools like minisatellites (Besse et al. 1993a), RFLPs (Besse et al. 1993b, 1994), mtDNA RFLPs (Luo and Boutry 1995), RAPDs and DAFs (Low et al. 1996; Venkatachalam et al. 2001), AFLPs (Lespinasse et al. 2000a) and SSRs (Besse et al. 1993b; Atan et al. 1996; Low et al. 1996; Roy et al. 2004; Saha et al. 2005) were developed and used in detection and increment of molecular markers in *Hevea*. All marker systems, except SNPs have been applied in *Hevea* so far. The following section deals with various aspects of application of aforesaid techniques in dealing with measurement of molecular diversity, formulation of gene linkage maps, detection of QTLs, and evaluation of laticifer specific gene expression.

13.11.1 Molecular Diversity

Initial studies on isozymic diversity showed the existence of three genetic groups and many new alleles could be found in the Amazonian populations (Chevallier 1988), which was later confirmed through molecular studies (Seguin et al. 1996b). These studies indicate that the genetic diversity available in Amazonian accessions is immense that are yet to be utilized at the molecular level to enrich the Wickham population. However, transfer of such diversity can only be accomplished through gene manipulations at molecular level. Further, analysis of isozymes that are proteic genetic markers were developed at CIRAD through formulation of a diagnostic kit with 13 polymorphic isozymic systems for clonal identification along with a clonal identification database. This kit is proved to be able to differentiate a large set of cultivated clones (Leconte et al. 1994). However, the analyses are to be carried out near the field sites due to fragility of isozymes to varied temperatures, or otherwise, the samples need to be freeze-dried and transported to the laboratory. Hence, initiating molecular studies, Low and Bonner (1985) characterized *Hevea* nuclear genome as containing 48% of most slowly annealing DNA (putative single copy) and 32% middle repetitive sequences with remaining highly repetitive or palindromic DNA. The whole nuclear genome size was first estimated as 6×10^8 base pairs. Estimation with flux cytometry demonstrated 2×10^9 base pairs for *H. brasiliensis, H. benthamiana, H. guianensis, H. pauciflora*, and *H. spruceana* (Seguin et al. 2003).

Fingerprinting through RFLP minisatellite probes could be more powerful and identification of 73 Wickham clones was done with 13 probes associated with restriction enzyme *Eco*RI (Besse et al. 1993b). RFLPs were also used for identification of progeny with two common parents such as PR 255 and PR 261; RRIM 901 and RRIM 905; RRIM 937 and RRIM 938 (Low et al. 1996). Furthermore, Besse et al. (1994) using 92 Amazonian and 73 Wickham clones did an assessment of RFLP profiles. RFLPs, as molecular genetic markers, were used with homologous

probes from a CIRAD *Hevea* bank that showed genetic enrichment brought by Amazonian collections to *Hevea* germplasm, following genetic structure based on geographical collection sites (Besse et al. 1993a; Seguin et al. 1996b). Exceptionally, a clone from Rondonia (RO/C/8/9) showed eight specific restriction fragments and a unique malate dehydrogenase (MDH) allele, indicating its interspecific origin.

In a comparative analysis of SSRs of 20 clones of *Hevea brasiliensis* and six allied species of *Hevea*, Low et al. (1996) measured polymorphism in *H. pauciflora, H. guianensis, H. camargoana, H. benthamiana* and *H. brasiliensis*. Three microsatellite sequences of the gene for hydroxyl methyl-glutaryl-coenzyme A reductase 1 (HMGR-1) were polymorphic. Amplification of $(GA)_9$ region with appropriate primer converted these regions into sequence tagged microsatellite sites (STMS). Polymorphisms in STMS were with regard to band number and band length. While intraspecific polymorphism (in clones of *H. brasiliensis*) was mainly with number of bands, both number and length of bands contributed to interspecific polymorphism. The intraspecific polymorphism must be due to allelic differences arising from recombination. The interspecific polymorphism is the result of DNA insertion/deletion and point mutations. On the other hand, DAF profiles were very distinct for vivid species (Low et al. 1996). These polymorphisms must have played a role in delineating species during the course of evolution. Microsatellites that are tandem repeats of short (2–6 bp) DNA sequences are high utility markers that are codominant, highly polymorphic, abundant and uniformly dispersed in plant genome. It allows precise discrimination of even closely related individuals (Mallet 1995). In a bid to select suitable parents for extending rubber breeding programs, Lekawipat et al. (2003) applied microsatellites in detecting diversity in 40 Wickham and 68 Amazonian accessions. This was accomplished with 170 alleles from 12 microsatellite markers spread among all genotypes. On average, 14 alleles were available per locus. Wickham clones were unambiguously less variable than Amazonian accessions. Also, microsatellites of wild accessions are more polymorphic than cultivated Wickham clones and could be divided into three clusters depending on geographical origin of collections such as Acre, Rondonia and Mato Grosso. This conforms to the earlier studies on isozymes and RFLPs. Two clones (RO/OP/4 20/16 and RO/A/7 25/133) were unique as they do not fall under any cluster due to high level of specific alleles (Lekawipat et al. 2003). A microsatellite-enriched library was constructed in *H. brasiliensis* involving four types of simple sequence repeats like $(GACA)_n$ (10%), $(GATA)_n$ (9%), $(GA)_n$ (34%) and $(GC)_n$ (9%) (Atan et al. 1996). In cooperation with the French 'National Centre for Sequencing', CIRAD developed different microsatellite-enriched libraries in order to identify a large collection of microsatellite markers. Two possible applications are: clonal identification with the advantage of leaf samples sent through normal mail from one site to a distant laboratory and parental identification of seeds collected from an open pollinated seed garden (Blanc et al. 2001).

The evolution of cytoplasmic genome in *Hevea brasiliensis* was slower, due to lack of genetic recombination through meiosis. The estimated mean molecular size of chloroplast DNA (ctDNA) is 152 kb (Fong et al. 1994). Mitochondrial DNA

(mtDNA) was also analyzed with heterologous probes from broad bean by CIRAD and CNRA (Luo et al. 1995, Luo and Boutry 1995). A high mtDNA polymorphism was found in Amazonian accessions. The diversity of mtDNA of 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. Mitochondrial DNA appears to be a valuable tool for studies on classification and phylogeny in plants, resulted more from DNA rearrangements rather than nucleotide substitutions (Palmer and Herbon 1988). Sequencing of a highly polymorphic mtDNA fragment from 23 genotypes showed real potential for phylogenetic analysis in *Hevea* (Luo and Boutry 1995). In chloroplast DNA analysis, much less polymorphism was found, therefore indicating the high level of conservation of this genome.

As a synthesis of these diversity studies, the *Hevea* genetic structure clearly appears as geographically structured (Besse et al. 1994), in relationship with the hydrographic network of Amazonian forest (Luo et al. 1995 and Seguin et al. 1996b). Good relationships are found between the results from 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 resulted from the fragmentation of the Amazonian forest during the pleistocene period, according to the refuge theory presented by Haffer (1982). The mtDNA of Wickham clones has lesser 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. Obviously, this is the reason for the mtDNA profile showing only two clusters (Priyadarshan and Gonçalves 2003). A possible explanation for greater polymorphism in mtDNA of wild accessions is that many must have evolved through interspecific hybridization. The mtDNA polymorphism in wild accessions needs to be exploited fully. A molecular survey of available Amazonian accessions and isolation of competent molecular variants in their progeny are the possible exercises that can give meaningful results.

13.11.2 Gene Linkage Maps and QTLs

The construction of molecular gene linkage map in *Hevea* requires specific methodology because of high heterozygosity. Unlike annual crops, a cross between two heterozygous parents in *Hevea* can yield information up to four alleles, which are segregated further. A comprehensive genetic linkage map of *Hevea brasiliensis* has been formulated recently with the help of RFLPs, AFLPs, microsatellites and isozyme markers (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. Furthermore, homologous markers segregating in both parents were ascertained and consensus map prepared. The

parents used were PB 260 (PB5/51 × PB 49) and RO 38 (F4542 × AVROS 363). F4542 is a clone of *Hevea benthamiana*. The F_1 synthetic map of 717 markers was distributed in 18 linkage groups. These comprised 301 RFLP, 388 AFLP, 18 microsatellite and 10 isozyme markers. Identification of loci was based on mobility of electrophoresis bands, necessitating verification of consistency of the location of alleles in both parental maps. The genetic length of 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 as reduced recombination frequency regions. Though the RFLP markers were well distributed all over the 18 linkage groups, these were insufficient to saturate the map. AFLPs and few microsatellites together facilitated saturating the map. However, these exercises are the initial steps for making a total genetic linkage map of *Hevea* in future. The isozymes were found to inherit following 1:1 ratio (Chevallier 1988). A partially non-random arrangement of duplicate loci observed in RFLP profiles indicates that they have homology descending from a common ancestor (Lespinasse et al. 2000a). The origin of such duplications is still unknown and *Hevea brasiliensis* continues to behave as a diploid.

As mentioned in the introduction, the upsurge of SALB looms over Asia and Africa as a potential threat for rubber plantations in future. Complete resistance to SALB was believed to be monogenic (Simmonds 1990). However, QTLs for resistance to SALB (*Microcyclus ulei*) were mapped using 195 F_1 progeny derived from a cross between PB 260 (susceptible) and RO 38 (resistant) clones (Lespinasse et al. 2000b), which was done in continuation to a genetic analysis done earlier (Seguin et al. 1996a). Eight QTLs were identified for resistance in RO 38 map through Kruskal–Wallis marker-by-marker test and interval mapping method (Lander and Botstein 1989; Oojen van et al. 1992) The F_1 consensus map confirmed results obtained in parental maps. It was further rationalized that the resistance (alleles) of RO 38 has inherited *H. benthamiana* from wild grandparent and no favorable alleles came from AVROS 363, the Wickham parent. Specificity to resistance to different strains was persistent. Two distinct forms of resistance were identified, that is, a complete resistance in the absence of sporulation lesions as in *H. benthamiana* and *H. pauciflora* and a partial resistance with a reduced rate of epidemic development (Le Guen et al. 2003). Investigations on the resistance mechanism were also conducted (Garcia et al. 1995). Le Guen et al. (2003) could detect one major QTL (*M13-lbn*) located in g 13 in RO 38 map responsible for 36–89% of phenotypic resistance. No QTL was detected for resistance against the most pathogenic isolate. Hence, a single isolate can thus completely bypass this polygenic resistance (Le Guen et al. 2007). The effect of QTL was large under natural conditions of French Guiana compared to controlled inoculation. This study should lead to marker-assisted selection (MAS), for identifying resistant genotypes with priority to geographical extent of efficiency and predictable durability. More durable resistance shall be available in other allied species and wild accessions of *Hevea* (Priyadarshan and Gonçalves 2003). However, the selection of clones having durable resistance with polygenic determinism is also important while undertaking such studies (Simmonds 1991). Darmono and Chee (1985) while studying lesion size on leaf discs identified SIAL 263, an illegitimate progeny of RRIM 501, as resistant to SALB.

13.11.3 Laticifer-Specific Gene Expression

Studies on rubber biosynthesis have gained momentum due to inquisitiveness to synthesize artificial rubber. Genes responsible for the key enzyme for polymerisation of polyisoprenes – the rubber transferase – is one of the most abundantly expressed genes in the latex. Genes expressed in the latex can be broadly categorized into three, based on their function: (a) defense genes, (b) genes for rubber synthesis, and (c) genes for allergenic proteins (Han et al. 2000). *Hevein*, a chitin-binding protein is one of the defense proteins that play a crucial role in the protection of wound sites from fungal attack. A cDNA clone (HEV 1) encoding *hevein* was isolated using polymerase chain reaction (PCR) (Broekaert et al. 1990). HEV 1 is of 1,018 base pairs and includes an open reading frame of 204 amino acids with a signal sequence of 17 amino acid residues followed by a 187 amino acid polypeptide. This polypeptide is found to contain striking features like an amino terminal region (43 amino acids) with homology to other chitin-binding proteins and amino acid termini of wound inducible proteins in potato and poplar. It was also seen that their genes were well expressed in leaves, stems and latex (Broekaert et al. 1990). Nearly 12.6% of the proteins available in the latex are defense related (Han et al. 2000).

Mainly three-rubber synthesis-related genes are expressed in the latex, namely, rubber elongation factor (REF) (Dennis and Light 1989; Goyvaerts et al. 1991), HMG CoA reductase (Chye et al. 1992) and small rubber particle protein (SRPP) (Oh et al. 1999). They constitute the 200 odd distinct polypeptides (Posch et al. 1997). The most abundantly expressed gene is REF (6.1%) and then SRPP (3.7%) (Han et al. 2000). These expressed sequences (Expressed Sequence Tags – ESTs) were compared with public databases of identified genes. About 16% of the database matched ESTs encoding rubber biosynthesis-related proteins. Analysis of ESTs revealed that rubber biosynthesis-related genes are expressed maximum followed by defense-related genes and protein-related genes (Han et al. 2000). 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. Studies on laticifer specific gene expression have important implications on selection and breeding. It would be worthwhile to use transcript levels as molecular markers for early selection (Kush et al. 1990). The transcript levels of hydrolytic enzymes, namely, polygalacturonase and cellulase shall be taken as indicators for better laticifer development. It is felt that extensive studies on expression of genes are mandatory to unravel the intricacy of latex production. Detection and evaluation of more molecular markers must also help to breed *Hevea* at molecular level.

13.11.4 Direct Gene Transfer

While the in vitro plant regeneration system in rubber is getting standardized in few laboratories worldwide, efforts have been made to transform *Hevea* cells

through *Agrobacterium tumefaciens* in order to complement plant breeding efforts to increase genetic variation (Arokiaraj et al. 1990, 1994). The anther-derived calli were transformed with *A. tumefaciens* harboring *gus* and *npt*II genes encoding ß-glucuronidase and neomycin phosphotransferase, respectively. Fluorometric assay and enzyme linked immunosorbent assay (ELISA) were performed to prove the expression of *gus* and *npt*II genes respectively in calli and embryoids (Arokiaraj et al. 1996). The expression of foreign proteins in *Hevea* latex was demonstrated in 1998 (Arokiaraj et al. 1998). This transformation appeared stable even after three vegetative generations with no chimeras, indicating a single transformed plant is sufficient to have a population achieved through budding. But this exercise would not be adequate enough to take care of the stock-scion interaction and ensuing yield variation in a clonal population. Transformation of *Hevea* cells with genes for apomixis might be an alternative to circumvent stock-scion interaction. Lately, genes for human serum proteins have been expressed in rubber latex through genetic transformation (Arokiaraj et al. 2002; Yeang et al. 2002). However, the aforesaid studies have to go a long way to significantly assist breeding new clones with traits like resistance and capability to produce secondary proteins.

Another important achievement is with the expression of cDNA for farnesyl diphosphatesynthase (FPS) from a rare rubber-producing mushroom, *Lactarius chrysorrheus*, in *Escherichia coli* (Mekkriengkrai et al. 2004). Such research has to go a long way ahead to commercially produce rubber in vitro or in vivo.

13.12 Conclusions and Future Outlook

It is quite evident that rubber breeding is time consuming and labor intensive. It is here the biochemical and biotechnological tools come handy in assisting the rubber breeder in deriving and evaluating new recombinants/clones in a shorter time span possible. The foremost and essential way to shorten the period taken to derive a clone is to standardize and implement a routine biochemical/molecular marker-assisted selection system to detect high yielding accessions at the juvenile stage. Though many efforts have been incurred in this direction, a dependable method is yet to emerge out of this research. Even if a real 'Marker-Assisted Selection' applied to rubber is still to be developed and validated, so contributing to early selection, it is very probable that molecular markers, especially microsatellites will be substantially used at different levels and will improve the efficiency of rubber breeding. Another emerging area of research is metabolic profiling, that can give insights into constraints like Tapping Panel Dryness and assist in implementing an efficient early selection system. However, this does not mean that the quality of field-testing, associated with the methodology of quantitative genetics and modern statistics must be overlooked.

Rubber is traditionally propagated through bud grafting. Variations among a bud-grafted population are significant that can influence the productivity levels. As in vitro techniques are yet to make a commercial impact in rubber, a propagation

system that can circumvent the influence of stock–scion interactions needs to be achieved. One way is to derive somatic seeds that can produce true-to-mother plants. Introduction of genes for apomixis is the only way to have a homogeneous population, however, one needs to wait further since genes for apomixis are yet to be characterized in other species.

Cryo-preservation of endangered seedling trees is yet another important aspect to be looked into urgently. Since the introduction of Wickham seedlings in Asia in 1876, rubber breeding in Southeast Asia was based on Wickham material with focus on yield improvement, while research in Latin America was devoted to create *Microcyclus*-tolerant and productive clones. This leads to two constant parallel ways of achieving clones suited to the needs of respective regions. Since *Microcyclus* is a threat to Southeast Asian countries, international multi-location experiments must be given priority.

As a perennial crop, rubber breeding has been influenced by the grafting technique, which permitted the development and multiplication of clones evolved from recombinants. In spite of the implementation of early selection techniques, rubber breeding is impeded by the time-length of the selection process, and the limited creation of full-sib families gained through low success rates of hand-pollination. Standardization of a rapid early selection technique is the immediate requisite.

In order to broaden the genetic base, varied attempts were made for introducing new germplasm to Asia, including species allied to *H. brasiliensis*, among which the international IRRDB collection was the most significant. But, due to low yield level of this germplasm and to the length of the breeding process, benefits will be distributed only over a long term. Apart from the creation of new clones for development, this research requires specific germplasm pre-breeding programs to produce new parents for recombination breeding prior to selection. The spectrum of useful genetic variation need to be enlarged, especially through utilizing variable cytoplasmic donors, since most oriental clones received cytoplasm either from PB 56 (through PB 5/51) or from Tjir 1. Biotechnological tools should assist in finding useful variation among the wild germplasm and allied species, especially cytoplasmic genetic diversity and QTLs for resistance to diseases.

There must be an effort to split Wickham population into different groups at the molecular level. This will pave the way delimiting the development of many related clones and of inbreeding depression. Also, with regard to rubber cropping in relation to overall economy (new locations, new objectives, and new economic constraints), rubber breeding needs to address derivation of larger scope of clones adapted to varied biotic or abiotic stresses, and to varied specifications including rubber quality. Consequently, it would be required not only to select elite clones but also to describe the behavior of a larger range of clones at small-scale experimental level and in different environments (development of clonal databases). There are newer clones derived by fast developing economies like China, Vietnam and Thailand. All these and the existing popular clones shall be enlisted in the clonal database, probably under the umbrella of IRRDB. Such databases should provide details of not only clones but also judicious tapping schedules and discriminatory fertilizer doses for new locations. Such a description will also help to suggest better arguments for the

diversification of recommended clones. More emphasis on eco-physiology research could provide the necessary results for achieving some or all of those goals.

Research devoted to SALB resistance involving recurrent backcrossing of Amazonian-resistant clones (mainly the *H. benthamiana* F 4542 or derived clones) with Wickham high-yielding clones to evolve different resistance sources (clone or polyclonal seedling population) needs to be augmented. This strategy could also be applied to specific programs aimed at selecting clones resistant to *Corynespora*, or *Oidium*, or other diseases within integrated approaches.

As rubber wood has become an ancillary source of income, rubber breeding now has to integrate new traits, especially traits based on architecture and on biomass production, in order to produce better-optimized 'latex-timber' clones.

Although in vitro culture still meets different obstacles, somatic embryogenesis is the gateway for the implementation of targeted genetic transfers, so accelerating genetic progress on agricultural traits or widening the scope such as the possible production of proteins in the latex.

International cooperation and the interface between research institutions and product transforming private sector must be promoted in order to amplify the efficiency and efforts of rubber breeders. Even if the needs of smallholders are addressed with priority, industrial estates have provided a significant contribution in land facilities and logistics for large-scale and long-term testing of clones, and would continue to do it. With many projects toward this direction, IRRDB can play a key role in this field of cooperation in rubber breeding.

Small holdings are the backbone of rubber economy. With fluctuations in domestic and international prices, it is highly essential to train the farmers on feasible investment procedures. This aspect should occupy a significant place in the participatory breeding programs that can immensely help the farmers to educate and innovate themselves.

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