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Chapter 5

Germplasm resources

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1. INTRODUCTION

The Para rubber tree has its origin in the Amazon forests. During the early stages of introduction, the exploration for the genetic material was limited to the Para region of Brazil. As such, this represented only a miniscule of the vast area where the tree has been distributed in its original home in the wild (Schultes, 1977). The locality mostly housed only *Hevea brasiliensis* and the collection did not represent much species diversity or naturally occurring hybrid progenies of the genus.

Only a limited number of genotypes was successfully introduced into other centres. As commercial cultivation progressed, only the very promising trees among the genotypes were propagated and used. When vegetative propagation was successfully introduced in *H. brasiliensis*, their number became further restricted. Crop improvement programmes were mainly oriented towards higher yield. These events led to the cultivation of only a few high yielding genotypes in the rubber growing regions.

Such directional breeding towards high productivity and monoculture of only the high yielding varieties resulted in further gene erosion, and it became imperative to widen the genetic base of *Hevea* in the East. International Board of Plant Genetic Resources has placed rubber among the crops to be given top priority for the conservation of its entire gene pool (IBPGR, 1984). Genetic variability being the back bone of crop improvement programmes in all species, collection and conservation of plant genetic resources are among the most important activities in plant breeding. The wild germplasm constitutes the basic raw material that not only sustains the current crop improvement programmes, but is also required in future to face the unforeseen challenges from virulent strains of pathogens and abiotic stresses like drought and cold. Enrichment of the existing genetic stock and building up of disease resistance necessitate the introgression of wild genes brought in from the primary centre of origin of *Hevea*. Moreover, all the available germplasm, both from the primary and the secondary diversity centres, has to be conserved and maintained as gene reservoirs for future utilization.

2. GENETIC BASE

All the eastern clones of *H. brasiliensis* originated from the relatively very narrow genetic pool referred to as 'Wickham base' (Simmonds, 1989). Though utilization of this base for crop improvement did achieve remarkable results during the early phases, it gradually recorded a slowdown in progress and more or less reached a plateau for productivity. The need for expansion of rubber cultivation under suboptimal conditions demanded an array of genetic materials possessing appropriate adaptability.

2.1 Habitat diversity

The different species of the genus occur in an area which covers the whole of the Amazon basin (Fig. 1), extends southwards into the foothills of the Mato Grosso region and northwards into the upper part of Orinoco basin, the lower slopes of the Guiana highlands and parts of the lowlands of the Guianas. This large area covers parts of Brazil, Bolivia, Peru, Colombia, Ecuador, Venezuela, French Guiana, Surinam and Guyana up to an altitude of about 800 m (Webster and Paardekooper, 1989). All the species of *Hevea* grow together here, and since there is no cytological barrier for hybridization between most species, natural hybrids and variants are also present in the population.

The genus *Hevea* exhibits a wide range of ecological preferences and grows in diverse habitats like moist but well-drained and seldom-flooded soils, often sandy and rocky soils, flooded swamp forests, in muddy banks and often flooded islands, regularly flooded sandy soils, quartzitic soils, dry savannahs and seasonal muddy swamps (Wycherley, 1977). The different species exhibit wide variation in growth (from giant trees to shrubs) and other morphological traits.

H. brasiliensis and *H. guianensis* mostly thrive well in well-drained, moist soils, while *H. pauciflora* usually prefers well-drained sandy or rocky regions. *H. benthamiana*, *H. spruceana* and *H. microphylla* are distributed along the swampy and flooded localities. Species like *H. nitida* and *H. rigidifolia* are seen mostly in the well-drained dry rocky and quartzitic soils, and *H. camporum* grows in the dry savannah.

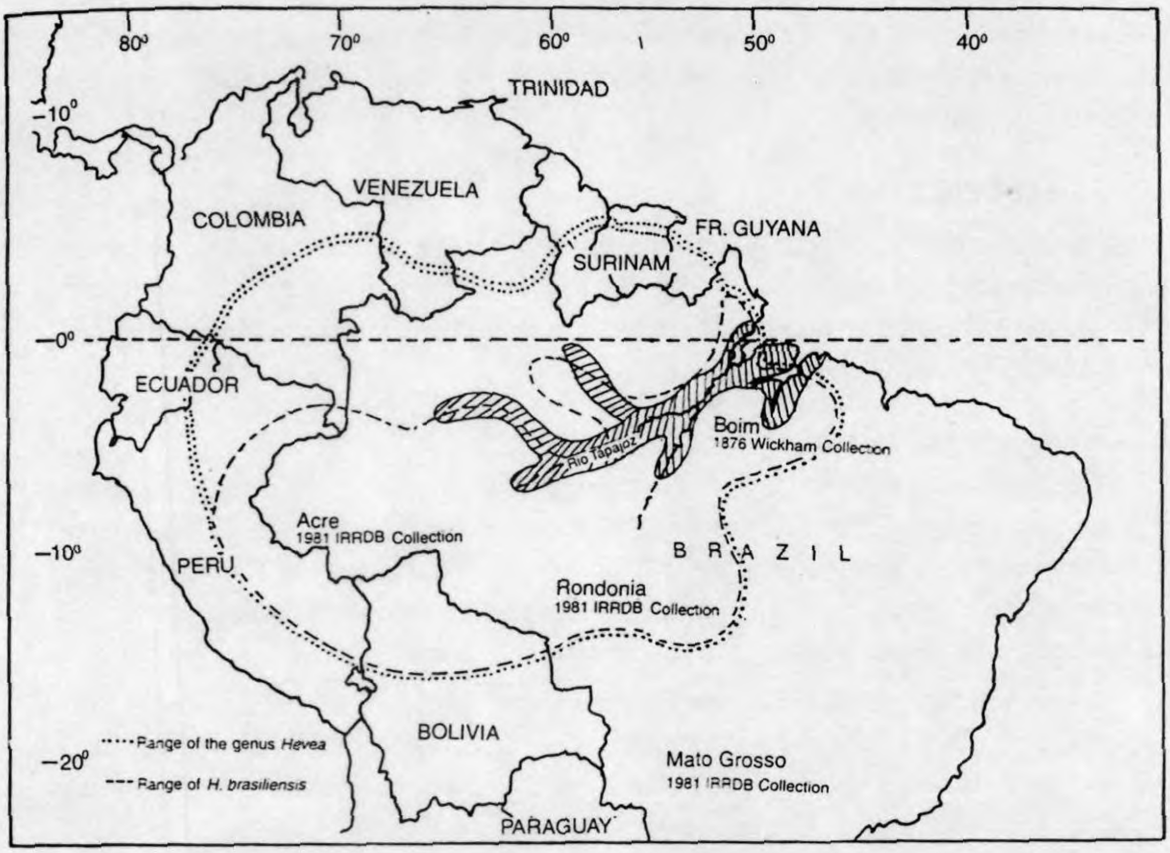

Fig. 1. Distribution of the genus *Hevea*

2.2 Potential threats

Though monoculture gives higher returns in terms of yield, it has serious negative implications. As all the plants have the same genetic constitution, such areas become highly vulnerable to disease outbreaks in epidemic proportions. Such instances have been reported in other crops and the outbreak of potato blight in Ireland in the 1840s, coffee rust in Ceylon in the 1870s, and wheat stem rust in USA in 1954, are some well-known examples (USDA, 1973). Had there been genetic variability, the genes for disease resistance would have buffered the effect of the attack and at least some of the plants would have withstood the pathogen. Rubber too is under the potential threat of the fungus *Microcyclus ulei*, causing the devastating South American leaf blight (SALB) which is specific to *Hevea* species (Chee and Holliday, 1986; Edathil, 1986). It is fortunate that the disease has so far been confined to the American hemisphere. None of the eastern clones are reported to possess resistance to SALB. There are also indications of erosion of genes controlling resistance to *Oidium* and *Gloeosporium* in the original Wickham material (Wycherley, 1977). Evolution of new strains of fungi and herbivorous pests (Thankamma, 1974; Jayaratnam *et al.*, 1991) also poses a threat. Instances of less serious diseases becoming more severe have also been reported. In Sri Lanka, *Corynespora* leaf spot disease, which was until then a minor disease of *H. brasiliensis*, suddenly assumed epidemic proportions and devastated large tracts of the clone RRIC 103 in the 1980s, resulting in its withdrawal from planting recommendations (Liyanage *et al.*, 1989). Similarly, in Malaysia, the clone GT 1 was also severely affected by this disease (Tan, 1990). Very recently, *Corynespora* has been observed to be a potential threat to clones like RRII 105 and RRIM 600 in restricted localities in Dakshin Kannada district of Karnataka in India (Jacob, 1997). As both the host and the pathogens coexist in the wild habitat, search for genes showing resistance should be made in the wild population.

3. COLLECTION

The main objective of germplasm collection is to make available maximum variability present in the crop species, its wild relatives and related species. The hunt should be aimed at capturing all the genetic diversity so that no valuable material is left behind (Arora, 1981).

3.1 Sources of germplasm

The spectrum of *Hevea* germplasm can broadly be grouped into those belonging to the primary centre of diversity and those in the secondary centres. The primary centre of diversity not only accommodates the wild genotypes of the genus but also the naturally occurring hybrids, morphotypes and variants. Commercial cultivars, selections and genetic variants form the genetic resources in the secondary centres.

In the primary centre *H. brasiliensis* occurs in almost half the range of the genus in geographical distribution, and the area comprises all of the Amazon basin south of the river and the headwater of tributaries of the Paraguay river. It spreads to the lowermost portions of the tributaries on the left bank, penetrates north of the Amazon river and the affluents on the right bank of the lowermost Negro river. About half of the natural range occurs in low areas subjected to annual floods, but the species also grows near flood banks. In the south-west sector comprising Peru, Bolivia, Acre, Rondonia and Mato Grosso, the species occurs in relatively high and well-drained plateaux between rivers (Schultes, 1987).

Commercial cultivation is very often restricted to high yielding cultivars which may become obsolete when newly improved ones are released. As such, it is not unlikely that the older materials disappear in course of time. These, however, are valuable genetic materials and most of the countries where *H. brasiliensis* is an introduced crop, maintain the variants, selections and cultivars as an insurance against gene erosion.

3.2 Plant quarantine

Plants, whether in the wild or under cultivation, are subject to the attack of diseases and pests. Some of the diseases and pests have limited distribution while many others have worldwide occurrence. Introduction of genetic materials from other countries can also introduce diseases and pests along with the materials if they are infected, and the problem has become complex with the quicker modes of transport available now. All countries have legal enforcement measures, known as plant quarantine regulations, to check this problem. Under the regulations, only permitted plant materials can be imported and they shall be accompanied by valid import permits, phytosanitary certificates and additional stipulated declarations. Domestic regulations are to be observed in the exporting country. In the importing country, post-entry inspection, disinfection treatments, *etc.* are to be done.

H. brasiliensis can be propagated both by generative and vegetative methods, the former through seeds and the latter through grafting of buds taken from budwood. As such, both seeds and budwood have to be handled in quarantine.

Detailed guidelines for importers on quarantine procedures and clearance of seeds/plants/plant materials for propagation/consumption, *etc.* have been issued by the Government of India (Ministry of Agriculture, 1997). Any plant material imported to India shall be free from diseases and pests. *Hevea* planting materials imported shall be accompanied by a special certificate indicating that the state of origin and the materials are free from *Microcyclus ulei* and *Sphaerostilbe repens.* Import of rubber (and all species of *Hevea*) into India from America or West Indies is prohibited (GOI, 1989). India is one of the signatories of the Asia and Pacific Plant Protection Commission Agreement, 1954 and it is obligatory to observe plant quarantine procedures.

3.3 Early introductions

After the Wickham material, there were, several attempts to introduce *H. brasiliensis* germplasm to the Asian centres. Introductions including those of other species, were made from Brazil and Surinam to Java in the 1890s and 1910s (Dijkman, 1951). However, these introductions were very few in number and did not contribute significantly to the gene pool in the East. It is generally agreed that the bulk of the eastern plantations have originated from the Wickham collection.

In Malaysia, seedlings of *H. brasiliensis, H. benthamiana, H. guianensis, H. spruceana* and *H. pauciflora* as well as hybrid seeds of different provenances, were imported from Brazil during 1951-52 (Brookson, 1956; Tan, 1987). Malaysia also imported IAN clones from Brazil via Sri Lanka in 1952, of which IAN 873 and IAN 717 have comparatively good yield and tolerance to *Colletotrichum* and to some physiological races of *Microcyclus* (Ong *et al.*, 1985). There were several other introductions of clones from Brazil to Sri Lanka and Malaysia during the 1960s.

3.4 Wild germplasm collections

Collections of wild *Hevea* germplasm were attempted from time to time. A few expeditions were made between 1943-46 in the Colombian forests, the most important of which was the Schultes' collection of 1946, when both seeds and budwood were collected (IRRDB, 1978). In Brazil, the first seed collection was made in Rondonia in 1945, which was however destroyed by fire in 1950. In 1952 and 1962, further collections were made in the same area (IRRDB, 1982). Budwood collections were made in the Amazonas, Acre, Para and Rondonia from 1972 to 1982, when different species including *H. brasiliensis* were collected (IRRDB, 1978; Goncalves *et al.*, 1983). A Franco-Brazilian prospection during 1974 in Acre and Rondonia, collected budwood from 60 high yielding trees. Out of this, 41 were later introduced into Ivory Coast (IRRDB, 1978). Ten attempts were made between 1945 and 1982 in the Amazon forests, in Acre, Amazonas, Para and Rondonia, by scientists of Brazil and the germplasm materials collected included *H. benthamiana, H. camargoana, H. guianensis* and *H. pauciflora* (Goncalves *et al.*, 1983).

The most important expedition was the large-scale collection of wild *Hevea* germplasm from Brazil launched by the International Rubber Research and Development Board (IRRDB) and EMBRAPA of Brazil in 1981, which can be considered as a major achievement in the history of the collection of rubber germplasm with a defined objective of strengthening and broadening the genetic base of *H. brasiliensis.*

3.5 1981 IRRDB collection

Hevea breeders have recognized the urgent need for collection and conservation of wild *Hevea* germplasm for quite some time. The Amazon wilderness, along with its natural vegetation, has been threatened by rapid deforestation and urbanization. Realizing the seriousness of the situation, the IRRDB organized a collection expedition to the Amazon rain forests in 1981. This was the first and the major collective attempt to enrich the rubber plantation industry through genetic improvement. This expedition, organized in collaboration with Brazil, collected 64736 seeds from the states of Acre, Rondonia and Mato Grosso (Fig. 1) and budwood from 194 high yielding trees (ortets), which were not affected by *Microcyclus* and *Phytophthora* (Ong *et al.*, 1983).

The collection was carried out from January to March 1981. The budwoods, seeds and seedlings were despatched to the primary nursery at Manaus in Brazil, where 50 per cent of the entire seed collection was retained by Brazil as per an agreement between the two organizations. Of the remaining, 75 per cent was sent to the Asian multiplication and distribution centre in Malaysia, while the remaining 25 per cent was sent to the African centre in Ivory Coast. Phytosanitary measures were taken at each stage to ensure that no pathogen was transmitted along with the material. After being multiplied at each of the distribution centres, budwoods of the various genotypes were distributed to member countries. The entire budwood collection of ortets, on the other hand, was budded and raised in the primary centre at Manaus. The materials thus established were maintained there for approximately 15 months. One hundred and fifty clones were successfully raised and the budwood was despatched to the secondary nursery at Guadeloupe which served as the intermediate quarantine station. From this station, budwood was despatched to the Malaysian and African centres. One hundred and thirty one ortets survived in Malaysia (Ong and Ghani, 1990).

Introduction of the 1981 IRRDB wild germplasm into India was effected from the Malaysian centre. The materials were received at Chennai (Tamil Nadu) and Calcutta (West Bengal) where port of entry inspection and quarantine treatments were carried out. The materials from the former were multiplied and established at the RRII Central Experiment Station at Chethackal (Kerala) and those from the latter at two Regional Research Stations, one at Agartala (Tripura) and the other at Guwahati (Assam). The total number of accessions established in the different centres in the country is 4967, 3617 being in Kerala and 1350 in the two north-eastern states.

4. INTRODUCTION OF CULTIVARS

The genetic resources in the secondary centres are also an important factor in broadening the genetic base. Rubber growers prefer modern clones to the older ones and during the process of replanting, older clones, some of which are the donor parents of modern high yielders, are discarded. This channel of gene erosion can be checked only by conserving the obsolete clones. Introduction of proven genotypes, besides broadening the breeders' stocks, makes available clones developed elsewhere for adaptability studies in the introduced countries. Being a perennial crop, where the breeding cycle is very long and has many other constraints, introduction of cultivars and genetic materials of breeding value from secondary centres is advantageous. India has introduced 127 clones (Table 1) till 1995 from eight secondary centres. These introduced clones, along with other indigenously developed ones, are being conserved in five germplasm gardens by the RRII (Plate 7. a).

Table 1. Introduction of clones into India from other countries

Year	Country of origin	Clones
Before 1956	Indonesia	Tjir 1, Tjir 16, AVROS 49, AVROS 255, AVROS 352, LCB 1320, PR 107, BD 5, BD 10
	Malaysia	PB 25, PB 86, PB 186, PB 5/60, PB 5/139, PB 6/9, PB 6/50, Pil B 84, Pil D 65, Gl 1, Ch 2, Ch 4, Ch 8, Ch 26, Ch 29, Ch 30, Ch 31, Ch 32, CHM 3, S. Reko 9, Lun N
	Sri Lanka	Mil 3/2, Hil 28, Hil 55, Wagga 6278, War 4
1956	Malaysia	RRIM 501, RRIM 526, RRIM 601, RRIM 602, RRIM 603, RRIM 604, RRIM 605, RRIM 607, RRIM 608, RRIM 609, RRIM 610, RRIM 611, RRIM 612, RRIM 615, RRIM 617, RRIM 618, RRIM 620, RRIM 621, RRIM 622, RRIM 623
	South America	IAN 45-717, FX 516, F 4542
1962	Malaysia	RRIM 513, RRIM 519, RRIM 600, RRIM 628, PB 206, PB 213, PB 217, PB 5/76
1963	Malaysia	RRIM 632, RRIM 636, RRIM 701, RRIM 707, PB 5/51, PB 5/63, PB 28/59, PB 28/83
	Indonesia	GT 1
1964	Malaysia	PB 215, PB 230, PB 235, PB 240, PB 242, PB 252, PB 253
1966	Malaysia	RRIM 703, RRIM 704, RRIM 705, RRIM 706, Ch 153
1969	Liberia	Harbel 1
	South America	IAN 45-713, IAN 45-873
1972	Sri Lanka	RRIC 7, RRIC 36, RRIC 45, RRIC 52, RRIC 89, RRIC 100, RRIC 102, RRIC 104, RRIC 105, Nab 17
1979	Malaysia	PB 260, PB 310, PB 311
1984	Thailand	KRS 25, KRS 128, KRS 163
	China	SCATC 88-13, SCATC 93-114, Haiken 1
1985	Malaysia	PB 255, PB 280, PB 312, PB 314, PB 330
1991	Ivory Coast	IRCA 18, IRCA 109, IRCA 111, IRCA 130, IRCA 230
1993	Malaysia	RRIM 712, RRIM 722, RRIM 728
1995	Indonesia	BPM 24, PR 255, PR 261
	Sri Lanka	RRIC 110, RRIC 130

5. CONSERVATION OF WILD GERMPLASM

Germplasm conservation aims at maintenance of the genotypes in a viable form so that it is available when needed. *In situ* conservation of the materials in their original habitat, and *ex situ* conservation where materials introduced from elsewhere are maintained in special nurseries or fields, are both possible in the case of *Hevea*. The former poses several sociological, economical and logistic problems and therefore, *ex situ* conservation is advantageous and generally adopted in the secondary centres. Cryopreservation and *in vitro* conservation, though followed in several field crops, are not commonly followed for *Hevea*. Irrespective of the method, it is important to assign a proper accession number to each genotype. Care should be taken to maintain the identity of the materials and ensure that there is no mix up.

Hevea germplasm has to be conserved in field gene banks, both in source bush nurseries and in gardens or arboreta. The former is intended for maintenance and multiplication while the latter is used mainly for breeding purposes.

5.1 Conservation nursery

All the genotypes introduced are vegetatively multiplied and maintained in insurance source bush nurseries, preferably in two geographically distant locations as a precaution against calamities. The spacing is adopted in such a way that a preliminary assessment is also possible at the nursery stage. It is also important to maintain a minimum number of plants of each genotype. The commonly adopted spacing is 1 m between plants and the minimum number is five per genotype. To ensure identity and accessibility, authoritative layout sketches, sign boards and registers are maintained.

5.2 Field conservation gardens

The field conservation gardens or arboreta are established along with the source bush nurseries, with the primary objective of providing the breeding material of genotypes identified as promising from the evaluation trials. Information on tree habit, flowering and wintering pattern, floral and seed morphology will also be generated much ahead of field trials. Such arboreta will also serve as an insurance against any loss in source bush nurseries. The spacing adopted for commercial and experimental planting is also used here, and a minimum of five trees per genotype should be maintained.

6. CHARACTERIZATION AND EVALUATION

While the wild genotypes are a treasure for the plant breeder, their effective utilization in crop improvement has to be preceded by characterization and evaluation. Proper documentation of all the data generated, preferably in a computerized form for easy retrieval, is essential. Characterization of all accessions is done on the basis of appropriately designed descriptors. Only highly heritable traits that are expressed in all environments are used. Evaluation of *Hevea* germplasm is carried out in two stages.

6.1 Preliminary evaluation

Preliminary evaluation is done for all accessions. An augmented design with five plants per genotype, is adopted. The wild accessions are not replicated, though three to five controls are planted at regular intervals. Characterization can be done at this stage.

Morphological features and growth characteristics which are easily measurable are given importance. Additional agronomic traits as desired by the users are also given due consideration. The important characters are plant height, diameter, vigour, growth habit, foliar features, anatomical features, disease incidence, *etc.* Juvenile yield is assessed by appropriate microtapping techniques wherever possible. All these together aid in preliminary identification of genotypes useful for specific purposes. Molecular markers are also helpful in identifying such genotypes. Identification of markers for *Oidium* resistance (Shoucai *et al.*, 1994) and *Phytophthora* resistance (Jacob, 1996) has been attempted using the random amplified polymorphic DNA (RAPD) technique.

6.2 Further evaluation

Only the genotypes found promising in the preliminary evaluation are subjected to further evaluation due to practical considerations. The general evaluation is done in an appropriate location, adopting a suitable statistical design. However, specific screening for any attribute is done in a location where the problem exists. Such hot spot screening alone can lead to identification of genotypes which have adaptability to tide over specific agro-environmental constraints.

Rubber cultivation under suboptimal conditions demands clones with specific attributes. Some of the wild genotypes may possess such traits, but proper evaluation and identification of the ideal genotypes are essential.

The wild germplasm of *Hevea* exhibits very high variability, both within and between the provenances from where they were collected, with respect to several characters (Plate 7. b-f). Studies conducted at the RRII on some of the genotypes at the nursery stage showed that apart from the wide variability with respect to vigour, juvenile yield and certain morphological characters (Annamma *et al.*, 1986; 1988), some accessions possessed superiority for desirable individual traits, though they were poor in juvenile yield (Abraham *et al.*, 1992; Madhavan *et al.*, 1996). Certain characters like bark thickness, number of latex vessel rings and density of latex vessels per ring had high heritability values which indicate their future promise. The genotypes from Mato Grosso were superior to those from Rondonia and Acre for many morphological, anatomical and growth characteristics (Abraham *et al.*, 1992; Reghu *et al.*, 1996). From the wild germplasm, genotypes with potential for resistance to *Phytophthora* and tolerance to drought have also been isolated. In the nursery stage, Mato Grosso genotypes were observed to show better tolerance to *Phytophthora* than the Acre or Rondonian genotypes (Mercy *et al.*, 1995). Two wild genotypes of Rondonian origin were distinct in growth pattern, colour of flowers, presence of basal disk in male flowers and in the shape of fruits and seeds (Plate 7. d-f) indicating a probable interspecific origin (Madhavan *et al.*, 1997). A field trial in French Guyana revealed the presence of continuous variability for resistance to *Microcyclus ulei* (Clement-Demange *et al.*, 1998).

Thirty three new alleles for 14 loci had been observed in the wild germplasm using electrophoretic methods (Chevallier *et al.*, 1988). Significant genetic distance, based on leaf morphology studies (Nicolas *et al.*, 1988) and isozyme markers (Clement-Demange and Nicolas, 1987), between the 1981 germplasm and the 1876 Wickham material had also been reported. These studies also revealed distinct differences between Mato Grosso and Acre, with Rondonia falling in between. Restriction fragment length polymorphism (RFLP) analysis of 164 wild and cultivated genotypes indicated that the wild germplasm has the potential for enrichment of genetic resources, though the cultivated clones have conserved a relatively high level of polymorphism despite the narrow genetic base and high level of inbreeding. Wild populations appeared to be more polymorphic than the cultivated ones, the Rondonia and Mato Grosso genotypes being the most variable with RFLP, and Acre genotypes being most variable with isozyme markers (Besse *et al.*, 1994). Evaluation of germplasm at RRII using RAPD techniques also indicate that there is considerable variability in the wild germplasm compared to the Wickham clones. Based on the genetic distance data, Mato Grosso genotypes were found to form a distinct cluster, well separated from the Acre and Rondonia clusters.

Preliminary tapping trials, over three years, of germplasm materials in Sri Lanka revealed that GPB 1 (42-552) is a promising accession for yield (RRISL, 1995). Rondonian (RO) genotypes, in general, ranked highest for yield potential from among the accessions from 16 provenances, both in the African centre and in the Malaysian centre (Clement-Demange *et al.*, 1998).

7. UTILIZATION

Value of the wild germplasm will remain little noticed and unexplored unless the potential ones are effectively utilized. Though the scope for direct selection for economic traits among the wild genotypes of *Hevea* is limited, their potential as breeders' stock is great, in view of the broad genetic base and posession of specific traits.

Despite non-synchronization in flowering, four cross combinations were carried out successfully between the 1981 Amazonian genotypes and improved cultivars in 1993 and 13 combinations in 1995 in Malaysia (RRIM 1994; 1996). In India, among 12 cross combinations involving wild genotypes and popular cultivars, juvenile yield at two years growth was highest in a hybrid involving RRII 105 and RO/JP/3/6 (RRII, 1994). Nine wild genotypes were utilized as parents for hand pollination with two popular cultivars in 1997 also (RRII, 1999). Eighteen potential genotypes have been identified and planted in hand pollination gardens in Vietnam (Vo Thu Ha *et al.*, 1994). Hybrid progeny from Wickham and unselected Amazonian clones in Ivory Coast recorded wide variability for tree growth and shape with probable heterotic effects for the former, but their yield potential was low.

In view of the increasing demand for rubber wood, screening for accessions with superior latex-timber traits in the wild germplasm is gaining importance. Twenty fast-growing genotypes were identified as potential clones for timber production, the total wood volume ranging from 1.44 to 2.52 m^3 per tree in Malaysia (RRIM, 1996). Fifty vigorous clones had been identified for timber value in Ivory Coast (Clement-Demange *et al.*, 1998).

Effective utilization of the 1981 Brazilian genotypes is only in the infant stage and several of them still remain to be characterized, catalogued and evaluated. Early indications, however, are promising, as genes for desirable attributes like vigour, disease resistance, *etc.* appear to be present in this collection.

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