Germplasm Conservation, Utilization and Improvement in Rubber

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Introduction

The para rubber tree of commerce, *Hevea brasiliensis Mull. Arg.*, produces around 99 % of the world's natural rubber, an industrial raw material of strategic importance. The rubber plantations in the east, producing a major share of the total production, are reported to have originated from a narrow genetic base. From this small genetic foundation, substantial improvement in productivity has lready been achieved. However, there has been a slow down in genetic advance in recent years, which is attributed mainly to the narrow genetic base. Hence action was initiated at the international level for enriching the original gene pool by introducing a large number of wild germplasm from the center of diversity, in Brazil. These are being conserved, catalogued, evaluated and documented in the different rubber producing countries with the objective of broadening the genetic base and thereby achieving further genetic improvement of the species.

The ten species reported in *Hevea* are *H.brasiliensis, H. benthamiana, H.guianensis, H.nitida, H.pauciflora, H.rigidifolia, H.camargoana, H.spruceana, H.microphylla* and *H.camporum* . The genus *Hevea* has its origin in the whole of the Amazon river basin in Brazil, covering parts of Brazil, Bolivia, Peru, Colombia, Ecuador, Venezuela, French Guyana, Surinam and Guyana, upto an altitude of about 800m. (Webster and Paardekooper, 1989). Since there is no cytological barrier among most of the species, natural hybrids and genetic variants are also found in this population. The different species exhibit wide variation in growth and morphological traits.

Origin and Distribution

Of the ten spices reported under *Hevea*, the commercially important species *Hevea brasiliensis* occupies about half the range of the genus, mainly in the region south of the Amazon, extending to Acre, Mato Grosso and Parana areas of Brazil, parts of Bolivia, Peru, north of the Amazon to the West of Manaus as far as the extreme south of Colombia. The species is now grown mainly in the tropical regions of Asia, Africa and America, in countries like Malaysia, Indonesia, India, Sri Lanka, Thailand, China, the Philippines, Vietnam, Kampuchea, Myanmar, Bangladesh, Singapore, Nigeria, Cameroon, Central Africa, the Ivory Coast, Ghana, Zaire, Liberia, Brazil and Mexico. However, the major share of the total production comes from tropical Asia.

The Genetic Base

The genetic base of *Hevea* in the east is reported to be very narrow, limited to a few seedlings originally collected by Sir Henry Wickham, referred to as the 'Wickham base'. (Simmonds, 1989).These few seedlings originate from a minuscule of the genetic range of the species in Boim, near the Tapajos river in Brazil (Wycherley, 1968). Thus, this collection lacked species diversity and also naturally occurring hybrids of the genus (Schultes, 1977).

Decrease in genetic diversity

The original gene pool was further narrowed down due to a number of factors, like a directional selection for yield, cyclical generation wise assortative breeding pattern and a wider adaptation of clonal propagation by budding. In all crop improvement programs till recently, the primary concern was productivity improvement. Such unidirectional selection for yield, over the years, ignoring the genetic variability with regard to secondary characters (Wycherley, 1969), has reduced the genetic variability in the population. Breeding in *Hevea* involves generation wise assortative mating (GAM), where the best clones in one cycle are used as parents for the next and so on (Simmonds, 1989). Hence ,the parentage of popular clones bred in various rubber growing countries can be traced back to just a handful of parent clones, like Tjir 1, Pil A 44, Pil B84, PB 24, PB 49, PB 56 and PB 86 (Tan,1987 and Varghese, 1992).

With the development of high yielding clones, extensive areas are being planted with a limited number of modern clones multiplied through vegetative propagation by budding, which also has had its share in decreasing the genetic base.

Broadening genetic base for biotic and abiotic stresses

Rubber plantations in the east are under the potential threat of *Microcyclus ulei*, prevalent in the American hemisphere, causing the devastating South American Leaf Blight (SALB) which is specific to *Hevea* species (Chee and Holliday,1986; Edathil, 1986). None of the Wickham clones have been reported to have resistance to SALB. There are also indications of erosion of genes controlling resistance to

Oidium and *Gloeosporium* in the original Wickham material (Wycherley, 1977). Besides, there is the threat of several minor diseases assuming epidemic proportions. The most serious problem reported is the severe incidence of *Corynespora* leaf spot disease, observed from 1985 onwards affecting the clones RRIC 103, KRS 21 and RRIM 725 in Sri Lanka. As a result, RRIC 103, one of the most popular high yielders planted extensively, had to be withdrawn from the planting recommendation necessitating replanting of vast areas under this clone (Liyanage *et al.*, 1991). In Malaysia, the popular clone GT1 was also severely affected by this disease (Tan, 1990). In India, the two major leaf diseases viz. abnormal leaf fall disease (*Phytophthora* sp.) and powdery mildew (*Oidium* sp.) cause considerable crop loss. Recent reports on the incidence of *Coryenespora* leaf spot observed in the popular clones RRII 105 and RRIM 600, in the rubber growing tracts of Karnataka and certain parts of North Kerala (Jacob, 1996), has caused serious concern over the possibility of the disease attaining epidemic proportions. In the case of the emergence of a virulent strain of a particular pathogen in favourable environmental conditions, the disease will spread and cause serious damage, if sufficient genetic variability is not available in the population.

Apart from the problem of disease susceptibility, variability for resistance/tolerance to various abiotic stress situations like drought, cold, high elevation, high velocity wind, low soil fertility etc. assumes much significance in the present day context of rubber cultivation extended to marginal and non-traditional areas due to non availability of land in the traditional rubber growing tracts. In order to develop/ select clones suitable for specific locations, the base material should contain ample genetic variability.

Considering all these factors which are essential for further genetic improvement of the crop, the breeders realized the need for broadening the genetic base of the crop by the introduction of fresh germplasm into the breeding cycle.

Germplasm Resources

The spectrum of *Hevea* germplasm can be broadly classified into (a) those existing in the primary centre of diversity in Brazil and (b) those developed in the centres of secondary diversity. The primary centre of diversity accommodates the wild genotype of the genus, along with naturally occurring hybrids, morphotypes and variants, while commercial cultivars, obsolete clones, cytotypes and genetic variants selected over the years are available in the secondary centres. All the available germplasm need to be conserved in a genepool, which can be utilized in breeding programmes as and when, required.

The genetic resources developed in the secondary centers also contribute to the broadening of the genetic base to a limited extent. Regular exchange of desirable genotypes between different countries, makes available potential clones developed elsewhere for adaptability studies besides broadening the genetic base for further utilization.

Early introductions

After the Wickham collection of 1876, there were a few attempts to introduce new germplasm to the centres of secondary diversity. These include introductions made in 1890s and 1910s from Brazil and Surinam to Java (Dijkman, 1951); five spp. and some of their hybrids from Brazil to Malaysia in 1951-52 (Brookson, 1956); 106 IAN clones, from Brazil via Sri Lanka to Malaysia, in 1952 (Baptiste, 1961) of which IAN 873 and IAN 717 were good yielders with tolerance to *Colletotrichum* sp. and *Microcyclus* sp.(Ong *et al.,* 1986; Ong and Tan, 1987) and the famous Schultes collection in 1943-46 in the Colombian forests (IRRDB, 1978). In Brazil, seed collections were made in 1945, 1952, and 1962 (IRRDB, 1982) and budwood of different species from the Amazonas, Acre, Para and Rondonia during 1972-82 (IRRDB, 1978; Goncalves *et al.,* 1983).

A Franco-Brazilian prospection in 1974 to the forests of Acre and Rondonia collected budwood from 60 high yielding trees 41 of which were later introduced into Ivory Coast (IRRDB, 1978). In general, all these introductions were of relatively small sample size and had low yield potential, when compared to the original Wickham collection and hence have contributed little to the plantation industry. (Simmonds, 1989).

1981 BIRRD (International Rubber Research and Development Board) Collection

Hevea breeders across the world stressed the need for broadening the narrow genetic base through introduction of wild germplasm into the breeding pool. There were also reports of constant threat to the survival of the species in the wild habitat due to urbanization of the region. The IRRDB organized a major collection expedition to the Amazon rain forests in 1981. This effort can be considered as one of the most significant events in the history of rubber germplasm collection, made with a view to ensuring a healthy future for the plantation industry (Varghese, 1992). This expedition organized jointly with the Brazilian Government, collected a total of 64736 seeds (Ong *et al.,* 1983; Mohd.Noor and Ibrahim, 1986) from the Brazilian states of Acre and Mato Grosso and the territory of Rondonia and budwood from 194 presumably high yielding seedling trees (ortets) which were not affected by *Microcyclus* and *Phytophthora* (Ong *et al.,* 1983).

The seeds and budwoods collected were despatched to the primary nursery at Manaus in Brazil, where 50% of the entire seed collection was retained, as per the agreement with Brazil. Of the remaining seeds, 75% was sent to the Asian multiplication and distribution center in Malaysia, and 25% to the African Center in Ivory Coast. From the seedlings raised at these centers, budwood were distributed to member countries. The entire budwood collection was raised in the primary center at Manaus, of which 150 clones were despatched to the secondary nursery at Guadeloupe from where it was distributed to the Malaysian and African centers. In Malaysia, 131 ortets survived (Ong and Ghani, 1990). Varying proportions of this fresh germplasm were distributed as per the requests from the IRRDB member countries.

In India, this new germplasm was imported from Malaysia during the period 1984 to 1990, was established at the RRII Central Experimental Station in South Kerala and in the North East. The total number of accessions established in the traditional and non-traditional areas in the country is 4967, the number in each location being 3617 and 1350 respectively.

Conservation of Germplasm

Both *in situ* conservation of genotypes in their original habitats and *ex situ* conservation in special nurseries or fields are feasible in *Hevea*, of which the latter is widely adopted due to practical considerations.

Conservation of genetic resources of *Hevea* is indeed elaborate, expensive and difficult. The germplasm collection, maintained in the field as 'active collection' for regeneration, multiplication, distribution and characterization, are exposed to natural calamities as well as pest and disease outbreaks. From this a 'core collection' or a condensed assembly of germplasm should be identified and established for efficient conservation as well as for elimination of redundancy in large collections. Finally a 'working population' to suit short term needs of individual breeders and breeding programs can be established for current use. (Varghese, 1992). Clement-Demange *et al.*, 1997 proposed a conservation scheme with two levels viz. (1) basic conservation of the whole germplasm in the existing budwood garden, with pruning reduced to a minimum frequency, and (2) conservation of a core collection in two replications at two different sites, and with regular cutting back of the two replicates on an alternate basis.

In India, the germplasm materials are established and maintained in conservation nurseries in the traditional and /or non-traditional areas with a spacing of 1m between plants. In source bush nurseries, each genotype is represented by 5-10 plants which are inter planted with the selected control clones, ensuring proper identity of the accessions. Selected genotypes are also being conserved and evaluated in a phased manner in field conservation gardens.

In the conservation nurseries at RRII, one plant per genotype is allowed to grow without regular pruning in order to gather early information on tree habit, flowering pattern, floral characteristics and seed morphology. Madhavan *et al.*, (1997) identified two off types exhibiting variations in flower colour, presence of a prominent disc at the base of the staminal column, shape of fruits and seeds.

Though cryopreservation protocols for the long-term storage of germplasm have been developed for various field crops, attempts in this direction are rather scanty in *Hevea*. IPGRI (International Plant Genetic Resources Institute) has recently reported the establishment of two efficient cryopreservation protocols for the embryogenic calli of *Hevea*. Under optimal conditions, high survival rate and rapid regrowth of cryopreserved somatic embryos, compared to the control was observed (Englemann *et al.*, 1997). Feasibility of this *in vitro* technique as a convenient and secure alternative to the present *ex situ* method of conservation of the large collection of *Hevea* germplasm, needs to be established.

Characterization and Evaluation of Germplasm

Studies on the 1981 collections established in conservation nurseries and/or field evaluation gardens, are underway in the different Institutes across the world, where they have been introduced and established. The genotypes are subjected to preliminary characterization and evaluation in source bush nurseries laid out employing augmented design. Selections for specific characters are then planted in field trials employing suitable statistical designs.

With the objective of characterization of the wild germplasm, a *Hevea* descriptor has been prepared for the first time in India. The descriptor includes passport data, plant type description at juvenile phase and data on relevant quantitative and qualitative characters expressed in subsequent phases of growth. Abraham *et al.*(1994) reported morphological characterization of the wild germplasm at the juvenile phase.

Based on D^2 analysis for yield and various yield components a set of 40 and 35 mature Wickham clones were grouped into eight (Mydin *et al.*, 1992) and nine (Abraham *et al.*, 1997) genetically divergent clusters. Data on early growth, dry rubber yield and important bark structural parameters of the five IRCA (Institute de Recherches Sur la Caoutchouc) clones from Ivory Coast indicate their superiority over the popular clone RRII 105, with IRCA 130 being the best among the five (Reghu *et al.*, 1997).

Preliminary studies at RRII, revealed wide variability in the wild germpalsm with respect to a set of morphological and structural parameters as well as juvenile yield (Annamma *et al.*, 1988;1989; Abraham *et al.*, 1992; Madhavan *et al.*, 1993; Mercy *et al.*, 1993). Genotype MT 999 has been identified as a superior one, with respect to the number of latex vessel rows and diameter and cross sectional area of laticifers (Abraham *et al.*, 1992; Reghu *et al.*, 1996) and the genotypes MT 1650, RO 1269, AC 2016 were more vigorous than the control clone RRII 105 (Mercy *et al.*, 1993). The data generated so far has also been analyzed to study the degree of association between different characters, and their direct and indirect effects on yield (Madhavan *et al.*, 1996). In general, the wild genotypes recorded poor yield, as compared to the domesticated control clones. Similar results have also been reported from Malaysia, (IRRDB 1996), Ivory Coast (Clement-Demange *et al.*, 1997) and Vietnam (Lam *et al.*, 1997).

Evaluation of provenance wise performance of the wild accessions for various morphological and anatomical traits and juvenile yield revealed that the genotypes from the provenance of Mato Grosso, were found to be superior in performance, compared to those from Acre and Rondonia (Reghu *et al.*, 1996; Mercy *et al.*, 1993). Similarly, in a source bush nursery, genotypes from Mato Grosso exhibited superior field tolerance against shoot rot disease caused by *Phytophthora* sp. compared to Acre and Rondonian genotypes (Mercy *et al.*, 1995). Lam *et al.*, 1997, also reported superiority of Mato Grosso genotypes over those from Acre and Rondonia, in Vietnam. In Malaysia, however, Rondonian genotypes recorded superior yield compared to the other two provenance (IRRDB, 1996).

A set of 175 wild genotypes were grouped into 3 distinct classes of low, medium and high performers using index-score method and metroglyph analysis based on certain growth characters. (Mercy *et al.*, 1993). Another set of 100 genotypes grouped into five clusters using D^2 analysis revealed that the genetic diversity is independent of the geographic origin. Acre genotypes had a wider distribution in the clusters compared to the other two provenance, indicating the existence of greater genetic diversity among the Acre genotypes (Abraham *et al.*, 1995)

Identification of desirable genotypes for qualitative and quantitative timber traits from the wild germplasm has been recognized as a priority area since rubber wood has been accepted as an alternate source of commercial hardwood. The search for vigorous genotypes, which have potential for timber and productivity, has been initiated in various countries (Clement-Demange *et al.*, 1997; Lam *et al.*, 1997; IRRDB, 1996). Screening of the wild genotypes for timber-latex traits has also been envisaged at the RRII In India, Acre and Rondonia genotypes were observed to be more vigorous with the main trunk growing straight with very little branching, which is a desirable feature for timber-latex clones whereas, the growth habit of genotypes from Mato Grosso resembled more to Wickham clones, with more intense branching and comparatively less vigorous growth. Similar observations were reported from Ivory-Coast also. (Clement-Demange *et al.*, 1997).

Selected accessions from the preliminary studies are being subjected to field evaluation, in a phased manner, for their performance in terms of growth characters, structural parameters, rubber yield, incidence of major diseases and other desired secondary parameters. The micromorphological and histological parameters also supplement the selection of potential genotypes for hot spot screening for resistance to various abiotic factors.

Documentation of Germplasm

Being the national agency dealing exclusively with all the research activities related to *Hevea* germplasm, RRII maintains a National Accession Register, with all the relevant basic data like accession number, genotype code, donor identification number and date of introduction. In order to store, use and retrieve information on the large volume of data being generated in the course of the elaborate evaluation process of *Hevea* germplasm, RRII has initiated steps to prepare an extensive and user friendly data base of *Hevea* germplasm.

Genetic Improvement

Heave breeding is aimed at synthesis of ideal clones with high production potential combined with desirable secondary attributes like initial vigour, smooth thick bark with a good latex vessel system, good bark renewal, high growth rate after opening, tolerance to major diseases, wind, tapping panel dryness(TPD), good response to stimulation and low frequency tapping (Varghese, 1992.). Since rubber is predominantly a small holder oriented crop, the breeding objectives should take care of their specific requirements too.

Utilization of Wickham germplasm

The conventional methods of genetic improvement viz., introduction, selection and hybridization are followed in *Hevea*.

Introduction of exotic clones

Clones introduced from other rubber producing countries form the base material for genetic improvement programmes so far in different Rubber Research Institutes.. Recent introductions under bilateral and multilateral clone exchange programmes organized by IRRDB (International Rubber Research and Development Board) and ANRPC (Association of Natural Rubber Producing Countries) are confined to potential clones of good performance. Popular clones of exotic origin are evaluated under the local agroclimatic conditions and promising selections are recommended for large scale planting. In India, a total of 127 domesticated clones, originating from eight different countries, form the exotic component of the original gene pool.

Ortet selection

Ortet selection or mother tree or plus tree selection is the oldest breeding method aimed at systematically screening for outstanding seedling genotypes resultant of natural genetic recombination. Screening of extensive plantations in Indonesia, Malaysia and Sri Lanka resulted in a good number of primary clones of importance like Tjir 1, PR 107, GT 1, BD 10, AVROS 255, Gl 1, PB 28/59, Mil 3/2, Hil 28, PB 23, PB 25, PB 86 etc. of which clones GT 1, PB 28/59 are still planted widely. In India, ortet selection resulted in the identification of 46 primary clones of RRII 1 series. Among these RRII 1, RRII 2, RRII 3, RRII 4, RRII 5, RRII 6, RRII 43, RRII 44 are the selections for high yield (Marattukalam *et al.*, 1980). RRII 33 is a clone, resistant to abnormal leaf fall caused by *Phytophthora* sp. Further, a total number of 195 selections from different estates have been established in the small-scale trials for preliminary evaluation (Mydin *et al.*, 1990). Since seedling orchards are increasingly being replaced with modern clones, further extensive screening for yield, resistance to disease and drought in traditional and non-traditional areas is given priority.

Hybridization and clonal selection

Controlled pollination was started in Malaysia (1918), Sumatra (1920), Brazil (1937), Sri Lanka (1939) and Indo China (1947) (Panikkar *et al.*, 1980). In India hybridization programmes were initiated in 1954 and the early hybrid clones developed include RRII 100, RRII 200 and RRII 300 series.(Annamma *et al.*, 1990). Among the RRII 100 series, RRII 105 is a very promising selection.(Nair and George, 1969; George *et al.*, 1980; Nazeer *et al.*, 1986), with wide popularity in the rubber planting sectors. Clones RRII 203 and RRII 208 are the best selections in RRII 200 series (Saraswathy Amma *et al.*, 1980) and RRII 300 and RRII 308 in the 300 series (Premakumari *et al.*, 1984). Among another recent hybrid series under evaluation trial, nine clones revealed comparable/marked heterotic improvement during the mature phase with a yield increase ranging from 4 – 50% over the high yielding standard clone RRII 105 (Licy *et al.*, 1996). In a recent report on 24 hybrid clones, evaluated at 44 months age, 12 clones exhibited a heterotic increase of over 20% for yield, which offers much scope for exploitation of hybrid vigour (Varghese *et al.*, 1997).

The Rubber Research Institute of Malaysia developed clones RRIM 500 to RRIM 1000 series, while Prang Basar Institute in the private sector selected certain PB clones of commercial significance. The Indonesian Research Institute for Estate Crops in Java and Sumatra (BPM) evolved PR, AVROS, BPM, LCB, PPN and RR clones.

Apart from the conventional breeding methods, special techniques in breeding like polyploidy, mutation and *in vitro* culture has also been attempted in *Hevea*. Induction of polyploidy and mutation has been attempted on a limited scale (Saraswathy Amma *et al.*, 1984). By crossing diploids and tetraploids, a triploid has been synthesized from the clone RRII 105 (Saraswathy Amma *et al.*, 1980) and a spontaneous triploid also has been identified (Nazeer and Saraswathy Amma, 1987). The progenies of a genetic variant with compact crown (John *et al.*, 1995) is being incorporated in breeding programmes for altering the tree architecture with the objective of combining yield and compact canopy for high density planting.

Prepotency

As prepotency is comparable to General Combining Ability, identification of prepotent clones has been given due importance in *Hevea* breeding., as these can produce superior seedling progeny under open pollination. Specially designed polyclonal seed gardens, with prepotent component clones, yield superior polycross progenies for use as planting material and also as base material for ortet selection. On the basis of these studies, nine prepotent clones viz. AVT 73, RRII 105, PB 28/83, Ch 32, PB 215, PB 242, BD 5 and PB 252 have been identified.(Mydin *et al.*, 1990)

Clonal composites

In India, in the past two decades, the outstanding high yielder RRII 105 was planted extensively in traditional rubber growing belts, leading to a tendency towards monoculture. Considering the serious consequences of monoculture, a strategy for multiclone planting with suitable clonal composites is being propagated. Rubber Board of India is now recommending to restrict the planting of RRII 105 to 50% of the total area and to plant the remaining area with promising clones in Category II (upto 35%) and Category III (upto 15%). Several experiments have been laid out with different clonal combinations in traditional and non-traditional rubber growing areas to identify suitable clonal composites. There is no doubt that development of clone blends of diverse genetic materials will offer better protection to the plantation industry from the possible disasters due to monoculture in future.

Utilisation of wild germplasm

Characterization and evaluation of the large number of wild germplasm resultant of the 1981 IRRDB (International Rubber Research Board) prospection is in progress in different countries. Due importance has been given for the best utilization of the wild genotypes identified for various desirable characters. Among 12 cross combinations involving wild genotypes and popular cultivars, juvenile yield at two years growth was highest in a hybrid from the cross RRII 105 X RO/87 (RRII, 1994). Nine wild genotypes identified for their superiority in growth/ anatomical characters and resistance to *Phytophthora* sp. have been used as male parents in a hand pollination programme incorporating Wickham and Amazonian clones. In Malaysia, progenies derived from crosses between oriental clones (RRIM 600, RRIM 701 and PB 5/51) and selected 1981 germplasm tapped for five cycles in the nursery, produced quite encouraging results for girth and yield. (IRRDB, 1996).

Resistance to biotic and abiotic stresses.

Scarcity of ideal land in the traditional rubber growing tracts has necessitated expansion of rubber cultivation to non-traditional and marginal lands, exposed to various biotic and abiotic stresses. Hence development or selection of location specific genotypes is of international importance.

The genetic potential of the wild germplasm for specific desirable traits has already been realized from the preliminary studies in India and other countries. Various fungal diseases limiting plant growth and yield in South East Asia, include those caused by *Phytophthora* sp., *Oidium* sp., *Corynespora* sp.and *Colletotrichum* sp. Identification of sources of resistance to all these diseases is the immediate priority. In India, screening of wild genotypes for resistance to major diseases caused by *Phytophthora* sp. and *Oidium heveae* are progressing. Due attention is being given to search for resistant sources for the *Corynespora* leaf spot disease, in the backdrop of the recent reports on the severity of the disease in certain parts of traditional rubber growing tracts. Laboratory screening of a population of 495 wild genotypes for resistance to *Phytophthora* sp. showed interesting results, with 53 genotypes showing high levels of resistance (RRII, 1997). Studies carried out in Indonesia on the wild germplasm have identified genotypes with resistance to *Colletotrichum* leaf diseases (Alwi and Suhendry, 1992).

Although SALB, the most disastrous disease of *Hevea* is presently confined to South America, the possibility of the disease entering the eastern hemisphere cannot be ruled out. Hence attempts are essential for identification of an array of genotypes having resistance to the various physiological races of the fungus. The existence of a general resistance to SALB in the Amazonian clones, compared to the total susceptibility of Wickham clones has been reported from French Guyana. In Ivory Coast, the hybrid population of Wickham X Amazonian crosses is reported to show continous variability for resistance to SALB (Clement-Demange *et al.*, 1997).

Abiotic stress conditions like drought, cold, high velocity wind etc. prevalent in the nor traditional and marginal areas warrants the necessity of developing clones capable of withstanding such extreme situations. In India, hot spot screening of preliminary selections of wild germplasm for drought tolerance is in progress. Screening of wild genotypes for membrane thermostability has indicated positive results with certain genotypes showing remarkable tolerance to water and temperature stresses.

Screening of the wild germplasm for cold tolerance revealed that 38 genotypes recorded higher tolerance than the Chinese cold tolerant line SLATC 93-114 . Similar evaluation of 5164 wild accessions revealed that 50 of them were more cold tolerant than SCATC 93-114 of which 10 survived a cold treatment even upto -2^0 C.(Lin *et al.* 1994)

Application of Molecular Techniques

Recent advances in molecular techniques like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), and Simple sequence Repeats (SSR) or microsatellites offer reliable, fast and attractive adjuncts to the elaborate conventional procedures. This is especially significant in vegetatively propagated perennial spp. like *Hevea* where conventional genetic analysis is difficult due to the long breeding and selection cycle and difficulties in raising the F2 progeny. Molecular genetics has an important role to play in many aspects of conservation such as the detection, characterization and evaluation of plant genetic diversity for purposes of improved acquisition, maintenance and utilization (Kresovich *et al.*, 1992; Karp *et al.*, 1997). These DNA based diagnostics are now well established as a means to assay diversity at the locus, chromosome and whole genome levels for screei nucleotide sequence based polymorphism.

In *Hevea*, studies on the application of molecular markers, have been initiated and a few reports on the potential use of this powerful technique are available. Genetic diversity among wild and cultivated populations of *Hevea* was assessed using izozymes, (Chevallier et al., 1988) RFLP analysis (Besse *et al.*, 1994) and mitochondrial DNA analysis (Luo *et al.*, 1995). Significant genetic distance, between the 1981 germplasm and the Wickham group using Isozyme or RFLP/ RAPD had also been reported. (Clement-Demange and Nicolas, 1987). Wild populations appeared to be more polymorphic than the cultivated ones, the Rondonia and Mato Grosso genotypes being the most variable with RFLP (Besse *et al.*, 1994). RFLP analysis on wild genotypes and cultivated clones indicated that the wild ones have led to the enrichment of genetic resources, and that the cultivated clones have conserved a relatively high level of polymorphism despite its narrow genetic base and high level of inbreeding. Variation in RAPD profile between TPD affected and normal plants

(Thulaseedharan *et al.*, 1994), *Oidium* resistant and susceptible genotypes (Shoucai *et al.*, 1994) and for *Phytophthora* leaf fall (Jacob, 1996) were observed.

In a recent study, the applicability of RAPD markers for genetic analysis in *Hevea* was evaluated using 42 informative primers in a set of 24 clones from the breeding pool of RRII (Varghese *et al.* 1997). Estimation of genetic distance among the tested clones facilitated identification of genetically divergent clusters. Among the different clones tested, RRIC 100 displayed the highest mean genetic distance of 0.616. Use of this clone as a parent in hybridization programs has resulted in highly heterotic hybrids (Licy *et al.*, 1996). Regular use of such techniques will be of great help in the management of large number of germplasm for assessment of genetic identity, structure and variability among accessions. Work on these lines has been initiated at Rubber Research Institute of India.

Future prospects

Recognizing the urgent need for broadening the original narrow base, in *Hevea*, a major expedition to the centre of diversity made in 1981, collected a large number of wild germplasm which is under conservation and evaluation in the different rubber producing countries. Presently these accessions are conserved *ex situ*, which, however, is prone to natural calamities. Hence feasibility studies on alternate *in vitro* methods viz. Cryo-preservation are envisaged. Similarly, for characterization and evaluation, an optimum number of descriptors, which are highly heritable should be utilized. A set of descriptors for *Hevea* has been suggested for the first time in India, which has to be updated/ modified from time to time so as to accommodate all relevant traits of importance. Attempts are underway to develop a computerized database of *Hevea* germplasm, which will be of much help in supplementing the elaborate manual documentation system.

In *Hevea*, hybridization between widely divergent genotypes has resulted in greater range of variability especially in view of the highly heterozygous nature of the crop. Early performance of the progenies of such crosses has indicated promise. However, further systematic studies are required for the identification of wild genotypes with desired traits for introgression into cultivated clones.

It is all the more significant for development of location specific clones capable of withstanding different constraints prevalent in non traditional areas, like extremes of temperature in winter and summer, prolonged drought, high velocity winds and occasional hailstorms, high altitudes, depleted and soils. In India, the non-traditional areas include the northeastern states like Tripura, Assam and Meghalaya, Jalpaiguri District of W.Bengal. Konkan region of Maharashtra , Goa and selected areas in the states of Orissa and Andhra Pradesh. Regional Research Stations have been established in most of these states, to give research back up for scientific rubber cultivation. Wild germplasm is definitely a source of many valuable genes capable of withstanding the above mentioned stresses. The challenge before the breeder lies in evolving strategies for identifying these genes and incorporating them into high yielding clones. Studies have been initiated in this direction.

In spite of the creditable improvement of productivity within a short span of time utilizing the Wickham base, theoretical conclusions indicate more potential in terms of yield. (Sethuraj *et al.*, 1981). Various factors responsible for this gap need to be identified and steps taken to overcome the constraints. Due to the occurrence of significant G X E interaction, trials should be conducted in diverse environments for proper evaluation of genotypes. In addition to utilization of wild germplasm, multidisciplinary investigations assume importance in order to bridge the gap between the theoretical maximum and the present day yield. The prepotency concept and the principle of clonal composites still hold much importance for the future rubber crop improvement in the country.

Molecular techniques like RFLP, RAPD and microsatellites have already been deployed for the analysis of the extent and distribution of genetic diversity among Wickham and wild germplasm. These markers provide reliable genetic information for molecular characterization, evaluation and utilization of germplasm resources. Data on genetic distance is required for identification of diverse sub-populations.

Application of molecular markers can be a valuable adjunct to conventional breeding for better exploitation of the available genetic variability in the Wickham gene pool and the wild germplasm to bring about further significant genetic improvement.

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