

## Review

# Natural rubber producing plants: An overview

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Currently, *Hevea brasiliensis* has been only one resource for commercial natural rubber production. Rubber tree, *H. brasiliensis* (Willd. ex A. Juss.) Muell. Arg., commonly known as the Brazilian rubber tree is native to the Amazon River basin. Attempts to develop alternative sources of natural rubber have been made at various times and no fewer than eight botanical families, 300 genera and 2500 species have been found to produce natural rubber in their latex. Only two species, in addition to the Para rubber tree, are known to produce large amounts of rubber with high molecular weight: a shrub named guayule (*Parthenium argentatum* Gray) and the Russian dandelion (*Taraxacum koksaghyz*). These plants were considered sufficiently promising as alternative rubber sources that several research programs have been conducted on these plants, especially during World War II. *Hevea* rubber has been an undeniably beneficial commodity for the past 100 years. The superior qualities of the natural elastomers produced by this tree have never been surpassed by any of the synthetic products. *Parthenium argentatum* (guayule) is an industrial crop, which is the best potential source of latex suitable for use in medical products, gloves etc., which does not cause allergic reactions in patients suffering from Type 1 latex allergy. The dandelion rubber will be tested for use in a variety of applications, but primarily for use in tires. Researchers hope the project will lead to the production of the country's first dandelion rubber commercial facility in the next five years. By 2020, they hope the plant will be producing 60 million pounds of natural rubber. Other alternative rubber producing plants like lettuce (*Lactuca serriola*) and fig tree (*Ficus bengalensis*); have not yet been sufficiently studied to establish their usability. The ideal rubber-producing crop would be fast growing plant species that can grow in any type of land across the world. The major objective of this review was to provide the information about cultivation, genetics and breeding aspects of *Hevea* and also other natural rubber producing species for alternative source of latex production in the near future.

**Key words:** Alternative rubber sources, biotechnology, breeding, *Hevea brasiliensis*, *Parthenium argentatum*, *Taraxacum koksaghyz*, *Ficus bengalensis*, *Lactuca serriola*.

## INTRODUCTION

The Para rubber tree (*Hevea brasiliensis* Muell. Arg.), which produces natural rubber is a tall tree (30 to 40 m high in the Amazonian forest, its natural habitat), deciduous, with orthotropic rhythmic growth, belonging to the family Euphorbiaceae. *H. brasiliensis* (Willd. ex A.

Juss.) Muell. Arg., whose center of diversity is the Amazon basin, is the major source of commercial rubber in the world. Natural rubber is synthesized in over 2000 plant species confined to 300 genera of seven family's viz., Euphorbiaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Moraceae, Papaveraceae and Sapotaceae (Cornish et al., 1993). Natural rubber, obtained almost exclusively from the Para rubber tree (*H. brasiliensis*), is a unique biopolymer of strategic importance that is, in

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many of its most significant applications, cannot be replaced by synthetic rubber alternatives. Several pressing motives lead to the search for alternative sources of natural rubber. The world's major rubber producing plant, *H. brasiliensis*, has been under cultivation on Hainan Island for several decades. However, diminishing acreage of rubber plantations and life-threatening allergies to the latex based products of rubber tree, coupled with increasing demand for high quality rubber, point to a need for alternative natural rubber resources.

In recent years, guayule (*Parthenium argentatum* Gray, Asteraceae), an alternate rubber source, a shrub native to Chihuahuan desert of Texas and North Mexico provides only 10% of the world's natural rubber. Guayule can withstand a temperature range of 18 to 49°C and can grow in well drained soils with an annual rainfall as low as 230 to 400 mm. The yield potential of guayule is only 600 to 900 Kg/ha (Estilai and Ray, 1991). However, guayule latex is useful for hypoallergenic latex products. Despite some limitation as an alternative rubber crop due to its slow volume growth and low abundance of rubber particles, guayule has been proposed as a viable commercial alternative for hypoallergenic latex (Cornish and Siler, 1996). Among the rubber producing species found in North and Northeast China, the rubber content of the *Euonymus alata* (Celastraceae) bark ranged from 10 to 16%. *Taraxacum koksaghyz* (Dandelion rubber), a species of economic importance, has been discovered in Sinkiang, China with high rubber content (Cheng, 1963). A few other rubber sources are: Ceara rubber (*Manihot glaziovii*), India rubber (*Ficus elastica* Roxb.), Panama rubber (*Castilla elastica* Cerv.), Lagos rubber (*Funtimia elastica* Stapf.) and Madagascar rubber (*Cryptostegia grandiflora* R. Br.). Besides these higher plants, it is noteworthy that some species of fungi genera *Lactarius*, *Peziza*, *Russula* and *Hygrophorus* have been reported to produce latex containing cis- polyisoprene of low molecular weight mass. *Lactarius chrysorrhoeus* is one of the *Lactarius* mushrooms which produce white latex that turns yellow on exposure to air (Mekkriengkrai et al., 2004). However, these species are not commercially exploited. Rubber is a hydrocarbon polymer constructed of isoprene units, and rubber is a secondary metabolite (cis 1, 4-polyisoprene) chiefly originating in the secondary phloem of the tree. No other synthetic substitute has comparable elasticity, resilience and resistance to high temperature (Davis, 1997).

It is quite exclusively cultivated over 10 million hectares in the world for providing the industry with natural rubber of 8.4 million tons per year. It is a renewable ("green") elastomer, and its production requires much less oil than that of synthetic rubber (one sixth). Natural rubber is produced in south-east Asia (92%), in Africa (6%), and in Latin America (2%). Rubberwood has generated a profitable industry mainly in Malaysia and Thailand, but also in India, Vietnam, Indonesia and Cambodia. This

review describes both wild and cultivated natural rubber producing plant species distribution, conservation and its molecular breeding aspects for future research applications.

## DISTRIBUTION

*H. brasiliensis* was introduced to tropical Asia in 1876 through Kew garden from the seeds brought from Rio Tapajo's region of the upper Amazon region of Brazil by Sir Henry Wickam (Dijkman, 1951). Kew gardens, UK played a special role in the domestication of wild plants. It was in the Kew Gardens, the planting materials are assembled from the native land, propagated and then distributed to other botanical gardens around the world (Baulkwill, 1989). The successful transfer of *H. brasiliensis* to Asia and the subsequent establishment of commercial rubber plantations were in response to the growing demand for this raw material. Natural rubber is produced in south-east Asia (92%), in Africa (6%), and in Latin America (2%). Mainly rubber producing countries are, by descending order, Vietnam, Thailand (2.9 million t in 2004), Indonesia, India and Malaysia, China and also Côte d'Ivoire, Liberia, Sri-Lanka, Brazil, Philippines, Cameroon, Nigeria, Cambodia, Guatemala, Myanmar, Ghana, D.R. of Congo, Gabon and Papua New Guinea.

The rubber balls were made from the latex of trees and its one of the species in the genus *Castilla*, which belongs in the Moraceae, or mulberry family. The rubber balls seen by Cortez in Mexico have been made either from *Castilla* or from the desert shrub known as guayule. Several of the *Castilla* species produce much more latex at a single tapping than any *Hevea* tree yield. However, they will not produce such yields if tapped more frequently than once or twice per year. *Castilla* rubber is of good quality and wild trees of this genus contributed substantially to the rubber supplies of the United States during World War II, when every available wild tree was sought out and tapped. *Castilla* could almost surely be greatly improved as a rubber producer through research. Wild trees of this genus still supply rubber for the local rubber needs in the tropics. Guayule, Manihot, Funtumia, *Cryptostegia*, Russian dandelion and many other plants have been exploited for rubber to some extent in the past, especially in times of high prices, but in the long run, none could compete with *Hevea*. Interest in guayule rubber has waxed and waned over the years and there has been a continuing production right up to the present day. Guayule rubber plant cultivation is once again attracting attention because its competitive position has improved with the rise in cost of raw materials needed for making synthetic rubber, and it can grow and produce rubber under lower rainfall conditions than many other crops. Several of the rubber-producing plants mentioned above have an interesting history, but this paper will deal only with some highlights in the story of *Hevea*.

**Table 1.** Properties and sources of natural rubber producing plants.

Natural rubber producing plant	Property	Source of rubber	Mw (kDa)	Production (Tones/Yr)	Content of rubber (%)
<i>Hevea brasiliensis</i>	<i>Hevea</i> , rubber yielding tree which is white or yellow latex occurs in latex vessels in the bark.	Bark	1,310	9,000,000	30-40
Guayule shrub <i>P. argentatum</i> Gray	A high protein guayule latex would also be a brown/green color	Root	1,280	10,000	3-12
Russian dandelion <i>Taraxacum (koksaghyz)</i>	It produces a milky fluid in its roots, which contains a high-quality rubber	Root	2,180	3,000	0-15
Fig tree ( <i>Ficus carica</i> )	Natue of latex pale grey in colour.	Bark, leaf	190	---	4

Until the early 1900's, there was still disagreement as to whether *Castilla*, *Hevea*, *Manihot* or some other plant should be chosen for cultivation on a large scale. The Southeast Asian experience with the Wickham progenies soon made it clear, however, that *Hevea* was the best choice. It also became clear that yields per tree and per hectare would have to be improved. British, Dutch, French, and local specialists, and later North and South American scientists as well, all had a part in developing better planting stocks and improving culture of the trees. The first unselected Wickham trees had a yield potential no higher than 225 kg per hectare per year, even with Ridley's best tapping methods. Crosses between some of these original trees gave encouragement to the early workers in the form of vigorous progenies that out yielded their parents. It has been suggested that a number, perhaps all, of the original Tapajoz seedlings were in fact inbreds, as a result of many generations of natural selfing of individual, isolated, wild forest trees.

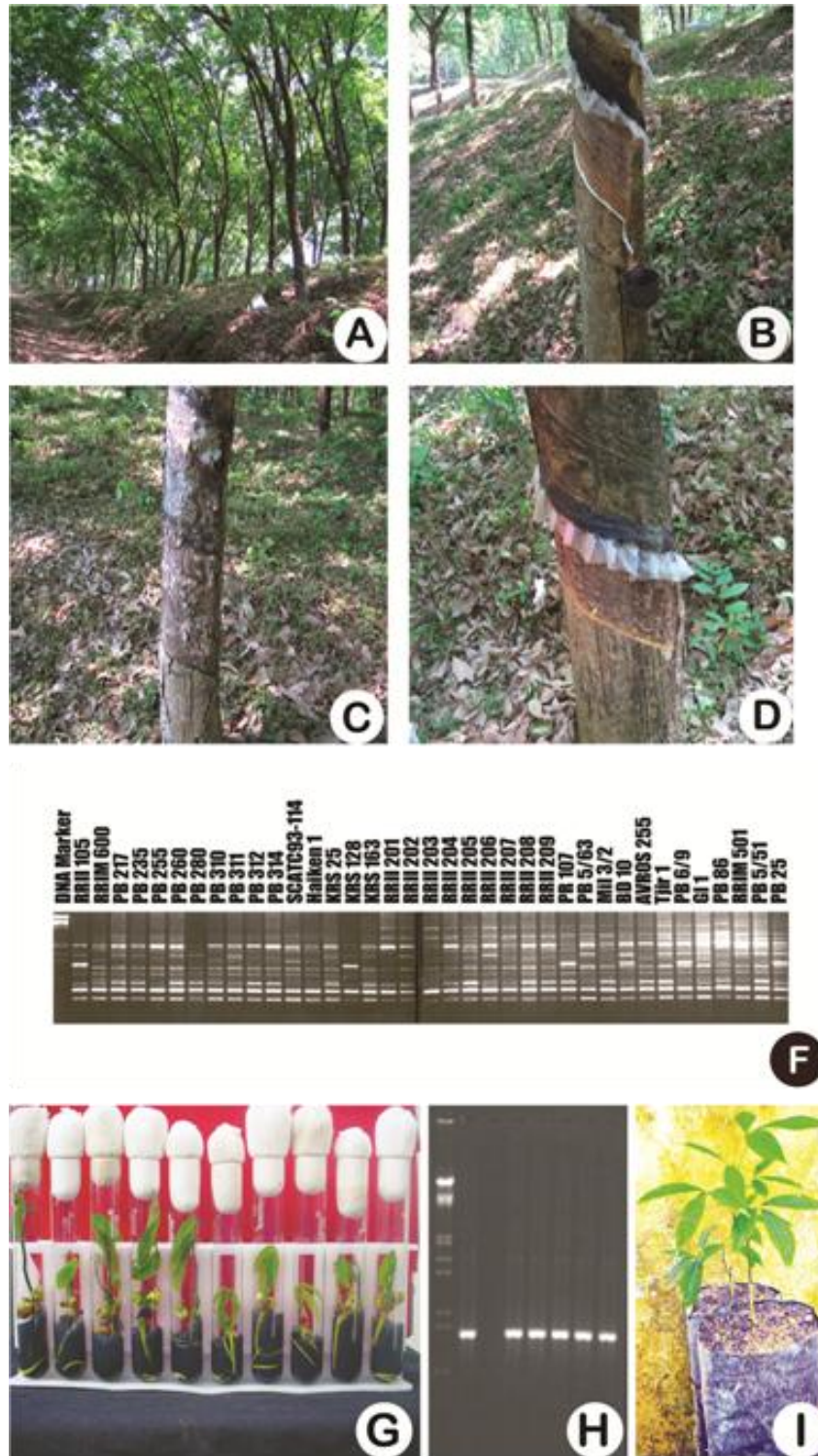
## BREEDING AND GENETICS

The global consumption of natural rubber is steadily increasing and the production has also been increased so as to meet the demand (Table 1). The major objective of *Hevea* breeding is to develop potential clones with high rubber yield combined with desirable secondary characters such as high initial vigor, smooth and thick bark with good latex vessel system, good bark renewal, high growth rate after initiation of latex harvest, tolerance to major diseases and wind (Annamma et al., 1990; Varghese et al., 1992). Recently importance has also been given to develop clones with tolerance to abiotic stresses such as drought, high temperature, cold, etc. (Thulaseedharan et al., 2000). Clones attaining early tapping girth and high initial yields are preferred to clones with higher yields in a later stage (Lim et al., 1973). In countries, where labor is cheap and small holding sector is predominant, clones capable of withstanding high tapping intensities are preferred. Besides high rubber

yield, superior technological properties of rubber, timber and its quality (latex-timber clones) and low incidence of tapping panel dryness (TPD) are also major breeding objectives (Venkatachalam et al., 2007, 2009a). TPD is the major issue to hinder the latex yield in rubber tree and recently, few genes associated with the onset of TPD was reported (Figure 1).

Rubber breeding aims at improving the potential of rubber clones for land and labor productivity, as well as their adaptation to the ecological conditions of the cropping areas (breeding objectives). Many factors such as latex yield components, growth of the tree, resistance to abiotic stresses (wind damage or tapping panel dryness), resistance or tolerance to leaf diseases, and tolerance to water deficit or to low temperature, must be addressed based on an in-depth understanding of the functioning of the tree. Low fruit set and its variation among clones, notably in the case of self pollination, may be regarded as a general characteristic of *H. brasiliensis* reproductive biology that is not confined to specific incompatible crosses (Hamzah et al., 2002), and it is a major limitation to genetic recombination in rubber breeding. Although it is not due to natural pollination deficiency (Warmke, 1952), fruit set success is generally higher in hand pollination. Pollen fertility, varying in a range from 50 to 98%, does not seem to be a limitation. The development of flowers to fruits is estimated to be very low, around 5% (Husin, 1990). In fact, fruit-set success rate, assessed by controlled pollination, varies widely, depending on the pollinated clones, from no success at all to a maximum of 5 to 10 % for the more fertile clones such as PB5/51 or PB260. The success rate varies from year to year with a coefficient of variation of 45% (Clément-Demange et al., 1995). A pre-zygotic or post-zygotic control exerted by the same incompatibility alleles and/or the inbreeding effects due to accumulation of homozygous loci in the embryo appear to be the reasons for low fruit set in self pollination.

In order to enlarge genetic variability of *Hevea*, some researches were carried out on mutation breeding (Ong and Subramaniam, 1973; Markose et al., 1977), and on



**Figure 1.** A, Rubber plantation from RR11, Kottayam, India. B, A healthy mature rubber tree after tapping for latex collection. C, Rubber tree tapping panel dried. D, A rubber tree affected by TPD with no latex flow. E, A schematic representation for TPD syndrome development in rubber tree (reproduced from Molecular Biotechnology, 2009). F, RAPD profile of 37 cultivated *Hevea* rubber clones generated by OPC-05 primer. G, Transformed embryo germination and plantlets development. H, Amplification of *uidA* gene fragments by PCR (600 bp). I, Somatic embryo derived plants established in polybags (Reproduced from Functional Plant Science and Biotechnology, 2007).

polyploidization of the '2n = 36 *H. brasiliensis* species' (Mendes and Mendes, 1963; Zheng et al., 1981). An artificial triploid has been produced by crossing a diploid and a tetraploid (Saraswathyamma et al., 1988). The existence of some putative genetically dwarf or semidwarf genotypes was mentioned (Ong et al., 1983); *H. camargoana* would have a dwarf growth habit (Gonçalves et al., 1982). It was attempted to associate some molecular genetic markers with the dwarfing trait (Venkatachalam et al., 2004).

Rubber experiences an initial growth phase varying generally from 5 to 7 years, depending on climate, soil conditions and management. Trees are tapped when their trunks attain 50 cm in girth: so, growth rapidity determines the end of the initial unproductive period and the earliness of "opening" the time when tapping begins, which is a very important factor for profitability. Tapping is a periodically renewed cut incised in the bark of the trunk, which generates latex flow (the cell cytoplasm containing rubber particles) throughout the year (Jacob et al., 1995). The combination of tapping frequency (tapping every two, three, four or five days) and stimulation intensity by the application of ethephon, an ethylene-generating compound (Abraham et al., 1968), determines tapping intensity. Ethylene is an important plant growth factor which, among other effects, is synthesized and acts as a response of the tree to wounding. Tapping could last for more than 40 years, but many different causes usually lead to the decision to stop tapping after 15 to 30 years and to fell the rubber plot for replanting or for another use of the land. Felling is decided more often when rubber yield has fallen below a critical level, mainly due to the regular or sudden decrease in the density of the tapped trees (wind damage, or other causes). The recent new possibility to benefit from selling the rubberwood has generated strong reasons for felling earlier (money is more valuable when earned earlier). There is no unique rationale for that, decrease especially when one considers the socio-economic differences between estates and smallholdings.

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. The locality mostly housed only *H. brasiliensis* and the collection did not represent much species diversity or naturally occurring hybrid progenies of the genus. Only a limited number of genotypes were 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 programs 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). All the eastern clones of *H. brasiliensis* originated from the relatively very narrow genetic pool referred to as 'Wickham base' (Simmonds, 1989).

Ten (10) species of *Hevea* have been identified in the genus, viz., *Hevea benthamiana*, *H. brasiliensis*, *Hevea camargoana*, *Hevea camporum*, *Hevea guinensis*, *Hevea microphylla*, *Hevea nitida*, *Hevea pauciflora*, *Hevea rigidifolia* and *Hevea spruciana* (Schultz, 1990). The chromosome counts made by various investigators showed variations and reported as 2n = 16, 34 and 36. However, detailed cytological investigations have confirmed the chromosome complement of *H. brasiliensis* in the somatic cells as 2n = 2x = 36 (Saraswathyamma et al., 1984). The chromosomes are small and vary in length and the total chromosome length of the species is 89.7 µm. Triploid plants with 2n = 3x = 54 (Nazeer and Saraswathyamma, 1987) and induced tetraploids with 2n = 4x = 72 by the application of colchicine (Saraswathyamma, 1990) were also reported.

Guayule lines (Figure 2) were also grown in Greece, Morocco and South Africa, but in these cases reports or publications are not publicly available. In Mexico cultivation experiments have been carried out with native germplasm (Jasso de Rodriguez et al., 2002; 2005). Guayule is the dominant perennial shrub found on the semi-arid limestone bajadas and hill sides of the Big Bend region of Texas and the Chihuahuan desert of north central Mexico, areas with a temperature range between -18 and 49.5°C. Wild guayule stands contain a natural polyploid series of diploids (2n = 2x = 36), triploids (2n = 3x = 54) and tetraploids (2n = 4x = 72); and under cultivation, individual plants have been identified with chromosome numbers up to octaploid (2n = 8x = 144). Diploids reproduce predominantly sexually, and polyploids reproduce by facultative apomixis. Guayule has been grown in desert-like environments, humid warm conditions, hot dry semi-arid conditions, and regions with moderate temperature and rainfall. Germplasm from the USA breeding program has been tested in Argentina (Coates et al., 2001), Australia (George et al., 2005) and Israel (Mills et al., 1990). Good seed quality is a prerequisite for good plant establishment in the greenhouse or in the field. Guayule seeds are very small (about 1000–1500 seeds per gram). Primary dormancy in guayule seeds is caused by an inner seed coat dormancy and embryo dormancy, but can be broken by seed treatment, with the advantage of uniform and fast germination (Foster and Coffelt, 2005). Currently, seedlings are grown in nursery trays in the greenhouse and fields are established using typical commercial transplanting systems.

Russian dandelion (*Taraxacum koksaghyz* Rodin)





a



b

**Figure 2.** Guayule (*Parthenium argentatum*). a) Photo courtesy ARS: Jack Dykinga. b) Photographer: Flaigg Norman G. Country: Pinal, State: AZ.



a



b

**Figure 3.** Russian Dandelion (*Taraxacum koksaghyz* Rodin). a) Photo source: Christian Schulze; b) Photo source: Ohio Agricultural Research Gronover and Development Center.

(Figure 3) was discovered in Kazakhstan in the course of a strategic program in 1931–1932 to develop a domestic source of natural rubber in the USSR. It was selected as the best candidate rubber crop out of almost 1,100 indigenous plant species tested. The roots of wild Russian dandelion contain 4 to 5% high quality rubber, produced in laticifers accompanying the vascular bundles throughout the plant (Kekwick, 2001). Russian dandelion was cultivated extensively in the USSR in the 1930s. In 1941, the 67,000 ha of Russian dandelion covered about 30% of the total USSR rubber consumption. Due to

natural rubber shortages during World War II, several other countries independently started growing Russian dandelion in emergency rubber programs: the USA (Whaley and Bowen, 1947), the UK, Germany (Heim, 2003), Sweden, Spain, and other countries (Polhamus, 1962). The best USA cultivation fields yielded about 110 kg rubber per hectare, while the best Russian yields were 200 kg rubber per hectare. Unfortunately, the agronomic properties of Russian dandelion were reported to be rather unfavorable. Cultivation was very labor intensive and expensive: because the seedlings were rather small



a



b

**Figure 4.** Fig tree (*Ficus carica*). **a)** Photo by C Novello. **b)** Photo courtesy of Petr Kocna.

they were outcompeted by weeds, making intensive tilling necessary. After World War II, cheap *H. brasiliensis* rubber became available again and in all countries the Russian dandelion programs were terminated. Cultivation of Russian dandelion continued in the USSR until the early 1950s, after which it was abolished, probably for economic reasons.

Fig tree (*Ficus carica*) (Figure 4) is cultivated for its fruit in southern parts of temperate zones. Development of fig tree as an alternative rubber crop is promising because it generates a large latex volume, has a fast growth habit and long life expectancy, and is suitable for vegetative propagation, a means for amplifying genetically engineered trees. However, there have been no reports on rubber biosynthesis in fig tree. Evaluating the quantity and quality of the natural rubber produced in fig tree, and characterizing the rubber biosynthetic activity in rubber particles and latex serum remain important objectives in the development of fig tree as an alternative rubber producing plant (Kang et al., 2000). Fig tree contains natural rubber comparable to many other rubber biosynthesizing temperate plants. The rubber particle proteins of fig tree are distinct from that of rubber tree, in that the major proteins tightly associated with the particles are unique and not related to any other rubber transferase. The present results also show that different physiological condition including divalent metal ions in the latex serum can be an important factor in determining different rubber biosynthetic activities in fig tree and rubber tree.

#### CONSERVATION AND PROPAGATION METHODS

*Hevea* germplasm conservation is to make available

maximum variability present in the crop species, its wild relatives and related species. 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*. Cryopreservation and *in vitro* conservation, though followed in several annual crops, are not available for *Hevea*. Irrespective of the method, it is important to assign a proper accession number to each genotype. *Hevea* germplasm has to be conserved in field gene banks, both in source bush nurseries and in gardens. Commercial cultivation is very often restricted to high yielding cultivars, which may become obsolete when newly improved ones are released. Much importance was conferred on a small number of 22 seedlings disseminated from Singapore to Malaysia after 1876; but a significant part of the Wickham seedlings, which germinated in Kew Gardens, was then sent to Ceylon (now Sri Lanka), raised and disseminated to different countries, especially India. Genetic diversity can now be compared to that of the available wild Amazonian populations by use of molecular genetic markers.

The diminishing acreage of rubber plantations, an increasing demand, and the lifethreatening latex allergy to *Hevea* rubber have prompted research interests in the development of alternative rubber sources. In recent years, guayule (*Parthenium argentatum* Gray), which accumulates rubber in the parenchyma cells and contains high molecular mass rubbers comparable to *H. brasiliensis*, has attracted research interest as an additional source for natural rubber (Bowers, 1990; Mooibroek and Cornish, 2000). In spite of some limitation



as an alternative rubber crop due to its slow volume growth and low abundance of rubber particles, guayule has been proposed as a viable commercial alternative for hypoallergenic latex (Mooibroek and Cornish, 2000). Despite some limitation as an alternative rubber crop due to its slow volume growth and low abundance of rubber particles, guayule has been proposed as a viable commercial alternative for hypoallergenic latex (Cornish, 1996). Therefore it is highly likely that guayule has the protein homologous to the SRPP. Singh et al. (2003) demonstrated by immunocytochemical analysis that the SRPP-antibody did not show any cross reactivity with the rubber particle proteins in *Ficus carica* and *Ficus benghalensis* that produce substantially lower molecular weight rubber compared with *H. brasiliensis*. Although the rubber content in the latex of fig tree is not as high as that in rubber tree and several other plants, its content is comparable to other rubber biosynthesizing temperate plants that accumulate about 1 to 3% rubber (Bowers, 1990). The amounts of latex in plants and rubber content in the latex vary depending on the physiological conditions of the plants.

*Parthenium argentatum* is an alternative source of natural rubber for biomedical applications. In Guayule, mature stem bark contains high rubber content and rubber producing potentials, younger stem contains low rubber content and lower potential for producing rubber. Currently, *P. argentatum* is used for the alternative source of natural rubber because it requires very small quantity for DNA barcodes analysis and also purity of breeding lines also determined at early stage of seedling growth. Barcodes would allow seed producers and breeders to discover seed lot contamination before advance breeding lines for latex production. It has the ability to remove contaminating lines, especially when they represent lower rubber lines; would improve the efficacy of breeding efforts. Several genera within the Asteraceae produce high molecular weight rubber in the cytosol, including *Lactuca sativa* and *Taraxacum koksaghyz*, and the species of interest to our studies. *P. argentatum* was determined by DNA barcoding study which forms the foundation for genetic identification of commercially important lines of natural rubber latex for biomedical applications (Kumar et al., 2009).

All the genotypes introduced are vegetatively multiplied and maintained in bush nurseries, preferably in two geographically distant locations as precaution against calamities. It is also important to maintain a minimum number of plants of each genotype. To ensure identity and accessibility, authoritative layout sketches, sign boards and registers are maintained. Many other introductions from Brazil to Asia and also Africa were carried out between 1896 and 1974, including some species different from *H. brasiliensis* (Dijkman, 1951; Ong and Tan, 1987). All collections were quantitatively rather limited, especially for non-*brasiliensis* species. In 1981, the International Rubber Research and Development

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

The field conservation gardens 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. The field evaluation of this wild Amazonian germplasm showed that the latex yield was as low as about 10% of GT1, one of the most cultivated clones. Attempts to improve it through Wickham × Amazonian crosses resulted in recombinants with a still 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, enabling the selection of very vigorous Wickham × Amazonian clones. A clear difference in branching habit could be observed between accessions from Acre and Rondonia, which more often have tall trunks with poor branching located at high height, and those from Mato Grosso, which display abundant branching at low height. Obviously, this wild Amazonian germplasm is bearing an important genetic burden in terms of unfavorable alleles. From the evaluation of the IRRDB 1981 germplasm in Ivory Cost, a working population of 287 accessions was selected, taking into account genetic diversity but mainly based on yield; the average yield level of this population is estimated at 36% of the level of GT1 (Clément-Demange et al., 1998; Nicolas et al., 1988). Four genetic groups of this population could be the base of a population pre-breeding work aimed at improving their yield level before testing them by crossing with the Wickham population.

*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. During the early years of rubber plantation industry, propagation of the crop was through seeds only. Later, vegetative propagation using buds became common. At present, seeds are utilized mainly for the production of root stocks. Special types of seeds known as polyclonal seeds are used directly for propagation. Polyclonal



seeds, which are hybrid seeds, are produced in plantations called polyclonal seed gardens. In these gardens, several clones are planted intermixed so as to maximize cross-pollination. Clones planted in these gardens should possess desirable characters like high yield, disease resistance, vigor, ability to produce good seedling families and profuse production of seeds. Rubber is currently propagated in nurseries through axillary bud-grafting (vegetative multiplication) and planted at a density of about 500 trees per hectare. The buds are collected from budwood grown in the budwood gardens which are developed for the recommended clones. The plants produced in the nurseries can be budded stumps grown in the soil, or budded plants grown in plastic bags. Rootstocks can be also grown directly in the plantation field at standard density, with budding carried out at field level. Under this system, there is no transplanting operation from the nursery to the field, but field maintenance before tapping lasts an additional year.

Plant propagation through asexual (vegetative) parts such as buds, leaves and stem cuttings is termed as vegetative propagation. Vegetative propagation of rubber is carried out mainly by budgrafting (budding). Propagation through rooted cuttings is possible in rubber but is not generally practised due to unsatisfactory development of the root system, especially tap root. Depending on the part of the stock where budding is carried out, buddings are classified into four types. These are, base budding, crown budding, over budding and high budding. Propagation of rubber is possible through tissue culture too. Tissue culture plants were developed via somatic embryogenesis using anther, leaf explants. Carron et al. (1995) compared the micropropagation capacities of clones with explants issued either from mature plants or from theoretically rejuvenated somaplants (plants from somatic embryos). The micropropagation capacity of explants from somaplants was much greater than that of explants from mature trees. This suggests that plants from somaplants are completely rejuvenated and behave as seedlings. Research on somatic embryogenesis from immature inflorescences was also reported by Sushamakumari et al. (2000). At the Rubber Research Institute of India (RRII), high frequency somatic embryogenesis and plant regeneration were achieved from immature anthers of Indian *Hevea* clones by Kumari Jayashree et al. (1999). Transgenic rubber plants were also developed successfully by Jayashree et al. (2003) and Rekha et al. (2006) (Figure 1). Chen et al. (2001) reported the production of somatic plants through embryogenesis issued from anthers explants, and their use as explants for microcuttage, to generate a new type of "self-rooting juvenile clones." As in the case of most other tree crops, multiplication rate in *in vitro* culture is very low in rubber crop.

Tissue culture techniques for asexual propagation of guayule have been developed in the 1980s (Radin et al.,

1982; Lovelace et al., 1982). The use of tissue culture may be important to maintain the genetic stocks of selected guayule germplasms and cultivars, because the identity of selected lines cannot be maintained in successive generations due to lack of control over sexual reproduction and apomixis. A new method for guayule tissue culture, using low light and ammonium was recently published (Dong et al., 2006). In genetic manipulation experiments, a resistance gene to the herbicide ammonium-glufosinate and various allylic diphosphate synthetase genes were also introduced in tissue-culture generated transgenic guayule plants (Veatch et al., 2005). Also, Russian dandelion is both amenable to tissue culture and can be relatively easily transformed, with the analysis of meaningful rubber phenotypes possible within six months after transformation (Van Beilen and Poirier, 2007).

## MOLECULAR MARKERS

The main aim of rubber breeding is to provide the farmers with adapted superior clones, currently represented by mature budded clones, primarily for latex production and secondarily for rubberwood production. New challenging objectives such as quality of the rubber product, contribution to the protection of the environment, as by carbon sequestration, are emerging. Nowadays, the impressive development of biotechnologies holds great promises, but their combination with conventional breeding in an efficient and integrated way is a major challenge; apart from contributing to latex and rubberwood production improvement, they could also lead to the production of proteins by the laticifer system used as a cellular plant at farm level or "pharming" (Yeang et al., 1998). The main objective of this review provides information to the researchers about historical background and a global view of rubber molecular breeding and genetics.

Molecular markers serve as useful aids in understanding the genetics of *H. brasiliensis* in the recent past. They can play an important role in assisting *Hevea* clonal identification and origin. For the last two decades, a large number of molecular markers and techniques have been applied in *Hevea* breeding. Nowadays many types of molecular marker techniques are available; the most widely used include Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), minisatellite fingerprints and microsatellites or Simple Sequence Repeats (SSR). These markers differ in the type and amount of variability they express, in their suitability for each particular question and in the ease and costs of their development and application. Among these, three major molecular marker techniques were applied on cultivar identification: RAPD, RFLP and AFLP. In addition to these methodol-

ogies, microsatellite markers, otherwise known as Simple Sequence Repeat Length Polymorphisms (SSRLP) are generated by highly specific PCR amplification. SSR are regions of short tandemly repeated DNA motifs (generally less than or equal to 4 bp) with an overall length in the order of tens of base pairs. SSR have been reported to be highly abundant and randomly dispersed throughout the genomes of many plant species.

Thus, SSRLP may occur even between closely related individuals. Microsatellite markers have been used in plants for fingerprinting, mapping, and genetic analysis. Though RFLPs are powerful for studying genetic diversity and mapping, this technology is not preferred now since it is labour intensive and requires large DNA samples. Its marker index value (expressed as the number of polymorphic products per sample) is also low with only 0.10 compared to PCR based marker systems like RAPDs (0.23), SSRs (0.60) and AFLPs (6.08) (Low et al., 1996). Initially, isozymes were utilized for clonal identification (Chevallier, 1988), subsequently other tools like minisatellites (Besse et al., 1993a), RFLPs (Besse et al., 1993b; 1994), mitochondrial and chloroplast RFLPs, RAPDs and DAFs (Low et al., 1996; Varghese et al., 1997; Venkatachalam et al., 2001; 2002; 2006; 2010), AFLPs (Lespinasse et al., 2000), and SSRs (Besse et al., 1993a; Atan et al., 1996; Low et al., 1996; Bindu Roy et al., 2004) were developed and used in detection of molecular markers in *Hevea brasiliensis* (Figure 1)

In literature, a dozen species of *Parthenium* were growing on the North American continent, which is native to *Parthenium* whereas *P. argentatum* is the only species with high amount of rubber production. Some species like *P. tomentosum* and *P. incanum* produce only primarily resinous materials. To enhance *P. argentatum* commercial viability, there are two approaches in Biotechnology; i) chloroplast metabolic engineering; ii) marker assisted breeding.

Chloroplast genomic sequences were developed DNA barcodes to discriminate at the species level. In order to differentiate three *Parthenium* species (*hysterophorus*, *schottii*, and *tomentosum*) from each other and from *P. argentatum* and *P. incanum*, a *matK* barcode was already developed. However, this barcode could not be used to differentiate *P. incanum* from *P. argentatum* or *P. argentatum* lines from each other. The barcode information of both *matK* gene and the *psbA-trnH* were combined to differentiate the *P. tomentosum* and cv. 11591 from the other two species (*P. argentatum* lines and *P. incanum*) (Kumar et al., 2009). These barcodes will be used in breeding program.

*Hevea* rubber tree (*H. brasiliensis*) is the only plant species being cultivated for commercial production of rubber in the world. It is therefore of great interest to study the regulation and the expression of the genes involved in natural rubber biosynthesis. To meet the ever increasing rubber demand, it is necessary to increase the latex production substantially by genetic manipulation

(Priya et al., 2006; Venkatachalam et al., 2009b). Rubber biosynthesis in *Hevea* has become a major field of research to understand the isoprenoid pathway. The general metabolic pathway of rubber biosynthesis is as follows: Sucrose from photosynthesis is actively transported into laticiferous cells through the plasmalemmic membrane, and is then hydrolyzed into glucose and fructose by invertase. These sugars are then converted into acetyl-CoA through glycolysis. Three molecules of acetyl-CoA are condensed into mevalonic acid which is converted to isopentenyl pyrophosphate (IPP). Polymerization of thousands of IPP molecules assisted by the action of the enzyme rubber transferase in association with REF, a molecule fixed on the rubber particles membranes leads to the formation of high molecular weight rubber. Genes expressed in the latex of *Hevea* can be divided into three groups based on the proteins they encode: (1) rubber biosynthesis-related proteins such as rubber elongation factor (REF), hydroxymethylglutaryl-coA reductase (HMGR), hydroxymethylglutaryl-coA synthase (HMGS), cis-prenyltransferase (CIS), geranylgeranyl diphosphate (GGPP) synthase, small rubber particle protein (SRPP), isopentenyl diphosphate (IPP) isomerase; (2) defense/stress-related proteins such as MnSOD, hevein, chitinase,  $\beta$ -1,3-glucanase and HEVER; and (3) latex allergen proteins such as Hev.b.3, Hev.b.4, Hev.b.5, Hev.b.7, etc. Biological functions of the allergenic proteins are largely unknown (Oh et al., 1999).

## COMMERCIAL APPLICATIONS

Natural rubber is a highly valuable and strategically important biomaterial; unlike most other biopolymers, it cannot be replaced by synthetic materials in many of its applications. For example, heavy-duty tires for trucks, busses, and airplanes, as well as many latex products for the medical profession, cannot be made exclusively from synthetic rubber, or only at a significantly higher cost. Approximately 10% of natural rubber is used as latex to produce gloves, condoms, catheters, and other medical products. Especially in the USA, but also in Europe and Japan, increasing numbers of people are allergic to proteins in *Hevea* rubber. Thus, alternative crops for natural rubber production would not only help to secure supply, but could also provide a source of hypoallergenic rubber. For all of the above reasons, there is interest in countries depending on imported natural rubber to develop large-scale alternative (domestic) natural rubber production (Mooibroek and Cornish, 2000). This has been recognized at various times in the past, leading to research and development programs during which many plants were investigated. Eight botanical families, 300 genera, and 1,800 species have been identified that produce natural rubber in their latex, but only a few of these are known to produce large amounts of high

molecular weight rubber (Bushman et al., 2006). Guayule, a shrub growing in semi-arid regions in Mexico and the Southern US, is the only non-tropical plant that has been used as a commercial source of natural rubber (early 20th century). Several other potential sources of natural rubber were investigated as well. One of these, the Russian dandelion, was promising enough to justify large research and development programmes in the USSR (1931 to 1950), in the US in the framework of the Emergency Rubber Program during WWII, and in other countries. Many other species have not been studied in sufficient detail to establish their utility or were found to contain rubber of low molecular weight. Until now, the most important product of the rubber tree was its latex and considerable efforts have been taken to improve the latex yield.

With the depletion of tropical forests leading to a shortage of timber for many industrial and engineering uses, attention has moved on rubber wood as an alternative source of timber for markets (Killmann, 2001; Arokiaraj et al., 2002). As the composition of wood is important for the pulp industry, genetic engineering to improve lignin content in rubber is a very active area of research that has been stimulated in recent years by the characterization of important genes controlling lignification. This tropical wood was originally used merely as a fuel for drying and smoking rubber and to provide a source of charcoal for local cooking. However, rubberwood has favorable qualities and light color, making it a good timber for furniture making and other applications. The export of rubber-wood from Malaysia rose from RM900 million in 1993 to RM3.7 billion in 1998 and subsequently to RM5.2 billion in 2001 (Arokiaraj et al., 2002). This figure clearly indicates how rubber wood is growing. In trees, only limited information is available about the process of differentiation and development that are involved in wood formation. Knowledge of what determines the pathway of differentiation that cambium cells undergo is essential to any attempt to design better wood characteristics and improve latex yield. In rubber tree, the homeobox (HB) gene has been isolated and it is presumed that HB genes may be involved in differentiation of cambium cells to form latex vessels (Arokiaraj et al., 2002). The research is important because it clearly demonstrates that modifying specific genes in the wood forming process can also potentially influence important tree characteristics and hence improve timber production.

## ADVANTAGES AND DISADVANTAGES

*Hevea* latex products are responsible for moderate to severe allergic reactions due largely to proteins naturally associated with the rubber particles. The incidence of such reactions has increased dramatically in the last 15 years and it is now accepted that 1 to 6% of the general

population suffer from latex allergies. Some studies have shown that up to 17% of healthcare workers are at risk of reactions (Bousquet et al., 2006). The new standard offers an opportunity for medical product manufacturers looking to develop safe protein-free high performance latex from alternative plant sources. *H. brasiliensis* latex allergens have also been reported to occur in the particulates derived from rub-off and wear of tires. The presence of allergens in particulates possibly worsens asthma symptoms in sensitized patients (Namork et al., 2006). Other research, however, indicates that dry rubber products (cut thread, hot water bottles and divers' flippers were tested) contain extremely low amounts of residual extractable protein.

While guayule rubber has the same molecular weight and general properties as *H. brasiliensis* rubber, it does not contain the proteins that can cause severe allergic reactions (Cornish, 1996). This development has revived interest in guayule rubber, and the US-based company Yulex is now building a small-scale production facility of low-protein guayule latex, providing a new source of hypoallergenic natural rubber gloves and medical items. Initial target applications for guayule rubber include gloves and other products for which the strength and resiliency of natural rubber is desired without the potential allergic reactions.

Dry guayule rubber isolated directly from the shrub may contain as much as 20 to 40% resin by weight. If the rubber is isolated by solvent extraction and deresinated, the bulk viscosities of the rubber are lower than those of *H. brasiliensis* rubber. Only selective coagulation to isolate the high molecular weight fraction of the guayule rubber produces material with properties similar to *Hevea* rubber (Schloman, 2005). *H. brasiliensis* rubber contains proteins and reactive functional groups that cause storage hardening with an increase in bulk viscosity when stored over long periods of time. This results from cross-linking reactions that produce branched polymer chains. In contrast, guayule rubber undergoes irreversible, heat-induced chain cleavage, thus, is less stable to oxygen and heat than *Hevea* latex. Unsaturated fatty acid triglycerides present in the resin facilitate oxidation of the polymer chain. Combinations of an amine antioxidant and a zinc dialkyldithiocarbamate have been used to counter these effects, improving guayule rubber stability (Schloman, 2005).

Laboratory tests on the physical and chemical properties of *T. koksaghyz* rubber showed that it was of excellent quality. Tires made from Russian dandelion rubber were as resilient as those made from *H. brasiliensis*, and better than guayule-based tires (Heim, 2003). One potential disadvantage of Russian dandelion is that rubber particles contain possibly more associated proteins than *H. brasiliensis* rubber particles, raising the possibility that people already sensitized to natural rubber from *H. brasiliensis* may also be sensitized to rubber from Russian dandelion (Cornish et al., 2005). Therefore,



Russian dandelion should be considered also for conventional, non-medical applications, such as tires and other dry rubber applications.

## FUTURE PROSPECTS AND CONCLUSIONS

In order to broaden the genetic base, attempts were made for introducing new germplasm to Asia, including species allied to *H. brasiliensis*, among which the 1981 International IRRDB collection was the most significant. Although, due to the low yield level of this germplasm and to the length of the breeding process, benefits will be distributed only over a long-period. Moreover, the micropropagation techniques are not yet sufficiently refined for commercial application. A number of large-scale field trials with *in vitro* derived clones are in progress and it is expected that these will reveal whether commercial application is possible or not. The field trials will determine the current potential of micropropagation techniques for commercial scale cultivation of rubber. *In vitro* approaches are presently being applied to *Hevea* rubber tree to achieve genetic transformations. These techniques are likely to play a vital role in future tree-improvement programs. Molecular investigations provide a basis for understanding the regulation of various genes and their potential involvement in the complex rubber biosynthetic pathway. Using the identified genes, it is possible to determine the rate limiting steps and the regulation points or controlling events in latex biosynthesis at least at the transcriptional level. Although considerable progress was achieved in annual crops during the last decade, many biotechnological approaches are at a preliminary stage in rubber tree. Moreover, the application of biotechnology to rubber tree requires further investigations on the risk of genetic instability in transgenic plants and on potential escapes of transgenes into wild plants.

A combination of breeding and molecular biology may synergistically provide great benefit to the improvement of natural rubber producing plants. All available rubber breeding tools must be employed to meet the challenges of sustainable agriculture and rubber security in the world. Scientists are investigating new biotechniques that may have great applications in the genetic enhancement of rubber tree. However, biotechnology should be integrated along with other existing techniques in current *Hevea* improvement programs. Today, molecular biotechnology offers to *Hevea* improvement programs; clean and fast multiplication of clones via micropropagation, and genetic markers for assisted selection. Rubber biotechnology has made a first phase impact. If current progress in *in vitro* culture and genetic transformation combined with molecular biology applications continues, the future may witness superior *Hevea* tree species tailored for special agronomic and economic characteristics.

Fig tree contains natural rubber comparable to many other rubber biosynthesizing temperate plants. The rubber particle proteins of fig tree are distinct from that of rubber tree, in that the major proteins tightly associated with the particles are unique and not related to any other rubber transferase. *Parthenium argentatum* Gray, commonly named as guayule, is a shrub belonging to Asteraceae family and it is native to the Southwestern United States and Northern Mexico. The Russian dandelion *Taraxacum koksaghyz*, which has long been considered as a potential alternative source of low-cost natural rubber, has a rapid life cycle and can be genetically transformed using a simple and reliable procedure. However, there is very little molecular data available for either the rubber polymer itself or its biosynthesis in *T. koksaghyz*.

To ensure a stable supply of natural rubber and to decrease our dependence on petroleum-based synthetic rubber, both development of alternative sources of natural rubber and improvement of the *Hevea* tree for higher productivity would be necessary. The development of alternative sources of natural rubber appears essential in the mid- to long-term to both secure our access to this essential polymer and decrease our dependence on fossil carbon for the production of synthetic equivalents. Although alternative sources for natural rubber were investigated in the past, recent progress in plant molecular sciences not only provide us with powerful tools, like genomics, metabolomics and proteomics, to scrutinize the mechanism of natural rubber synthesis, but also provide us new methods for improvement, like marker-assisted breeding.

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