Series of Crop Specific Biology Documents

BIOLOGY OF *HEVEA BRASILIENSIS* (RUBBER)

Phase II Capacity Building Project on Biosafety

सत्यमेव जयते

UNEP

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भाकृअनुप ICAR

Ministry of Environment, Forest and Climate Change
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Biology of *Hevea brasiliensis* (Rubber)

Prepared by:
Ministry of Environment, Forest and Climate Change (MoEF&CC)
and Rubber Research Institute of India, Rubber Board, Kottayam
under UNEP/GEF supported Phase II Capacity Building Project on Biosafety

Complied by:

Dr. James Jacob
Director (Research)
Rubber Research Institute of India
Rubber Board, Kottayam

Dr. A. Thulaseedharan
Joint Director (Biotechnology)
Rubber Research Institute of India
Rubber Board, Kottayam

Dr. R.G. Kala
Principal Scientist (Biotechnology)
Rubber Research Institute of India
Rubber Board, Kottayam

Dr. C. Narayanan
Senior Scientist (Clone Evaluation)
Rubber Research Institute of India
Rubber Board, Kottayam

Dr. Kavitha K. Mydin
Joint Director (Crop Improvement)
Rubber Research Institute of India
Rubber Board, Kottayam

Mr. Sabu P. Idicula
Joint Director (Crop Protection)
Rubber Research Institute of India
Rubber Board, Kottayam

Reviewed by:
Dr. Ranjini Warrier
National Project Coordinator, Phase II Capacity Building Project on Biosafety
Advisor, MoEF&CC

Dr. O. P. Govila
Former Professor of Genetics, Indian Agriculture Research Institute

Dr. Michael Wach
Senior Scientist, Program Manager,
Centre for Environmental Risk Assessment-ILSI Research Foundation

Dr. Vibha Ahuja
Chief General Manager, Biotech Consortium India Limited (BCIL)

Assisted in co-ordination & printing by:
Dr. P. Hareesh Chandra, Project Consultant and Ms Sonia Kaushik, Senior Project Executive, BCIL
Project Coordination Unit, Phase II Capacity Building Project on Biosafety

For further information, please contact:
Ministry of Environment, Forest and Climate Change
Government of India
Indira Paryavaran Bhawan
JorBagh Road, Aliganj
New Delhi 110003
Email: biosafety-mef@nic.in

प्रकाश जावडेकर
Prakash Javadekar

राज्य मंत्री (स्वतंत्र प्रभार)
MINISTER OF STATE (INDEPENDENT CHARGE)
पर्यावरण, वन एवं जलवायु परिवर्तन
ENVIRONMENT, FOREST & CLIMATE CHANGE
भारत सरकार / **GOVERNMENT OF INDIA**

Message

I am happy to learn that the Ministry of Environment, Forest & Climate Change (MoEFCC) as part of the initiative under the UNEP GEF supported "Phase II Capacity Building Project on Biosafety" has developed eight crop specific biology document on Chickpea, Mustard, Papaya, Pigeon-pea, Potato, Rubber, Sorghum, and Tomato.

I am happy to note that the documents have been prepared with support from seven research institutions namely Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research.

While Bt cotton is the only genetically modified (GM) crop approved for commercial cultivation in India, there are several crops under various stages of research, development and field trials. The present set of crop specific biology documents aims to provide scientific baseline information of a particular plant species that can be used as credible source of information for conducting safety assessment of GM plants.

I would like to congratulate all those who were involved in preparing these documents and those involved in steering this initiative.

I am confident that these biology documents will serve as a valuable tool for regulators, scientists, crop developers, policymakers, academicians and other stakeholders who are involved in the safety assessment of GM plants. I am also hopeful that baseline information provided in the biology document would further enhance awareness on biosafety aspects of GM crops.

(Prakash Javadekar)

Paryavaran Bhawan, Jor Bagh Road, New Delhi-110 003
Tel.: 011-24695136, 24695132, Fax : 011-24695329
www.youtube.com/prakashjavadekar www.facebook.com/prakashjavadekar
www.twitter.com/prakashjavadekar e-mail :mefcc@gov.in
website : www.prakashjavadekar.com

Hem Pande
Special Secretary &
Chairman, GEAC

सत्यमेव जयते

भारत सरकार
पर्यावरण, वन एवं जलवायु परिवर्तन मंत्रालय
GOVERNMENT OF INDIA
MINISTRY OF ENVIRONMENT, FORESTS AND CLIMATE CHANGE
इंदिरा पर्यावरण भवन, जोर बाग रोड,
नई दिल्ली-110 003
INDIRA PARYAVARAN BHAWAN, JOR BAGH ROAD,
NEW DELHI-110 003
Website : moef.nic.in

PREFACE

India is an agriculture based economy with abundance of genetic base, diverse agro-climatic zones and highly qualified manpower which provides a rich scope for technological advances in agricultural biotechnology. The shortage of healthy seeds/planting material, lack of disease resistant clones, crop damage by insects, pests etc. have often affected the Indian agricultural economy adversely and therefore the role of new technologies assumes significant importance for Indian economy.

With significant advances in the field of agricultural biotechnology the regulatory system has to deal with multiple crops integrated with multiple traits. In order to streamline the process of safety assessment, the Ministry of Environment, Forest & Climate Change (MoEF&CC) under the UNEP-GEF supported "Phase II Capacity Building Project on Biosafety" has prepared a set of crop specific biology documents namely Chickpea, Mustard, Papaya, Pigeon-Pea, Potato, Rubber, Sorghum, Tomato with support from six Indian Council of Agriculture Research (ICAR) institutions and Rubber Research Institute of India.

The biology documents provides an overview of baseline biological information of a particular plant species such as taxonomy, the centres of origin, its related species including wild relatives, general description of their morphology, reproductive biology, biochemistry, potential for gene introgression, biotic and abiotic interactions. Such species specific information is expected to serve as a guiding tool for use in risk assessment of genetically modified (GM) plants.

The documents has been prepared through a consultative approach and comments received from several organizations have been extremely useful in validating this document. I express my deep appreciation for the support provided by Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research in preparing these documents. I would also like congratulate Dr. Ranjini Warrier, Advisor, (MoEFCC) and Dr O.P Govila (Former Professor, Department of Genetics, IARI) for their sincere efforts and the consultative approach adopted in finalizing the biology documents.

I am confident that these crop specific biology documents would be of immense value for researchers, regulators and industry in planning for the safety assessment of GM crops.

Hem Pande

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BIOLOGY OF
Hevea brasiliensis (WILD. Ex Adr. De Juss.) Muell. Arg. (RUBBER)

1. INTRODUCTION

1.1 Classification and Nomenclature

Hevea brasiliensis (Wild.ex Adr. de Juss.) Muell. Arg. (Para rubber tree), commonly known as "rubber tree" belongs to the family Euphorbiaceae. The taxonomic position of *Hevea brasiliensis* is as follows.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Euphorbiales
Family	Euphorbiaceae
Sub-family	Crotonoideae
Tribe	Micrandreae
Sub-tribe	Heveinae
Genus	*Hevea*
Species	*brasiliensis*

1.2 General Description of *Hevea brasiliensis*

H. brasiliensis is a sturdy, quick-growing, erect tree with a straight trunk and an open leafy crown. The bark is usually grey and fairly smooth. The bark of the trunk is the part from where rubber is harvested (Fig.1). In the wild, the trees may grow to over 40 m with a life span of more than 100 years. However, cultivated plants rarely grow beyond 25-

Fig.1: Rubber plantation (Inset: Trunk of a single mature tree showing latex collection)

30 m in height because of the growth reduction due to harvesting of latex by tapping (Webster and Paardekooper, 1989). Moreover, the trees are usually replanted after about 30 years when yield falls to an uneconomic level. The young plants show characteristic growth pattern of alternating periods of rapid elongation and consolidation. The tree is deciduous with annual leaf fall. Refoliation and flowering follow wintering. The leaves are arranged in groups or storeys. From each storey, a cluster of spirally arranged, trifoliate glabrous leaves is produced. The petioles are long, usually about 15 cm, with extra floral nectaries present in

the region of insertion of the leaflets (Premakumari and Saraswathyamma, 2000). The trees develop a strong tap root and extensive lateral roots forming the whole root system comprising about 15 percent of the total dry weight of the mature rubber tree.

1.3 Economic Importance

H. brasiliensis is the major source of commercial natural rubber. Chemically, natural rubber is *cis*-1,4-polyisoprene, having molecular weight ranging from 200,000 to 8000,000 and with visco-elastic properties. Natural rubber produced in the milky cytoplasm (latex) of specialized cells called laticifers, is one of the most important biological macromolecule, used as industrial raw material for the manufacture of about 50,000 products. Although natural rubber has been found in the latex of over 2,000 plant species belonging to 311 genera of 79 families, *H. brasiliensis* remains the only cultivated species as commercial source of natural rubber because of its abundance in latex, high quality and convenience of harvesting.

The higher strength, low heat buildup and better resistance to wear and flex cracking make natural rubber a suitable raw material for manufacture of automobile tyres. A major quantity of natural rubber produced is consumed by the automobile tyre industry. Natural rubber is a good insulator and can be easily manipulated. Being water resistant, it finds use in the manufacture of water proofing materials. In addition to automobile tyres, more than 50,000 rubber based products such as hand gloves, toys, balloons, hoses, footwear, etc. are manufactured from rubber. It is also useful in soil stabilization, in vibration absorption, road surfacing etc. There is hardly any segment of life, which does not make use of rubber-based material.

The economic production of latex is for about 30-35 years, after which the old rubber trees are felled and become a source of timber. Rubber wood has high environmental acceptability both in domestic and international markets. Processed rubber wood has a wide range of applications like furniture, panelling, table top, flooring, household articles etc. The estimated total availability of rubber wood was 2.30 million m^3 during 2012-2013, of which, stem wood accounted for 1.38 million m^3, Rubber tree is also a source of nectar. It is found at the extra floral nectary glands at the end of the petiole where the leaflets join. Honey can be collected by maintaining hives in the rubber plantations. However, nectar is produced by the rubber plants only for a short period in a year, when refoliation occurs. Rubber seed is a minor source of non-edible oil in India. Seed production is not stable every year. An estimated 45,000 tonnes of seeds are produced in a year. But only a fraction of the seeds are collected and processed. About 10% of the collected seeds are used for raising stock seedlings.

2. AREA, PRODUCTION AND PRODUCTIVITY

2.1 Geographic Distribution

The genus *Hevea* occurs naturally in the entire Amazon Valley, the upper Orinocco basin, the Guianas and the Mato Grosso region of Brazil (Schultes, 1977). Different species of *Hevea* prefer varying habitats. However, all the species are found in Brazil. *Hevea* is also found growing naturally in Bolivia, Colombia, Ecuador, French Guiana, Guiana, Peru, Surinam and Venezuela (Wycherley, 1992). A map showing the regions of the world where *Hevea* is naturally present is given in Fig.2.

Fig.2: Detailed map showing regions where *Hevea* species are found naturally.

The global area under rubber cultivation is about 9.6 million hectares producing 9.2 million tons annually (IRSG, 2014). The major rubber producing countries are Thailand, Indonesia, India, Malaysia and Vietnam. Rubber is also commercially grown in Sri Lanka, Philippines, Nigeria, Cameroon, Ivory Coast, Liberia, Brazil, Mexico etc. A world map indicating rubber cultivating countries is given in Fig.3.

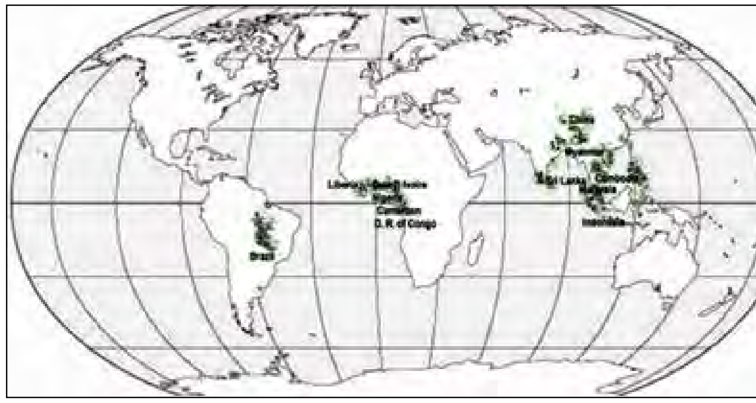


Fig.3 : World map showing rubber cultivating countries

2.1.1 Related species of *Hevea*

Different species of *Hevea* have been reported from Brazil, which is considered as the centre of origin. Apart from *H. brasiliensis*, nine other species have also been identified based on their taxonomic description (Schultes, 1970; Pires, 1981). Except *H. brasiliensis*, other species are not grown in India. The other nine species are:

1) *Hevea benthamiana*

H. benthamiana is a medium sized tree, usually attaining 20 to 24 m in height. Flowers are yellowish and seeds are small, ovoid and grayish with rich brown mottling. It hybridizes freely with other species of *Hevea*. It produces white latex and the rubber is of good quality.

2) *Hevea camargoana*

This is the most recently reported species and is restricted to the Marajo Island in the Amazon delta. The trees are small to medium in size and grow in transition savannahs and woodlands near swamps and streams. Flowering occurs within one year of planting. Flowers are whitish with rose-red colouration at the base and have one whorl of three to five anthers. Crossing with other species is not reported.

3) ***Hevea camporum***

H. camporum is endemic to far South Amazon tributaries. The trees are small, usually attain a height less than 2 m and grow in dry savannahs. Flowers are yellowish. The seeds are the smallest in the genus and are grey in colour with blackish spots.

4) ***Hevea guianensis***

H. guianensis grows in well-drained areas, from sea level up to 760 m. It is tall, attaining a height of 24 to 27 m with a cylindrical trunk. Branches are formed at a higher level and the crown is compact. *H. guianensis* and *H. guianensis* var. *lutea* have five anthers, arranged in one regular whorl in the former but in one irregular or two regular whorls in the latter.

5) ***Hevea microphylla***

Trees are slender, reaching a height of about 21 m with a whip-like trunk slightly swollen at the base and sparse crown. It grows along deeply flooded river banks. *H. microphylla* has the largest female flowers in the genus, with a distinctive, greatly swollen torus. Unlike other species, the capsule opens slowly and the seeds are not ejected violently. Latex is white and watery with very little rubber.

6) ***Hevea nitida***

This is a small to medium-sized tree, usually growing in sandy areas. It has a cylindrical trunk with characteristic red brown bark. Flowers are pale yellow or whitish yellow. The fruits have reddish tips and the seeds are angular in shape. Latex is pure white in colour with high resin and low rubber content and has anticoagulant properties. *H. nitida* var. *toxicodendroides* is a shrub of about 2 m tall and has smaller seeds than *H. nitida*.

7) ***Hevea pauciflora***

H. pauciflora is a stout tree growing to a height of about 27 m, with a cylindrical trunk and brittle, dark brown bark. It prefers well-drained rocky hill slopes. Fruits have reddish tips and seeds are comparatively large in size. It produces a white or dull, sticky latex, rich in resins and containing a weak and hardly elastic rubber. *H. pauciflora* is a rare tree of the Rio Negro, though the variety *coriacea* is widely distributed. The flowers of *H. pauciflora* var. *coriacea* have a reddish colour at the base, and the seeds are markedly smaller.

8) ***Hevea rigidifolia***

This is a rare species in the genus endemic to the uppermost Rio Negro, Brazil. The tree, which grows to a height of 12 to 18 m, has a slender cylindrical trunk and a small crown. Flowers are large, usually with six anthers in two irregular whorls. Fruit tips are red in colour. The white latex contains resins and does not yield good quality rubber.

9) ***Hevea spruceana***

H. spruceana usually grows on river banks prone to flooding. The tree is medium sized with a swollen trunk and a comparatively dense crown of membraneous leaves with whitish pubescence on the underside. Flowers are large, red or brownish purple in colour and excessively pungent. Fruits open slowly. The seeds, which are the largest in the genus, are ventrally compressed, long and angular. Latex is white and watery.

2.1.2 History of Domestication

H. brasiliensis is one of the recently domesticated crops in the world. Before the commercial cultivation of *H. brasiliensis* started, the major sources of natural rubber were *Ficus elastica* and *Castilla elastica* which grew wild in the forests of Central and South America, India, Africa, Madagascar etc. In India, the major contribution was from *Ficus elastica* (Assam rubber). The rubber was promoted as a plantation agriculture crop in South East Asia to feed raw material for the rubber based industries located in Europe in late 19th Century. (Thomas and Panikkar, 2000). Attempts to domesticate *H. brasiliensis* in the South East Asia were started with the arrival of 2000 seeds, collected from the centre of origin at Kew gardens, Kew, during June 1873. The initial attempt to propagate these plants through cutting failed as the climate was unfavorable.

Later a shipment of 1919 seedlings packed in portable greenhouses was sent to Ceylon of which 90 per cent survived; of these 18 seedlings were sent to the Botanic Gardens at Bogor, Indonesia and only two survived. Fifty seedlings were sent to Singapore and only one survived (Dean, 1987). During 1876, seedlings from Kew Gardens were received in Sri Lanka, Java, Singapore and subsequently Malaya and another 22 plants were sent to Singapore in 1877 (Wycherley, 1959). During the early days of rubber cultivation, Sri Lanka became the centre of activity with the Heneratgoda Botanic Gardens in Colombo being the major supplier of rubber seeds and seedlings for domestic distribution and for export.

2.1.3 Establishment of Rubber Plantations in India

The growth of the Indian rubber plantation industry has been mainly through the expansion of rubber cultivation in Kerala. The cultivation of rubber in India actually started in 1878 from the rooted cuttings imported from Royal Botanic Gardens, Heneratgoda, Sri Lanka (Dean, 1987). During 1878-87, many consignments of seeds and rooted cuttings had been sent to Nilambur in Kerala. Originally, rubber was planted as a forest crop in the teak plantations of Nilambur valley under the Forest Department of the Government of Madras. In June 1879, twenty eight *H. brasiliensis* plants, out of the 33 received from Sri Lanka, were planted at Nilambur near the Government teak plantations.

The British planters initiated rubber cultivation on a plantation scale and the state administration encouraged them by providing land, labour, capital and trade facilities. The price boom during the early 1900s attracted foreign investors to India in estate enterprises. Planting in the first rubber estate in Travancore was initiated in 1902 at Thattekad on the bank of Periyar River. In 1904, planting began in Yendayar, Eldorado and Mundakayam estates aggregating to a total of 240 acres in Mundakayam region (Speer, 1953). Subsequently, the Central Travancore Rubber Company and Mundakayam Valley Rubber Company were formed in 1906. By 1910, Travancore had become the lead state of rubber planting in India with 18252 acres and Mundakayam became the main centre (Sarma, 1947) of rubber planting with about 10000 acres.

2.2 Distribution in India Including Regions of Cultivation and Existence of Naturalized Populations

In India rubber is cultivated in about 7.35 lakh hectares with an annual production of about 9.1 lakh metric tonnes (Indian Rubber Statistics, 2012). Kerala and the Kanyakumari district of Tamil Nadu together constitute the traditional rubber growing region of the country. About

85 % of the rubber cultivation is in the traditional region and the remaining is in Karnataka, and the North Eastern States and a very minor contribution from Andaman & Nicobar Islands, Goa, Maharashtra, Orissa, West Bengal and Andhra Pradesh. The rubber cultivating areas in India is given in Fig.4.

Fig.4: Rubber cultivating areas in India.

3. GEOGRAPHIC ORIGIN, GENOMIC EVOLUTION AND CHROMOSOME NUMBER

3.1 Centers of Origin and Diversity

The different species of the genus occur naturally in an area which covers the whole of the Amazon basin, extends southwards to the foothills of the Mato Grosso region and northwards into the upper part of Orinoco basin, the lower slopes of the Guiana Highlands and parts of the lowlands of the Guianas. This large area covers parts of Brazil, Bolivia, Peru, Columbia, Ecuador, Venezuela, French Guiana, Surinam and Guyana up to an altitude of about 800m (Webster and Paardekooper, 1989) (see also Fig.2). All the species of *Hevea* grow together here, and since there is no cytological barrier for hybridization between most species, natural hybrids and variants are also present in the population.

3.2 Genomic Evolution

Based on the chromosome count made by several investigators, the chromosome number of all *Hevea* species is established as 2n= 36 with the exception of one triploid clone, *H. guianensis* (2n = 54) and another species, *H. pauciflora* with 2n = 18 (Polhamus, 1962; Baldwin, 1947; Majumder, 1964). Although *Hevea* behaves as a diploid, some investigators consider it as an amphidiploid with 2n = 36 and x = 9, that stabilized during the course of evolution (Raemer, 1935; Ong, 1976; Wycherly, 1976). Natural triploid plants with 2n = 3x = 54

(Nazeer and Saraswathyamma, 1987) and induced tetraploids with 2n = 4x = 72 by the application of colchicine (Saraswathyamma and Sethuraj, 1992) were also reported. Estimated haploid genome size of *H. brasiliensis* is 4×10^9 (≈ 4 gb) base pairs considering its disomic nature (Roy *et al.*, 2004). *Hevea* nuclear genome contains 48% of slowly annealing DNA (putative single copy) and 32% middle repetitive sequences with remaining highly repetitive or palindromic DNA (Low and Bonner, 1985) The complete chloroplast genome has been sequenced and found to be 161,191 bp in length including a pair of inverted repeats of 26,810 bp separated by a small single copy region of 18,362 bp and a large single copy region of 89,209 bp. The chloroplast genome contains 112 unique genes, 16 of which are duplicated in the inverted repeat (Tangphatsornruang *et al.*, 2011).

The predominantly out breeding nature of *H. brasiliensis* has resulted in the generation of tremendous genetic variability within the species. Hybridization aids recombination of superior genes and clonal selection among the hybrids leads to fixation of desirable recombinants. Apart from hybridization and clonal selection, the importance of polycross populations generated through polyclonal seed gardens have also contributed to the genetic variability in *Hevea* (Tan 1987; Simmond, 1989). The genetic divergence among the cultivated *Hevea* clones of different countries of origin through phenotype assessment was done in the early 1990s and using molecular markers recently, revealed considerable clonal variation. The extent of diversity among forty clones originated from India, Malaysia, Indonesia and Sri Lanka, in respect of rubber yield and various physiological, morphological and structural attributes were assessed and the existence of considerable genetic diversity among the clones were observed. The clones were found to belong to eight genetically different clusters. It is also observed that geographical diversity had no relation with genetic diversity (Mydin *et al.*, 1992). Later genetic diversity analysis using molecular markers such as RAPD, AFLP, microsatellites and SNPs have also revealed similar results (Venkatachalam *et al.*, 2002; Roy *et al.*, 2004).

3.3 Genetic Diversity of Indian Germplasm

The genetic base of *Hevea* in the east is very narrow, limited to a few seedlings originally collected from a minuscule of the genetic range in Brazil referred to as the "Wickham base" (Simmonds, 1989). Using this gene pool, substantial improvement in productivity has already been achieved over the past few decades. The original narrow base has further narrowed down through the unidirectional selection for yield, a cyclical generation-wise assortative mating pattern and a wider adaptation of clonal propagation by budding. A cyclical breeding pattern with the best genotypes in one breeding cycle used as parents for the next, has led to the selection and release of clones that are more or less related. The parentage of popular clones bred in various rubber growing countries can be traced back to a handful of parent clones (Tan, 1987; Varghese, 1992; Mydin and Saraswathyamma, 2005).

Commercial cultivation is very often restricted to high yielding cultivars which may become obsolete when newly improved ones are released. As such, it is not unlikely that the older materials disappear in course of time. These, however, are valuable genetic materials and most of the countries where *H. brasiliensis* is an introduced crop, maintain the variants, selections and cultivars as an insurance against gene erosion. Now about 423 *Hevea* cultivars are maintained by the Rubber Board of India, which include the recently developed high yielding popular clones also (Mydin, 2014). Among these, 57 are indigenously evolved clones.

Hevea breeders have recognized the urgent need for collection and conservation of wild *Hevea* germplasm for quite sometime. The Amazon wilderness, along with its natural vegetation has been threatened by rapid deforestation and urbanization. Realizing the seriousness of the situation, the International Rubber Research and Development Board, Malaysia had organized a collection expedition to the Amazon rain forests in 1981. This was the first and major collective attempt to enrich the rubber plantation industry through genetic improvement. This expedition, organized in collaboration with Brazil, collected 64736 seeds from the states of Acre, Rondonia and MatoGrosso and budwood from 194 high yielding trees (Ortets), which are not affected by *Mycrocyclus* and *Phytophthora* (Ong *et al.*, 1983). India's share of the wild germplasm was received between 1984 and 1990 in batches. A total of four thousand five hundred and forty eight seeds have been received and are being conserved at two locations – one in traditional region in Kerala (3576) and the other in the non-traditional region of North East (972) in conservation nurseries.

4. REPRODUCTIVE BIOLOGY

4.1 Growth Stages of Rubber Plant

The seeds are large usually weighing 3.5 to 6 g and ovoid in shape with the ventral surface slightly flattened. The seed coat or testa, is hard and shiny, brown or grey brown with numerous darker mottles or streaks on the surface. The embryo is situated in the middle of the endosperm with the radicle pointing towards the micropyle. The two white, veined cotyledons are pressed against the endosperm and enclose the plumular end of the axis of the embryo. The endosperm forms 50-60 percent of the weight of the seed (Webster and Paardekooper, 1989). The seeds lose viability and deteriorate rapidly after dispersal.

Germination is hypogeal and mostly occurs within a week of sowing. The radicle breaks through the testa at the hilar depression and very soon produces a ring of primordia which rapidly grow out as lateral roots. Further development of radicle is briefly delayed until the laterals have grown about 2 cm, after which it grows rapidly to form the primary tap root. The cotyledons remain within the seed but their stalks elongate and emerge with the plumule between them. Initially the emerging plumule is bent like an inverted U, but it soon withdraws its tip from within the seed, straightens up and grows vigorously. It produces the first pair of leaves about eight days after germination. Subsequently an internode is grown and the first flush of three leaves is produced above it (Fig.5). Simultaneously, further lateral roots develop below the first ring of laterals on the primary tap root, which continue rapid growth, and are well provided with root hairs near its tip (Gomez, 1982).

Growth in length of the stem is discontinuous, being characterized by rapid elongation of the internode towards the end of which a cluster of leaves is produced, followed by a rest period at a node during which scale leaves develop around the terminal bud. By repetition of this sequence, leaves are produced in tiers, storeys or whorls separated by internode. The leaves are glabrous with long petioles (about 15 cm) which bear three extra floral nectaries at the point where they give rise to the leaflets (Premakumari and Saraswathyamma, 2000).

For raising elite planting material for commercial cultivation, seedlings from 3 months to one year old are used as stock plants for grafting buds from elite clones. After successful bud intake, the shoot above the bud union will be removed, the stumps are pulled out and either directly planted in the field or planted in polybags and raised in the nursery. The nursery raised polybag plants are field planted when the plants attain two to three leaf whorled stage. Under favorable conditions the bud grafted rubber plants may grow to about 2 m in height every year for the first 3 years attaining a height of about 25-30 m in 30 years. During cultural operations, branching is induced at a height of about 2.5 m to enhance girthing of the trunk, enhance the crown size as well as to reduce wind damage. Flowering occurs in trees after the 4th year of field planting. Latex harvest will be initiated by the 7th year when the tree attains a girth of 50 cm at a height of 150 cm from the bud union. However, the period varies depending upon the soil and environmental conditions. Sustainable latex harvest will be continued for about 25 yrs (Fig.5).

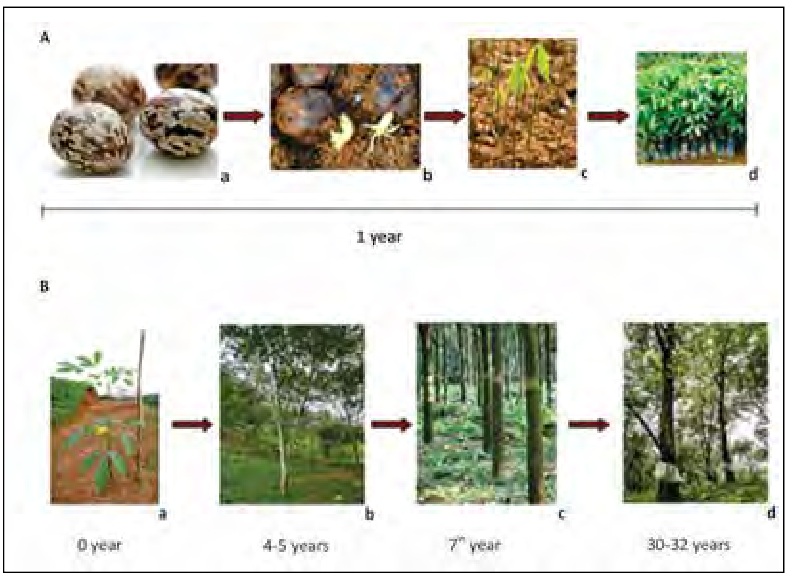


Fig.5: Growth stages of rubber plants. **A**-development of bud grafted plants for field planting; a- seeds; b- early stage of seed germination; c-seedling with fully developed leaf; d-bud grafted plants ready for field planting. **B**-Different growth stages in the field; a-bud grafted plants just field planted; b-plants at the flowering stage; c- a closer view of the main trunk of the plants in the field which are ready to initiate tapping; d-30-32 years old plants which are to be felled for replanting.

4.2 Reproduction

The economic life span of rubber trees under managed condition is about 30-35 years. Rubber is a deciduous tree that winters from December to February in South India. Refoliation and flowering follow wintering. Inflorescences are borne in the axils of the basal leaves of the new shoots that grow out after wintering. Trees older than 4 years after planting are subjected to wintering. *H. brasiliensis* is monoecious with diclinous flowers arranged in a pyramid shaped panicle. The inflorescence is a many- branched, shortly pubescent panicle bearing flowers of both sexes. The larger female flowers are borne at the end of the central axis and main branches of the inflorescence while the smaller and much more numerous male flowers on other parts of the panicle. Flowers of both sexes are short stalked, scented and have a greenish- yellow, bell-shaped calyx with five triangular lobes, but no petals. The male flowers are about 5 mm long have a slender, white staminal column bearing 10 very small, sessile anthers in 2 rings of 5, one above the other. The female flowers are about 8 mm long and have a green, disc-like base surrounded by a three celled, shortly pubescent, conical ovary with 3 short, white, sticky, sessile stigmas. Rubber is a naturally cross pollinated plant. In rubber, flowering usually lasts only for about two weeks on any one tree. In an inflorescence, some male flowers open first and after one day the female flowers open and remain open for 3-5 days, after which the remainder of the male flowers opens. Thus there is incomplete protandry, and a relatively large proportion of cross pollination usually occurs in a plantation (Webster and Paardekooper, 1989; Premakumari and Saraswathyamma, 2000). Fertilization takes place 24 hours after pollination and unfertilized female flowers quickly wither

Fig.6.a : Panicle of *H. brasiliensis* showing immature flower buds

Fig.6.b : Female and male flowers of *H. brasiliensis*

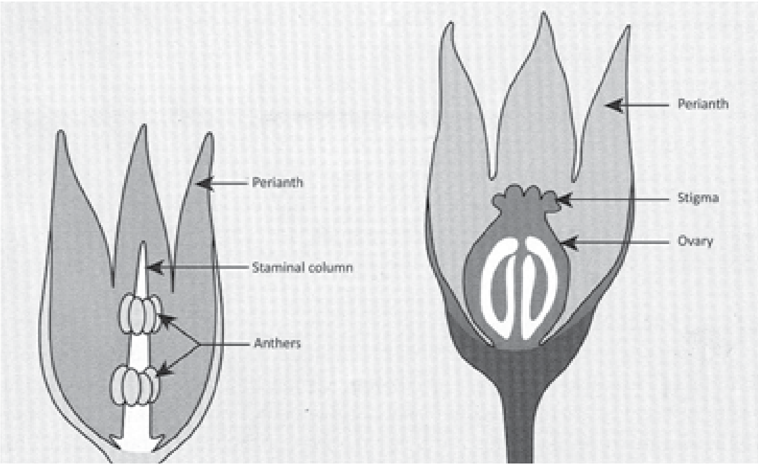


Fig.6.c: Longitudinal section of male flowers (left) and female flowers (right) of *H. brasiliensis.*

and fall. Based on isozyme analysis, the natural out-crossing rate in rubber has been estimated to be 64%. The species also exhibits an inbreeding coefficient of 22% (de Pavia *et al.*, 1993; 1994).

4.3 Methods of Pollination, Known Pollinators and Pollen Viability

Pollination in *Hevea brasiliensis* is by insects. Ceratopogonid midges of *Dasyhelia* and *Forcipomyia* are the major and effective pollinators in India. Although, honey bees visit the trees, they are mainly found to feed on the liquid exudation present seasonally in the tip of leaf petiole only and they rarely visit the flowers (Jayarathnam, 1965). The viability of pollen grains can be as high as 90%, but on an average is only about 50% (Gandhimathi and Yeang, 1984; Sowmyalatha *et al.*, 1997). Pollen trap experiments show that, pollen could not be carried to more than 15 m by wind. For practical purpose the maximum distance *Hevea* pollen can travel is assumed to be 100 m, although there is no evidence for it. Therefore, in seed gardens a boundary belt of that width should be planted with one of the rubber clones as isolation distances and no seed should be collected from this area (Anon, 1965). In trials of genetically modified rubber, pollen flow can be effectively controlled by maintaining a distance of 100 m from the periphery of the trial.

4.4 Potential for Gene Transfer to other Plants

H. brasiliensis is reported to cross only with other *Hevea* species either naturally or artificially. In India *H. brasiliensis* is growing only under cultivated conditions. No wild relatives with the potential for gene transfer are reported in India. Therefore, in India, there is no potential for gene flow to other plants from *H. brasiliensis*.

4.5 Seed Production and Dispersal

4.5.1 Seed production

Only a small proportion of the female flowers set fruit and the majority of the unfertilized flowers are soon shed (Webster and Paardekooper, 1989). Mature fruits containing less than three seeds are rare, suggesting that all three ovules of the female flower must form seeds for the fruit to develop to maturity. Therefore, for successful fruit development, pollen must germinate on all three stigmas of a flower (Gandhimathi and Yeang, 1984). After fertilization, the ovary develops into a three lobed dehiscent capsule, regma with three large mottled seeds.

The seeds are large usually weighing 3.5 to 6 g and ovoid in shape with the ventral surface slightly flattened. The seed coat or testa, is hard and shiny, brown or grey brown with numerous darker mottles or streaks on the dorsal surface. The hilum can be seen as a shallow, approximately circular depression on the ventral surface and the micropyle is adjacent to it. A papery integument lines the inside of the testa and encloses the endosperm, which fills the seed. The embryo is situated in the middle of the endosperm with the radicle pointing towards the micropyle. The two white, veined cotyledons are pressed against the endosperm and enclose the plumular end of the axis of the embryo. The endosperm which forms 50-60 percent of the weight of the seed contains a semi drying oil (Webster and Paardekooper, 1989). The seeds lose viability and deteriorate rapidly after dispersal.

4.5.2 Seed dispersal and viability

About 20-24 weeks after pollination the ripe, dry capsules dehisce explosively with the endocarp splitting into six pieces and forcefully dispersing the seeds to a distance of about 15 m from the tree. There is no dormancy period for the seeds and under natural conditions the viability will be lost within 15-25 days. For storage, seeds can be mixed with sawdust and kept at 7^0 C to prolong the viability up to four months (Ang, 1976).

4.6 Volunteers and Weediness of *Hevea brasiliensis*

Since there is no dormancy period for the seeds, the viable seeds will germinate immediately after dispersal in the field itself, if not collected. These seedlings cannot compete with the cultivated plants and are eventually destroyed. The *H. brasiliensis* plants have no weedy tendencies and they do not grow in unmanaged habitats in India.

4.7 Potential for Vegetative Propagation

No natural methods of asexual reproduction are known in *H. brasiliensis*. The commercial method of multiplication of elite planting material is vegetative by budding a dormant bud (bud patch) taken from the clone to be multiplied (scion) to a seedling (stock) resulting in a new two-part tree, comprising a root system belonging to the stock plant and a shoot system contributed by the donor of the bud. Depending on the color and age of the buds, there are two types of budding viz. brown and green budding. In brown budding, vigorously growing seedlings of about one year of age are used as stock plants with brown buds collected from mature shoots of the same age. In the green budding, three to five month old, healthy, vigorous seedlings are used as stock and tender green coloured buds are used as scion. Stock-scion union takes place in about three weeks (Marattukalam and Mercykutty, 2000). Nevertheless, asexual reproduction or vegetative propagation has not so far been reported either in its natural areas of distribution or in the cultivated regions.

5. HYBRIDIZATION AND INTROGRESSION

5.1 Naturally Occurring Interspecific Crosses

Based on indirect estimates collected from seeds in a natural population of rubber, rubber exhibits more than 60 % natural, intraspecific out crossing (de Pavia *et al.*, 1993; 1994). However, there is not much information about natural interspecific crosses, although, all the species can be crossed interspecifically by artificial pollination. Some natural crossing may also occur in the wild (Webster and Paaradekooper, 1989). There is evidence for natural hybridization of *H. camargoana* with *H. brasiliensis* which grows in the same area. However, *Hevea species* other than *H. brasiliensis* are not cultivated in India. Other species are also not growing wild in India.

5.2 Experimental Interspecific Crosses

There are reports on interspecific crosses among different species of *Hevea*. Interspecific crosses between *H. camargoana* and *H. brasiliensis* as well as *H. benthamiana* and *H. brasiliensis* have been carried out and viable progenies have been generated (Goncalves *et al.*, 1980). However, these hybrids are not cultivated commercially and are not existing as free living populations.

5.3 Genetic Introgression

Introgression between cultivated and wild species has not been documented so far. The back crossing of the F1 with either parent is not encouraged, since being a predominantly out crossing species, this may lead to inbreeding depression. Under experimental conditions, back crossing to the high yielding parent is considered in disease resistance breeding programmes.

6. KNOWN INTERACTIONS WITH OTHER ORGANISMS IN MANAGED AND UNMANAGED ECOSYSTEMS

6.1 Interactions in Unmanaged and Managed Ecosystems

Among crop plants, rubber is an exception with regard to the attack of pests. One reason attributed to the un-attractiveness of rubber to the pests is the presence of latex all over the plant from root tip to the shoot tip, which may coagulate and block the mouth parts of insects and other animals. But a few insects and non-insect pests do overcome this problem and feed on rubber plants (Jayarathnam, 1992). Rubber is growing wild/unmanaged only in its center of origin and interaction with other animals in the unmanaged system is not documented. Rubber plants are also visited by honey bees for a short period in a year, when new flushes come after wintering, to collect nectar produced in the extra-floral nectarines present in the region of insertion of the leaflets to the petiole. They rarely visit the flowers. Ceratopogonid midges of the *Dasyhelia* and *Forcipomyia* genera also visit the rubber plants in the flowering season. Two parasitic flowering plants, *Dendrophthoe* and *Cuscuta* are also found growing on rubber plants. However, this is not prevalent in India. Rats (*Bandicota Spp*.) occasionally feed on kernels of seeds sown in germination bed and also on young plants they gnaw the roots and damage the plants. Porcupines (*Hystrix sp*.) and Wild boars (*Sus spp*.) pull out young plants and feed on tap roots in plantations near natural forests. The details of the common insect pests and disease causing agents are given in sections 6.2 and 6.3. Certain beneficial micro organisms associated with rubber are given in Table.1.

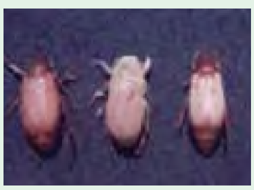
Table 1: Beneficial micro organisms associated with *H. brasiliensis*

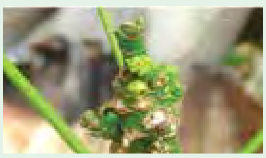
Name	Nature of Interaction
Azotobactor spp.	Rhizosphere nitrogen fixing bacteria
Azospirillum spp.	Root colonizing nitrogen fixing bacteria
Bacillus spp.	Root colonizing phosphate solubilising bacteria
Pseudomonas spp.	Root colonizing growth promoters.

6.2 Important Insect Pests and their Nature of Damage

The important insect pests and their nature of damage are given in Table.2

Insects	Nature of insects and nature of damage
Scale insect	
Saissetia nigra 	Small insects with an outer black, dome-shaped covering. Occur on leaflets, petioles and tender shoot portions and suck the sap; severely affected portions dry up and result in mortality. Spray organophosphorus insecticides like 0.1% malathion or 0.1% quinalphos or 0.03% imidacloprid.

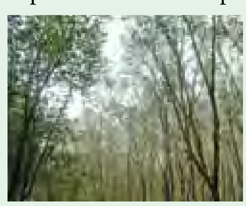
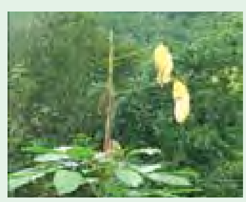
Mealy bug	
Ferrisiana virgata 	Soft-bodied small insect with white mealy outer covering. Occurrence and damage similar to scale insects.Control measures same as for scale insect.
Termite (white ant)	
Odontotermes obesus   Termite attack on plants	Feeds on the dead bark of trees and young plants. Builds covered passage ways of soil on the tapping panel and collection cup. Sometimes young plants dry up due to attack.Drenching 0.1% chlorpyriphos is recommended for control.
Cockchafer grub (White grub)	
Holotrichia serrata *H. rufofluva Anomala varians*  Grub  Adult	Feeds on the roots of seedlings in the nursery and young plants. More prevalent near forest areas and in loose soil areas. The affected plants droop and fall off. Drenching soil with 0.03% imidaclopridor 0.075% quinalphos controls the grubs.
Bark feeding caterpillar	
Aetherastis circulate   Gallery on trunk	*A. circulate* and *P. rosaria* are two genera of bark feeding caterpillars found in rubber trees. The former is more damaging. The caterpillars build galleries with faecal matter and silk all over the trunk region and branches of trees. Generally feed on dead bark and occasionally on live bark causing exudation of latex. Deep scaris found at the regions of feeding. Application of 20% fenvalerate dust @ 7kg/ha or spraying trunk with 0.02% fenvalerate is recommended.
Ptochoryctis rosaria 	

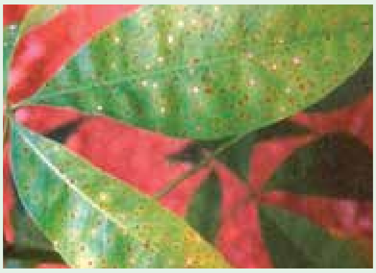
Borer beeteles	
Heterobostrychus aequalis, Sinoxylon anale, Dinoderus bifoveolatus, Xylothrips flavipes, Platipus latifinis, Minthea rugicollis, Xyleborus perforans  Symptoms of borer attack  Larvae of beetle  Borer beetle	Various borer beetles attack on partially dried bark of rubber and make tunnels inside wood. The trees may fall due to trunk snap. Application of 0.5% carbaryl + 0.25% quinalphos on infested trunk portion reduces further damage.
Slug and snail	
Mariaell adussumieri, Cryptozona(Xestina) bistrialis  Slug attack in seedlings  On tapping panel	These molluscs feed on latex by lacerating the tender leaves and buds and side shoots develop giving a bunchy appearance. Slugs drink latex from the tapping cut and collection cup also. Broadcasting 2.5% metaldehyde bait pellets gives control. To repel slugs and snails, brush Bordeaux paste 10% around the stem above the bud union to a length of 30 cm. Repeat the application after 30-45 days

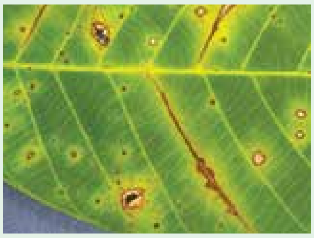
6.3 Important Diseases and their Causal Agents in Managed Ecosystems

The important diseases of *H. brasiliensis* and their causal agents is given in Table.3.

Table 3: Important diseases of *H. brasiliensis* and causal agents and their control in managed ecosystems

Diseases	Casual agents	Symptoms
Abnormal leaf fall	*Phytophthora spp*	Lesions develop on the midrib and leaf blades followed by premature leaf fall in large numbers. A black lesion may develop on the petiole with a drop of latex. Fruits will also rot. 
Shoot rot	*Phytophthora spp*	The tender green shoots rot, mostly in the nursery seedlings and young plants in the field. Noticed during south west monsoon period. 

Patch canker or Bark canker	*Phytophthora spp*	Bark bulging and bursting with oozing of amber-coloured liquid are common. In most cases oozing of latex is observed. Infection mostly during the wet weather on the tapping panel or anywhere on the stem including the collar region and occasionally on the roots 
Black stripe, Black thread or Bark rot	*Phytophthora spp*	Small bark depressions are formed in the renewed bark region due to localised rotting and drying of the bark which get adpressed to the wood. When scraped, deep vertical black lines running downwards into the renewed bark are noticed. Prevalent during the rainy season 
Powdery mildew	*Oidium heveae*	In tender leaves ashy coating and curling, crinkle and edges roll inwards and fall, leaving the petioles attached to the twigs. On older leaves, white patches later causing necrotic spots reducing photosynthetic efficiency. Infected flowers and tender fruits drop affecting seed production.  
Birds eye spot	*Drechslera heveae (petch)* M.B. Ellis	Small necrotic spots with dark brown margins and pale centre on the lamina. A hot weather disease, damaging young plants in the field and nursery. 

Corynespora leaf disease	*Corynespora cassiicola*	In leaves large spots with brown margins and pale centre which fall off forming short holes causing defoliation in nursery plants. Disease incidence occurs during November – May. In mature trees, during refoliation the light green leaves are more susceptible. Disease spreads along the veins and infection on the midrib or base of the leaf causes leaf abscission.  
Pink disease	*Corticium salmonicolor*	White or pink-coloured cob-web mycelial growth on the bark surface with streaks of latex oozing out from the lesions. Rotting, drying up and cracking of the affected bark follow. The distal portions of branches dry and dried leaves remain intact on the dead branches. 
Dry rot, Stump rot, Collar rotor, Charcoal rot	*Ustulina deusta*	Affects roots, collar, trunk and branches with copious exudation of latex from the lesions. Affected bark and wood become soft and powdery with double black lines in the wood. Grey crust-like flat fructifications which later coalesce and turn black are seen on the bark. Affected trees or branches are blown over. 
Brown root disease	*Phellinus noxius*	A general yellowish discolouration of the foliage and unhealthy condition. Affected roots, show encrustation of soil, sand and fungal hyphae cemented to the root and brown lines appear in the roots. 

Poria root disease	*Poria vincta*	Same as above 
Colletotrichum leaf disease	*Colletotrichum acutatum, C. gloeosporiodes*	Infects tender leaves mostly at the leaf tip or margins where numerous spots coalesce and dry up leading to defoliation. The infected leaves often crinkle and become distorted before shedding. 

6.4 Free-living Populations

No natural / free living populations exist in India. The rubber plants grow only under managed conditions.

7. AGRONOMIC PRACTICES

7.1 Soil

Rubber trees can grow on a wide range of soils. However, deep (1 m) well-drained soils free from underlying sheet rocks are best suited (Karthikakuttyamma *et al.*, 2000).

7.2 Sowing Season

The planting material for commercial cultivation is bud grafted plants raised in polybags. The field planting of bud grafted plants is done during May-August when sufficient rain fall is available.

7.3 Land Preparation and Planting

The land is cleared of all vegetation followed by a light burn which retards regeneration of weeds. This is followed by lining the area based on plant spacing and planting density to be adopted. Rubber can be planted by adopting square (4.6X4.6 m) or rectangular (6.7 X 3.4 m) planting systems. Square planting is suitable for level lands and rectangular system can be adopted in slopes. Proper drainage is facilitated by clearing natural waterways available or drains are dug at an interval of 100-200m depending on the slope and drainage problem. Silt pits of about 120 cm length, 45 cm width and 60 cm depth are taken along the contour (150-250/ ha) to check erosion and to conserve water and soil. Construction of stone bunds is another method to check erosion in steep slopes. Rubber is planted in pits (75x75x75 cms) refilled with top soils. The normal planting density is 420 to 500 plants per ha. Polybag plants with fully matured top whorl of leaves are used for field planting.

7.4 Manure and Fertilizer Requirement

The nutrient requirements of rubber plant vary considerably during the immature and mature stages. At the time of pit filling before planting, 12 kg of compost or well rotted cattle manure and 200 g of rock phosphate is incorporated. NPKMg (10-10-4-1.5) or NPK (12-12-6) mixture is applied in Mg deficient and sufficient soils respectively as shown in Table 4.

Table 4: Quantity and the schedule of fertilizer application during the first four years

Months after planting	Dose of mixture per plant (g per plant)	
	10-10-4-1.5 (NPKMg)	12-12-6 (NPK)
3	225	190
9	450	380
15	450	380
21	550	480
27	550	480
33	450	380
39	450	380

The fertilizer requirements of rubber for 5-7 years depend on the cultivation practices especially when the establishment of leguminous ground covers improves the soil conditions and availability of plant nutrients, particularly nitrogen. The fertilizer recommendation from the 5th year of planting for a well maintained field where mulching is practiced and leguminous ground cover is established and maintained is 30, 30, 30 NPK kg/ha. For areas where no legume ground covers are established and no mulching practiced during the initial years, the recommendation is 60, 40, 24 NPK kg/ha. For mature trees under tapping the general recommendation is 30, 30, 30 NPK kg/ha, given in square or rectangular patches in between rows, each patch serving four trees.

7.5 Intercultural and Weed Control

Hevea cultivation requires only minimum intercultural practices. However, weeds compete with rubber for light, moisture and nutrients particularly during its initial years and these can be controlled either manually, chemically or mechanically. The leguminous cover crops, if grown, will smother the weeds in the inter- rows till they get shaded out. After fourth year, weed growth is not a serious problem because by that time the rubber plants close their canopy. So in well maintained fields, weeding need to be carried out only in planting strips from 5th year onwards. The most common cover crops in rubber plantations are *Puraria phaseolides*, *Mucuna bracteata, Calpogonium mucunoides* and *Centrosema pubescens*. Since rubber is planted at wider spacings intercrops such as banana, pineapple etc. can be grown during the initial years. Shade tolerant perennial crops with canopy underneath such as *Coffee, Vanilla* on *Glyricidea* support, *Garcinea* and cocoa can also be cultivated along with rubber without adversely affecting growth and yield.

7.6 Harvest and Post- Harvest Practices

The major harvested crop from rubber tree is latex, a colloidal suspension in which 30-45% is natural rubber (*cis*-1,3-polyisoprene), present in the form of particles. Latex is harvested by the controlled excision of thin bark strips (shaving) from the main trunk using a specialized knife, a process known as "tapping". During tapping latex vessels are cut and latex runs through the channel formed by the cut and collected in collection cups.

Fig.7: Main trunk of a mature tree showing the tapping panel and latex harvest

A downward half spiral tapping cut is made in the bark at an angle of 30^0 to the horizontal on the main trunk from 125 cm height. The tapping is done at a depth of 1.0 mm close to the cambium but without damaging the cambium for the regeneration of the bark. The same cut is reopened during the next tapping to remove coagulum of rubber along with bark shaving. This process continues till the tapping cut reaches the bud union in the trunk (Fig.7.). Further, the tapping can be continued on the other half on the opposite side and later on the renewed bark. Thus four panels and high panels can be tapped in the economic life time of a plant. From renewed bark stage of basal panel, virgin bark above 25 cm height is tapped employing Controlled Upward Tapping (CUT). The normally recommended tapping system is half spiral tapping once in three days (1/s2d3 6d/7). Low frequency systems are useful for cost reduction as well as long-term harvesting.

Latex collected after tapping in cups or other containers are pooled into buckets and processed immediately into marketable forms and for safe storage. The important forms, in which the crop is processed and marketed are 1) preserved field latex and latex concentrates, 2) sheet rubber, 3) crepe rubber and 4) block rubber.

7.7 Seed Production (see also section: 4.5.1)

Seeds for stock plant nurseries are collected mostly from monoclonal and polyclonal plantations. With an aim to produce vigorous hybrid seeds for stock plants, polyclonal seed gardens have been established in a few locations in India. Due to natural cross pollination among different chosen clones planted in specific crossing layouts, the seeds produced are expected to possess more hybrid vigour and hence the stock plants would be healthier with high vigour.

8. BREEDING OBJECTIVES

8.1 Milestones in Breeding

Crop improvement programmes in India were initiated in 1954 and a large number of progenies were evolved and evaluated to identify potential recombinants. Rubber Research Institute of India, (RRII) Kottayam, came out with a series of clones starting from the early RRII 100 series to the most recent RRII 400 series. Early hybrid clones developed by RRII include RRII 100 (Nair and Panikkar, 1966; Mydin *et al.*, 1994) RRII 200 (Saraswathyamm *et al.*, 1990) and RRII 300 (Mydin *et al.*, 2005) series. Among clones of RRII 100 series, RRII 105 is a highly successful and popular clone in terms of realized and potential yield. This clone occupies about 85 per cent of rubber grown area, till recently. Clones RRII 203, RRII 208, RRII 300 and RRII 308 are selections from the 200 and 300 series respectively. The most recent RRII 400 series, from a set of 23 hybrid clones of cross combination RRII 105 x RRIC 100 of which 9 clones were selected based on their early performance at the age of four and a half years, have shown promising trends in the mature phase too and recorded significant yield increase in the range of 23-46 per cent over RRII 105 during first 8 years of tapping in small-scale trials (Licy *et al*., 2003; Saraswathyamma, 2003) and large-scale trials (Mydin *et al*., 2011). Of nine high yielding hybrid clones, two clones viz., RRII 414 and RII 430 were included in category I of the planting recommendations of Rubber Board and released for commercial planting during 2005. Two more clones viz. RRII 417 and RRII 422, were released during 2009.

8.2 Advancements and Challenges

Hevea breeding is aimed at the synthesis of ideal clones with high production potential combined with desirable secondary attributes, such as initial vigor, 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, good response to stimulation, and low frequency tapping. Clones with early attainability of tapping girth and high initial yields are preferred over clones with higher yields in later phases of exploitation. Specific objectives, however, vary depending on specific agroclimatic and socio economic requirements. Marginal and nontraditional areas demand priority for development of clones resistant to prolonged drought, high summer and low winter temperatures, strong winds, altitudes etc. Such situations also demand genotypes responding well under high-density planting, poor soil fertility and low-input agriculture based on sustainable farming systems (Varghese and Abraham, 2006; Mydin, 2014).

The major limitations in rubber breeding and quick release of cultivars include the narrow genetic base, heterozygous nature of the crop, seasonal and non-synchronous flowering pattern, low fruit set, long breeding and selection cycle and lack of fully reliable early selection parameters (Varghese and Mydin, 2000; Mydin, 2014).

8.3 Zonalization of Varietal Testing System

Rubber Research Institute of India (RRII) conducts multilocational trials to identify clones suitable for different locations based on agroclimatic zonation. Accordingly, clones have been identified for the traditional region (Saraswathyamma *et. al.* 2000) as well as non-traditional regions like Tripura (Priyadarsan, 2003), West Bengal, Assam (Varghese, 2003), Meghalaya (Reju*et al*., 2002) and the dry regions of Dapchari in Maharashtra (Chandrasekhar et al., 1998) and Dhenkanal in Orrissa (Gupta and Edathil, 2001). Planting recommendations drawn up for the traditional and non-traditional regions vary based on the differential response of the clones to various environments.The RRII also has specific breeding programmes for evolving rubber clones suitable for the varied environments. Conventionally, there have been two approaches for developing new rubber clones, namely classical breeding and ortet/plus tree selection. The genotypes thus evolved are screened for specific traits and test tap yield of rubber in a nursery. Field trials for evaluation of newly evolved clones are taken up following the basic principles of field experimentation viz, randomization, replication and local control. The popular check clone is included for comparison in each stage of evaluation. The selected genotypes are then cloned/vegetatively multiplied and evaluated

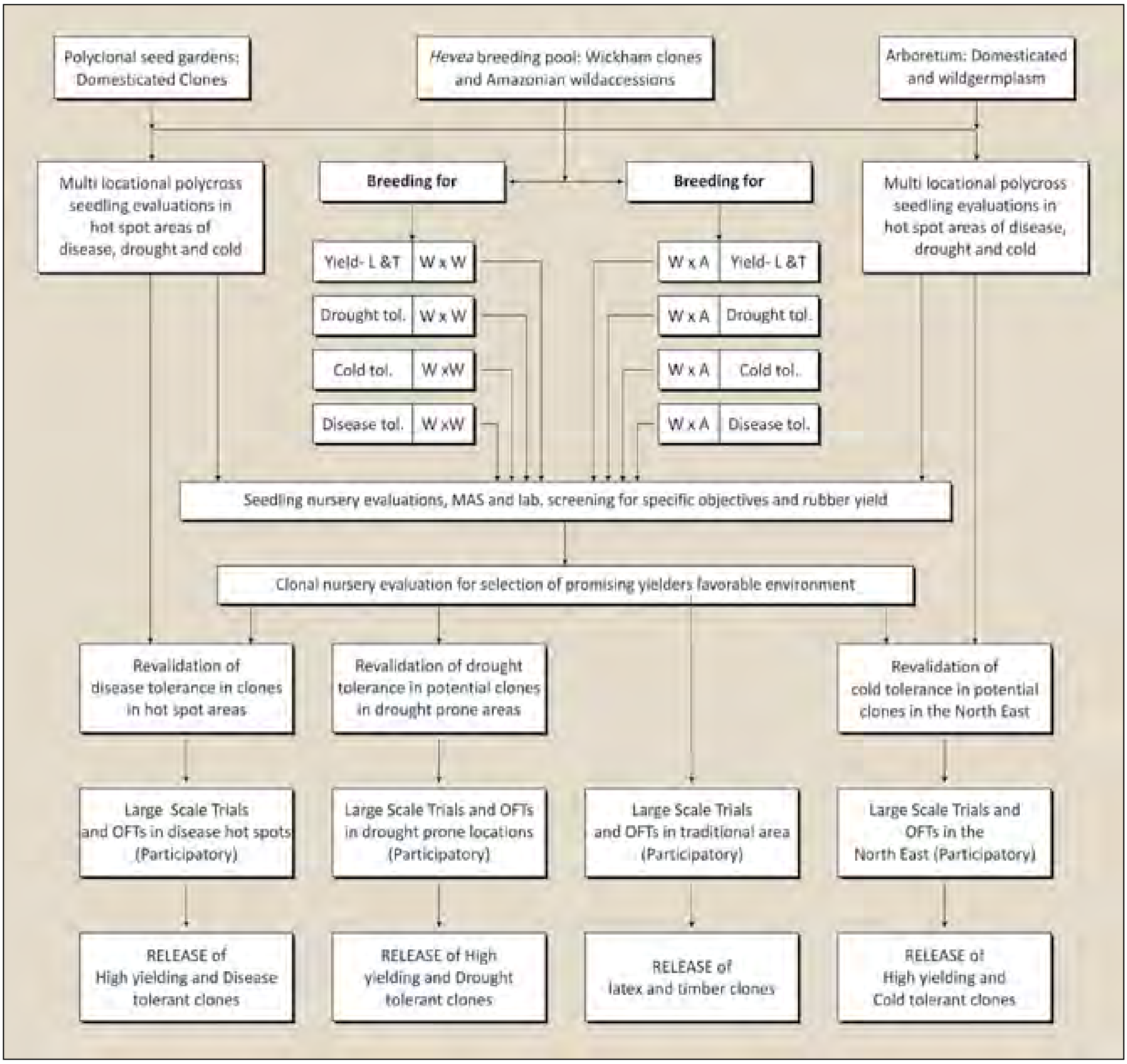


Fig.8: *Hevea* breeding strategy for the varied environment of India

in a phased manner.The three phases of field evaluation consist of Small Scale Trial (SST), Large Scale trial (LST) and farmer participatory multilocational On-Farm Trials (OFT). (Mydin and Saraswathyamma, 2005). A time span of over 20 years is required for completion of the clonal selection procedure.

Small scale trials are essentially rough 'sorting trials' in which numerous entries are evaluated in plots of 5 trees per clone with three replications. Based on the performance of the 15 trees of each clone, over a period of 4-6 years the best ones are selected for evaluation in large scale trials. In a large scale trial each clone is planted in plots of 16 trees with 3 replications. Thus, 48 trees per clone are evaluated for a minimum period of 12 years. Simultaneously, farmer participatory On-Farm Trials (OFT) of batches of selected clones in the pipeline are laid out with clonal plots of 50 trees in various locations.

The clones that perform best in terms of yield and other specific desired attributes in the LST and OFT are finally released for cultivation as described in the flow chart. Region specific clone recommendations are evolved on the basis of the farmer participatory on-farm evaluations. *Hevea* breeding strategy for the varied environment of India is given in Figure 8 .

9. HUMAN HEALTH CONSIDERATIONS, ANY KNOWN ENDOGENOUS TOXINS, ALLERGENS OR ANTI-NUTRIENTS

The major harvested product is latex containing rubber which is an industrial raw material. No endogenous toxins causing human health concerns are reported. There is no documentation of any medicinal property of this tree.

10. BIOTECHNOLOGICAL INTERVENTIONS

Genetic improvement in tree species by conventional methods is a slow and rather difficult process. The transfer of selected genes in a single generation by genetic transformation is especially interesting for the species *H. brasiliensis*, since its improvement is limited by long breeding cycles and high levels of heterozygosity. Initial focus on *Hevea* genetic transformation was to optimize the *Agrobacterium*- and biolistic-mediated genetic transformation systems in rubber trees. Different workers employed GUS as the marker gene under the control of cauliflower mosaic virus (CaMV) 35S promoter and the selectable marker gene used was the sequence coding for neomycin phosphotransferase II (*npt*II) (Arokiaraj *et al.*, 1994; 1996; Montoro *et al.*, 2000; 2003; Blanc *et al.*, 2006). Arokiaraj et al. (1998) observed GUS expression in the leaves and latex of transgenic plants. Transgenic plants integrated with the cDNA coding for human serum albumin (HAS) (Arokiaraj *et al.*, 2002) and for a single chain variable fragment antibody (ScFv4715) against the coat protein of an oral dental bacterium, *Streptoccocus sanguis* under the control of CaMV 35S promoter have been developed (Yeang *et al.*, 2002), to explore the possibility of producing the recombinant proteins in the latex. Analysis of the recombinant proteins at different growth stages of the transgenic plants indicated an increasing trend in the concentration of the recombinant proteins in the latex as the plants aged.

RRII has started active research in the development of transgenic plants integrated with genes for desired agronomic traits. Very efficient *Agrobacterium* mediated genetic transformation systems as well as methods for high frequency transgenic plant regeneration have been developed (Jayashree *et al.*, 2000; 2003; Sobha *et al.*, 2003). Initial focus was to develop transgenic plants tolerant to abiotic stresses like elevated temperature and light, drought and tapping panel dryness. Transgenic plantlets integrated with the superoxide dismutase gene (isolated from *H. brasiliensis*) under the control of different promoters such as CaMV 35S promoter and FMV 34S were developed separately from different cell lines obtained through independent transformation events. The stable integration and copy number of the transgene were confirmed through molecular analysis (Jayashree *et al*., 2000; 2003; Sobha *et al*., 2003a). Physiological and molecular analysis under drought condition showed enhanced SOD expression and significant reduction in oxidative stress in the transgenic plants compared with the control (Jayashree *et al*., 2011). The recovery of the transgenic plants on re-watering after induction of drought was also better with transgenic plants (Sumesh *et al*., 2014). With the objective of enhancing rubber biosynthesis in Hevea, the plants were transformed with the gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A reductase 1 (hmgr1) and transgenic plants were developed and established (Jayashree *et al*., 2010). Transgenic plants integrated with the osmotin protein were developed which provide abiotic as well as biotic stress tolerance in *Hevea brasiliensis* (Rekha *et al*., 2013).

Leclercq *et al*. (2012) developed *H. brasiliensis* plants integrated with a cytosolic isoform of the CuZnSOD gene from *H. brasiliensis* which changed its response to water deficit. Transcript abundance and proline content were also found to be more in the transgenic plants on induction of water stress. In their study also, SOD transgenic plants survived better under water deficit conditions. Sunderasan *et al*. (2012) developed transgenic *Hevea* plants were integrated with a gene encoding human atrial natriuretic factor (HANF), a peptide hormone regulating cardiac blood pressure. HANF transcripts were detected in the leaf samples and western immunoblot detected the recombinant protein.

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Important Contacts: **NATIONAL PROJECT DIRECTOR**
Shri Hem Kumar Pande, Special Secretary

NATIONAL PROJECT COORDINATOR
Dr. Ranjini Warrier, Advisor

Ministry of Environment, Forest and Climate Change
Indira Paryavaran Bhawan, Ali Ganj, Jor Bagh Road, New Delhi - 110003

In association with

Rubber Research Institute of India
P.O. Kottayam, Kerala - 686 009

For further information, please contact at E-mail: biosafety-mef@nic.in