



Rubber Research Institute of India

The Rubber Research Institute of India (RRII), under the Rubber Board (Ministry of Commerce and Industry, Government of India), had its inception in 1955. With a very modest beginning, the RRII is now capable of handling most of the problems associated with natural rubber (NR) production technology, primary processing and product development. The steady growth of RRII in its scientific worth and research contributions has won it the recognition as an International Centre of Excellence in NR research.

Location

The RRII is located on a hillock 8 km east of Kottayam town in Kerala State and is easily accessible by road. Kottayam is connected to all major cities in the country by rail. There are two International Airports, one at Thiruvananthapuram, 160 km south and the other at Nedumbassery, 95 km north of RRII.

Organization

For the efficient discharge of its functions, the RRII has established major research divisions and research supporting sections at its headquarters and regional research establishments at appropriate locations where *Hevea brasiliensis* is commercially grown or is likely to be grown.

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**RUBBER RESEARCH INSTITUTE OF INDIA
RUBBER BOARD**

(Ministry of Commerce & Industry, Govt. of India)

KOTTAYAM-686 009, KERALA, INDIA

E-mail: rrii@rubberboard.org.in

Website: www.rubberboard.org.in

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THE RUBBER BOARD



The Indian Rubber Board was constituted under the Rubber (Production and Marketing) Act, 1947, which came into force on 18 April 1947. This Act was amended in 1954, 1960, 1982 and in 1994. The Act was further amended by the Rubber (Amendment) Act, 2009 which came into force on 22 January 2010.

Organization

The Chairman is the principal executive officer and exercises control over all departments of the Rubber Board. The Research Department, (Rubber Research Institute of India) works under the administrative control of Chairman.

Chairman

Mr. Sajen Peter IAS
(up to 4th November 2010)
Ms. Sheela Thomas IAS
(From March 2011 onwards)

Rubber Research Institute of India

Dr. James Jacob
Director

Crop Improvement

Dr. Kavita K. Mydin
Joint Director

Botany

Deputy Director (vacant)

Biotechnology

Dr. A. Thulaseedharan
Deputy Director

Genome Analysis

Dr. Thakurdas Saha
Senior Scientist

Germplasm

Dr.C.P. Reghu
Deputy Director

Crop Management

Joint Director (vacant)

Agronomy

Dr. M.D. Jessy
Deputy Director

Crop Physiology

Dr. R. Krishnakumar
Joint Director

Plant Physiology

Dr. K. Annamalaiathan
Deputy Director

Crop Protection
Mr. Sabu P. Idicula
Joint Director

Plant Pathology
Dr. Jacob Mathew
Deputy Director

Economics Research
Dr. Tharian K. George
Joint Director

Latex Harvest Technology
Dr. K.U. Thomas
Joint Director

Rubber Technology
Dr. K.T. Thomas
Joint Director

Rubber Technology
Dr. Rosamma Alex
Deputy Director

Technical Consultancy
Dr. Siby Varghese
Deputy Director

Central Experiment Station, Chethackal
Dr. P. Mallinath Priyadarshan
Deputy Director

Regional Research Station, Guwahati
Dr. Dhurjati Chaudhuri
Project Co-ordinator (NE)

Regional Research Station, Agartala
Dr. Sushil Kumar Dey
Deputy Director

Regional Research Station, Nagrakatta
Dr. Gitali Das
Deputy Director

Regional Research Station, Tura
Dr. A. P. Thapliyal
Deputy Director

Regional Research Station, Dapchari
Dr. Meena Singh
Plant Physiologist (Officer-in-charge)

Regional Research Station, Dhenkanal
Dr. Balkrishan
Scientist C (Officer -in-charge)

Regional Research Station, Padiyoor
Dr. Radha Lakshmanan
Senior Scientist (Officer -in-charge)

Hevea Breeding Substation, Nettana
Dr. T.R. Chandrashekar
Senior Scientist (Officer -in-charge)

Hevea Breeding Substation, Paraliar
Dr. T.A. Soman
Scientist C (Officer -in-charge)

Administration
Mr. Raveendran Nair K.
Deputy Secretary

Finance & Accounts
CA. Zachariah Kurian
Deputy Director

ADVISORY PANEL OF EXPERTS

CROP IMPROVEMENT

Dr. K.U.K. Namboothiri

Director
M.S. Swaminathan Research Foundation
Phulabad, Jeypore (R.S.) - 764 002
Koraput, Orissa.

Dr. K.J. Madhusoodanan

Deputy Director (Rtd.)
ICRI, Spices Board, Kavakattu House
Nedumkandam P.O., Idukki Dist. - 685 553

Dr. George Thomas

Interfield Laboratories
XIII/1208, Interprint House
Kochi-682 005, Ernakulam.

Dr. Krishna Reddy

Senior Scientist, Division of Plant
Pathology
Indian Institute of Horticultural Research
Hessaraghatta Lake P.O.
Bengaluru-560 089.

CROP MANAGEMENT

Dr. Reena Mathew

Associate Professor
Rice Research Station
Kerala Agricultural University
Moncompu, Thekkekkara P.O.
Alappuzha - 688 503

Dr. P. Sureshkumar

Professor and Head
Radiotracer Laboratory
Kerala Agricultural University
Vellanikkara, Thrissur - 680 656

Dr. P. Rajendran

Director and Ex-Officio Additional
Secretary
Agriculture PPM Cell
Government Secretariat
Thiruvananthapuram - 695 001

BIOTECHNOLOGY, GENOME ANALYSIS, MOLECULAR PHYSIOLOGY, MOLECULAR PLANT PATHOLOGY

Dr. K. Nataraja Karaba

Associate Professor
Department of Crop Physiology
University of Agricultural Sciences
GKVK Campus, Bengaluru- 560 065.

CROP PROTECTION

Dr. Jim Thomas

Professor & Head
Department of Entomology
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur - 680 656.

Dr. C. Gokulapalan

Professor & Head
Dept. of Plant Pathology
College of Horticulture, Vellayani. P.O.
Thiruvananthapuram-695 522.

Dr. D. J. Bagyaraj

41, R.B.I. Colony
Anandanagar, Bengaluru-560 024.

Dr. V.U.M. Rao

Project Co-ordinator (Agrometeorology)
CRIDA
Santosh Nagar, Hyderabad-500 059.

CROP PHYSIOLOGY

Prof. T.G. Parasad

Professor Emeritus
Department of Crop Physiology
University of Agricultural Sciences
GKVK Campus, Bengaluru-560 065.

Dr. D. Venkataramanan

Joint Director (Res)
Central Coffee Research Institute
No.82, 9th Main, 5th Cross, Coffee Board Layout
Kempapura, Bengaluru-560 024.

LATEX HARVEST TECHNOLOGY

Prof. A. Narayanan

Emeritus Scientist (ICAR)
#19, Phase 5, Maharani Avenue
Vadavalli, Coimbatore-641 041.

ECONOMICS RESEARCH

Dr. D. Narayana

Fellow
Centre for Development Studies
Medical College P.O., Prasanth Nagar
Ulloor, Thiruvananthapuram - 695 011.

Prof. K. Nagaraj

Asian College of Journalism
Second Main Road
(Behind M. S. Swaminathan Research
Foundation), Taramani, Chennai- 600 113.

RUBBER TECHNOLOGY/ TECHNICAL CONSULTANCY

Dr. R.K. Matthan

3A Regent Place
20 Habibulla Road
Chennai - 600 017.

Dr. S.S.Bhagawan

Professor
Department of Chemical Engineering
Amrita Viswa Vidyapeetham
Ettimadai P. O.
Coimbatore-641 112.

Dr. Rani Joseph

Professor
Department of Polymer Science and
Rubber Technology
Cochin University of Science and
Technology
Kochi - 682 022.

DIRECTOR'S REVIEW

Rubber industry is one of the major industries in the world and the demand for natural rubber (NR) is expected to go up in the coming years. Natural rubber sector will face many challenges in the coming decades. Unpredictability of climate and the consequent fall in latex yield, poor performance of world economy, shortage of skilled manpower and rising labour/input cost, pests and diseases menace, increasing extent of senile holdings, non-availability of suitable land for extending rubber cultivation, land fragmentation, alternative land uses, etc. are some of the main challenges. Increasing dependence on agro-climatically less suitable non-traditional locations for rubber cultivation has inherent risks such as lower yield, longer gestation period and lack of skilled entrepreneurs and workers. These regions which lack the best agro-climatic conditions require suitable clones and farm techniques.

Shortage of skilled tappers is a major challenge facing the NR plantation sector in the traditional region. RRII is in the process of developing a tapping knife which could be easily operated by an unskilled person. Wider adoption of scientific tapping practices, especially low frequency tapping, use of good quality planting materials and farm mechanization are priority areas. Controlled upward tapping is one practice to increase latex yield from senile trees. Low frequency tapping can help reduce cost of production. Ensuring sustained profitability is required for rubber growers to stay in business.

The major thrust areas of research in Botany Division are evolving new and improved rubber clones incorporating a wider genetic base, breeding for drought

tolerance, investigations on propagation as well as anatomy of bark and wood. A large number of promising selections continued to out-yield the check clone, RRII 105.

Nine cross combinations were attempted in 2179 pollinations. A few trial clones worth watching ahead are 93/98, 93/172, 93/48 and 89/27. Eleven elite mother trees selected from South Andamans were multiplied. Mean yield data over 10 years of RRII 400 series clones planted in 1993 indicated their superiority over RRII 105. Clone PB 280 registered a record high annual mean yield of 136.4 g/t and summer yield of 108 g/t and had a girth of 105 cm after 14 years of tapping. In many onfarm trials also this clone maintained high yield. The clone D111, a drought tolerant ortet selection from Dapchari recorded highest yield of the 107.8 g/t and exhibited no signs of TPD.

As part of the post-release follow up on RRII 400 series clones, several small holdings growing these clones were inspected. The RRII 400 series clones maintained their superiority in growth and yield. However, there was some regional variation in their yield performance which is being monitored. Phase II of participatory evaluation of clones was initiated with planting 14 pipeline clones in eight locations and an LST at Central Experiment Station (CES) along with three check clones. Growing polybag plants in



polyhouse gave good quality planting materials.

Conservation of domesticated gene pool, its evaluation, screening for diseases/stress resistance and timber latex traits, utilization of wild germplasm in crop improvement, formulation of DUS norms and generation of mapping population for *Hevea* were major activities of Germplasm Division. Among the wild accessions, AC 166 had the highest dry rubber yield (63.67 g/t/t) and this was on par with RRJ1 105. Along with this promising wild accession, three IRCA and three check clones were planted in five locations. The wild accession, MT 4788 showed promising potential for drought tolerance. Eighty four progenies of inter-specific crosses between RRJ1 105 (*Hevea brasiliensis*) and F 4542 (*Hevea benthamiana*) were screened using 12 RAPD primers to confirm their hybridity.

Studies conducted by the Biotechnology Division showed that embryo recovery was best achieved with hormonal combination of 3.0 mg/L zeatin and 1mg/L GA₃ and the recovered plants were established in the field. Pollen protoplast culture studies have been initiated to develop homozygous lines in *Hevea*. Transgenic plants (Mn SOD) were multiplied by bud grafting the originally transformed lines for field planting at RRS, Dapchari (after obtaining permission from the competent authority). Regeneration of transgenic rubber plants integrated with *hmgR* 1 gene has been achieved. Haploid genome of *Hevea* contained four copies of *cis*-1 and two copies *cis*-2 of the *cis* prenyl transferase gene.

Genome Analysis Laboratory's research included development of molecular markers for genetic characterization of popular clones and wild accessions and also construction of a genetic linkage map for marker-assisted selection in *Hevea* breeding. More than one hundred genomic and genic SSR markers were generated. SNPs were detected in latex biosynthesis genes regulating latex yield in *Hevea*. In linkage analysis, marker segregation

data are being continuously integrated into the mapping data to populate the linkage map of rubber. Cloning and characterization of a functional resistance gene analogue showing significant over-expression in *Corynespora* infected tolerant rubber clones was carried out. Besides working on biotic stress, characterization of stress-tolerant clones of *Hevea* through transcript profiling was performed in response to cold and several stress responsive genes were identified. Epigenetic modifications (DNA methylation) in the regulatory sequence of latex biosynthesis genes under cold stress were also studied. For improving wood quality through enhanced lignin biosynthesis in rubber, efforts were made on the functional characterization of genes involved in lignin production.

Agronomy/Soils Division's research programmes focused on development and refinement of agro-management practices to improve growth and yield of rubber, reduce cost of cultivation and sustain soil quality. Combined application of chemical fertilizer and organic manure gave better growth and improved soil properties. Skipping fertilizers did not affect growth and yield in mature holdings. Intercropping of coffee, vanilla, *Garcinia* and medicinal plants like *Alpinia calcarata* and *Strobilanthes cuspidata* in mature rubber generated additional incomes without adversely effecting growth and yield of rubber and soil properties. Indiscriminate cultivation of pineapple reduced growth of rubber and resulted in a in soil pH.

In soils deeper than one meter, the size of planting pits needs to be only as large as the size of the polybag. Planting of direct seeded green-budded polybag plants enhanced growth compared to green budded stumps raised in polybags. Experiments on developing techniques to mitigate adverse effects of drought were initiated. Development of rubber information system using remote sensing and GIS was continued.

Economic and eco-friendly management of diseases and pests of rubber, evaluation of sprayers and spray oils, yield loss assessment due to diseases, disease response of new clones, studies in TPD, bee keeping and treatment of sheet processing effluent were the focus areas of research by the Pathology Division. Two rounds of spraying COC:Spray oil at 1:5 ratio (4:20 per ha. round) gave better control against Abnormal Leaf Fall disease. A study on the genetics of resistance to *Phytophthora* and biocontrol potential of antagonistic endophyte was initiated. Occurrence of entomopathogenic nematode was reported in rubber soils for the first time. *Cyclea peltata* in coconut/neem oil and mixture of neem oil and citronella oil (1:1) were effective as leech repellants. Efforts are on for finalizing a mini tractor-mounted mist blower and modifying other sprayers and accessories. Hybrid anaerobic digester-reed bed system reduced COD by 95% and BOD by 94% in latex processing effluents and the reactor yielded 3.5 m³ biogas per day.

Physiology of growth and yield, environmental and stress physiology, tapping panel dryness (TPD), gene expression studies, secondary metabolites and ecological impact of NR cultivation were the focus in Physiology Division. Drought tolerance potential of RRII 400 series clones and selected germplasm accessions was studied. On the basis of PS II activity and stress protein expression, clones RRII 430 and RRII 600 are expected to be more drought tolerant clones. Quantification of transcripts under tapping panel dryness, drought and cold stress indicated up and down regulation of several genes. Using an eddy covariance system, ecosystem level carbon dioxide and water flux were measured. The annual net ecosystem exchange by a 5-year-old rubber ecosystem was 12 gCO₂/m²/day equivalent to 36 tons of CO₂/ha/year.

Clones PB 235 and PB 260 consistently registered better shoot biomass increment as well as yield and hence they can be called latex-timber clones. High concentration of

ATP was observed in latex of high yielding clones. Protein biosynthesis can be an indirect measure of latex regeneration capacity. Studies indicated that *prenyl transferase* enzyme could be an indicator of yield potential in *Hevea*. Comparatively less stress symptoms and high rubber production was seen with stimulant application away from tapping cut.

During the last 54 years (1956-2009), T_{max} and T_{min} increased at the rate of 0.05°C/yr. and 0.03°C/yr., respectively at RRII. A multiple linear regression (MLR) analysis of impact of weather parameters on rubber yield indicated that 1°C rise in maximum and minimum temperatures in the traditional rubber growing regions may reduce rubber yield by more than 10%.

The Latex Harvest Technology Division continued research activities on various aspects of crop harvesting. A comprehensive review of the past experimental results under d4 and d7 tapping frequencies indicated their better performance in the experimental fields. Phase II of the collaborative programme on low frequency (d3) tapping was commenced in 2010. Fixing of a mini rainguard of 3 inch wide polythene strip in the month of August-September above the normal rainguard (fixed during April-May) ensured complete leak proofing and regular tapping and thus prevented crop loss. Biodegradable polythene for rain guarding is being evaluated. Efforts were continued for development of a mechanized tapping machine. Crop loss under non-rainguarded d3 tapping can be partially compensated by yield stimulation. Controlled upward tapping introduced in various Regional Research Station was successfully continued which showed its potential for substantial yield improvement of senile trees in the non-traditional regions also.

Studies in Economic Research Division included (i) farm management, (ii) primary processing and marketing of NR, (iii) rubber products manufacturing industry and

foreign trade, (iv) intercrops and by-products and (v) inter-divisional collaborative projects. During the reporting period, the Division highlighted the issues in farm management in the traditional and non-traditional rubber growing regions. In the traditional region, among the attributes of crop loss, the single major factor was wind damage (79%) and life cycle (25 years) tree loss was 84. Economic loss per tree and hectare were estimated to be Rs.1874/- and Rs.1,57,393/-, respectively. NR cultivation in non-traditional area like Tripura led to higher income as well as lower income inequality among rubber growers. However, its linkage effects on the regional economy were seriously constrained by the nature of human capital, availability of infrastructural facilities and access to the same.

The Rubber Technology Division focused on evolving improved techniques in primary processing like low temperature preservation of latex to produce ISNR 3L grade, deproteinization of natural rubber latex using the proteolytic enzyme papain and developing an easy process for the skim latex processing for latex industry. Studies on RVNRL/H5BL blends (90/10) resulted in improved modulus and tensile strength and were used for the preparation of latex threads. Reinforcement of natural rubber using polymeric fillers was comparable with that of conventional HAF filler system and its suitability for various dry rubber products has been established. Latex filler masterbatch with carbon black/silica dual filler system with improved dynamic and mechanical properties could be prepared with almost no process loss of the fillers. A methodology to prepare nanosilver rubber nanocomposites was developed with improved dynamic properties of the nanocomposites. Studies on latex blends nanocomposites in which suitable nanoclays

were dispersed in XNBR followed by the blending of the same in RVNRL resulted in improved tensile strength and higher solvent resistance. Studies have shown that scorch control in peroxide vulcanization could be achieved by using stable free radical TEMPO and the combination with suitable co-agents could regain the loss of cross-linking. A joint patent application on 'Development of silica-filled tyre compounds based on ENR' was filed. Collaborative programs on development of footwear sole for physically handicapped and development of 'Hurth coupling membrane' for Indian Railways are currently under progress.

Technical Consultancy Division concentrated on providing consultancy services for rubber industries. Services include product development, quality control and certification, trouble shooting, training and capacity building, etc. Evaluation of different types of rubbers, fillers and rubber chemicals for their performance in various rubber products was one of the major services rendered by the Division. In addition, the Division was also involved in industry-oriented research and development activities.

RRII played host to several meetings and conferences during this year. In the month of June 2010, a one-day grower conference was organized as part of the grower-scientist interaction programme. An international workshop on "Climate change and natural rubber cultivation: Research and development priorities" was held in July 2010. The institute hosted the XIXth Plantation Crops Symposium during December 2010. The Institute published 26 research articles, 8 book chapters, 5 books/reports/directories/working papers and 39 popular articles during 2010-2011. In addition, 130 papers were presented in various national and international conferences and one patent was registered during the period.

AGRONOMY/SOILS DIVISION

The various research programmes of the Division are aimed at development and periodic refinement of agromanagement practices to improve growth and yield of rubber in different agroclimatic regions, reduce cost of cultivation and sustain soil quality. Research programmes are also undertaken to increase the resilience of rubber cultivation in the context of climate change. Experiments on nutrient management in nurseries, young and mature rubber were continued. Various experiments on cropping systems and mixed planting with timber trees were in progress. A multi-species cropping system experiment was initiated in Tamil Nadu with locally preferred crops to identify a cropping system for the region. An experiment was initiated to study the effect of mechanized land preparation on soil properties. Experiments to develop an agronomic package for reducing the gestation period of rubber was continued. Development of the rubber information system using remote sensing and GIS is being continued. Estimation of area under rubber and rubber area distribution in relation to soil and landscape attributes were in progress. The Division also functions

as a centre for dissemination of knowledge on various soil and crop management techniques.

1. Nutrient management

Experiments in seedling nurseries to revise the general fertilizer recommendation were continued. Ten months after planting, significantly higher buddability (brown) was noticed in the treatment which received N and P @ 250 kg/ha as ammophos (20-20) + K @ 50 kg/ha and it was on par with standard practice and N and P @ 150 kg/ha as ammophos (20-20) + K @ 30 kg/ha in combination with PGPR (Table Ag.1).

In the experiment on biofertilizer application in seedling nursery, growth of plants was comparable in standard practice, standard practice + biofertilizer (BF) and 50 per cent N and P + full K + biofertilizer treatments and was significantly superior to control and biofertilizer alone treatments. Treatment difference was recorded in the buddability and budding success per cent also (Table Ag. 2). In control and BF alone treatments, the budding success was very poor.

Table Ag. 1. Buddability 10 months after planting

Treatment	Buddability (%)
Control	47.66
Standard practice (NPK @500-250-100kg/ha, N&P as urea and RP)	53.57
N&P@ 150 kg/ha (ammophos) + K @ 30 kg/ha	49.77
N&P@ 250 kg/ha (ammophos) + K @ 50 kg/ha	53.81
N&P@ 150 kg/ha (ammophos) + K @ 30 kg/ha +PGPR	52.94
SE	1.08
CD (P = 0.05)	3.23

Table Ag. 2. Effect of biofertilizer application on growth, buddability and budding success

Treatment	Diameter (mm)		Buddability (%)	Budding success (%)
	Dec.-10	Jan.11		
Control	5.20	5.71	51.10 (45.60)	75.75 (50.11)
Standard practice	5.70	6.64	73.90 (59.47)	89.00 (54.02)
BF alone	5.19	5.52	47.18 (43.37)	81.50 (51.70)
Standard practice+ BF	6.16	6.88	68.58 (55.99)	88.00 (53.75)
50% N & P+ Full K + BF	5.92	6.70	63.40 (56.57)	91.00 (54.64)
SE	0.19	0.19	1.94	0.79
CD (P = 0.05)	0.59	0.57	6.01	2.44

BF- biofertilizer Figures in parentheses are arc sine transformed values

The experiments initiated in 2008 at CES, Chethackal and Puthukkad estate, Trichur to develop an integrated nutrient management package for young rubber with cover crop were continued and no significant difference was observed between treatments. Similarly in the experiment to develop an integrated nutrient management package for rubber-banana system at CES, Chethackal also there was no significant difference between treatments.

The project on management of active and microbial carbon pools at Pottamkulam Estate, Yendayar was continued. Girth of the plants was not significantly influenced by the different treatments. Soil C and N status was significantly higher in treatments with *Mucuna* + *Glyricidia* and natural cover + *Glyricidia* compared to treatments with *Mucuna* and natural cover alone.

The field experiment to study the effect of long-term application of inorganic and organic manures on the growth and yield of rubber and on the physico-chemical properties of soil was continued. Application of 25 per cent chemical fertilizer and 75 per cent farm yard manure recorded significantly higher girth compared to all other treatments. Yield recording was commenced in the experiment area (Table Ag. 3).

Table Ag. 3. Effect of integrating with inorganic fertilizers organic manures on the growth of rubber

Treatment	Girth (cm)
No fertilizer/no manure (control)	51.95
Farm yard manure (FYM) alone	55.29
Chemical fertilizers (Standard recommendation)	54.41
25% Fertilizers +75% FYM	58.51
50% Fertilizers+50% FYM	55.18
75% Fertilizers+25% FYM	54.05
SE	0.76
CD (P = 0.05)	2.28

The field experiment to study the effect of C POM as soil amendment in marginal soils, at Thanneermukkom, Cherthala was continued. Girth of plants did not show significant difference among treatments. The growth of rubber plants and cover crop, *Pueraria phaseoloides* was satisfactory in marginal areas.

The field experiment at Malankara estate, Thodupuzha, to study the effect of zinc on the growth of rubber was continued. Application of zinc did not influence growth of rubber.

The experiment on sequential skipping of fertilizer application in mature rubber is being continued. Significant difference was not noticed among the treatments either in

Table Ag. 4. Effect of continuous application of K and Na on forms of K (mg/100g soil)

Treatments (kg/ha)		Water soluble K	Exchangeable K (Ammonium acetate)	Fixed K (1 N HNO ₃)	Total K (1:1HCl)
K ₂ O	Na ₂ O	0-30 cm	0-30 cm	0-30 cm	0-30 cm
30.0	0	2.03	7.71	10.29	111.08
30.0	7.5	1.64	6.80	5.80	65.92
30.0	15.0	1.64	8.80	6.15	52.00
22.5	0	0.96	6.79	4.64	107.92
22.5	7.5	1.15	6.66	4.52	59.67
22.5	15.0	1.18	4.71	7.99	63.17
15.0	0	0.77	6.33	4.16	60.18
15.0	7.5	0.98	6.13	4.63	54.67
15.0	15.0	0.81	5.93	3.08	42.13
0	0	0.69	4.48	2.14	40.08
CD (P = 0.05)		0.89	2.30	NS	11.38

yield or in girth increment (2002-2011) in the ninth year also.

Experiment to explore the possibility of substituting potassium (K) fertilizer with sodium chloride (NaCl) in mature rubber plantation at Malankara estate, Thodupuzha was concluded. Soil samples were analysed for available nutrients, water soluble, exchangeable, non-exchangeable and total K. Among the treatments, the highest amount of water soluble K was recorded in the treatment which received full dose of K (30 kg/ha). Exchangeable K increased in all the treatments at 0-30 cm. Total K was significantly higher in all the treatments except in the treatment with substitution of K with 50 per cent Na compared to control (Table Ag. 4).

Continuous application of Na @ 7.5 and 15 kg/ha for 10 years did not affect different fractions of Na in rubber growing soils. The average yield data for the last three years indicated that among the treatments the highest yield of 66.88 g/t/t was recorded in T₄ (50% K₂O + 25% Na₂O) and it was comparable with that of T₁ and T₃ (Table Ag. 5).

Table Ag. 5. Effect of different combinations of K and Na on yield (g/t/t)

Treatment (kg/ha)	Treatment		Mean yield (average of 3 years)
	K ₂ O	Na ₂ O	
T1	30.0	0	57.36
T2	30.0	7.5	54.55
T3	30.0	15.0	51.45
T4	22.5	0	51.80
T5	22.5	7.5	57.27
T6	22.5	15.0	53.39
T7	15.0	0	50.50
T8	15.0	7.5	66.88
T9	15.0	15.0	52.89
T10	0	0	39.77
Mean			53.58
SE			3.93
CD (P = 0.05)			11.67

The experiment on nutritional management of TPD was continued.

2. Soil and water conservation

In the experiment to evaluate the effectiveness of various biological bunds for soil and moisture conservation in rubber plantations, vetiver, guinea grass and pine-apple established well. The growth of

rubber was not significantly influenced by planting vegetative hedges one year after planting of rubber and the soil erosion from all treatments was comparable during the first year of establishment of hedges.

3. Intercropping and cropping systems

Experiment to find out the feasibility of growing perennial intercrops in rubber was continued. Coffee, vanilla, *Garcinea* and nutmeg are being evaluated in the normal system of planting and paired-row system of planting of rubber. In the paired-row system of planting, a strip of 1.0 m width was available at the centre of wide inter-row area without shading for intercropping. There was no significant difference between treatments with respect to girth and girth increment in the paired row system of planting. However in the normal system of planting, girth was significantly higher in all the intercropped plots except rubber with coffee (Table Ag. 6).

Garcinea has started to die off in the normal system of planting due to the intense shade and yield was the highest in the paired-row system of planting. Yield of vanilla was higher under intercropping. Yield of coffee was also higher in the paired-row system of planting.

A project on development of a multi species rubber-based cropping system for Tamil Nadu with locally-preferred-crops was initiated in collaboration with Tamil Nadu Agriculture University, Regional Station, Pechiparai, Kanyakumari district. Three varieties of banana, and one variety each of cocoa, cinnamon, and pine apple were established as intercrops.

The experiment initiated in Mundakayam estate during 2009 to evaluate the performance of shade-tolerant medicinal plants in mature rubber plantation was in progress. The medicinal plants which are being evaluated are *Adathoda beddomei*, *Alpinia calcarata*, *Andrographis paniculata*, *Asparagus racemosus*, *Desmodium gangeticum*, *Holostemma ada-kodien*, *Piper longum*, *Pseudarthria viscida*, *Rauwolfia serpentina* and *Strobilanthus cuspidata*. Among the medicinal plants, growth and yield of *Alpinia calcarata* and *Strobilanthus cuspidata* are comparatively better. Yield of rubber was not influenced by intercropping with medicinal plants.

The experiment to find out the feasibility of inter planting timber yielding trees with rubber was continued. The girth of rubber was not significantly influenced by row spacing, type of timber intercrops and their interactions.

Table Ag. 6. Influence of intercropping on growth of rubber (cm)

Treatments	Paired row system of planting	Normal system of planting
	Girth	Girth
	2011	2011
Rubber alone	56.93	54.35
Rubber + <i>Garcinea</i>	60.72	62.52
Rubber + Coffee	58.82	57.58
Rubber + Vanilla	57.08	59.42
Rubber + Nutmeg	57.09	58.88
SE	1.53	1.25
CD (P = 0.05)	NS	3.85

Experiment initiated in 2003 to monitor the changes in soil physical and chemical properties due to intercropping in rubber plantations and on growth of rubber was in progress at Travancore Rubber and Tea estate, Mundakayam. Among treatments the highest average girth (45.68 cm) and percentage tappareability (43.75%) were recorded in banana-intercropped area after 6.5 years followed by cover crop established area. In pineapple-intercropped area only 17 per cent trees could achieve tappareability during this period (Table Ag. 7).

Table Ag. 7. Effect of intercrops on girth of rubber and tappareability 6.5 years after planting

Intercrop	Girth (cm)	Tappareability (%)
Cover crop	44.00	37.83
Tapioca	42.29	37.82
Pineapple	39.29	17.02
Banana	45.68	43.75
CD (P = 0.05)	1.91	

Change in soil nutrient status due to cultivation of intercrops and cover crop was studied after 6.5 years (Table Ag. 8). Organic carbon content significantly improved in cover crop and banana area after 6.5 years over initial status while it was not significantly influenced in tapioca and pineapple area. An increase in available P was noticed in all the systems and the difference was significant in tapioca and pineapple intercropped areas at 0-30 cm depth. A significant increase in available potassium (K) was noted in banana area while a decrease was noted in other systems and the decrease is significant in tapioca and pineapple intercropped systems. A significant increase in pH was noted in banana area (5.01) compared to the initial value of 4.81 while a decrease from 4.94 to 4.82 was noted in pineapple area.

Table Ag. 8. Change in soil nutrient status due to planting intercrops and cover crop

OC status (%)			
Intercrop	Initial	After 6.5 years	Significance
Cover crop	1.39	1.79	**
Tapioca	1.42	1.22	NS
Pineapple	1.55	1.32	NS
Banana	1.08	1.86	**
CD (P = 0.05)	NS	NS	
Av. P status (mg/100g soil)			
Intercrop	Initial	After 6.5 years	Significance
Cover crop	0.58	1.14	NS
Tapioca	0.51	1.42	**
Pineapple	0.25	1.03	**
Banana	0.51	0.91	NS
CD (P = 0.05)	NS	NS	
Av. K status (mg/100g soil)			
Intercrop	Initial	After 6.5 years	Significance
Cover crop	10.32	10.01	NS
Tapioca	15.38	9.62	**
Pineapple	16.44	6.88	**
Banana	11.23	22.63	**
CD (P = 0.05)	NS	9.10*	
Av. Ca status (mg/100g soil)			
Intercrop	Initial	After 6.5 years	Significance
Cover crop	14.4	12.41	NS
Tapioca	12.68	12.23	NS
Pineapple	10.57	6.88	NS
Banana	10.37	9.78	NS
CD (P = 0.05)	NS	NS	
Av. Mg status (mg/100g soil)			
Intercrop	Initial	After 6.5 years	Significance
Cover crop	2.22	2.66	NS
Tapioca	2.44	2.29	NS
Pineapple	2.75	1.97	NS
Banana	2.42	2.33	NS
CD (P = 0.05)	NS	NS	
pH of soil			
Intercrop	Initial	After 6.5 years	Significance
Cover crop	4.95	4.94	NS
Tapioca	4.94	4.93	NS
Pineapple	4.94	4.82	*
Banana	4.81	5.01	**

*Significant at 1 per cent

** Significant at 5 per cent

4. Weed management

The project on comparison of rubber plantations with and without control of weed flora at Pathampuzha village in Kottayam District was continued. Soil samples were analyzed for C and N. Soil moisture content at 0-15 cm and at 15-45 cm was higher in plots with no control of weed flora (Table Ag.9). C and N contents were also significantly higher in fields where no control of weed flora was undertaken.

Table Ag. 9. Soil moisture content in fields with and without control of weed flora

Soil depth (cm)	Gravimetric moisture (%)		Significance
	Weeds controlled	Weeds not controlled	
0-15	10.13	12.24	*
15-45	11.97	14.65	*

* Significant at 5 per cent.

5. Planting techniques

The experiment to study the effect of planting densities viz; 420, 479, 549, 638 and 749 trees/ha, on growth and yield of rubber was continued. Plants in the lowest density of 420 trees/ha recorded significantly higher yield (g/t) than other treatments. The annual yield per hectare was higher in the highest density (749 t/ha).

The experiment to study the effect of different planting geometries on canopy development, growth and yield of rubber was in progress. Growth of rubber in the triangular system of planting and twin system of planting is superior to that of control (square system of planting). The canopies in altered planting systems started to exhibit asymmetrical pattern of growth.

As a follow up of earlier experiment, a multi-localational trial was initiated in three

agro-climatic zones to study the effect of size of planting pits on establishment and growth of rubber in soils with a depth of more than 1m. All plants planted with and without pits established well and three months after planting, the growth of rubber was not significantly influenced by size of planting pit in all the locations.

An experiment was initiated in 2010 to evaluate the effect of mechanized land preparation on soil quality and growth of rubber. The treatments include manual pitting and terracing (control), pitting by tractor and mounded hole digger and manual terracing by earth mover and tilling the entire land, pitting and terracing by earth mover.

6. Development of agromanagement technique for reducing the gestation period

The field experiment initiated to develop an agronomic package to reduce the immaturity period of *Hevea* at Malankara estate was continued. Growth of rubber was positively influenced by the management practices. The girth of the plants throughout the experimental period under integrated management which is a combination of selective manuring, enhanced nutrient application, conservation-oriented tillage and irrigation was significantly superior to all other treatments (Table Ag.10).

Another experiment initiated at CES, Chethackal on development of agromanagement practices for reducing the gestation period is being continued. There was significant difference in the performance of the two types of planting material. The growth of direct-seeded green-budded polybag plants was found to be superior to

Table Ag. 10. Effect of agromanagement practices on growth of rubber (5 years after planting)

Treatment	Girth (cm)
Standard practice	40.44
Selective manuring	43.55
Enhanced nutrient application	43.44
Conservation-oriented tillage	45.84
Irrigation	46.20
Irrigation+ Enhanced nutrient application	47.04
Integrated management	50.19
SE	0.64
CD (P = 0.05)	1.95

that of green budded stumps raised in polybags. The effect of agromanagement practices was reflected in the growth of the plants. The girth of the plants under integrated management was found to be superior to that of respective type of planting material under standard practice.

The experiment on the effect of different types of planting material (polybag - one-whorl, two-whorl and three-whorl and root trainer - one-whorl, two-whorl and three-whorl) on growth of rubber was continued. There was significant difference in the girth of the plants between different stages of the same type of planting material (polybag - one-whorl, two-whorl and three-whorl and root trainer - one-whorl, two-whorl and three-whorl). Three-whorl plants were significantly superior to two-whorl which in turn were superior to one-whorl plants irrespective of the type of planting material.

The experiment on age of root stock and quality of planting materials initiated in 2008 was continued. The girth increment of different types of planting materials was significantly influenced during the initial 6 months of growth. During the later phase of growth (6-12 months) significant difference in girth increment was not

noticed among different types of planting materials.

7. Stress management

The glass house experiment to study the effect of silicon and potassium in alleviating the adverse effects of drought stress in young rubber plants was continued. Observations on chlorophyll content index and leaf water potential indicated positive effect of silicon and higher dose of potassium (N: K = 1:1) in overcoming moisture stress effects. After imposing water stress for 35 days, all the plants were irrigated uniformly and it was observed that plants supplemented with higher dose of K recouped faster and showed better survival percentage.

A field experiment was initiated at Puthukkad estate, Trichur, a comparatively drought-prone area in the traditional rubber growing region to develop agromanagement techniques to mitigate the adverse effects of drought. Different types of planting materials, polybag plants raised with budded stumps, *in situ* budded polybag plants and root trainer plants are being compared. Other treatments include higher levels of potassium, hydrogel and tillage of plant basin at the end of the rainy season.

Initiated a polybag nursery experiment to study the effect of silicon nutrition on controlling powdery mildew in young rubber plants at two locations, CES, Chethackal and HML, Mundakayam.

8. Rubber growing soils

The study on comparison of six soil ecosystems *viz.* mature rubber, rubber-*Mucuna*-cover cropped, rubber-pineapple intercropped, cassava monocrop, teak mature plantation and forest in Kottayam

Table Ag. 11. Total carbon content (g C / 100 g soil) and its distribution to the three physical size fractions of different soil systems

System	Carbon content in different fractions (g C/100g soil)			Total carbon in soil (g C / 100 g soil)
	2000-250 μ	250-53 μ	< 53 μ	
Rubber	0.3213	0.1940	1.6282	2.14
Rubber + <i>Mucuna</i>	0.6373	0.4309	1.7505	2.82
Rubber + Pineapple	0.3107	0.3354	1.5287	2.17
Cassava	0.2461	0.1689	0.9788	1.39
Teak	0.6931	0.4867	1.3944	2.57
Forest	2.1873	1.2821	0.7591	4.23
CD (P = 0.05)	0.139	0.156	0.371	0.371

district was continued. Soil samples from these systems were analyzed to quantify organic matter, C and N contents in the three physical size fraction, viz. 2000-250 μ , 250-53 μ and < 53 μ . Organic matter, C and N contents in these size fractions were widely varying in soils of different system (Table Ag. 11 and Ag.12). In forest soils, major portion of the organic matter, C and N contents was associated with bigger physical fractions (2000-250 μ & 250-53 μ) or particulate organic matter (POM), while in all cultivated soils and very prominently in rubber soil, the observation was *vice versa*, indicating that easily mineralizable carbon compounds are more in forest soil.

Mucuna-cover cropped soil had higher WSOC, HWEC, POM and higher N

mineralization rate and bacterial population than the pineapple-intercropped soil. In all the cultivated soils where fertilizer application was a regular practice, soil pH decreased. The N mineralization rate of different systems was found to be positively and significantly influenced by the C content in POM or bigger size fractions (2000-250 μ & 250-53 μ) as well as soluble C components of a soil.

The observational trial on establishment of *Mucuna* (cover crop) and fodder crops in the later immaturity phase of rubber, after the removal of pineapple intercrop was continued. It was observed that *Mucuna* began to spread in the immature field (6th year). Among the different fodder crops, *Stenotaphrum*

Table Ag. 12. Total nitrogen content (g N / kg soil) and its distribution to the three physical size fractions of different soil systems

Systems	N content in different fractions (g N/kg soil)			Total Nitrogen in soil (g N / kg soil)
	2000-250 μ	250-53 μ	< 53 μ	
Rubber	0.18	0.10	0.97	1.26
Rubber + <i>Mucuna</i>	0.75	0.23	1.49	2.47
Rubber + Pineapple	0.27	0.19	1.46	1.92
Cassava	0.10	0.01	0.81	0.96
Teak	0.31	0.37	1.35	2.03
Forest	2.05	0.71	0.63	3.39
CD (P = 0.05)	0.31	0.13	0.27	0.57

continued to perform well even in the 6th year of plantation. But the growth of guinea grass was less in the 6th year than that in the previous.

9. Development of rubber information system using remote sensing and GIS

Project on developing rubber information system using remote sensing and GIS was continued. Soil series attribute data build up and vectorization of road network, rail network, important locations and water bodies of Kerala state and Kanyakumari district of Tamil Nadu were

completed. GPS reading of rubber plantations and other vegetation was collected from all fourteen districts of Kerala and Kanyakumari districts of Tamil Nadu for rubber area mapping and accuracy assessment. Preparation of rubber distribution maps and accuracy assessment is in progress.

Initiated a sub-project on Geospatial analysis and soil nutrient dynamics of rubber plantations in relation to growing environment. Rubber plantations were identified in Kanyakumari and Kasargode districts for recording soil nutrient status and yield of trees.

BIOTECHNOLOGY DIVISION

Genetic improvement of *Hevea brasiliensis* using modern tools is the major goal of Biotechnology research at RRII. The major ongoing research programmes in Biotechnology Division are 1) Development of *in vitro* propagation methods for elite *Hevea* clones 2) Development of transgenic *Hevea* plants for better adaptation to environmental stress, tapping panel dryness, latex yield and disease tolerance 3) Development of *in vitro* fertilization techniques & embryo rescue to complement conventional breeding programmes 4) Study of molecular mechanism and characterization of genes controlling tolerance to diseases, abiotic stresses, latex biosynthesis and characterization of related genes and 5) Study of laticifer cell specific gene expression and characterization of laticifer cell specific promoters.

1. Somatic embryogenesis

Experiments were carried out to refine the somatic embryogenesis and plant regeneration pathway developed earlier from *ex vitro* leaf explants of *Hevea* clone RRII 105. Embryo induction was tried with embryogenic callus obtained from proliferated friable callus of newly initiated leaf cultures. Variation was observed in texture of the embryogenic callus obtained from cultures initiated during different seasons and its response in terms of embryogenic competence. Experiments were also carried out to increase the rate of embryo induction from the proliferated embryogenic calli. The concentration of calcium nitrate, phytohormones and phytagel were varied during further subcultures according to the texture of the callus to be subcultured. Accordingly, the

hormonal combinations and concentrations were also changed with inclusion of ABA (0.2 mg/L) along with polyethylene glycol (5.0 g/L) and increasing the concentration of phytigel. Rate of embryogenesis could be increased to 80 per cent. The influence of different gelling agents on embryogenic callus formation and embryo induction was experimented. Bacto agar (15.0 - 25.0 g /L), phytigel (3.0-10.0 g/L) and agar (10.0 -20.0 g/L) were tried. It was observed that bacto agar was not suitable for both embryogenic callus formation and embryo induction, while phytigel (5.0 g/L) and agar (18.0 g/L) were equally good. Experiment was also done to find the effect of charcoal on embryogenic callus formation and embryo induction. It was observed that inclusion of charcoal in the medium was necessary for embryogenic callus formation. Embryo induction was obtained in medium with and without charcoal. In medium with charcoal, embryo induction was obtained after 50 days. In medium without charcoal, embryo induction took more time (3-4 months) but the rate of embryo induction was high with the whole clump getting converted into embryos. Embryo maturation and plant regeneration were also obtained from the induced embryos.

Leaf explants collected from physiologically juvenile source plants gave good response to *in vitro* culture. Experiments were done to find out the molecular factors if any, related to tissue juvenility and *in vitro* response. Work was done to identify the presence of genes controlling phase changes in plants. PCR was attempted with specific primers designed based on genes controlling phase changes in other plant species using genomic DNA as the template. The gene

coding for chlorophyll a/b binding protein (CAB) was successfully amplified, cloned and sequenced. RT PCR with cDNA obtained from tissues with different levels of maturity showed that the gene is differentially expressed with more expression in juvenile tissues.

Attempts were continued to develop plantlets from unfertilised ovules and to use the embryogenic callus as a target tissue for genetic transformation experiments.

2. *In vitro* approaches to complement conventional breeding programmes

2.1. *In vitro* fertilization and embryo rescue

Refinement experiments by manipulating the media components, for the rescue of immature embryos (1-5 week) were continued. More promising results were obtained when kinetin was replaced with zeatin in combination with GA₃. Results of the experiment with zeatin are given in Table Biotech.1. A steady increase in the embryo

Table Biotech. 1. Effect of zeatin concentration on embryo recovery

Zeatin (mg/L)	Embryo recovery (%)
1	16.2 (4.02)a
2	22.4 (4.73)b
3	42.2 (6.4)c
4	25.6 (5.06)d
5	20.4 (4.64)e

Means followed by common letters are not significantly different at P<0.05. The values in parenthesis are transformed values.

recovery was observed with an increase in the concentration of zeatin up to 3.0 mg/L. Further increase in zeatin concentration reduced the embryo recovery. Maximum embryo recovery (42%) was achieved with a hormonal combination of 3.0 mg/L zeatin and 1.0 mg/L GA₃. Plants were recovered and established in the field.

Experiments for the induction of polyembryony were also continued. A protocol for the induction of polyembryony has been developed. Multiple seedlings of single origin were developed, hardened and field planted. Molecular analysis was carried out in these plants along with the female parent (RR11105). RAPD and microsatellite analyses could prove the genetic uniformity as well as zygotic origin.

Experiments were also conducted for the induction of embryogenic calli from the immature fruits. Embryogenic calli could be successfully induced from immature ovules. These calli could be continuously multiplied. Genetic transformation was attempted using this calli as the explant. High frequency transformation was obtained. Transgenic cell lines and embryos were obtained with different gene constructs.

2.2. Isolation and culture of pollen protoplasts

The lengthy and tedious process of creating a pure line in an open-pollinated, highly heterozygous tree species like *Hevea* can be overcome by culturing haploid tissues like pollen grains and unpollinated ovules and subsequent diploidization of the

regenerated tissue. Proliferation and further development of microspores in culture has been observed to be extremely difficult, probably the main reason being the presence of exine, the tough outer coat. Pollen protoplast culture is an attractive option for developing homozygous lines in *Hevea*.

As an initial step towards this end, the effect of different levels of enzymes and osmotica on pollen protoplast isolation was experimented. The yield of protoplasts was highest when a mixture of 0.5% cellulase and 0.05% pectolyase was used in the presence of 0.6M mannitol and 0.3% sorbitol. For purification of the released protoplasts, two methods *viz.* density gradient centrifugation and sieving were attempted. Density gradient centrifugation was not effective for separation of the pollen protoplasts whereas the protoplasts could be partially purified by sieving through a 64 μ m mesh. These protoplasts were cultured in the nutrient medium. Further work needs to be done for optimization of the media as well as a suitable nurse tissue promoting cell division and further proliferation. The regeneration potential of pollen at various developmental stages also needs to be assessed.



Fig. Biotech. 1. A-Protoplast release from pollen; B-Partially purified protoplasts; C- Viable pollen protoplasts

3. Genetic transformation

Genetic transformation experiments were continued to develop transgenic plants with increased tolerance to abiotic stresses, tapping panel dryness and higher latex yield. The different constructs used were superoxide dismutase gene under the control of CaMV 35S and FMV 34S promoters, sorbitol-6-phosphate dehydrogenase, isopentenyl transferase, *hmg1* and osmotin protein. Extensive optimization experiments were carried out to improve the transformation efficiency and regeneration of transgenic tissues. New *Agrobacterium* infections were performed using different explants viz. embryogenic callus of anther, ovule and polyembryony derived callus.

Attempts were continued to develop more transgenic plants incorporated with *MnSOD* gene under the control of CaMV 35S promoter, from different transformation events. In order to understand the effect of pre-culture of target tissues in liquid medium, the embryogenic callus derived from immature anther and ovule were cultured in liquid medium for different time intervals (1-5 days) and used as the target tissue for *Agrobacterium* infection. Liquid pre culture of the embryogenic callus improved the frequency of transformation (35%). Emergence of new transgenic cell lines was observed from the cultures. GUS positive ones were further sub-cultured and proliferated.

Effect of L-cysteine on transformation frequency was studied by supplementing five different concentrations of cysteine (0, 50, 100, 200 & 300 mg/L) in the co-cultivation medium. Results showed that the inclusion of L-cysteine in co-cultivation medium had a positive influence on transformation

frequency. Among the five concentrations tried, maximum cell lines emerged in the medium containing 100 mg/L cysteine. More or less same frequency was obtained at higher concentrations also. At lower concentrations, induction of new transgenic cell lines was low. After two months, 50 GUS positive cell lines were obtained.

Experiments were done to improve the transgenic callus proliferation rate and texture, which in turn improve the transgenic plant regeneration efficiency. Effect of different concentrations of 2, 4-D (0.0-2.0 mg/L) and NAA (0.0-1.0 mg/L) was assessed on callus proliferation in modified MS medium. Callus proliferation was favored by the addition of 2,4-D (0.4-1.4 mg/L) and NAA (0.1- 0.7 mg/L), while higher concentrations of 2,4-D (above 1.4 mg/L) induced hard and compact callus. A callus proliferation frequency of around 80 percent was obtained with a growth regulator combination of 2, 4-D (0.8 mg/L) and NAA (0.4 mg/L). It was also noticed that the proliferation rate of transformed callus was less compared to the control callus. The combined effect of auxin and cytokinin (2, 4-D, NAA and BA) on friable callus induction was also studied. The proliferated transformed calli were subcultured on media fortified with different concentrations of 2, 4-D (0.0-1.0 mg/L) and BA (0.0 to 0.5 mg/L) keeping the

Table Biotech. 2. Effect of 2, 4-D and BA in presence of 0.4 mg/L NAA on friable callus induction

2,4-D (mg/L)	BA mg/L				
	0.1	0.2	0.3	0.4	0.5
0.2	10	15	25	15	5*
0.4	15	20	35	20	10
0.6	35	40	70	55	30
0.8	25	30	45	25	20
1.0	10	25	30	15	10

concentration of NAA constant (0.4 mg/L) (Table Biotech.2.). Friable callus induction frequency of 70 per cent was obtained with the hormonal combination 2, 4-D (0.6 mg/L), NAA (0.4 mg/L) and BA (0.3 mg/L).

The transgenic cell lines obtained earlier from the *Agrobacterium* infected immature anther derived callus were proliferated and sub-cultured for embryogenesis in medium supplemented with PEG and ABA and transgenic embryos were induced at a frequency of 75 per cent. In a separate experiment, for the induction of embryogenesis from the proliferated embryogenic calli, the induction medium was desiccated with different concentrations of agar (0.3-0.5%). Embryos were induced with a frequency of 50 per cent. Mature embryos were transferred to plant regeneration medium. Plantlets obtained were transplanted to small polybags filled with sand, soil and soilrite (1:1:1) for hardening.

Agrobacterium mediated genetic transformation with binary vectors containing *MnSOD* gene under the control of FMV 34S promoter was also carried out using embryogenic calli derived from the polyembryonic tissues. Effect of vacuum infiltration on transformation frequency was assessed using this system. Vacuum infiltration was carried out for different time intervals (5, 10 and 20 minutes) and also at different vacuum pressures (20-40 psu). The results revealed that 10 minutes vacuum infiltration at 30 psu was ideal for getting transformed cell lines. The infected calli after three days co-culture were transferred to selection medium and the calli were sub-cultured on fresh antibiotic medium at three weeks interval. Transgenic cell lines emerged 40 days after *Agrobacterium* infection and transformation frequency was

also enhanced. The GUS positive cell lines were sub-cultured for further proliferation.

Northern blot analysis was repeated to assess drought-induced over expression of *MnSOD* gene in the transgenic plants. Transgenic plants (L1 & L2) developed earlier, non-transgenic plants raised through somatic embryogenesis and RRII 105 plants were multiplied by bud grafting and maintained in polybags (filled with garden soil) were used in this study. During summer season, the plants were grouped into two sets, one set was irrigated daily and another set was subjected to water stress by withholding irrigation for two weeks. At the end of the drought treatment, leaf samples were collected for molecular analysis. Although equal amount of RNA (30µg) was loaded in each lane, significant difference in the *MnSOD* mRNA level was observed among the two transgenic and control plants. Transgenic plant L1 showed a higher *MnSOD* transcript level than L2, whereas the expression pattern was very low in the bud-grafted RRII 105 plants and in the somatic plants of the same clone which served as the control. The transgenic plants L1 and L2 were multiplied by bud-grafting along with control plants for initiating a field trial, Biosafety Research Level 1 (BRL1) at the Regional Research Laboratory, Dapchari, Maharashtra.

Attempts were also continued to develop an *Agrobacterium* mediated genetic transformation and plant regeneration protocol using leaf explants as the target tissue for *Agrobacterium* infection. The protocol developed for somatic embryogenesis and plant regeneration from leaf explants was used for the regeneration of transgenic tissues also, with suitable modification. Earlier, the infection, co cultivation and selection medium were

modified by including certain factors like L-cysteine, silver nitrate and the surfactant pluronic F 68, that help to increase the transformation efficiency, improve the texture of the newly emerging transgenic cell lines and to control the bacterial overgrowth. The same work has been repeated and found to be reproducible. The transgenic embryogenic callus obtained earlier incorporated with MnSOD, isopentenyl transferase (*ipt*) and TB antigen genes were further sub cultured for embryo induction and plant regeneration. Embryo induction and maturation was obtained from three transgenic lines incorporated with *ipt*, two with MnSOD gene and one with TB antigen gene. Ten transgenic plantlets incorporated with *ipt*, three with MnSOD and one plantlet incorporated with TB antigen genes were regenerated and to be hardened. The results showed that callus derived from leaf explants can be used as target tissue for genetic transformation.

The effect of ABA, sucrose and maltose on embryogenesis was evaluated individually and in combinations. The transformed cell lines integrated with sorbitol -6- phosphate dehydrogenase gene were cultured in the medium containing five different concentrations of ABA (0, 0.25, 0.5, 1.0 and 2.0 mg/L) and sucrose (2, 4, 6, 8 & 10%). The effect of maltose was monitored by replacing it for sucrose (1, 2, 3, 4 & 5%). Combined effect of sucrose and maltose was studied by using five different combinations of sucrose and maltose (0.5 + 0.5 %, 1+1 %, 1.5 + 1.5 %, 2.0 + 2.0 %, and 2.5 + 2.5 %) in the medium. From each treatment, torpedo / heart shaped embryos were separated. Inclusion of ABA in the embryo induction medium positively influenced somatic embryogenesis and maximum number of embryos was obtained at 2.0 mg/L. Among

the combinations of sucrose tested, maximum embryo induction was obtained at 4% sucrose. By increasing the sucrose level to 6% and above, embryogenesis was reduced. When maltose was used as the carbohydrate source, a negative response was observed at all concentrations tested. The heart-shaped embryos obtained from the above treatments were matured upon transferring to fresh embryo induction medium. The matured embryos were sub-cultured to the plant regeneration medium containing 1.0 mg/L BA and 2.0 mg/L GA₃.

However, full plant regeneration was not satisfactory. PCR analysis was carried out from the GUS positive transgenic embryos and also from the non-transgenic ones. Using S6PDH gene specific primers; 360 bp fragment was amplified in the transformed tissues. Integration of the *uid* A and *npt* II gene was confirmed in the transformed tissues by the presence of 650 and 800 bp fragments respectively.

Work was continued to develop transgenic rubber plants integrated with *hmg* 1 gene under the control of super promoter, using the embryogenic callus derived from the polyembryonic tissue. The screening of the transformants was performed with hygromycin. Proliferated transgenic cell lines on frequent sub-culturing produced transgenic embryos at a frequency of 80 per cent. Transgenic plantlets were produced from these embryos in the germination medium. The frequency of plant germination was 28 per cent (Table Biotech.3). The transgene integration was confirmed using PCR analysis and the plantlets were successfully acclimatized and maintained in polybags. This is the first report on the regeneration of transgenic plants integrated with the *hmg* 1 gene in *H. brasiliensis*.

Table Biotech. 3. Effect of growth regulators on embryo induction and plant germination on transgenic embryogenic callus integrated with *hmgR1* gene

Treatment	Growth regulators (mg/L)			Embryo germination (Mean %)	Embryo Induction (Mean %)
	GA ₃	Kin	BA		
T1	0.1	0.1	0.2	32.75	7.62
T2	0.2	0.25	0.25	66.62	13.0
T3	0.3	0.5	0.3	80.75	16.87
T4	0.4	0.75	0.35	68.25	19.75
T5	0.5	1.0	0.4	45.62	27.5
T6	0.6	1.5	0.45	27.0	13.87
CD (P = 0.05)				2.05	1.52

Five transgenic lines integrated with *hmgR1* were developed earlier from the *Agrobacterium* infected immature anther derived callus. On repeated sub-culture, one line became embryogenic with emergence of transgenic embryos. The transgenic embryos were sub-cultured to the maturation medium and embryos showing bipolar differentiation were observed.

Genetic transformation experiments were carried out using different explants for the integration of osmotin gene following a modified procedure. Transformation frequency has been enhanced to 40 per cent by adopting liquid co-culture. From the 70 transgenic cell lines obtained, 50 could be proliferated in MS medium supplemented with 1.0 mg/L NAA along with 1.5 mg/L

2,4-D. From the proliferated cell lines, five lines were randomly selected and PCR analysis was carried out using gene-specific primers and all the lines tested were found to be positive. Experiments were performed by manipulating the media components for embryo induction. Embryo induction frequency was enhanced from 36 to 50 per cent when the media was additionally supplemented with Kin and ABA. A growth regulator combination of 0.3 mg/L Kin, 0.1 mg/L ABA and 2.0 mg/L NAA along with 10 g/L mannitol was found to be ideal for embryo induction (Table Biotech. 4). Embryos were matured (15%) and a few has been germinated (2%) and are in the process of hardening. More embryos are being sub-cultured for further development and planting out.

Table Biotech. 4. Effect of Kinetin and ABA on embryo induction (%) in presence of NAA (2.0 mg/L) and mannitol (10g/L)

ABA (mg/ L)	Kinetin (mg/ L)				
	0.1	0.2	0.3	0.4	0.5
0.1	09.00 (17.20)*	28.5 (32.21)	50.00 (45.00)	42.0 (40.40)	37.50 (37.76)
0.2	15.25 (22.98)	30.5 (33.50)	42.00 (40.39)	39.5 (38.94)	26.25 (30.76)
0.3	20.00 (26.56)	34.5 (35.97)	39.50 (38.09)	33.5 (35.30)	21.50 (27.62)
0.4	17.00 (24.32)	27.0 (31.30)	32.75 (34.91)	24.5 (29.66)	20.00 (26.55)
0.5	12.50 (20.69)	16.0 (23.55)	28.50 (32.26)	15.0 (22.76)	12.00 (20.23)
CD (P = 0.05)					1.5

*The values in parenthesis are sine transformed values.

4. Molecular studies

4.1. Molecular mechanism of disease tolerance

The earlier studies on isolation and characterization of β -1,3-glucanase gene involved in abnormal leaf fall disease tolerance lead to the identification of five isoforms of β -1,3-glucanase gene in a single clone RR11 105. Attempts were continued to study the functional properties of these different promoters to drive the respective genes by developing promoter: GUS fusion binary vectors and measuring the GUS expression in the heterologous system through transgenic approaches. In order to study the efficiency of promoters of different forms of glucanase gene, upon induction and non-induction, the vector

pCambia 1381 Z has been chosen for developing Promoter: GUS fusion binary vectors for *Agrobacterium* mediated genetic transformation in tobacco. This binary vector features a promoterless version of GUS A with the catalase intron immediately downstream of a truncated lacZa containing the pUC 8 polylinker. It is having the hygromycin as plant selection gene and chloramphenicol / kanamycin for the bacterial selection. The promoter fragments has been cloned in pCambia 1381 Z TDNA vector upstream to the GUS A gene (Fig Biotech.2.). All the five promoters identified were cloned in to the binary vector with and without deletion. Total eleven binary vectors with different sizes of the characterized promoter were developed for tobacco transformation.

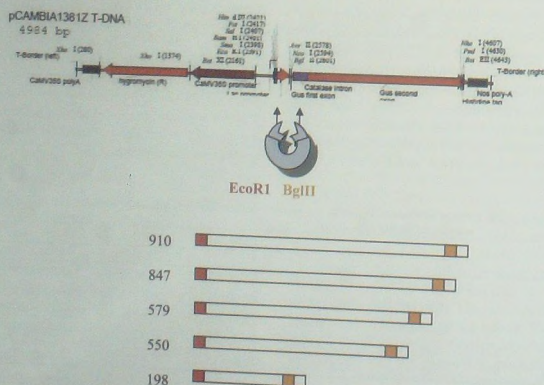


Fig. Biotech. 2. Schematic representation of cloning of promoter: GUS fusion in binary vector

After confirming the presence of the inserts of different lengths in the above mentioned binary vector, the constructs have been transformed into the competent *Agrobacterium* strain EHA 105 and grown in LB agar plates containing 50 µg/ml kanamycin and 20 µg/ml rifampicin. The positive transformants has been confirmed through colony PCR for the presence of vector constructs with the insert. Later tobacco transformation was carried out and the results awaited.

4.2. Tissue specific gene expression and characterization of promoters

4.2.1. Characterization of *cis* prenilyltransferase gene isoforms from *Hevea*

The relative copy number of *cis*-prenilyltransferase gene isoforms 1 and 2 genes was studied through $-C_T$ method in a high yielding *Hevea* clone, RR11 105 and a low yielding clone RR11 33. A C_T difference of "1" was observed between *cis*-1 & 2 gene isoforms. *Cis*-2 gene has shown a high C_T value indicating that the copy number of *cis*-2 is only half of *cis*-1.

The absolute copy number of *cis*-1 and 2 gene isoforms were studied through standard curve method. Plasmid carrying

cloned *cis*-prenilyltransferase1 gene was used as the standard. The mass of the plasmid was calculated from the formula:-

$$m = [n] [1.096 \times 10^{-21} \text{ g}]$$

where n = genome size (bp)

$$\text{bp} \quad m = \text{mass}$$

$$e^{21} = \times 10^{21}$$

A single plasmid contains a single copy of our target gene. Using the molecular mass of a single plasmid total copy number in the sample is calculated. Serial dilutions of DNA with 300,000, 30,000, 3,000, 300 and 30 copies respectively were made and used to develop a standard curve. Twenty nanogram of genomic DNA from the clone RR11 105 was used along with the plasmid standard to find out the copy number. *Cis* 1 and *cis* 2 were amplified together in the standard curve experiment with plasmid and the copy number of *cis*1 was found to be double of *cis* 2. According to Leitch *et al* (1998), the genome size of *Hevea* is 4.3 pg (2C). Therefore, the haploid genome of *Hevea* contain four copies of *cis*-1 and 2 copies of *cis*-2.

An attempt has been made to PCR amplify *cis*-prenilyltransferase gene isoforms from different *Hevea* clones. Genomic DNA

Table Biotech. 5. The sequence identity of different cloned fragments of *cis*-prenilyltransferase gene

Clones	Colony No.	Similar genes in GenBank / cultivar	% Similarity	NCBI Acc. No. & References
RR11 105	1	HRT2 / RRIM 600	100	ABO64661.2, Asawatreratanakul <i>et al</i> , 2004
	2	<i>cis</i> -prenilyltransferase2/ RR11	100	EU675683.1, Saleena <i>et al</i> , 2007
RR11 33	1	<i>cis</i> -prenilyltransferase1/ RR11 105	100	EF387242.1, Saleena <i>et al</i> , 2007
	2	HRT1/ RRIM 600	99	ABO61234.2, Asawatreratanakul <i>et al</i> , 2004
RR11 430	1	HRT2/ RRIM 600	99	ABO64661.2, Asawatreratanakul <i>et al</i> , 2004
	2	hpt9/ PR 255	99	AY124472.1, Coldren <i>et al</i> , 2002
RR11 414	1	HCP22/ RRI 600	99	ABO61237.2, Asawatreratanakul <i>et al</i> , 2001
	2	<i>cis</i> -prenilyltransferase1/ RR11 105	99	EU675683.1, Saleena <i>et al</i> , 2007
RR11 Tjir 1	1	HRT2 / RRIM 600	100	ABO64661.2, Asawatreratanakul <i>et al</i> , 2004

was isolated from RRII 105, RRII 33, RRII 430, RRII 414 and Tjir1. To amplify *cis*-prenyltransferase 1 gene, primers were designed based on the sequence deposited earlier in GenBank (NCBI acc no: EF587242).

The amplified fragment was cloned in plasmid vector. Two bacterial colonies each

from the cloned bacterial plates of clones RRII 105, RRII 33, RRII 430, RRII 414 and Tjir1 were randomly selected and sequenced. Upon sequencing it was found that the screened colonies contained different *cis*-prenyltransferase genes. The results are given in Table Biotech.5.

BOTANY DIVISION

Participatory programmes for evaluation of promising clones in the pipeline with the involvement of rubber planters in various agro-climatic zones of the traditional area was continued. Evolving new and improved rubber clones incorporating a wider genetic base, breeding for drought tolerance, need-based investigations on propagation aspects and studies on the anatomy of bark and wood were the thrust areas of work in the Botany Division. Post-release follow up observations on the yield of the RRII 400 series clones planted in small holdings were also continued.

1. Evolving high yielding clones for the traditional area

1.1 Hybridization and clonal selection

In order to widen the genetic base of breeding populations Wickham x Amazonian hybrids (W x A) with the RRII 400 series clones and RRII 105 having high yield, hand pollinations were carried out during the year 2011 (HP 2011). Female parents used were RRII 105, RRII 414, RRII 422, RRII 429 and male parents include three W x A hybrids viz., 90/10, 90/34 and 90/274. Nine cross combinations were attempted in 2179 pollinations (Table Bot.1).

Table Bot. 1. Details of hand pollination

Female	Male (WXA)		
	90/10	90/34	90/274
RRII 414	476	54	240
RRII 422	92	118	162
RRII 429	0	202	0
RRII 105	488	347	0
Total	1056	721	402

Among the 42 promising selections from 200 hybrid clones resultant of the 1986 hybridization programme which were in the 13th year of tapping in four small-scale trials laid out at CES, 37 hybrid clones maintained higher annual mean yield and summer yield than RRII 105.

Out of the 37 promising selections among 196 hybrid clones under observation in eight small scale trials planted in 1990, 21 maintained higher yield than RRII 105 in the 12th year of tapping. Of the 21 hybrid clones under evaluation in two small-scale trials planted in 1992, five clones recorded superiority in yield over the check (RRII 105) in the 11th year of tapping.

In the SST of 34 W x W hybrids planted in 1995 at CES, Chethackal, nine clones recorded higher yield than the control RRII 105 in the 8th year of tapping. Yield and girth of promising clones are given in Table Bot. 2.

Table Bot. 2. Yield and girth of promising hybrids^a

Clone	Girth (cm)	Yield (g/t)
89/7	77.66	87.94
89/27	84.58	123.32
89/79	68.24	80.91
89/95	72.31	81.01
89/102	68.39	86.13
89/124	70.79	93.04
89/309	70.71	84.82
89/349	72.54	77.42
89/356	74.00	77.70
RRII 105	57.13	74.04

^aCES 1995 planting

Among the 42 W x A hybrid clones under evaluation in two SSTs planted in 1995, 16 clones showed higher yield than the control clone RRII 105 (Table Bot. 3.) in the 8th year of tapping.

Table Bot. 3. Mean girth and yield of promising W x A clones

Clone	Girth (cm)	Yield (g/t)
90/10	82.58	89.48
90/21	86.39	50.51
90/25	64.59	56.09
90/29	89.75	64.26
90/34	77.00	48.78
90/170	72.88	63.60
90/171	86.88	53.57
90/174	85.17	56.81
90/193	76.03	78.35
90/271	80.21	82.63
90/340	61.96	59.14
90/352	71.25	52.60
90/55	85.50	46.88
90/109	80.00	65.87
90/132	74.08	47.40
90/274	71.58	44.81
RRII 105	56.46	39.01

^aCES 1995 planting

In the clonal nursery planted at CES in 2003, based on test tap yield and secondary characters, clones 97/4, 97/47, 97/182, 97/213

and 97/300 were identified as promising selections and source bush nursery of these clones were planted for further participatory field evaluation trials.

Among the 21 hybrid clones and their parents under evaluation in the two trials planted in 1998, two clones were superior and 13 clones were on par with RRII 105 in terms of mean yield over six years. Of the top six clones, clone 93/98 had summer yield comparable to and peak season yield significantly higher than the parent clones (Table Bot. 4).

Table Bot. 4. Yield (g/t) of the clones in 1998 SST^a

Clone	Summer season yield (g/t)	Peak season yield (g/t)
93/56	33.4 ^{ab}	57.4 ^{cd}
93/98	38.9 ^a	120.4 ^a
93/194	20.8 ^b	34.6 ^d
93/163	19.0 ^b	46.7 ^{cd}
93/243	38.1 ^a	66.2 ^{bc}
RRIM 703	41.4 ^a	53.6 ^{cd}
RRII 105	—	79.3 ^b
GM	31.9	65.5
CD (P = 0.05)	15.9 [*]	24.8 [*]
CV (%)	27.4	21.2

^aCES 1998 planting

In the 1998 SST, 14 clones had significantly superior girth than the check clone RRII 105. The yield data showed that the clones 93/37 and 93/2 had significantly higher summer yield than the check clone RRII 105 and other parent clones (Table Bot 5). Nine clones (93/2, 93/7, 93/37, 93/39, 93/48, 93/172, 93/247, 93/250 and 93/263) showed comparable peak season yield to the check clone RRII 105.

In the clonal nursery evaluation (2007 B) at RRII, 24 clones which are the selections from Paraliar: 10 (Hybrids-5, Orlets-5);

Table Bot. 5. Girth and yield of the clones in 1998 SST⁺

Clone	Girth (cm)	Summer yield (g/t/t)	Peak season yield (g/t/t)
93/2	72.20 <i>bsde</i>	28.3 <i>de</i>	61.0 <i>absde</i>
93/5	63.50 <i>bs</i>	18.0b <i>bsde</i>	46.5 <i>bsde</i>
93/7	64.70 <i>gh</i>	22.2 <i>bs</i>	71.3 <i>absde</i>
93/17	74.20 <i>bs</i>	22.7 <i>bs</i>	49.8 <i>bsde</i>
93/22	67.40 <i>gh</i>	06.9 <i>f</i>	29.3 <i>ff</i>
93/27	74.00 <i>bs</i>	22.9 <i>bs</i>	53.2 <i>bsde</i>
93/37	67.20 <i>gh</i>	35.5 <i>a</i>	62.8 <i>absde</i>
93/39	69.10 <i>bsde</i>	26.9 <i>abc</i>	77.5 <i>absde</i>
93/45	71.50 <i>bsde</i>	19.1 <i>bsde</i>	57.1 <i>bsde</i>
93/48	80.60 <i>a</i>	27.3 <i>abc</i>	94.8 <i>de</i>
93/172	76.50 <i>de</i>	21.4 <i>bs</i>	98.3 <i>a</i>
93/179	61.10 <i>ff</i>	14.7 <i>bsde</i>	42.5 <i>bsde</i>
93/247	59.70 <i>ff</i>	15.1 <i>bsde</i>	77.8 <i>absde</i>
93/248	68.10 <i>gh</i>	14.7 <i>bsde</i>	20.9 <i>f</i>
93/250	69.50 <i>bsde</i>	17.8 <i>bsde</i>	67.1 <i>absde</i>
93/263	74.30 <i>bs</i>	17.2 <i>bsde</i>	81.9 <i>bs</i>
AVT 73	63.90 <i>ff</i>	09.8 <i>ff</i>	38.2 <i>ff</i>
PB 252	58.90 <i>ff</i>	11.5 <i>bsde</i>	60.7 <i>absde</i>
RRII 105	59.60 <i>ff</i>	16.4 <i>bsde</i>	70.3 <i>absde</i>
RRII 118	68.90 <i>ff</i>	18.3 <i>bsde</i>	44.2 <i>bsde</i>
RRIM 600	57.20 <i>ff</i>	09.6 <i>ff</i>	48.2 <i>bsde</i>

Table Bot. 6. Mean girth of ortets and hybrids⁺

Clone	Mean girth (cm)	Clone	Mean girth (cm)
Par O 10	19.13	Gw 5	12.65
Par O 18	17.71	Par Hy 14	12.14
Par O 11	15.65	Pad 1	12.01
Par Hy 3	15.08	Gw 3	11.98
Gw 1	14.66	Gw 6	11.50
Par O 17	14.60	Irity	10.50
Par Hy 19	13.85	Pad 213	9.99
Par Hy 4	13.74	Pad 280	9.54
Par Hy 15	13.61	RRII 414	15.07
Gw 9	13.31	RRII 430	13.01
Gw 4	13.22	RRII 105	10.58
Pad 270	13.05	GM	13.31
Par O 9	12.83		

⁺ RRII 2007 planting

In the SST 2001 (Trial I) clone 95/131(RRII 105 X PB 235) registered high yield of 55.6 g/t/t. Growth of all clones in the ninth year after planting was found superior than control RRII 105. Clone 95/63 (PB 242 X RRII 105) recorded the highest girth of 62.6 cm compared to that of RRII 105 (46.1cm). Data on growth of 14 hybrid clones in the SST 2001 (Trial II), in the 9th year, showed that 12 hybrid clones were superior to control (RRII 105).

Among the 22 clones of full-sib and half sib origin generated during the 2002 hybridization and polycross breeding programmes, which are under evaluation in a clonal nursery planted in 2008 along with the check clones RRII 105, RRII 414 and RRII 429, three clones of the cross combination of PB 330 x RRII 414 showed highest growth (20.8, 19.7 and 19.4 cm) in the second year after planting (Table Bot. 7). They exhibited good secondary traits also.

Thirty five promising progenies from the hybridization programme 2005 were

Guwahati: (6 ortets) and 4 ortets from Padiyoor (Ambalavayal), are being evaluated with three check clones RRII 105, RRII 414, and RRII 430. Selections from Paraliar (ortets and hybrids) recorded high juvenile vigour. Three polyclonal selections from Guwahati also recorded high vigour. (Table Bot. 6).

Data on growth and yield of 24 hybrid clones in the SST 1999 at 11th year showed that two hybrid clones (94/92: RRII 105 x Mil 3/2; 94/87: RRII 105 x RRII 703) were significantly superior to control (RRII 105) which recorded an yield of 76 g/t/t and 88 g/t/t respectively which was higher than RRII 105 (36 g/t/t).

Table Bot. 7. Girth of clones in clonal nursery trial 2008

Clone code	Parentage	Girth (cm)
02/286	RRII 105 OP	16.0
02/683	PB 330 x RRII 414	17.3
02/700	PB 330 x RRII 414	15.4
02/638	PB 330 x RRII 414	19.7
02/688	PB 330 x RRII 414	20.8
02/477	RRII 414 x PB 330	16.1
02/575	RRII 414 OP	12.3
02/690	PB 330 x RRII 414	19.4
02/822	PB 330 OP	16.6
02/512	PB 330 x RRII 414	17.4
02/514	PB 330 x RRII 414	16.6
02/881	PB 330 x RRII 429	16.3
02/701	PB 330 x RRII 414	14.8
02/704	PB 330 x RRII 414	14.6
02/712	PB 330 x RRII 414	17.2
02/445	RRII 429 OP	15.8
02/342	RRII 414 OP	13.9
02/844	PB 330 OP	18.4
02/848	RRII 429 OP	7.3
02/843	PB 330 OP	12.0
02/335	RRII 414 OP	16.3
RRII 105		12.6
RRII 414		18.0
RRII 429		17.4
PB 330		16.1
CV	12.4	
CD (P = 0.05)	3.2	

* CES 2008 planting

selected based on growth and test tap yield in the seedling nursery stage. The selections were multiplied for further evaluation.

1.2. Ortet selection

Timber yield in terms of clear bole volume of trees of 14 ortets planted in 1993 was computed. It was found that 10 ortet clones recorded better timber yield compared to RRII 105 with the highest

Table Bot. 8. Girth, crotch height and timber yield of ortets

Clone	Girth (cm)	Crotch height (m)	Timber yield (m ³)
KnO 28	90.31	3.97	0.28
KnO 11	83.29	4.30	0.19
KnO 59	77.51	3.43	0.14
KnO 54	87.60	4.05	0.18
KnO 63	66.07	3.18	0.11
KnO 75	85.93	3.63	0.19
KnO 22	72.51	3.97	0.15
KnO 25	70.58	3.52	0.11
KnO 01	63.54	3.43	0.09
KnO 12	81.22	3.50	0.18
KnO 39	84.00	4.08	0.21
KnO 74	77.43	3.83	0.14
KnO 27	71.17	3.20	0.11
KnO 19	76.21	4.57	0.18
RRIM 600	71.13	4.00	0.12
RRII 105	67.39	3.67	0.11
SE	5.96	0.35	0.030
CV	13.48	16.25	38.48

timber volume of 0.2 m³ recorded by the clone KnO 28 (Table Bot. 8). Crotch height of the highest yielding clone KnO 39 was 4.08m, whereas crotch height of RRII 105 was 3.67m. Consequently, timber yield of the KnO 39 was 0.21m³, with an improvement of about 90% over RRII 105.

Source bush nursery of five potential ortet selections from PCK Kodumon Estate was established at CES Chethackal. Budwood of eight promising ortet selections from small holdings, HML Kaliyar estate, Thodupuzha and Vimala estate, Kasargod was generated for raising source bush nurseries. The promising latex-timber clone O 73 (selection from Aanathan Estate, Kanjirappally) and O 72 (selfed progeny of RRII 105) maintained better performance in terms of yield and girth during the sixth year of tapping also. Among nine clones under evaluation in the Kuthattukalam

Table Bot. 9. Mean girth and yield in the 6th year of tapping

Clone	Girth (cm)	Yield (g/t/t)
O 32/6	59.69	51.24
RRIM 600	57.23	51.84
RRIC 110	69.27	58.64
O 12	76.10	87.83
PR 261	57.57	52.71
O 4	73.00	69.23
BPM 24	60.10	63.98
O 33/8	80.64	74.02
O 35	65.11	73.40
RRII 105	68.10	63.34

estate at Chittar, five clones showed higher yield than the control clone RRII 105 in the 6th year of tapping (Table Bot. 9).

Yield and girth of 23 finally selected elite mother trees from South Andamans, the oldest PB 161 seedling plantation in India planted during the year 1965-'68 are given in Table Bot. 10. Budwood could be generated from 13 trees of which 11 trees were successfully multiplied. The polybag plants were maintained under polyhouse established at CES Chethackal.

2. Evaluation of clones

2.1. Large-scale evaluation

Yield of the 21 clones in the two LSTs of RRII 400 series clones planted in 1993 was recorded at fortnightly intervals during the 10th year of tapping. There was significant clonal variation in annual mean yield in both the trials. Clones RRII 417 and RRII 403 in Trial 1 and clones RRII 430 and PB 330 in Trial 2 were comparable in yield to RRII 105, in the 10th year of tapping. In general, RRII 105 performed better than in the previous years, with a yield of 72 g/t/t. Mean yield data over 10 years of tapping, proved that RRII 414 and RRII 417 in Trial 1 and RRII 430 and RRII 422 in Trial 2 were

Table Bot. 10. Yield and girth of selected mother trees from Andamans

Tree No.	Girth (cm)	Average yield (g/t/t)
1	120	100.00
2	172	107.22
3	149	130.56
4	203	143.33
5	150	127.78
6	140	132.22
7	167	148.57
8	139	147.06
9	150	134.44
10	142	144.38
11	135	112.67
12	140	123.53
13	145	114.44
14	140	142.94
15	180	175.50
16	188	104.00
17	170	137.89
18	124	150.00
19	175	118.42
20	148	151.50
21	152	159.50
22	168	150.71
23	149	135.26

significantly superior to RRII 105. The clones in general showed TPD incidence to the tune of 11% in Trial A and 10.5% in Trial B. Clone RRII 429 had the highest incidence of 27.8 % affected trees, followed by RRII 52 and RRII 417 with 17% TPD affected trees. The TPD incidence in RRII 430, RRII 414 and RRII 422 were comparable with that of RRII 105 where the mean TPD incidence in the two trials was 10.2%.

In the 1999 LST, girth and monthly yield were recorded from the nine clones under evaluation. Yield in the third year ranged from 26.8 (clone 26) to 46.3 g/t/t (clone 12). Average girth of the clones ranged from 49.3 to 64.8 cm. Average girth and yield in the third year of tapping is as shown in Table Bot. 11.

Table Bot. 11. Mean girth and yield in the third year of tapping

Clone	Girth (cm)	Yield (g/t/t)	
	3 rd year	3 rd year of tapping	
	Girth	Clone	Yield
4	64.84 ^a	12	46.3 ^a
12	61.2 ^{ab}	RRII 105	43.6 ^a
RRII 105	57.07 ^{bc}	22	40.4 ^{ab}
11	55.29 ^{cd}	4	38.3 ^{ab}
46	51.75 ^{de}	46	36.4 ^{ab}
BPM 24	51.73 ^{de}	RRIM 600	34.5 ^{ab}
26	51.41 ^{de}	BPM 24	33.5 ^{ab}
RRIM 600	50.54 ^e	11	31.7 ^{ab}
22	49.33 ^e	26	26.8 ^b

The averages followed by the same letter do not differ statistically at 5% level of probability.

In the multidisciplinary evaluation of 26 clones in the 13th year of tapping in two large-scale trials planted in 1989, in Trial 1, RRII 118 (70.3 g/t/t) was the highest yielder followed by RRII 105 (65.5 g/t/t) and RRII 5 (64.3 g/t/t). In Trial 2, the yield performance of all the clones except KRS 128 was better than RRII 105. PB 255, PB 235 and PB 280 were the top yielders. In another large-scale trial of the same clones at CES, clone PB 280 was superior to the rest of the clones in both annual mean yield (136.4 g/t/t) and yield in summer (108.0 g/t/t). This clone recorded a high girth of 105 cm after 14 years of tapping. Clones PB 314, PB 311 and PB 217 also recorded higher annual mean and summer yields compared to RRII 105 which yielded 61.9 and 44.3 g/t/t respectively in the 14th year of tapping.

Clone 86/44, which recorded highest yield in large-scale trial at CES was found to be the highest yielder at RRS Padiyoor too, recorded 68.1g/t/t in the third year of tapping. In the trial, RRII 105 recorded 59.4 g/t/t. (Table Bot. 12). Clone 86/61 recorded the highest girth of 76.3 cm with comparable yield of RRII 105.

Table Bot. 12. Mean yield and girth of clones*

Clone	Girth (cm)	Yield (g/t/t)
75	55.98	46.20
428	67.97	52.58
191	64.05	55.04
109	64.40	50.79
300	56.50	54.44
86/44	61.37	68.13
70	57.19	40.03
RRII 105	61.98	59.45
68	63.45	43.11
86/61	76.35	53.71
244	61.86	43.98
59	72.45	44.92
400	66.04	57.31
CD (P = 0.05)	6.92	13.40

*RRS, Padiyoor

In another LST comprising 12 clones at RRS Padiyoor, clone HP 468 continued to be performing better (64.2 g/t/t) than RRII 105 (62.6 g/t/t). In the fifth year of planting, among 12 ortets selected from HML Koney and Mundakayam estates, MO 28 recorded the highest girth with 40.1cm. ChyO 48 and ChyO 35 are the two vigorous ortet clones selected from HML, Cheruvally estate with 41.0 cm and 40.4 cm respectively.

2.2. On-farm evaluation

In the on-farm trial at Sasthamkotta, nine clones including 2 indigenous and 7 introduced

Table Bot. 13. Mean girth and yield over 8 years

Clones	Mean Girth (cm)	Mean yield (kg/ha/yr)	
		8 th year	Over 8 years
RRII 105	61.7	2579	2230
GT 1	63.8	1992	1526
RRII 203	66.3	2433	1713
PB 217	64.7	1927	1618
PB 255	69.6	2268	2029
PB 260	62.9	2047	1761
PB 311	63.4	2486	1985
PB 314	61.7	2291	2127
PR 255	65.7	2348	2008

clones were evaluated. Mean yield over eight years is given in Table Bot.13. RRII 105 was the highest yielder followed by PB 314.

In an on-farm evaluation (1992) at Shaliacary estate Punalur, PB 280 registered an yield of 3109 kg/ha followed by PR 255 (2720 kg/ha) while RRII 105(control) gave 2138 kg/ha in the 11th year of tapping. In another on-farm evaluation (1993) in the same estate, of the five clones, RRII 176 and RRII 51 showed more yield compared to RRII 105 (2822 kg/ha) in the 13th year of tapping. Clone PB 280 recorded higher yield (1451 kg/ha) compared to RRII 105 (1214 kg/ha) in the third year of tapping in an on-farm evaluation at Punalur.

On-farm evaluation for the post-release follow up on the RRII 400 series clones in small-holdings was undertaken in North, Central and South Kerala. In North Kerala, at Pallarmangalam (Ottapalam), RRII 417 showed better yield and growth (39.2 g/t/t) compared to RRII 105 (35.4 g/t/t) in the first year of tapping. At Malappuram and Kunnankulam, among the RRII 400 series clones, RRII 414 showed better performance than RRII 105.

Data on girth, yield and disease incidence were collected from five small growers' fields in Central Kerala. In Malayattoor, clones RRII 417, RRII 422 and RRII 429 recorded higher yield than RRII 105. In Onnukal, RRII 429 and RRII 430 were found better than RRII 105. In Ponkunnam, tapping was initiated in 100 % trees of RRII 414 in the fifth year after planting and yield was promising while RRII 105 (control) trees did not attain the required tapping girth.

In South Kerala, at Ayur the five RRII 400 series clones along with RRII 105 were under evaluation. In general, the RRII 400 series registered better growth and yield

compared to RRII 105. Yield of clone RRII 422 was the highest (80 g/t/t), while RRII 105 recorded 45 g/t/t. In another on-farm trial at Kanjirappally, RRII 422 recorded the highest girth and uniformity followed by RRII 430 and RRII 414 whereas, only 20 % of check clone (RRII 105) attained tappareability.

2.3. Genetic studies and investigations on genotype x environment interactions

In order to assess genetic variation and heritability of wood traits, wood core samples were collected from full-sibs and their parent clones. The wood samples were used for estimation of wood specific gravity of the progenies and their parental clones. It ranged from 0.63 to 0.70 in the progenies compared to mid-parental values of 0.63 to 0.68. Narrow sense heritability (h^2) for wood specific gravity based on parent-offspring method was estimated as 0.32 based on mid-parent value. This showed appreciable level of genetic control for this trait, and this can be used for genetic improvement. The wood cores will be used for analyzing the fibre characteristics. Data was also collected on the occurrence of TPD among the full-sibs and their parent clones. The observation will be repeated for further statistical analysis.

Two clonal nursery experiments were laid out at RRS, Agartala and RRS, Dapchari to assess performance of 47 pipeline clones under cold and drought conditions, respectively.

Performance of clones in the multi location trials laid out in 1996 incorporating RRII 400 series clones continued to show the same trend during the seventh year of tapping in three locations viz. Kanyakumari, Agartala and Nagrakata. In Kanyakumari, RRII 203 and RRII 417 were the top yielders with 73.9 and 71.1 g/t/t followed by RRII 105 (69.9 g/t/t). RRII 422

showed the highest yield (64.1 g/t/t) in Agartala whereas in Nagrakata RRII 429 recorded the highest yield (76.02 g/t/t). In Padiyoor, during the fifth year of tapping, RRII 430 was significantly superior to that of RRII 105. At Bhubaneswar, RRII 430 and RRII 429 were the top yielders followed by RRII 600 and RRII 417 during the second year of tapping.

3. Participatory evaluation of rubber clones

Under Phase 1 of the project, 20 pipeline clones and three high yielding check clones, viz. RRII 105, RRII 414 and RRII 430 are under evaluation in 14 field trials spread across 13 locations. Girth of clones was recorded in the third year after planting. Observations on diseases were recorded by the crop protection team and soil samples

collected from the various locations were analysed by the crop management team. Among the check clones, RRII 414 was superior in girth in all the 13 locations. Whereas superior growth was recorded by RRII 430 in 11 out of the 13 locations. Clones P010, P 026, P 067, P 072 and P 074 exhibited good growth in most of the locations.

Phase 2 of the project was initiated in the year 2010 with planting of 17 clones comprising 14 pipeline clones and three check clones in on-farm trials in eight locations and in the Central Large-Scale Trial at CES.

4. Breeding for other specific objectives

4.1. Compact canopy

In the 2001 SST, clonal progenies of crosses between compact canopy mutant and popular clones (RRII 118, RRII 600)

Table Bot. 14. Yield of hybrid clones over 2 years of tapping

Clone code	Cross combinations	Girth (cm) 2011	Mean yield 2010 (g/t/t)	Mean yield Over 2 yrs (g/t/t)
CV(C)	Genetic variant	19.3	0.0	9.6
RRII 118 (C)	RRII 118	62.5	36.5	30.8
94/76	RRII 105 x Genetic variant	60.5	19.4	16.1
94/70	RRII 600 x Genetic variant	51.1	33.8	16.9
94/210	RRII 600 x Genetic variant	45.9	18.3	13.3
94/600	Genetic variant x RRII 118	50.8	35.0	33.6
94/179	Genetic variant x RRII 118	54.5	11.1	13.7
94/82	RRII 105 x Genetic variant	57.9	27.4	22.2
RRII 105 (C)	RRII 105	51.0	47.2	45.9
94/171	Genetic variant x RRII 118	64.9	31.4	26.7
94/147	RRII 600 x Genetic variant	49.8	16.2	14.5
94/20	RRII 600 x Genetic variant	57.0	29.2	24.4
RRII 600 (C)	RRII 600	55.4	38.8	32.9
94/170	Genetic variant x RRII 118	55.0	30.3	27.5
94/587	RRII 600 x Genetic variant	52.4	15.0	7.5
94/24	RRII 600 x Genetic variant	41.7	27.9	21.7
94/86	RRII 105 x Genetic variant	49.0	22.6	20.5
94/78	RRII 105 x Genetic variant	62.9	32.4	29.9
CV		10.9	25.9	55.3
CD (P = 0.05)		9.4	11.3	15.1

*CES, 2001 planting

were under evaluation. Trees exhibited normal growth with compact canopy in the third year after tapping (Table Bot. 14).

4.2. Drought tolerance

There was significant clonal variation with respect to annual mean yield, summer yield and girth in the two SSTs planted in 1998 at CES. Yield in the sixth year of tapping ranged from 38.2 to 70.9 g/t. One clone, 93/214 in Trial A recorded superior yield than RRII 105 whereas performance of eight hybrid clones were comparable over six years of tapping. The parent clones PB 217, RRIC 104, and PB 260 recorded higher yield than RRII 105 in the summer months. In Trial B, one clone, 93/58 was superior to RRII 105 in terms of annual mean yield over 6 years of tapping and clones 93/92, 93/110, 93/75 and 93/272 were comparable. In terms of summer yield, all the clones were comparable to the check.

Data from the 56 clones under evaluation in the 1999 SST revealed significant clonal variation for yield and girth. Among the hybrids, 15 clones and RRII 118 were significantly superior in terms of annual mean yield and 18 hybrid clones along with RRII 118 were superior in terms of summer yield compared to the high yielding check clone RRII 105 in the fifth year of tapping. Annual mean yield of the 56 clones ranged from 26.8 to 98.2 g/t/t with a general mean of 47.44 g/t/t. Significant clonal variation was evident for mean yield over five years of tapping and 13 clones were superior in yield to clone RRII 105.

Among the 14 hybrid clones from the 1994 HP progeny under evaluation along with six parental clones in Small Scale Trial 1 planted in 1999 at CES, in the fifth year of tapping, three clones (94/23, 94/44 and 94/50) exhibited promising yield compared to

RRII 105 and three hybrid clones *viz.* 94/90, 94/23 and 94/44 recorded significantly superior girth over RRII 105 (Table Bot. 15).

Table Bot. 15. Girth and yield of clones

Clone	Mean girth (cm)	Clone	Yield (g/t/t)
94/90	73.54 ^a	94/23	74.7 ^a
94/23	72.55 ^a	94/44	71.9 ^{ab}
PB 260	71.33 ^{ab}	94/50	68.5 ^{ab}
94/44	70.75 ^{abc}	RRII 105	60.5 ^{ab}
94/50	63.89 ^{abcd}	PB 217	59.9 ^{ab}
PB 217	61.75 ^{abcde}	94/101	58.2 ^{ab}
94/19	61.39 ^{abcde}	RRIM 703	56.6 ^{ab}
94/6	61.00 ^{abcde}	94/90	52.0 ^{bc}
94/101	58.50 ^{cde}	94/66	51.3 ^{bc}
94/41	57.75 ^{de}	94/25	41.1 ^{cd}
RRII 105	57.61 ^{de}	94/8	39.9 ^{cd}
94/25	56.69 ^{de}	94/84	38.3 ^{de}
AVT 73	54.61 ^{de}	94/41	37.8 ^{de}
94/34	54.46 ^{de}	94/12	37.1 ^{de}
94/12	53.83 ^{de}	94/19	34.6 ^e
RRII 208	52.94 ^{de}	94/34	33.4 ^e
94/84	52.53 ^{de}	RRII 208	31.4 ^e
RRIM 703	50.25 ^e	PB 260	31.1 ^e
94/66	48.91 ^e	AVT 73	21.0 ^e

Means followed by a common letter are not significantly different at 5% level

Among the drought tolerant ortets from Dapchari, being evaluated for yield potential at CES there was significant clonal variation in annual mean and summer yields in the sixth year of tapping and in terms of the pooled mean over six years. The clone D 111 recorded the highest yield of 107.8 g/t/t followed by RRII 105 (99.2 g/t/t). Clone D 111 recorded a significantly high summer yield compared to the check and exhibited no signs of TPD. D 37, D 173, D 95, D 236 and D 35 were the other high yielders. Two clones, D 111 and D 37 recorded high volume of latex *i.e.*, 140 and 126 ml/t/t as compared to 103 ml/t/t recorded for RRII 105 during the peak season.

4.3. Polycross progeny evaluation

Significant variation existed among and within progenies in the polycross progenies comprising 150 clones which were in the 10th year of evaluation. A total of 69 clones among the 150 exhibited a higher yield than RR11 105. The mean yield of the progenies in Trial 1 in the 10th year ranged from 52.2 to 71.3 g/t/t. A progeny of clone PB 215 gave the highest mean yield, followed by that of PB 242 and RR11 105. The mean yield of the progenies in Trial 2 ranged from 46.1 to 88.8 g/t/t in the 10th year of tapping. The highest mean yield and recovery of high yielding clones was recorded by the progeny of PB 252, followed by that of Ch 26.

Twenty four clones selected from half-sib progenies were in the third year of growth in a clonal nursery planted in RR11 in 2007. Progenies of LCB 1320 and BD 5 continued its superiority in growth when compared to the check clones (RR11 105, RR11 414, RR11 430 and PB 235).

In the observational trial of polycross progenies planted in 2005 at CES, girth recording was continued. With reference to growth parameter, polyclonal seedlings showed better girth (43.6 cm at 50 cm height and 32.9 at 150 cm height) compared to RR11 105 (27.1 cm) in the fifth year of planting.

5. Anatomical investigations

For the first time, a time dependant chaotic and linear spatial displays of patterns were observed in the sieve tubes of *H. brasiliensis*. A large number of globular objects with kinetics were found. Sieve tubes recently differentiated from the cambial zone in the tree trunk of *Hevea* showed uniform distribution of globular objects in a continuous state of motion, when 40-50 μ m

thick tangential longitudinal sections of both fresh and long-term preserved bark were viewed under light microscope. The object with an average diameter of 1.6 μ m dispersed with a density of 200 per 1000 μ m² in a viscous medium. At a time, patterns developed at different loci were different and were not repeated and but across the loci the displays were chaotic. As the pattern repeats intermittently in a regular manner within a block, the motion along the block was linear. The direction of the moving objects to form intermittent oscillations in a block gave an indication of the direction of spatial pattern to be formed in the forthcoming block. The moving objects (average diameter of 2.1 μ m) were found in the sieve tubes away from the cambium, within the soft bark of the same bark identified as starch grains.

A simple and specific stain was used to identify the sieve tubes in cross sections of the bark. Bark samples from mature trees were collected and fresh sections of 20-30 μ m thickness were cut and fixed in 4% glutaraldehyde for 1-2 h, under 4°C refrigeration. Sections were then treated with a mixture of O-dianisidine (1mg/ml) and 1% hydrogen peroxide in 0.1M phosphate buffer (pH 7) for 0.5-1 minute. The present technique facilitates an easy localization of sieve tubes in the bark.

6. Studies on propagation

Plants raised from various budwood stocks showed high variability compared to plants from young budwood stocks though the mean girth of plants is comparable in the fourth year after field planting irrespective of the age of the stock plant. Plants developed from light green buds taken from the brown budwood and buds directly taken from mature trees also showed

lower girth coupled with higher variability than other types of buds.

Two separate polybag nursery experiments (using green-budded stumps and brown-budded stumps) were laid out at the Central Experiment Station at Chethackal with the objectives to compare sprouting and growth of green and brown-budded stumps planted in polybags kept in polyhouse and outdoor. Budded stumps generated from both green

and brown-budding techniques recorded early emergence of buds and better growth of scion in polyhouse conditions (Table Bot. 16). Plants in polyhouse imparted better growth in terms of height of scion, diameter of stem, number of whorls, number of lateral roots and total biomass and showed lesser disease incidence than those grown in outdoor conditions. The study has shown that growing plants inside polyhouse could improve the quality of planting materials.

Table Bot. 16. Characteristics of plants after seven months

Parameter	Types	Experiment No. I			Experiment No. II		
		Mean	CV	SE	Mean	CV	SE
Height of scion (cm)	Polyhouse	53.1**	23.13	3.55	57.20**	17.48	3.16
	Outdoor	26.75	36.13	2.77	34.10	36.10	3.61
Diameter of scion(mm)	Polyhouse	7.45	22.38	0.48	8.04	25.77	0.60
	Outdoor	6.16	38.16	0.68	7.48	30.28	0.66
No. of whorls	Polyhouse	2.47*	26.56	0.19	2.58	19.93	0.15
	Outdoor	1.83	39.15	0.21	2.17	33.13	0.22
No. of lateral roots	Polyhouse	29.04**	15.02	1.24	29.70**	8.41	0.79
	Outdoor	21.17	27.70	1.69	23.41	13.44	0.98
Total biomass (gm)	Polyhouse	126.38**	24.39	8.91	162.08*	22.80	10.67
	Outdoor	75.83	31.05	6.80	126.67	28.48	10.41

* Significant at $P = 0.05$, ** Significant at $P = 0.01$

GERMPLASM DIVISION

The 1981 IRRDB wild *Hevea brasiliensis* germplasm collection, domesticated clones derived from the original Wickham origin and five other *Hevea* species are being maintained at RRII. Apart from the conservation of the germplasm, its agronomic evaluation, screening for diseases, abiotic (drought and cold) stress resistance and timber latex traits, and utilization of the useful wild germplasm in crop improvement, are the major activities of the Division Formulation of

Distinctiveness, Uniformity and Stability (DUS) testing norms for *Hevea*, and generation of a mapping population for *Hevea* are other activities.

1. Introduction, conservation and documentation

1.1. Domesticated genepool (Wickham collection) from secondary centers

One hundred and eighty three Wickham clones are being conserved in field

gene banks with one clone museum at RR11 Farm, Kottayam, and two germplasm gardens at CES, Chethackal.

Significant clonal differences were recorded for yield, girth, bole height and bole volume in the Germplasm Garden IV consisting of five IRCA clones (Table Ger.1). IRCA 130 continued to be far superior to all the other clones in terms of yield, while IRCA 111 and IRCA 109 were on par with RR11 105. IRCA 111 and IRCA 130 were superior to all others for girth and bole volume.

Clones	Girth in the 19 th year of planting* (cm)	Bole volume** (m ³)	Dry rubber yield** (9 th year of tapping) (g/t/t)
IRCA 130	75.91	0.25	84.75
IRCA 109	65.77	0.17	43.27
IRCA 111	77.27	0.21	50.60
IRCA 18	69.88	0.16	35.89
IRCA 230	66.36	0.14	39.20
RR11 105	69.84	0.12	56.90
CD (P=0.05)	6.70	0.05	14.65

** Clonal differences significant at P = 0.01

The superior clones such as IRCA 130, IRCA 111 and IRCA 109 identified from the above study were planted in five on-

farm evaluation trials at five different locations.

In the Germplasm Garden V, among the 20 clones, RR11 23, RRIM 609 and RRIC 100, continued to be the best clones in terms of yield (79.4 – 63.7 g/t/t), while the control clone RR11 105 had an yield of 54.8 g/t/t (Table Ger. 2). These three clones also had higher girth ranging from 92.8 cm (RRIC 100) to 88.8 cm (RRIM 609), while the control clone RR11 105 had a girth of 71.6 cm.

In order to formulate Distinctiveness, Uniformity and Stability (DUS) norms, 50 Wickham clones were multiplied and raised in polybags at CES, Chethackal, RRS, Dapchari and RRS, Agartala, for a multilocation project. This is to validate the traits identified for DUS testing and also to identify additional traits for this purpose and to document a database of the popular clones. Data were recorded on the wintering pattern in a set of 25 mature clones.

1.2. IRRDB 1981 wild gene pool

Wild accessions numbering 3576 are being maintained in different field conservation-cum-source bush nurseries (SBNs). Re-establishment of the

Clone	Yield ** (g/t/t)	Girth ** (cm)	Clone	Yield ** (g/t/t)	Girth ** (cm)
RR11 23	79.4	88.8	PR 255	37.9	63.0
RRIM 609	70.9	86.7	Haiken 1	37.8	76.0
RRIC 100	63.7	92.8	RR11 15	37.8	76.3
RRIC 148	56.6	88.1	RR11 27	34.0	62.7
RR11 105	54.8	71.6	RR11 22	25.0	58.1
RR11 178	54.4	87.7	SCATC 88-13	19.8	74.9
RRIC 102	53.3	76.9	PB 255	17.5	91.3
SCATC 93-114	47.3	67.4	RRIM 618	13.9	64.3
RR11 20	44.6	70.7	RR11 12	12.2	76.0
RRIC 36	39.9	71.9			
RR11 108	38.1	75.3	CD (P=0.05)	24.94	12.69

** Significant at P = 0.01

conservation nurseries has been carried out. The first three re-established nurseries (SBNs 2003, 2004 and 2005) comprising of 550, 975 and 701 wild accessions respectively, were cut back, after ensuring proper identity. The fourth set of 806 accessions planted in conservation nursery 2006 in an augmented RBD with four controls is maintained properly. Second round of test tapping in 63 accessions of this nursery having more than 50% test tap yield with that of RRII 105 will be done during next year for reconfirmation of their yield potential. The conservation nursery 2007 comprising of 500 accessions was monitored for early growth and recorded test tap yield. The accessions AC 4130, RO 2008 and RO 857 recorded the highest girth (27 – 27.2 cm).

1.3. Other *Hevea* species

The arboretum comprising six species of *Hevea* and their hybrids available at RRII (*H. benthamiana*, *H. spruceana*, *H. nitida*, *H. camargoana* and two accessions of *H. pauciflora*, two *H. brasiliensis* clones, five natural putative interspecific hybrids and an F₁ 516, an inter-specific cross between *H. brasiliensis* and *H. benthamiana*) planted at CES in 2006, was maintained.

2. Characterization and preliminary evaluation

In PET (Ortets) 99, none of the wild accessions was high yielding. The controls yielded an average of 104.0 g/t (PB 260), 53.9 (RRII 105) and 49.3 (RRIM 600). The best wild accessions were OM 1116 and OM 1109 with yield levels of 36.7 and 25.1 g/t/t respectively (Table Ger. 3). Intra-clonal variation was high for this trait.

Among the 46 wild accessions, OM 1105 had the highest girth, followed by OM 1107. OM 1105 also had good timber clone

Table Ger. 3. Top ranking wild accessions for yield and girth in ortets 99

Accession	Yield (g/t/t) 2 nd year of tapping	Accession	Girth (cm) 12 th year of growth
PB 260	104.0	OM 1105	85.3
RRII 105	53.9	PB 260	83.8
RRIM 600	49.3	OM 1107	80.0
OM 1116	36.7	RRII 105	76.5
OM 1109	25.1	RRIM 600	74.9
		OR 1149	73.5
		OM 1109	72.8
		OM 1116	55.3
		OR 1166	57.8

characteristics (straight trunk and clear bole volume), though the yield was very poor. The girth of the controls ranged from 83.8 cm (PB 260) to 74.9 cm (RRIM 600). OR 1149 (73.5 cm) and OR 1109 (72.8 cm) also had relatively high girth among the wild accessions.

Six out of 47 wild accessions in preliminary evaluation trial (PET) 2000 had girth ranging from 65.0-77.4 cm, while that of the controls RRII 105 and RRIM 600 were 58.6 and 45.5 cm respectively. The two accessions RO 4599 and MT 4788, continued to show promising yield levels compared to RRII 105 (Table Ger. 4).

Monthly yield and annual girth recorded in PET 2000A at RRS, Padiyoor. The accession AC 2537 (59.75 cm) recorded

Table Ger. 4. Performance of top accessions in PET 2000

Accession	Girth (cm)	Accession	Yield (g/t/t)
MT 4740	77.4	RO 4599	44.4
MT 4763	71.0	MT 4788	41.2
RO 4612	67.3	RRII 105	23.0
AC 4637	67.0	AC 4937	22.0
MT 4861	65.0		
RO 4891	65.0		
RRII 105	58.6		
RRIM 600	45.5		

maximum girth followed by RO 2171 (55.75 cm) and 3609 (53.50 cm), while the control clones RR11 105, RR11 208 and RR11 600 recorded 37.91, 45.20 and 40.07 cm respectively. In PET 2000 B at RRS, Padiyoor, monthly yield was recorded in 166 wild accessions. Accession MT 4351 (26.38 g/t/t) was the highest yielder followed by AC 2004 (22.5 g/t/t). These two accessions recorded 80% yield of RR11 105 in which the monthly yield recorded was 29.8 g/t/t. Five accessions (AC 341, AC 3638, RO 3795, AC 1049, RO 210) recorded more than 50% yield of RR11 105. Annual girth was the highest in the accession RO 2883 (63.25 cm) followed by MT 39 (60.0 cm), which indicated the timber potential of these accessions whereas the annual girth of RR11 105 was 48.48 cm. In PET 2002, AC 567 recorded the highest girth followed by AC 1964 and AC 824.

3. Further evaluation and selection

3.1. Clonal nursery evaluation

Potential half-sibs (115) were derived from family blocks of nine pre-potent clones of polyclonal seed garden at HBSS, Nettana, which were identified as having high summer test tap yield. Out of this, 50 were multiplied and raised in polybag nursery during last year at RRS, Dapchari. They were field-planted during this year along with three checks viz. RR11 105, RR11 430, RR11 414 at a spacing of 2.5 x 2.5 m. These clones will be evaluated for their drought tolerance potential for five years and the selected ones will be subjected to large-scale evaluation at RRS, Dapchari which is a drought-prone area.

Another clonal nursery evaluation trial comprising of 31 clones selected from the above set of 115 potential half-sibs was planted last year at RRS, Padiyoor a

drought prone area in the traditional belt adopting a spacing of 2.5 x 2.5 m.

The third clonal nursery evaluation at CES, Chethackal, comprising 15 selected wild accessions from SBN 2004 having 50-80% test tap yield of RR11 105 was planted along with three check clones at a spacing of 2.5 x 2.5 m for evaluating their yield potential.

3.2. Further evaluation trials

The annual girth, dry rubber yield and volumetric timber (bole) yield were recorded and analyzed at the age of 16 years (7th year after tapping) in FET 95. Girth was maximum for the accession MT 1032 (79.61 cm) and minimum for MT 188 (39.5 cm). The mean girth of RR11 105 was 58.51 cm. Seven accessions (MT 1032, MT 941, MT 1674, MT 1599, MT 915, MT 1707 & MT 919) showed significantly higher girth than that of the control clone (Table Ger. 5). Sixty seven

Table Ger. 5. Girth of potential wild accessions

Accession	Girth (cm)
MT 1032	79.61
MT 941	79.35
MT 1674	75.55
MT 1599	75.22
MT 915	74.06
MT 1707	71.21
MT 919	70.29
RR11 105	58.51
CD (P = 0.05)	12.32

accessions had the girth statistically on par with the control clone.

Among the accessions AC 166 had the highest dry rubber yield (63.67 g/t/t) which is on par with the yield of RR11 105 (63.47 g/t/t) in the seventh year of tapping. This accession showed 99.70% of the yield of the

control clone RRII 105. Five accessions showed above 80 % of the yield and 10 accessions had 50% to 74% of the yield of RRII 105 (Table Ger. 6). AC 166 was multiplied for the on-farm evaluation trials

in five locations under different environments.

Four accessions (MT 941, MT 1032, AC 650 & MT 399) showed significantly higher volumetric timber in terms of bole volume than that of RRII 105 (Table Ger. 7).

Table Ger. 6. Accessions showing better performance for dry rubber yield

Accession	Dry rubber yield (g/t)	% of the yield of RRII 105
AC 166	63.67	99.70
AC 2004	57.90	90.65
RO 2385	57.09	89.38
RO 2908	54.84	85.89
AC 155	54.18	84.83
MT 1020	47.25	73.98
AC 650	46.25	72.41
AC 635	46.02	72.05
AC 638	45.72	71.58
MT 922	42.74	66.92
AC 655	42.68	66.82
AC 692	40.49	63.34
AC 685	32.88	51.00
MT 941	31.93	50.00
MT 999	31.76	50.00
RRII 105	63.87	50.00
CD (P = 0.05)	17.99	

Table Ger. 7. Accessions showing potential for high timber yield

Accession	Bole volume (m ³)
MT 941	0.16
MT 1032	0.16
AC 650	0.15
MT 399	0.14
RO 141	0.13
MT191	0.13
AC 638	0.13
MT 999	0.13
RRII 105	0.08
CD (P = 0.05)	0.05

Girth was recorded in the eighth year of growth in the FET 2003, comprising 22 wild accessions and three controls. RO 2629 (54.25 cm) recorded the highest girth followed by AC 4149 (50.19 cm) and AC 626 (47.17 cm). Significant clonal differences were seen for girth in FET 2005 comprising 22 wild accessions and three controls. AC 2004 and MT 4788 had the highest girth in the sixth year of growth (40.9 and 35.6 cm respectively), on par with the controls PB 235 (36.5 cm) and RRIM 600 (31.3 cm), while MT 185, RO 2731, MT 182 and RO 3012 (33.3 – 31.6 cm) were also on par with the best control.

Accession MT 77, RO 2846 and AC 4104 recorded the highest girth out of the 26 wild accessions in FET 2008.

Thirteen wild accessions in FET 2010A, selected from SBN 2004 having more than 80% test tap yield of RRII 105 were field planted at CES, Chethackal along with the check clones RRII 105, RRII 430 and RRII 414 and were maintained properly. Twenty two selected wild accessions along with three control clones were planted in simple lattice design at CES, Chethackal (FET 2010B) and another set of 11 selected wild accessions along with two control clones were planted in RBD at RRS, Dapchari (FET 2010C) and maintained with proper care.

3.3. On-farm evaluation trials

One promising wild accession (AC 166) and three IRCA clones (IRCA 130, IRCA 111, IRCA 109) were field-planted along with

three check clones RR11 105, RR11 430 and RR11 414 at five locations viz. B.C. Cheruvally estate, Erumely, Malankara estate, Thodupuzha, Mooply estate, Trissur, Calicut estate, Kozhikode and Bethany estate, Kanyakumari for evaluating their performance in different agroclimates.

4. Screening for stress tolerance

4.1. Abiotic stress resistance

4.1.1. Drought tolerance

Seven potential accessions out of 130 identified based on drought related growth parameters and rubber yield from the field screening trial 2003 at RRS, Dapchari were field-planted along with check clones RR11 105, RR11 414, RR11 430, RR11 600 and Tjir 1 for further evaluation of their drought tolerance potential in the drought-prone area at RRS, Dapchari.

In the further field evaluation of selected *Hevea* clones at RRS, Dapchari in collaboration with Botany Division, the growth during the summer and peak period of growth was assessed in the 34 selected *Hevea* clones comprising 23 wild accessions, 5 HP clones and 6 check clones viz., RR11 430, RR11 414, RR11 105, RR11 600, RR11 208 and Tjir 1. Among the 23 wild accessions, accession MT 4788 recorded the highest girth and it showed consistent performance throughout the growth period. After experiencing summer periods of 2010, eight wild accessions out of 23 and one among the hybrid clones (93/270) recorded higher girth than the proven drought tolerant clone RR11 600. Among the three year old plants of wild accessions, the highest girth was obtained by MT 4788 (18.55 cm) followed by MT 1616 (16.46 cm) whereas the clone RR11 430 had the girth of 17.57 cm. Among the five hybrid clones, clone 93/270 exhibited the highest girth

(16.23 cm). Among the six check clones, RR11 430 was superior in terms of absolute girth at the age of three years.

4.1.2. Cold tolerance

A total of 64 wild accessions are being evaluated for cold resistance in two trials at Regional Experiment Station, Nagrakata, West Bengal. Girth of the eleven-year-old accessions was recorded during pre- and post-winter period and it showed significant variation. Monthly yield was also recorded. Higher annual girth was observed in RO 2902 (77.92 cm), MT 5105 (76.92 cm) and RO 2387 (76.31 cm) as compared to clones SCATC 93/114 (64.64 cm) and RR11 600 (62.04 cm) in trial 1. In trial 2, clone MT 915 recorded the highest girth of 79.43 cm followed by RO 2727 (78.88 cm) and MT 900 (75.03 cm), while the controls Haiken 1 and RR11 600 recorded 73.90 cm and 61.60 cm, respectively.

5. Screening for timber characteristics

5.1. Field screening

Annual girth, monthly yield and timber volume were estimated at the age of 10 years. Among the wild accessions, 11 accessions showed girth on par with RR11 105; one accession on par with PB 235; two accessions on par with PB260; four accessions on par with RR11 33; six accessions on par with RR11 118 and 15 accessions on par with RR11 600. Three accessions had the timber volume significantly higher than that of PB 260, RR11 33, RR11 118, RR11 105 and RR11 600 (Table Ger. 8).

6. Utilization of *Hevea* germplasm

6.1. Hand pollination programmes

A hand pollination programme conducted during 2009 at CES, Chethackal involving three wild accessions showing

Table Ger. 8. Performance of wild accessions for girth, yield and timber volume

Accessions	Girth (cm)	Dry rubber yield (g/t)	Bole volume (m ³)
MT 941	55.36	6.43	0.10
MT 915	54.73	11.51	0.10
AC 650	54.23	1.01	0.10
MT 922	53.72	12.63	0.05
RO 255	53.26	26.69	0.05
MT 919	52.31	7.40	0.06
MT 1032	50.35	8.35	0.04
AC 635	50.35	14.25	0.08
MT 999	49.58	1.95	0.06
AC 1021	49.21	14.04	0.06
RO 322	49.06	3.85	0.07
MT 1020	48.80	6.99	0.04
MT 935	48.35	4.02	0.04
AC 655	45.07	1.93	0.03
AC 707	43.87	37.26	0.04
AC 637	41.41	1.73	0.03
AC 685	39.41	45.61	0.03
AC 651	37.88	2.50	0.03
RO 879	34.63	1.00	0.03
Wickham clones			
PB 235	62.17	63.22	0.08
PB 260	61.36	25.28	0.05
RRII 33	60.80	36.18	0.08
RRII 118	59.12	30.01	0.06
RRII 105	53.76	47.26	0.06
RRIM 600	50.00	40.46	0.06
CD (P = 0.05)	14.56	22.22	0.03

potential for yield and six popular Wickham clones resulted in 10 cross combinations. Seventy five successful hybrid progenies were established in the field at CES, Chethackal and early growth was monitored. Twenty nine progenies derived from two crosses and 25 OP seedlings are maintained in the seedling nursery at RRS, Padiyoor, which will be subjected to test tapping during next year.

6.2. Generation of mapping population

The progeny of the interspecific cross between the popular *H. brasiliensis* clone RRII 105 and the disease tolerant *H. benthamiana*

clone F 4542 were raised in a seedling nursery. Eighty four progenies were screened using 12 RAPD primers to confirm their hybridity, and three non-hybrids were identified and removed.

7. Other studies

7.1. Feasibility of ratooning in *Hevea*

Ratoons continued to be superior to their corresponding polybag grown counterparts in the tenth year of growth in terms of girth. (Table Ger. 9)

Table Ger. 9. Growth and yield of ratoon plants

	Ratoons plants	Polybag
Number	137	187
Girth range (cm)	15-104	18-78
Mean girth (cm)	69.4	47
>50 cm	134 (97.85%)	71 (38%)
Yield (g/t/t)	45.3	31.2
Range (g/t/t)	158.7- 5.4	55.6- 3.8

7.2. Effect of stimulation in the laticiferous tissues of *H. brasiliensis*

Bark samples were collected from BO-1 and B1-2 panels before and after stimulation (Clone-RRII 105) and recorded the bark thickness, number of functional and disorganized laticifers. The bark thickness increased in BO-1 panel and decreased in B1-2 panel during stimulation. The number of both disorganized laticifers increased in BO-1 panel and decreased in B1-2 panel due to stimulation whereas the number of functional laticifers and total number of laticifers decreased in both panels due to stimulation.

7.3. Evolving location specific *Hevea* clones through ortet selection programme

The objective of the study was

screening and identification of potential ortet clones from the selected poly-clonal private holdings of selected farmers in Rampachodavaram, Andhra Pradesh (Collaboration with Rubber Production Department).

7.4. Assessment of the performance of new rubber plantations of ITDA, AP.

This is a collaborative project with ITDA, RC Varam, AP and RRII (Germplasm Division and Agronomy/Soils Division). The study was taken up with the objective of monitoring growth of new large scale plantings in Andhra Pradesh by the ITDA in 2008. Twelve farmers' fields were inspected in association with the soil scientist, GPS readings recorded and soil profiles collected to estimate soil fertility status.

8. Participatory clone evaluation

8.1. On-farm evaluation of pipeline clones-Phase I

Twelve pipeline clones and three controls were evaluated in AVT, Shaliacary

estate, Punalur. Clone P 66 recorded the highest girth of 18.48 cm followed by P 15 (17.88 cm), P 72 (16.83 cm), while the control clones RRII 414, RRII 430 and RRII 105 recorded girth 20.88 cm, 18.15 cm and 13.44 cm, respectively. Among the 11 test clones at Mooply estate, clone P 15 recorded the highest girth of 18.88 cm at third year of planting and among the three check clones, clones RRII 430 and RRII 414 recorded girth 19.1 cm and 19.03 cm respectively. Disease incidence was evaluated in the test clones and three controls, with RRII 105 being used for covariance analysis in each plot. Among the 11 clones at TR&T estate, P 74 and P 10 had the highest girth (19.2 and 18.3 cm respectively), while the control clone RRII 430 had the highest overall girth (19.4 cm).

8.2. On-farm evaluation of pipeline clones-Phase II

Field planting of 14 test clones along with three check clones (RRII 105, RRII 414 and RRII 430) was carried out at B. C. Cheruvally estate, Erumeli and Malankara Estate, Thodupuzha.

PLANT PATHOLOGY DIVISION

The Division is primarily concentrating on the economic and eco-friendly management of diseases and pests of *Hevea* using chemical and biological agents. Evaluation of sprayers and spray oils, assessment of yield loss due to diseases, evaluation of newly evolved clones for disease resistance, studies on tapping panel dryness, bee keeping in rubber plantations, use of beneficial microorganisms for plant growth and treatment of sheet

processing effluents were the other areas of research.

1. Leaf diseases

1.1 Abnormal leaf fall disease

A field experiment was initiated at Chimony Estate, Trichur to improve the disease control efficiency of the clones RRII 105 and RRII 600. First year's result

indicated that two rounds of spraying at 1:5 ratio (COC: spray oil) at 4 kg: 20 L per hectare per round yielded better leaf retention in the clones studied.

A study on the genetics of resistance in rubber plants to *Phytophthora* was worked out based on the response of a common set of *Phytophthora* isolates to two fungicides viz. metalaxyl (Ridomil) and copper oxychloride (Fytran) and their virulence on host genotypes. Certain isolates were sensitive to Ridomil but resistant to Fytran. The isolates showed varying sensitive/tolerance reaction to both the fungicides. Variation in the aggressiveness of different isolates of *Phytophthora* was also observed. As both mating types have been reported from *Phytophthora* infecting rubber, a correlation with the mating type and the evolution of virulent isolates was also attempted to gain a better understanding of whether virulence is evolving through sexual recombination.

For developing a SCAR marker for *Phytophthora* tolerance in *Hevea*, seven clones were selected. Among the clones, GI1, RRII 105 and F 4542 are *Phytophthora* tolerant lines and Tjir 1, RRIC 100, RRII 600 are susceptible lines. DNA was isolated and RAPD analysis was carried out with 120 random primers. Of the 120 primers screened, OPA 4 and OPF 13 showed

polymorphic banding pattern with susceptible parent (Fig. Path.1).

To check the biocontrol potential of antagonistic endophyte *Alcaligenes* sp. against *P. meadii*, bioassay was done with mature leaves of tolerant clone RRII 105 and susceptible clone RRII 600. *In vitro* assay showed less infection of *P. meadii* in leaf tissues of antagonist-treated plants (lesion size, 3.30 mm) compared to media-sprayed control (lesion size, 5.89 mm) and untreated control (lesion size, 6.30 mm). *In vivo* assay showed no significant difference in per cent leaf fall by *P. meadii* between endophyte inoculated and control RRII 105 plants (Chi-square value 0.53) while in RRII 600, endophyte inoculated plants showed significant difference with control plants (Chi-square value 5.38, significant at 10% level).

The biochemical compound extracted from potato dextrose broth (PDB) culture supernatant of the antagonistic endophyte using diethyl ether showed growth inhibition of *P. meadii* under *in vitro* conditions. The compound was separated by thin layer chromatography (TLC) and one of the component showed antifungal activity against *P. meadii*.

The experiments laid out in 2004 to assess the impact of abnormal leaf fall (ALF) disease in clones like RRII 414, RRII 422, RRII 429 and PB 260 are in progress. The assessment of disease situation indicated mild severity of ALF (under sprayed situation) in test clones, moderate severity of *Oidium* in RRII series clones, but very severe in PB 260. The growth assessment indicated possibility attaining tappable girth by seventh year in all clones.

The experiment on crown budding to avoid spraying against ALF disease in

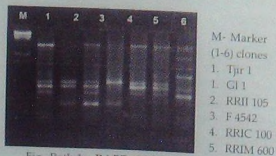


Fig. Path.1. RAPD OPF-13

clone PB 311 at Malankara estate resulted good protection of canopy from the disease in trees crown-budded with disease-tolerant clones like RR11 33 and Fx516. Another experiment initiated in 2004 in clone PB 260 for testing the feasibility or crown budding at nursery stage indicated growth parity in field in crown-budded trees compared to control (no crown budding).

1.2. Powdery mildew disease

The study at New Ambadi Estate, Kanyakumari to evaluate crop loss due to powdery mildew disease in the clones RR11 105 was continued for the third year. One plot each was maintained as dusted and undusted. Application of sulphur was undertaken at recommended dose at an interval of 7 - 10 days in the dusted plots. Very light and light to moderate disease intensity was observed in dusted and undusted plots, respectively during the third year.

1.3. *Corynespora* leaf disease

Co-existence of different pathogens in rubber plantations of Karnataka, with severe *Corynespora* disease during the season (January to March 2011) was assessed. The fungal pathogens *Corynespora cassiicola*, *Colletotrichum acutatum*, *C. gloeosporioides*, *Alternaria alternata* and *Guignardia* were isolated from the leaves showing disease symptoms. The frequency occurrence of *C. cassiicola*, *C. acutatum*, *C. gloeosporioides*, *A. alternata* and *Guignardia* was 75.39, 5.41, 6.72, 12.10 and 0.37 per cent, respectively.

Chitinase gene from GT 1, the tolerant clone was cloned in expression vector PET 32a⁺ and the orientation was confirmed through sequencing. The expression vector

was transformed in the strain BL21(DE)/BL 21(DE 3). The expression of targeted protein was induced by addition of IPTG to the growing culture. Control colonies containing the vector without insert were also maintained. The induced cells were harvested after 3 h.

The targeted recombinant protein was purified from the cells using His Bind purification kit (Novagen, USA). The purified protein was tested on 10 per cent SDS PAGE. In pET vector a very high induction of the fusion protein (Trx +target gene) was detected A ~ 49 kD band was observed in all induced samples (Fig. Path.2).

The antifungal activity of purified recombinant chitinase protein against *C. cassiicola* was assayed on PDA plates. Distinct growth inhibition zones of

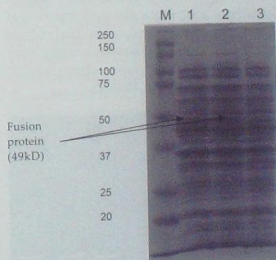


Fig. Path. 2. PAGE analysis of total cell protein from induced and uninduced samples showing recombinant protein expression with pET vector in BL21, DE3 host cells. M - Protein marker; Lane 1 - Induced (3h); Lane 2 - Induced (1.5h); Lane 3 - Uninduced control

C. cassicola were displayed around the filter paper discs soaked with 10 µg of recombinant protein compared to the control discs dipped with protein extraction buffer (Fig. Path. 3).



Fig. Path. 3. The antifungal activity of purified recombinant chitinase protein against *C. cassicola*

Among the 21 endobacterial isolates from culture collection of RRII, the isolates Jptf, Jpte and Gpta showed antifungal activity against *C. cassicola* in dual culture and were identified as *Bacillus subtilis* (Gpta), *Pseudomonas fluorescence* (Jpte) and *P. fluorescence* (Jptf) (Fig. Path. 4).



Fig. Path. 4. 16s rDNA of antagonistic endophyte
M- Marker, 1- Gpta, 2- Jpte, 3- Jptf

1.4. Colletotrichum leaf disease

For genotyping and DNA barcoding for species identification of *Colletotrichum acutatum* and *C. gloeosporioides*, causing Colletotrichum leaf disease, phylogenetically informative regions (ITS, Actin, β -tubulin, Calmodulin, Glutamine synthetase, Glyceraldehyde 3-phosphate dehydrogenase) were determined. Sufficient sequence data are being generated for validating both these species. Multigene phylogenetics are being attempted, followed by searching for a suitable gene for barcoding and developing barcodes with low heterogeneity within species, yet with enough variation to allow maximum separation of different species.

2. Participatory clone evaluation for disease assessment

Powdery mildew, Colletotrichum leaf disease, shoot rot and pink disease assessment in the participatory clone evaluation trial at six locations was carried out. Powdery mildew disease was mild in all the locations. Colletotrichum disease was not observed in Tharuvayar estate, Kanyakumari. Mooply and Calicut estates recorded moderate to severe disease intensity. All the other locations recorded mild disease intensity. Very mild incidence of shoot rot was recorded in Mooply estate. About 5–10 per cent incidence of pink was observed at Calicut and Mooply estates.

3. Determination of the etiology of Tapping panel dryness (TPD) of rubber

Plants budded with scion taken from TPD-affected trees as well as healthy trees were test-tapped to study the appearance of TPD symptoms. The results of one-year

Table Path.1. Occurrence of entomopathogenic nematodes in rubber growing soils of Kerala

District	No. of samples	No. of samples positive for EPN		Total
		<i>Heterorhabditis</i> spp.	<i>Steinernema</i> spp.	
Thiruvananthapuram	40	06 (15%)	2 (5%)	08 (20%)
Kollam	25	03 (12%)	—	03 (12%)
Pathanamthitta	45	04 (09%)	—	04 (09%)
Kottayam	35	07 (20%)	2 (6%)	09 (26%)
Ernakulam	35	02 (06%)	—	02 (06%)
Thrissur	35	10 (29%)	2 (6%)	12 (34%)
Palakkad	40	10 (25%)	3 (8%)	13 (33%)
Kozhikode	30	09 (30%)	1 (3%)	10 (33%)
Malappuram	30	07 (23%)	2 (7%)	09 (30%)
Idukki	30	03 (10%)	—	03 (10%)
Total	345	61 (18%)	12 (3%)	73 (21%)

* Figures in parenthesis are per cent values.

tapping showed occurrence of TPD in both group of plants, namely scion taken from TPD as well as healthy trees. Out of eight partial TPD trees in the TPD scion group, five turned to full TPD whereas, in the case of plants with healthy scion, a reduction in TPD was observed. Out of the 11 TPD trees none of them turned to full TPD after one year.

4. Pests of rubber

Standing rubber trees in the field partly affected by panel dryness, lightning injury, root and stem diseases were invaded by certain insect borer beetles viz. *Heterobostrychus aequalis*, *Synoxylon conigerum*, *Dinoderus bifoveolatus*, *Xylotrips flavipes* and *Platypus latifinis*. Experiments conducted at Palampra estate, Kanjirappally with different combinations of insecticides to control these beetles showed that a single swabbing of the combination of carbaryl 0.50% and lamdacyhalothrin 0.02% gave satisfactory control (97.00%) than single insecticides. The attack was predominant in the post-monsoon season until October.

Survey conducted in different locations recorded the occurrence of entomopathogenic

nematodes in rubber growing soils (Table Path.1) for the first time.

The results of the study on the management of leeches using repellants at Rajagiri Estate, Pathanamthitta indicated that *Cyclops peltata* (Padakizhangu) in coconut oil, *C. peltata* in neem oil, odomos (direct application), mixture of neem oil and citronella oil(1:1) were the effective repellents, where the leech didn't climb on legs. Materials viz. sodium hypochlorate, glyphosate and copper sulphate reduced the number of leeches in the soil for one week.

5. Improvement of spraying technology for pest and disease management of natural rubber

Mini tractor-mounted mist blower (Fig. Path. 5) was compared for its efficiency to manage the ALF disease prophylactically with the conventional micron sprayer. The leaf retention after the disease season was assessed and found to be comparable with the conventional micron spraying. However to improve efficiency further and to obtain maximum throw height, certain



Fig. Path. 5. Mini tractor-mounted mist blower

suggested modifications were to be implemented before further trial.

A single man carrying mist blower (Fig. Path. 6), developed by M/s. Oleo-Mac Company Ltd. was also tested. The throw height was found to be above 45 ft. in the field conditions.

Battery-operated back pack sprayer (Fig. Path. 7) developed by M/s. ASPEE group of Companies, Mumbai, was field tested and



Fig. Path. 7. Back pack sprayer



Fig. Path. 6. Mist blower

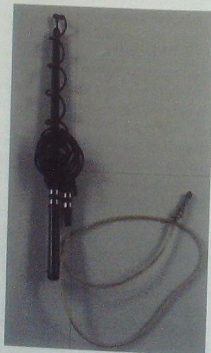


Fig. Path. 8. Telescopic lance

found to be effective in managing nursery diseases.

A telescopic lance (Fig. Path. 8) was also tested for the spray height and found to obtain 15 ft.

6. Bee keeping in rubber plantations

Conducted a field study on the effect of supplementary off-seasonal feeding on brood rearing activities of Indian honey bee *Apis cerana indica* in rubber plantations. The highest colony population was (8919) recorded in colonies fed with sugar and pollen (SPF). The population in colonies fed with sugar (SF), pollen (PF) and control averaged 8620, 7290 and 5477, respectively. The comb-building and brood-rearing activity was noticed higher in SPF-fed colonies. A percentage increase of 29.20 in

comb building and 65.24 in brood-rearing activity was recorded in SPF-fed colonies over control. The workers which received SPF and SF were more active in collecting pollen and nectar and in comb-building activities than the other treatments. The queens in these colonies were also more active (Table Path.2 & 3).

7. Microorganisms for improving growth of rubber and cover crops

Biochemical characterization of three bacterial isolates that nodulate *M. bracteata* selected from North East India and Kerala viz. NE 2 (from Agartala), NE4 (from Tura), and RR11-M (Kerala) was carried out by studying their reaction to 18 biochemical tests. The north east isolate NE 4 was multiplied and inoculated to *Mucuna* plants

Table Path. 2 Effect of supplementary feedings on comb-building and brood-rearing activities of *A. cerana indica* during off season

Treatment	Average area before feedings (sq.cm)		Average area after 6 feedings (sq.cm)		Average development percentage		Average area after 12 feedings (sq.cm)		Average development percentage	
	Comb area	Brood area	Comb area	Brood area	Comb area	Brood area	Comb area	Brood area	Comb area	Brood area
SF	227	170	278	210	22.46	23.53	280	219	23.34	28.82
PF	244	170	264	200	8.1	17.64	289	205	22.54	20.58
SPF	226	164	280	264	23.89	60.98	292	271	29.20	65.21
Control										
(no feeding)	224	176	226	63	0.8	-64.20	259	65	-15.62	-63.06

SF - sugar feeding; PF - pollen feeding; SPF - sugar and pollen feeding

Table Path. 3. Effect of supplementary feedings on the bee population in *A. cerana indica* colonies during off season

Treatment	Colony population (number of bees)			
	June	July	August	Mean
SF	7500(25.67)	8860(76.69)	9500(74.31)	86.20(57.38)
PF	6620(10.92)	7500(49.61)	7750(42.50)	7290(33.10)
SPF	6685(12.01)	9607(91.64)	10465(92.01)	8919(62.84)
Control (no feeding)	5968	5013	5450	5477

Figures in parenthesis are sine transformed values.

raised in polybags. Inoculated plants showed more growth than the uninoculated in polybags. They were field-planted (100 plants/acre) to study their performance under Kerala conditions.

A standard isolate of *Azospirillum* (*A. brasilense* CD4) was collected from IARI and a polybag study was initiated to compare the efficiency of two selected RRII isolates of *Azospirillum* with the standard in promoting the growth of rubber seedlings at different levels of nitrogen fertilizer. Periodic girth and height of the plants were recorded.

The beneficial activities of 12 selected rhizobacteria from rubber plantations were compared with the standard PGPR isolate (*Bacillus* sp R5) received from IARI. Nine isolates produced more indole acetic acid in sucrose minimal salt media. Seven isolates had more phosphate-solubilising efficiency (SE%) than the standard PGPR isolate. The SE% of four isolates was comparable to the standard phosphobacteria (*Pseudomonas striata* M27). A polybag study was initiated to compare the efficiency of RRII isolates with the standard to promote the growth of rubber seedlings upon inoculation.

The intrinsic antibiotic resistance levels of the various isolates used in different experiments to the antibiotics - kanamycin and rifampicin were studied to monitor their survival in soil. Four *Pseudomonas* isolates were tolerant to higher concentrations (>300 ppm) of kanamycin while others were sensitive even to 5 ppm of the antibiotic. The isolates were generally sensitive to rifampicin even at 5 ppm.

The various antagonistic bacterial isolates of rubber pathogens were compared with the standard antagonist of fungal pathogens (*B. subtilis*). Isolates having

more growth inhibition of the pathogens than the standard in dual culture plates were selected for further studies.

The SE% of nine phosphofungi from rubber plantations varied from 100 to 220% (Table Path. 4). Solubilization of FePO_4 was more by the standard isolate *Aspergillus awamori* while RRII isolates showed better solubilization of AlPO_4 and $\text{Ca}_3(\text{PO}_4)_2$. The isolate Pf4 only showed solubilisation of Rajphos, the sparingly soluble form of phosphatic fertilizer. A poly-bag study was initiated to compare the efficiency of RRII isolates with the standard in promoting the growth of rubber seedlings.

Table Path. 4. Phosphate solubilization by phosphofungal isolates ($\mu\text{g P}$ released/ml culture broth)

Isolate	SE%	FePO_4	AlPO_4	$\text{Ca}_3(\text{PO}_4)_2$	Rajphos
Pf 1	128	-	35	136	-
Pf 2	196	184	-	31	-
Pf 3	197	207	73	24	-
Pf 4	150	143	-	94	13
Pf 5	111	-	18	233	-
Pf 6	190	170	-	86	-
Pf 7	100	-	-	171	-
Pf 8	220	149	-	135	-
Pf 9	157	173	-	108	-
Std Pf	127	286	-	77	-

Annual growth measurements of the plants in the biofarming trial were recorded. The girth and height of the plants in the integrated treatment were more than bio and chemical treatments after one year of planting. The plants were also protected from shoot rot and Colletotrichum leaf fall, during the rainy season. For the plants in the integrated treatments, alternate application of chemical and bio-agent was given. Disease incidence was very less in all the treatments.

Bacterial isolates (122 nos.) collected from Padiyoor and Andhra Pradesh were checked for ACC deaminase activity which give stress tolerance to inoculated plants. Five isolates were selected for further studies.

8. Waste management in rubber processing

The evaluation of integrated waste water treatment system at Palakkad was continued. Hybrid anaerobic digester-reed bed system combination was under evaluation. Hybrid reactor in its second season of operation could reduce chemical oxygen demand (COD) by 94.65% and biological oxygen demand (BOD) by 94.37%. The pH after biomethanation was 7.0-7.2. The reactor could reduce total solids by 73.12% and dissolved solids by 75.30%. The reactor yielded 3.5 4 m³ biogas/day.

Initially the system showed high degrees of fluctuations in its performance in terms of pollution reduction of the effluent; hence some correction measures were taken. Modifications made in the reed bed system improved its functioning, even though the system was partially

operational. The reed bed could reduce BOD and COD at an average efficiency of 54.88% and 63.20%, respectively. The pH after the treatment ranged between 7.1-7.4. The entire system was running with zero energy consumption and zero operational cost. To improve the efficiency of reed bed system, a new improved design was adopted and based on that new system was constructed which is yet to be evaluated.

8.1 Integrated waste water treatment system

The integrated waste water treatment system (IWWTS) consisted of initial filtration unit, hybrid reactor and the reed bed could reduce COD by 97.86% and BOD by 98.22% of the raw effluent, i.e. final strength of 245 mg/l and 99 mg/l, respectively. The BOD value was not within the limit specified for safe disposal, and the COD was marginally inside the limit. The pH of the treated water was in the range of 7.1-7.4, within the limit for safe disposal. The overall reduction in solids was 78.18% (TS) and 77.78% (DS). These two parameters were not in the limit for safe disposal (Table Path. 5).

Table Path. 5 Performance of the integrated waste water treatment system (Initial filtration+ Hybrid reactor + Reed bed)

Parameter	Raw effluent	Filtered effluent	Post biometanation	Efficiency of hybrid reactor (% reduction)	Post reed bed treatment (Final)	Efficiency of reed bed (% reduction)	Overall efficiency of the system. (% reduction)
pH	5.4-6.0	5.5-6.1	7.0-7.2	—	7.1-7.4	—	—
COD	11463	10153	543	94.65	245	54.88	97.86
BOD	5563	4776	269	94.37	99	63.2	98.22
TS	14427	12623	3393	73.12	3148	7.23	78.18
DS	13172	11728	2897	75.30	2927	-1.04	77.78

Units are in mg/l, except pH

PLANT PHYSIOLOGY DIVISION

The major research areas of Crop Physiology Division are environmental physiology, physiology of growth and yield, stock-scion interaction, tapping panel dryness, nutrition physiology, gene expression studies, secondary metabolites and ecological impact of natural rubber (NR) cultivation.

1. Environmental physiology

1.1. Structure and function of photosynthetic apparatus of natural rubber in relation to its adaptation to high light and drought stress

Expression of a chloroplast stress protein in six clones (RRII 105, RRIM 600, RRII 414, RRII 417, RRII 422 and RRII 430) was analyzed under normal and early drought conditions. A 23 k Da chloroplast stress protein level was more prominent in drought tolerant clones like RRIM 600 and RRII 430 than susceptible clones. Further protein purification steps were initiated to raise antibodies against this protein. The effective quantum yield of PSII (Φ PSII) was also checked in these clones. The clones RRII 430 and RRIM 600 recorded less inhibition in PSII than RRII 105 and RRII 414 under drought conditions. From this study on the

basis of PS II activity and stress protein expression the clones such as RRII 414, RRII 417 and RRII 422 were shown to be drought susceptible.

1.2. Molecular basis for drought tolerance in *Hevea*

Three year old field grown plants of clones RRII 105, RRIM 600, RRII 208 and RRII 430 at Regional Research Station, Dapchari, were subjected to drought stress for fifteen days during the summer period of 2010. Leaf samples were collected after confirming the magnitude of stress effect on these plants by measuring gas exchange parameters like stomatal conductance (gs) and net CO₂ assimilation rate. This was followed by mRNA isolation, cDNA synthesis and qPCR. The qPCR analyses were carried out for seven genes which were identified to be drought responsive in array hybridization analyses. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as endogenous control for normalization and quantitative expression of each gene had been analyzed with reference to irrigated plants of RRII 105. Statistical analysis was performed with the relative quantification

Table Phy. 1. Relative quantification of gene expression of seven genes under drought conditions (D) with reference to irrigated plants (C) of RRII 105 (RQ values)

Genes	RRII 105 C	RRII 105 D	RRIM 600 C	RRIM 600 D	RRII 208 C	RRII 208 D	RRII 430 C	RRII 430 D	C/D (P=0.05)
HbDRT 5b	1	1.04	1.44	14.26 **	1.11	7.16 **	0.61	4.30 **	2.10
HbTPD 24	1	0.89	1.34	14.41 **	0.69	3.82 **	0.60	3.47 **	1.90
HbTPD27	1	1.83	2.99	1.99	4.58 **	4.97 **	3.04	9.83 **	3.50
HbDRT50	1	1.65	4.58 **	3.75 *	3.90 *	3.15	2.20	4.72 **	2.41
HbNRC18	1	0.47	4.34	5.23 **	74.39 **	7.22 **	3.25	29.65 **	3.63
HbNRC 21	1	1.28	4.93	3.23	6.24 **	4.27	5.39 **	16.45 **	4.13
HbDRT 82b	1	0.99	1.40	1.37	2.50	1.10	0.95	1.66	-

data using ANOVA. The ratio with a P-value d^* 0.05 was adopted as significant for down- or up-regulation.

The qPCR data of seven gene transcripts are given in Table Phy.1. Among the seven transcripts analysed, NAC transcription factor (HbDRT 5b and HbTPD 24) was found to be up-regulated significantly in drought experienced plants of RRIM 600, RRIM 208 and RRIM 430 when compared to RRIM 105 control plants (Table Phy.1). This significant level of up-regulation indicated a close association of NAC transcription factor with drought stress. Expression of HbTPD 27 was significantly high only in drought treated plants of RRIM 430 and RRIM 208. All the six genes except HbDRT 82b were significantly up-regulated in drought imposed plants of RRIM 430 compared to RRIM 105 control plants (Table Phy.1).

1.3. Molecular studies on cold stress in *Hevea*

Attempts were made to investigate the molecular basis of cold tolerance in *Hevea* with special reference to identification of genes/regulatory factors associated with cold tolerance that could be employed for selection of genotypes suitable for low temperature regions of India. Six month old plants (grown in polythene bags) of clones RRIM 105 (cold susceptible) and RRIM 600 (cold tolerant) were exposed to low temperature in a growth chamber. The cold stress was confirmed by considerable reduction in photosynthesis by using a portable photosynthesis system. cDNA obtained from mRNA of leaf samples were used as template for quantitative PCR analysis employing GAPDH as endogenous control gene (Table Phy.2).

Out of the twenty genes attempted expression of LEA 5 protein and peroxidase was found to be up-regulated in RRIM 600

Table Phy. 2. Relative gene expression (fold change) of twenty one genes in low temperature treated plants of RRIM 105 and RRIM 600 (LT) using RRIM 105 control (C) as calibrator

Gene	RRIM 105 Control	RRIM 105 LT	RRIM 600 Control	RRIM 600 LT
WRKY II	1	164.3	64.3	5.00
ABCT	1	3.19	3.65	9.77
TMBF	1	6.13	0.64	0.89
LEA5	1	1.48	5.98	58.9
CRT/DREBF	1	0.57	0.44	0.03
GPX	1	4.51	3.16	1.90
33HP	1	10.48	2.26	0.97
DnaJ protein	1	1.36	1.53	0.85
PEROXIDASE	1	9.48	19.87	158.3
ETR1	1	1.38	4.00	15.97
ETR2	1	1.18	2.13	5.13
ACO2	1	2.37	17.85	12.86
20 HP	1	1.88	1.02	1.14
22 HP	1	2.82	1.58	2.06
Cysteine protease	1	0.82	4.46	3.20
Chitinase	1	2.13	0.57	0.72
Annexin	1	3.10	1.31	4.18
SUMO	1	2.03	0.84	1.49
DRT 5b	1	5.20	0.60	15.48
TPD 24	1	4.34	0.53	9.86
DRT 50	1	16.03	12.13	9.87

when compared to RRIM 105, indicating that these proteins might be contributing for cold tolerance in clone RRIM 600. ETR1, ETR2 and NAC transcription factor (DRT 5b and TPD 24) were also found to be highly up-regulated in tolerant clone.

1.4.1. Developing a microarray of stress responsive genes from *Hevea brasiliensis*

The main objective of the study is to identify and characterize genes expressed during drought and low temperature stresses in *Hevea* and to develop a microarray using these genes for the eventual identification of candidate genes

that are responsible for imparting tolerance to various stresses. For this purpose, leaf samples were collected from drought and cold treated samples of clone RRIM 600 and mRNA isolation was carried out. Arrangements are made to get the transcriptome sequencing done with an aim to sequence and identify stress responsive transcripts and eventually to synthesize corresponding oligo probes for spotting on microarray.

1.4.2. Investigations on microRNAs in *Hevea brasiliensis*: Role in gene regulation during abiotic stresses

This project is conceived with an aim to isolate and identify if microRNAs are involved in gene regulation during abiotic stress conditions in *Hevea*. For this purpose, plants were (RRII 105 and RRIM 600) subjected to drought stress and miRNA isolation was attempted in leaf samples using mirVana miRNA Isolation Kit (Ambion, USA). The miRNA obtained were gel-eluted and adapted with ligator. Amplification of miRNA from adaptor ligated miRNA is being standardized.

1.5. Measurement of CO₂ and water vapour flux in rubber

Ecosystem level carbon dioxide (Fc) and water flux (LE) were continuously measured (April 2010 to March 2011) by using an eddy covariance system inside a five year old immature rubber plantation (Table Phy.3). The annual mean net ecosystem exchange (NEE) by the rubber ecosystem was 12 g CO₂/m²/day which is equivalent to 36 tons CO₂/ha/year. Most of the days recorded CO₂ influx in to the plantation; except a few heavy rainy days with net carbon efflux from the plantation to atmosphere. The evapotranspiration (ET) rate was calculated from the latent heat of

Table Phy. 3. Daily net carbon dioxide (g/m²/day) and water vapour (mm/day) flux in a five year-old immature rubber plantation at CES, Chethackal

Monthly mean	Carbon dioxide flux (Net ecosystem exchange) NEE (g/m ² /day)	Water vapour Flux Evaporation (mm/day)
April 2010	15.3 ± 0.9	3.80 ± 0.20
May	17.6 ± 1.0	3.50 ± 0.20
June	09.6 ± 0.6	3.90 ± 0.30
July	13.0 ± 1.2	3.30 ± 0.34
August	11.0 ± 1.5	2.80 ± 0.20
September	13.0 ± 1.1	2.90 ± 0.15
October	15.0 ± 0.8	2.90 ± 0.20
November	12.6 ± 0.9	2.30 ± 0.25
December	10.2 ± 1.2	2.35 ± 0.20
January 2011	11.0 ± 1.0	2.60 ± 0.15
February	10.0 ± 0.9	2.70 ± 0.10
March	14.3 ± 1.2	3.50 ± 0.25

vapourization (LE) and the annual mean was 3.2 mm/day in the immature plantation.

1.6. Studies on adaptive mechanisms in *Hevea* for drought and cold stresses

Under the current fluctuating climatic conditions, an understanding of the common mechanism of stress tolerance is very much essential to know the responses/behavior of the plant species under different environmental conditions. A polybag nursery of eight different *Hevea* clones (RRII 414, RRII 430, RRII 429, RRII 105, RRII 208, RRIM 600, and RRIC 100 & SCATC 88/13) was established at RRII. Drought stress was imposed and physiological measurements were taken for assessing the plant performance. Leaf water potential (Ψ_L) was found significantly decreased in drought imposed plants. Significant reduction was observed in dark Fv/Fm and effective quantum yield of PSII (Φ PSII) upon exposure to drought in all the clones.

1. 7. Evaluation of modern *Hevea* clones for drought tolerance

(a) Physiological evaluation of RR11 400 series clones for drought tolerance-Field trial at CES Chethakkal

Gas exchange and chlorophyll fluorescence measurements of RR11 400 series clones (one year old) were carried out in field grown plants at CES Chethakkal. RR11 414 and RRIM 600 showed maximum photosynthesis (P_n) and stomatal conductance (g_s) under field conditions during stress free period. RR11 105 showed the highest level of chlorophyll content whereas RRIM 600 had the lowest chlorophyll content. Soil moisture content in the field was around 20 % under stress free conditions. Due to pre-field monsoon showers during this year, photosynthetic measurements could not be carried out for drought period.

(b) Studies on RR11 400 series clones for drought tolerance-Plants in polybag

Polybag plants of five modern *Hevea* clones viz. RR11 414, RR11 417, RR11 422, RR11 429 and RR11 430 and three check clones RR11 105, RRIM 600 and Tjir 1 were subjected to drought by withholding irrigation continuously for 10 days while irrigated plants of the respective clones served as control. Plants were grown both in open and glass house conditions.

Among the 400 series clones, RR11 422 had the highest CO_2 assimilation (P_n) and stomatal conductance (g_s) while clone Tjir 1 recorded the lowest values. Similar results were obtained in the glass house experiment also. Effective quantum yield (Φ_{PSII}) values were higher for 400 series clones under drought compared to the check clones. The Φ_{PSII} was comparatively stable in clones such as RR11 430 and RRIM 600 under drought condition. Chlorophyll content decreased in all the clones under drought however, there was no significant variation among clones.

(c) Studies on drought effects on *Hevea* in relation to oxidative stress antioxidant responses

A study is being carried out to find out the role of xanthophylls cycle pigments in non photochemical quenching (q_n) during drought in *Hevea* clone. Leaf pigments were extracted from control and drought imposed plants of two *Hevea* clones (RR11 105 and RRIM 600) and subjected to HPLC analyses. The photosynthetic pigment separation method was standardized. Peak identification was attempted based on spectrum of each individual pigments, neoxanthin, chlorophyll *a* & *b*, α -carotene. Identification violaxanthin and lutein peak was found difficult due to overlapping spectrum. However, under drought conditions prominent zeaxanthin peak was identified. Pigments were separated into different fractions and work is in progress for quantification of the epoxidation level of the xanthophylls cycle pigments.

1.7.1. Photosynthetic measurements at RRS, Dapchari

Parameters such as photosynthetic CO_2 assimilation (A) rate and stomatal

Table Phy. 4. CO_2 assimilation (A) and stomatal conductance (g_s) of two year old field grown ortet and check clones under irrigated and drought treatment

Clone	CO_2 Assimilation ($\mu mol\ m^{-2}\ s^{-1}$)		Stomatal conductance ($\mu mol\ m^{-2}\ s^{-1}$)	
	Control	Drought	Control	Drought
RR11 430	10.8	10.2	0.28	0.20
RRIM 600	10.6	7.5	0.27	0.09
RR11 105	9.4	5.5	0.21	0.16
RR11 208	11.0	7.5	0.31	0.18
OS 135	11.8	10.2	0.29	0.16
OS 136	12.0	6.0	0.23	0.09
OS 137	12.0	5.7	0.43	0.08
SE(\pm)	0.6	0.8	0.03	0.01
CD ($P = 0.05$)	1.6	2.3	0.09	0.04

conductance (gs) were analyzed in two year old field-grown ortet and check clones under irrigated and drought treatment at RRS Dapchari during May 2010. Data revealed that ortet OSI35 followed by RRII 430 recorded maximum A and g_s under field conditions. Other two ortets tested were also on par with the drought-tolerant clone RRII 600 (Table Phy. 4).

1.7.2. Drought survey

With the objectives of finding out the susceptibility of young plants to drought incidence in the changing climatic scenario and study various management practices carried out by farmers to combat the drought situation, a survey was conducted in one year old young plantations during March 2011 in the northern Kerala under Sreekantapuram, Taliparamba and Thalassery Regional offices of Rubber Board. Information from each holding was collected using a standard questionnaire by visiting the plot and by visual scoring of the drought injury parameters. Nearly hundred holdings were surveyed at random in which about 25 per cent of the holdings resort to irrigate their young rubber plants in summer months.

1.8. Rapid screening of *Hevea* germplasm lines for intrinsic drought tolerance traits

1.8.1. Laboratory screening of germplasm accessions for intrinsic drought tolerance

Around 55 top and 11 bottom ranking germplasm accessions were identified from SBN 2003 for intrinsic drought tolerance based on field scoring parameters such as leaf yellowing and leaf senescence. They were further subjected to laboratory screening at CES, Chethackal. The leaf discs punched from middle leaflets were incubated in 60% polyethylene glycol (PEG) solution and exposed to light (350 $\mu\text{mol}/\text{m}^2/\text{s}$) for four hours and respective control leaf discs were incubated in water. The effective quantum yield of PS II (Φ PSII) of PEG treated and untreated samples were measured using PAM 2100 chlorophyll fluorometer. The per cent reduction in Φ PS II in drought stressed leaf samples was estimated and the accessions were sorted and ranked. The extent of reduction varied from 5% in accession AC 3964 to 84% in accession MT 41 (Table Phy.5). Those accessions exhibiting comparatively a small

Table Phy. 5. Ranking of germplasm accessions for intrinsic drought tolerance by lab screening method

Top ranking accessions			Bottom ranking accessions		
Rank	Accessions	% reduction in Φ PS II	Rank	Accessions	% reduction in Φ PS II
1	AC 3964	5.4	1	AC 4149	82.8
2	RO 2432	6.1	2	RO 2286	81.0
3	RO 3157	8.1	3	RO 1495	75.1
4	RO 3976	8.2	4	AC 4067	64.3
5	RO 3261	9.4	5	RO 2487	61.3
6	MT 62	9.6	6	RO 4363	55.6
7	RO 1425	9.9	7	RO 3662	40.9
8	RO 3271	10.1	8	AC 164	38.6
9	AC 1840	13.0	9	MT 76	33.2
10	RO 4393	13.3	10	AC 4409	26.2

reduction under *in-vitro* drought were selected as most intrinsic tolerant ones and *vice-versa*. The top ten most tolerant and bottom ten most susceptible accessions are given in Table Phy. 5.

1.8.2. Field scoring of RR11 400 series clones for intrinsic drought tolerance traits at CES, Chethackal

Five clones of RR11 400 series and two check clones (RR11 105 and RRIM 600) were planted at CES, Chethackal in 2009. They were empirically scored for intrinsic drought tolerance traits *viz.* leaf yellowing and leaf senescence in summer. The scored values were sorted and ranked accordingly. Clones that were exhibiting low drought injury *i.e.* less yellowing and senescence were selected as top ranking drought tolerant clones and *vice-versa*. Among the seven clones screened, clone RRIM 600 followed by clone RR11 430 emerged as more intrinsically tolerant than clones RR11 105 and RR11 414 in field scoring (Table Phy.6).

Table Phy. 6. Ranking of RR11 400 series clones based on empirical scoring for leaf yellowing and leaf senescence in summer

Yellowing	Senescence	Combined ranking
RR11 430	RRIM 600	RRIM 600
RRIM 600	RR11 430	RR11 430
RR11 417	RR11 417	RR11 417
RR11 429	RR11 429	RR11 429
RR11 422	RR11 414	RR11 422
RR11 105	RR11 422	RR11 105
RR11 414	RR11 105	RR11 414

1.8.3. Field scoring of wild germplasm accessions for drought tolerance traits at RRS, Dapchari

The germplasm accessions selected from field by empirical scoring at RRS Dapchari,

a non-traditional drought prone region were further evaluated for leaf chlorophyll reduction during summer period. The total chlorophyll content in leaves was measured both in stress free period (October) and in stressed period (April) using a chlorophyll content meter (CCM-200). The chlorophyll content index measured was converted to actual chlorophyll content using a regression equation. The chlorophyll reduction in summer ranged from 9.9% in accession MT 1627 to 32.8% in clone RR11 105. Based on per cent reduction in chlorophyll content the accessions were sorted out and ranked for intrinsic tolerance. Accessions exhibiting less chlorophyll reduction in summer were identified as the most tolerant ones and *vice versa*. Among 20 tested, five tolerant accessions and three susceptible accessions were selected for further evaluation at nursery stage (Table Phy. 7).

Table Phy. 7. Variation in chlorophyll content of wild germplasm accessions

Rank	Accessions	Chlorophyll, mg g ⁻¹ fr.wt.		% reduction
		Control	Stressed	
Top ranking accessions				
1	MT 1627	2.68 ± 0.042	41 ± 0.03	09.9
2	RRIM 600	3.10 ± 0.032	60 ± 0.03	16.5
3	MT 1623	3.21 ± 0.072	61 ± 0.02	18.8
4	RO 2387	2.88 ± 0.032	30 ± 0.02	20.7
5	RO 2153	2.97 ± 0.042	32 ± 0.02	22.0
6	HP 105	3.05 ± 0.052	31 ± 0.01	24.2
Bottom ranking accessions				
1	RR11 105	3.31 ± 0.062	22 ± 0.02	32.8
2	MT 41	2.98 ± 0.032	04 ± 0.02	31.5
3	RO 85	3.05 ± 0.032	14 ± 0.06	29.8
4	MT 1616	3.10 ± 0.052	34 ± 0.05	27

1.9. Experimental cultivation of high yielding clones at high elevation in Kerala

In Haileyburia Tea Estate Ltd., Elappara, a high altitude location in the

traditional belt, two field trials were initiated during 2006 and 2007, respectively to evaluate the performance of *Hevea* plants in high elevation. The clones PB 260 and GT 1 showed higher girth followed by clone RRIM 600 whereas PR 261 recorded the least girth in clone trial 2006 (Table Phy.8). In trial 2007 the clone RRIM 600 and RRII 208 reached maximum girth (13 cm) whereas clone RRII 105 and RRII 414 were the poor (7 cm) performers (Table Phy.9). The polyclonal seedlings planted in tea plantation measured 15 cm girth after five years whereas the traditional shade trees, silver oak showed more than 25 cm growth.

2. Physiology of growth and yield

2.1. Studies on yield and yield components

Growth and yield of 12 clones were evaluated at CES, Chethackal and shoot biomass accumulation was studied in untapped trees. Girth and biomass of tapped trees were always lesser than untapped trees irrespective of clones (Table Phy.10). Clone PB 235 followed by clone RRII 118 attained the highest girth and biomass per tree. There was 57% reduction in biomass in clone RRII 105 and 8% in clone

Table Phy. 8. Clonal variation in growth (fourth year) at Haileyburia Tea Estate Ltd., Elappara

Clone	Girth (cm)
PB 260	16.7 ± 0.26
RRIM 600	15.0 ± 0.27
PB 235	13.2 ± 0.26
GT 1	16.0 ± 0.25
PR 261	11.3 ± 0.28

Table Phy. 9. Clonal variation in growth (third year) at Haileyburia Tea Estate Ltd., Elappara

Clone	Plant girth (cm)
RRIM 600	12.6 ± 0.28
RRII 208	13.3 ± 0.50
RRII 105	06.6 ± 0.28
RRII 422	07.2 ± 0.51
RRII 414	06.3 ± 0.38
PB 311	09.7 ± 0.54
PB 260	10.5 ± 0.44
PB 235	07.6 ± 0.48
GT 1	07.1 ± 0.53
PR 261	07.5 ± 0.71

PR 107 due to tapping. For evaluation of long term yield out put the trees were tapped initially for 16 years under the S/2 d2 6d/7 system of tapping and shifted to controlled upward tapping (CUT) with stimulation. A considerable increase in yield was observed

Table Phy. 10. Comparison of girth, shoot biomass of 12 clones in tapped and untapped trees

Clone	Girth (cm)		Biomass (kg/tree)	
	Tapped	Untapped	Tapped	Untapped
RRII 300	097.6 ± 4.2	120 ± 10.0	970.0 ± 101	1735.3 ± 400
PB 235	109.3 ± 6.3	137.5 ± 7.0	1492 ± 265	2394.4 ± 327
RRII 105	085.2 ± 4.0	116.9 ± 8.0	681.5 ± 67.9	1601.9 ± 302
RRIM 600	095.5 ± 3.3	105.1 ± 8.0	936.0 ± 93.5	1209.7 ± 244
GT 1	091.3 ± 3.3	115.6 ± 5.0	819.3 ± 80.9	1493.3 ± 202
PR 107	083.1 ± 3.0	085.6 ± 8.1	633.4 ± 78.6	690.4 ± 162
GI 1	085.4 ± 2.5	095.9 ± 5.4	661.5 ± 57.1	900.8 ± 121.6
RRIM 501	073.3 ± 2.3	082.5 ± 6.6	436.3 ± 53.6	588.6 ± 142
RRII 118	108.9 ± 3.6	113.9 ± 9.0	1317 ± 109	1476.1 ± 332
RRIM 703	080.2 ± 3.5	093.8 ± 4.6	583.5 ± 78.9	824.5 ± 86.3
Tjir 1	083.6 ± 3.8	086.6 ± 10	656.7 ± 94.7	755.1 ± 290
RRIM 612	091.2 ± 4.4	-	869.1 ± 117	-

Table Phy. 11. Comparative yield in twelve clones in the fourth year of tapping under CUT

Clone	Mean yield (g/t/h)		
	CUT 1 st year HO- 1(I)	CUT 4 th year HO- 1(I)	% reduction
RRII 300	093.6 ± 08.1	24.7 ± 0.74	73.5
PB 235	187.2 ± 17.9	32.3 ± 0.83	82.7
RRII 105	097.5 ± 10.2	43.4 ± 1.50	55.4
RRIM 600	090.8 ± 07.3	49.0 ± 1.10	45.9
GT 1	132.3 ± 10.7	61.4 ± 1.50	53.5
PR 107	062.5 ± 04.2	38.1 ± 1.00	39.0
GI 1	071.0 ± 03.5	38.5 ± 1.10	45.6
RRIM 501	067.0 ± 06.2	25.8 ± 0.80	61.4
RRII 118	136.6 ± 08.2	80.1 ± 1.90	41.3
RRIM 703	067.5 ± 05.9	31.6 ± 1.10	53.0
Tpr 1	074.0 ± 08.7	34.6 ± 1.10	53.1
RRIM 612	056.3 ± 06.1	48.0 ± 1.80	13.0

in the first year of CUT and thereby a decrease in yield was noticed (Table Phy.11). An overall 43% increase in yield from normal tapping and 55% reduction from the first year of CUT was noticed. Clones RRIM 612, RRII 118 showed maximum increase in yield where as, clones RRII 105, PB 235 and RRII 300 showed considerable reduction in yield. However, CUT trees continued to record higher yield over normal tapping even in the fourth year of tapping.

2.2. Investigations on the mechanism of tapping induced loss of biomass

The mechanism of tapping induced unaccountable biomass loss is being

investigated in five clones of *Hevea* at HBSS, Nettana. The clone RRII 105 is proved to be a tapping sensitive clone as it recorded consistently less annual biomass increment upon tapping by d2 system. It lost around 39% of shoot biomass in d2 system compared to the respective untapped and d3 system of tapping (Table Phy.12). The annual biomass increment in d3 tapping system was higher than d2 system in clones such as RRII 105, PB 235, RRII 300 and PB 311. PB clones recorded better shoot biomass increment than RRII clones upon tapping. The clones PB 235 and PB 260 consistently recorded better shoot biomass increment as well as high yield, therefore they are likely to be L-T clones for the South Karnataka region.

2.3. On-farm trial for the selection of latex-timber clones

With an objective of selecting L-T clones, four clones planted during 1993 and 1995 were selected in Malankara estate to find out the existence of any relationship between yield and annual girth increment. The clones were under tapping on S/2 d3 system. The annual girth increment and annual block yield during 2010-2011 were recorded. In general, RRII 105 was the highest yielder in previous years. However, during the reporting period, PB 235 was

Table Phy. 12. Difference in annual biomass increment (kg/tree) between tapped and untapped trees (2010) in HBSS, Nettana

Clone	HBSS, Nettana			Standing shoot biomass			% standing biomass loss with respect to	
	Annual shoot biomass increment			(kg/tree)			untapped trees	
	untapped	d/2	d/3	untapped	d/2	d/3	d/2	d/3
				714	436	513	39	28
RRII 105	23.5 ± 4.0	14.0 ± 2.5	25 ± 3	547	427	560	22	0
RRII 300	19.5 ± 7.0	15.5 ± 4.0	26 ± 5	1196	890	924	27	23
PB 235	51.0 ± 7.8	50.0 ± 8.0	54 ± 4	1010	709	702	30	31
PB 260	42.0 ± 9.0	41.0 ± 7.5	45 ± 3	807	597	610	26	24.5
PB 311	42.0 ± 11	39.0 ± 4.0	46 ± 9					

± SE is shown

shown to be the highest yielding clone followed by PB 217 (Table Phy.13). The annual shoot biomass increment was also the highest in PB 235.

Table Phy. 13. Annual shoot biomass increment and rubber yield in four clones under d3 tapping system

Clone	Shoot biomass increment (kg/tree)	Annual block yield (kg/ha)
	2010-11	2010-11
RRII 105	12 ± 1.2	1843 ± 160
PB 235	24 ± 1.8	2116 ± 17
PB 260	16 ± 1.6	1857 ± 80
PB 217	22 ± 3.0	1984 ± 40

n=75

2.4. Relationship of latex ATP status, luteal membrane composition and ATPase activity with rubber yield

In continuation of the studies on correlation of latex ATP in young plants with their mature yield, analysis of ATP in 10 clones with different yield potentials (five year old plants) were done and correlated with mature yield data of same clones over five years. The data showed high latex ATP in high yielding clones and medium yielding clones compared to low yielders.

Table Phy. 14. Latex ATP content in different clones (five year old plants)

Clone	Latex ATP (µM)	Yield (g/t/t) (Mean over five years)
RRII 105	357.4	64.17
GT-1	349.3	45.29
PB 260	307.5	66.34
RRIM 600	300.3	46.32
PB 235	299.8	70.90
Tjuri	270.1	36.48
PB 217	252.8	47.32
PB 5/51	200.9	29.63
RRII 33	192.4	15.84
RRII 38	176.8	11.72
CD (P = 0.05)	21.8	4.83

Positive correlation ($r^2=0.70$) between latex (ATP) of immature plants and yield of mature trees of the same clones was also observed (Table Phy. 14).

2.5. Clonal variation on latex regeneration mechanism

Protein biosynthetic capacity of latex in high and low yielding clones was studied by measuring the incorporation of ^{14}C labeled amino acids (Leucine, Arginine, Lysine and Phenylalanine) into cytosol proteins. Incorporation of ^{14}C labeled amino acids into protein in the C-serum showed differences between clones. The highest incorporation was noticed in clones RRIM 600, GT 1 and PB 260 compared to RRII 105. Low yielding clones RRII 33 and RRII 38 showed relatively low protein biosynthetic capacity (Table Phy. 15). Protein biosynthesis is an indirect measure of latex regeneration capacity.

Table Phy. 15. Incorporation of ^{14}C amino acids into protein in different clones

Clone	Square root transformation values	Counts/minute
RRII 105	51.20	2634.3
RRIM 600	56.03	3153.0
GT 1	55.51	3130.6
PB 217	51.59	2690.0
PB 260	59.39	3539.0
RRII 33	43.38	1885.0
RRII 38	41.72	1740.6
CD (P = 0.05)	9.42	

2.6. Studies on rubber biosynthesis in *Hevea* clones

Prenyltransferase enzyme activity in the whole latex was studied using ^{14}C -labelled IPP as the substrate in mature trees of *Hevea* clones which showed varying yield potential. The clones selected were five high yielding (RRII 105, RRIM 600, PB 217, PB 235

and PB 260), three medium yielding (GT 1, Tjir 1 and PB 5/51) and two low yielding (RRII 33 and RRII 38). Along with this, five RRII 400 series clones were also included. Prenyltransferase enzyme activity was significantly high in high yielding clones (RRII 414, RRII 429, RRII 105) and low in low yielding clones (RRII 33, RRII 38). The high yielding clones had 3-6 fold increase in the enzyme activity than the low yielding clones and RRII 429 recorded the highest prenyltransferase enzyme activity. Studies revealed that prenyltransferase enzyme could be used as an indicator of yield potential in *Hevea*.

2.7. Cloning and production of HMG-CoA protein of *Hevea* for Immunoassay analysis

Investigations are progressing well by studying the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR enzyme) in the bark tissues of different *Hevea* clones in relation to varying yield potential. The coding region of HMGR from *Hevea* was PCR amplified, ligated into a TA cloning vector and transformed into *E. coli* cells. The transformed colonies were confirmed by colony PCR analysis and the confirmed colonies were sequenced. After analyzing the sequencing results one clone is selected and isolated the plasmid. The plasmid (pGEMT/ Hmgr) and expression vector (pRSET- A) was double digested with specific restriction enzymes. Attempts are being made to get the protein purified from the clones selected.

3. Tapping panel dryness

3.1. Effect of ethylene compounds on yield and stress responses in tissue

Effect of long term stimulation with ethephon was studied in the renewed and virgin tapping panels of the clone RRII 105.

Trees under regular tapping (S/2 d3; B1-2 panel) and trees of the same age and clone which were newly opened for tapping (BO-1 panel) were used in this experiment. One set of trees tapped in BO-1 and B1-2 panels were stimulated with 2.5% ethephon (bark application) as per the stimulation schedule viz. May, November, and April during 2010-2011. One month after every stimulation bark samples were collected from these trees for biochemical and histological/histochemical analyses. The soft bark tissues were analysed for various abiotic stress components such as H_2O_2 , PX, MDA, CN and β -CAS. Tapping observations on latex yield and TPD incidence were recorded at monthly intervals in both experimental and control trees.

The stress components like cyanide (CN), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the bark tissue were increased significantly due to stimulation both in BO-1 and B1-2 panels compared to their corresponding unstimulated controls. Peroxidase enzyme (PX) activity decreased in the stimulated bark tissues. On the contrary to the increased tissue CN, the cyanide scavenging enzyme, β -cyanolalanine synthase (β -CAS), activity was decreased in stimulated bark tissue. β -CAS activity was significantly high in the unstimulated trees. The data showed that the stimulation with ethephon reduces the levels of cyanide detoxifying enzyme and therefore accumulating the cyanide in the soft bark tissue.

Stimulated virgin panel (panel BO-1) always showed higher latex yield compared to renewed panel (panel B1-2) which showed an initial marginal increase in latex volume that subsequently declined. It seems that compared to virgin panel, stimulation could not induce a sustainable yield response in the renewed tapping panel.

The bark thickness of the tapping panel increased significantly during stimulation in both BO-1 and B1-2 panels. Both the functional and disorganized latex vessel rows (LVRs) number increased significantly in the panels due to stimulation effect. However, total LVRs increased in BO-1 panel but remained unchanged in B1-2 panel.

3.2. Stimulation and latex yield

Panel application of 2.5% ethephon three times per year is the present recommended stimulation method adopted in trees tapped under S/2 d3 frequency of latex harvesting system. Apart from wounding stress, stimulated trees are exposed to ethylene-mediated stress responses in the tapping panel that may eventually lead to metabolic disorders including impaired rubber biosynthesis. An experimental study was therefore, carried out aiming to reduce the ethylene-mediated stress responses in the tapping panel by applying the ethylene compounds away from the tapping area without compromising latex yield. In the present experiment bark application of 5% ethephon was made 1 inch wide around the tree trunk in the following regions: a). 150 cm above from the bud-union of the tree; b). on the bud-union; c). both the regions as mentioned in a and b. All the three regions of stimulant application were far away from the tapping panel. Trees with panel application of 2.5% ethephon and unstimulated trees were the controls.

The maximum yield response was noticed in trees stimulated at both regions away from the tapping area (150 cm up from the bud union). The rubber yield was significantly higher than that present in trees stimulated with standard recommended method. The yield

enhancement in trees with the modified stimulation method may be due to the increased drainage area achieved through applying ethephon at both side of the latex production area. Though there was general adaptation reaction, observed by accumulating stress components like H_2O_2 , cyanide, MDA etc. in the tapping panel of the trees with modified stimulation methods (bark application away from the tapping area), the stress components present in the tissue were less or comparable with the standard stimulation method (panel application). Any of the trees stimulated with ethephon had not shown abnormal late dripping of latex or tapping panel dryness (TPD) as a consequence of severe oxidative stress.

The present study therefore evidences comparatively less stress symptoms in the tapping panel area of trees stimulated with the modified stimulation method. Moreover, the sustainable latex production with high rubber yield could also be achieved in trees stimulated by this method.

3.2. Molecular basis of TPD

3.2.1. Investigations on the molecular physiology of tapping panel dryness syndrome (TPD) in *Hevea brasiliensis*

This study involves relative quantification of selected Tapping Panel Dryness (TPD) specific genes under different stages of TPD development. Bark samples were collected from three trees each from a group of healthy, late dripping and TPD trees (10, 25, 50, 75 and 100 % TPD) frozen in liquid N_2 in the field and eventually stored in -80° freezer. mRNA isolation and cDNA synthesis were performed as per standard protocols. A set of 20 genes was selected for the present study from *Hevea* EST (SSH data) for the quantitative expression analysis.

Table Phy. 16. Relative quantification (fold change) of transcripts under TPD and healthy condition

Gene	Normal tree	Late dripping	10% TPD	20-30% TPD	40-60% TPD	80% TPD	100% TPD
Annexin	1.00	1.14	0.97	1.22	2.24	1.10	2.22
SUMO	1.00	2.26	2.25	2.62	2.79	0.96	1.94
ETR1	1.00	1.86	0.36	0.94	0.86	0.54	1.64
ETR2	1.00	1.70	0.90	0.83	1.53	0.75	1.49
ACO2	1.00	1.26	0.62	0.63	0.29	0.02	0.08
20 HP	1.00	1.93	1.28	1.00	2.40	1.96	1.76
22 HP	1.00	2.53	1.75	1.69	1.02	2.42	1.16
33 HP	1.00	0.12	0.31	0.71	0.95	1.08	1.95
Cys Protease	1.00	1.08	0.64	0.08	0.24	1.10	0.11
Chitinase	1.00	1.15	0.63	0.18	2.34	0.34	0.99
DRT 5b	1.00	9.02	2.73	0.48	0.53	32.3	10.7
TPD 24	1.00	5.34	1.97	0.39	0.58	16.4	7.88

GAPDH was used as endogenous control for the relative quantitative PCR analysis.

Quantitative gene expression analysis was eventually carried out by using Applied Biosystems' 7500 Real Time PCR System. Out of the 20 genes attempted (Table Phy. 16), four genes were found highly up-regulated and one down-regulated remarkably. Peroxidase and Transcription factor MBF (tf MBF) showed significant level of up-regulation in the TPD trees. The significant up-regulation of peroxidase and tf MBF found in TPD trees indicates the possibility of increased levels of reactive oxygen species in TPD trees. NAC transcription factor (Nac tf) like TPD 24 and DRT 5b were also up-regulated in TPD trees when compared to healthy trees. WRKY transcription factor (WRKY tf) showed increased levels of expression in the initial stages of TPD whereas CRT/DRE binding factor showed down regulation.

3.3. Molecular and biochemical basis of ethylene-induced latex production (Ethylene receptors and signal transduction mechanism)

A new ethylene receptor was identified from the bark tissues in clone RR11 105. The

work on getting the full length sequence of this gene using 3' 5' RACE is in progress. Quantitative expression analysis of the two receptors (Hb ETR1 and new ETR) indicates that these two receptors were prominently up-regulated six days after stimulant application.

4. Gene expression studies

4.1. Construction of an expression vector for over-expression of chitinase in endophytes.

In connection with the over-expression of chitinase in *Bacillus subtilis*, attempts were made to get the protein expressed in transformed cells. A mutated strain of *Bacillus subtilis*, WB800N (obtained from Mobitec, Germany), which is suitable for over expression of gene of interest in pHT43 vector was used for transformation. The transformed cells were selected after confirming by DNA sequencing. Since these cells lack extracellular protease production, the protein produced and targeted externally would not be deteriorated. Attempts are being made to check the protein expression and to improve the level of protein production in the endosymbionts.

5. Secondary metabolites

5.1. Quantification and identification of inositols

The experiment was extended for identification and isolation of other forms of inositols from *Hevea latex*. It was found that in addition to L-quebrachitol, latex contains myo-inositol. However, the amount of myo-inositol was negligible compared to L-quebrachitol. In the case of patent, further steps were initiated for licensing the technology.

5.2. Water relations of latex with reference to the contents of inositols and sugars in the latex during drought

The study was continued for latex osmolytes and their role in latex water relations. Enzymes involved in the pathway of osmolyte accumulation such as invertase and sucrose were also included. Method for the separation of osmolyte was standardized. Latex invertase activity was high during peak yielding as well as stress season in relatively drought tolerant clones indicating the role of this enzyme in osmoregulation of latex.

6. Ecological impact of natural rubber cultivation

6.1. Impact of climate change on Indian plantation sectors with special reference to natural rubber

6.1.1 Historic climate change that has occurred in the rubber growing regions of India

Daily rubber yield (g/t/t) for several years was regressed with the weather parameters for the corresponding years separately for the different agro-climatic regions. The multiple linear regression (MLR) models obtained for the individual regions were $Y = 433.43 - 7.87T_{max} - 4.83T_{min}$ (CES, 9° 26'N to 76° 48' E), $Y = 171.01 - 2.54$

$T_{max} - 1.71T_{min}$ (Padiyoor, 11° 58'N to 75° 36' E), $Y = 204.98 - 1.01T_{max} - 5.51T_{min}$ (Dapchari, 20° 04'N, 72° 04'E), $Y = 41.25 + 0.67T_{max} - 1.13T_{min}$ (Agartala, 23° 50'N, 91° 16'E) and $Y = -24.85 + 3.58T_{max} - 2.59T_{min}$ (Tura, 25° 30'N, 90° 13'E). From these five models, the change in yield when both T_{max} and T_{min} concomitantly increased by 1°C was calculated. A reduction in yield was noticed in CES (16.23%) for 1°C rise in maximum and minimum temperatures. In Dapchari the yield reduction was 11.25% followed by 8.43% in Padiyoor. In other regions like Agartala and Tura in NE India, where winter temperatures are very low, the impact of warming was found negligible. In Agartala the yield reduction was about 1.17% and on the contrary in Tura there was an increase in the yield by 2.72% for 1°C rise in maximum and minimum temperatures. Thus, small rise in temperature in regions like Tura may have positive effect on rubber yield (Table Phy.17.).

During the last 54 years (1956-2009) T_{max} and T_{min} in RRII have increased at the rate of 0.05 °C/yr and 0.03 °C/yr, respectively. Extrapolating this data, the rise in T_{max} and T_{min} in the next 10 years was calculated and the same was used to estimate the expected reduction in productivity after 10 years at CES. The yield reduction after 10 years will be 6.90% in CES. In Padiyoor the rate of increase in T_{max} and T_{min} during the period 1998-2009 was 0.01 °C/year and 0.11 °C/year respectively and this may result in the reduction in yield by 4.23% after 10 years. In the case of Dapchari, Maharashtra during the period 1994-2009 the rate of increase in T_{max} was much higher (0.08 °C/year) but the minimum temperature increased by 0.03 °C/year where the reduction in the yield will be 3.70%

Table Phy. 17. Percentage change in the future yield for one degree (1°C) rise in temperature and predicted yield for the next 10 years with the current warming trends

Research Stations			MLR		R^2	% Change (for 1°C rise)	% Change (for next 10 years)	yield (g/t/t)
			Coeff.	Intercept				
Tura	2003-08	T_{max}	3.58	-24.85	0.23	2.72	11.25	35.8
		T_{min}	-2.60					
Agartala	2003-08	T_{max}	0.67	41.25	0.07	-1.17	-1.10	37.9
		T_{min}	-1.13					
Chethackal	2003-08	T_{max}	-7.87	433.43	0.29	-16.23	-6.90	73.0
		T_{min}	-4.83					
Padiyoor	2007-08	T_{max}	-2.54	171.01	0.19	-8.72	-4.23	48.6
		T_{min}	-1.71					
Dapchari	2007-08	T_{max}	-1.01	204.98	0.50	-11.25	-3.70	57.7
		T_{min}	-5.51					

for the next decade. In Agartala, the reduction in yield in the next 10 years will be very small (1.10%) extrapolating the rise in T_{max} (0.02 $^{\circ}\text{C}/\text{year}$) and T_{min} (0.06 $^{\circ}\text{C}/\text{year}$) during the period 1984-2007. For the last 12 years (1992-2003) T_{max} in Tura increased by 0.12

$^{\circ}\text{C}/\text{year}$. But the minimum temperature increased by 0.05 $^{\circ}\text{C}/\text{year}$ in this region. The cumulative effect of the expected changes in T_{max} and T_{min} in Tura region could lead to an increase in the yield by 9.36% in the next 10 years.

RUBBER TECHNOLOGY DIVISION

The activities of the Division were focused mainly on evolving improved techniques in deproteinisation of latex, chemical modification of NR, polymeric fillers for reinforcement of NR, scorch control of peroxide vulcanisation and rubber nanocomposites.

1. Primary processing

1.1. Low temperature preservation of latex

The technology for low temperature preservation of fresh natural rubber latex was modified and the latex was processed into high quality technically specified rubber of ISNR 3L grade.

1.2. Deproteinisation of latex

Standardised a method for production of deproteinised natural rubber (DPNR) using a protein hydrolysing enzyme. The process involved treatment of NR latex with papain, which is a proteolytic enzyme. The enzyme treated latex was further processed by two methods. In one method, the rubber recovered from the latex was subjected to an alkaline hydrolysis followed by washing and drying (Sample 1). In the other method, latex was subjected to a creaming process, followed by coagulation and drying of the recovered rubber (Sample 2).

Table Chem. 1. Raw rubber properties of DPNR

Parameter	Sample 1	Sample 2	Sample 3	Sample 4
Nitrogen content, %	0.16	0.14	0.16	0.40
Ash content, %	0.39	0.12	0.43	0.24
P ₉₀	45	30	36	48
PRI	74	17	19	85
ASHT (+ P ₉₀)	5	4	2	25
Mooney viscosity, ML (1+4) 100°C	78	68	68	81

The raw rubber properties of Sample 1 and 2 in comparison with commercial DPNR (Sample 3) and ISNR 5 (Sample 4) are shown in Table Chem. 1. It was observed that the nitrogen content could be reduced to the level required for DPNR by the both methods. The raw rubber properties were also comparable.

1.3. Skim latex processing

A new method of easy processing of skim latex using a creaming technique, in the absence of protein hydrolysing enzymes, was developed. Large scale trials (2000 L) were carried out, using the creaming technique in liaison with a private latex processing industry. The rubber recovered by this process had a lower nitrogen content compared to the conventional skim rubber. The quality of rubber was evaluated using conventional formulation for a carbon black filled mix (Table Chem. 2) and the mechanical

Table Chem. 3. Mechanical properties of carbon black-filled skim rubber-based mixes

Parameter	Skim rubber by new method	Skim rubber by conventional method
Modulus 100%, MPa	1.94	6.35
Modulus 200%, MPa	4.46	10.5
Modulus 300%, MPa	8.2	-
Tensile strength, MPa	24.10	12.75
Elongation at break, %	580	225
Hardness, shore A	58	80
Compression set, %	30	33
Heat build-up, ΔT , °C	23	33

properties so obtained are given in Table Chem. 3. It is found that it was easier to recover rubber and the rubber recovered by the new method had superior mechanical properties compared to rubber recovered by the conventional method.

2. Latex technology

2.1. Radiation vulcanised latex (RVNRL)

Trials were conducted to improve the modulus of RVNRL by blending with high styrene content styrene butadiene copolymer (HSBL) latex. The modulus of the films increased by several folds as the concentration of HSBL increased but simultaneously the tensile strength decreased (Table Chem. 4). Comparatively very good modulus and tensile strength were obtained when RVNRL and HSBL were mixed in 90/10 proportion based on dry rubber content and these properties increased further after leaching the films in calcium nitrate solution. This improvement

Table Chem. 2. Formulation of the carbon black-filled skim rubber mixes

Ingredient	Concentration
NR	100
ZnO	5
Stearic acid	1
Antioxidant HS*	1.0
HAF black	40
Process oil	-
Sulphur	2.5
CBS	0.7

*2, 2, 4 - trimethyl 1,2- dihydroquinoline

Table Chem. 4. Effect of concentration of HSBL on mechanical properties of RVNRL

Parameter	100/0*	90/10 *	75/25 *	60/40 *
Modulus 300%, MPa	1.02(1.07)	1.74(1.79)	3.56(3.62)	7.76(6.83)
Modulus 500%, MPa	1.40(1.49)	2.51(2.65)	5.71(6.99)	12.30(-)
Modulus 700%, MPa	2.60(2.73)	4.85(5.08)	9.65(12.68)	(-)
Tensile strength, MPa	23.0(26.66)	22.05(25.55)	20.13(15.62)	15.12(8.84)
Elongation at break, %	1398(1350)	1260(1270)	1000(400)	540(320)

*values in the parentheses are for the samples leached in 2.5 % calcium nitrate solution for 1 hour

is attributed to the factors that the two latices can mix homogeneously to a good extent as the polarity difference between the two is not very high and there can be entanglement of grafted n-butyl acrylate chains with HSBL polymer chains.

It was found that latex thread of good modulus could be prepared using this latex blend.

3. Rubber technology

3.1. Reinforcement

3.1.1. NR/Polymeric filler system

Two polymeric fillers were identified that could reinforce NR, named as PF system 1 and PF system 2. The effect of different proportions of HAF loading in NR/PF system 2 in comparison with a 50 phr HAF-filled NR compound was studied. Twenty phr HAF-filled NR/Polymeric filler 2 system showed comparable results with 50 phr

HAF-filled NR. Evaluated PF system 1 in typical formulation of products like tyre side wall, conveyor belt, microcellular soles etc. Satisfactory results were obtained for NR/PF system 1 in comparison with conventional filler incorporated formulations.

3.1.2. Latex - filler master batch

Latex master batches containing dual filler systems (carbon black/silica) were prepared. It was observed that there was almost no process loss for the fillers. Higher modulus, better tear strength along with a lower heat build-up was observed for the dual filler master batches as compared with mill mixed compounds. It was observed that the dual filler master batch had better filler dispersion as compared to mill mixed compounds (Fig. Chem. 1) as revealed from the data obtained using a Dispersion Analyser and the improvement in mechanical properties could also be attributed to this.

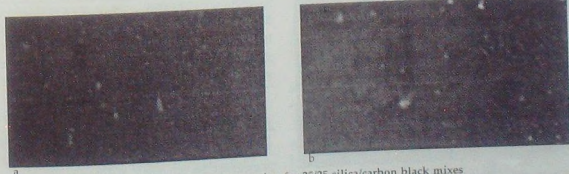


Fig. Chem. 1. Dispersion rating for 25/25 silica/carbon black mixes

a. Master batch X = 8.8 Y = 9.3 b. mill mixed X = 7 Y = 9.2 (Y = 10 denotes no agglomerate) X denotes filler dispersion and Y denotes agglomerate dispersion. Particles above 23 microns are considered as aggregates

Table Chem. 5. Formulation of the mixes for production of nanosilver composites

Ingredient	Control	RSnC1	RSnC2	RSnC3	RSnC4
ISNR 5	100	100	100	100	100
Stearic Acid	2	2	2	2	2
Zinc oxide	5	5	5	5	5
Antioxidant HSL	1	1	1	1	1
HAF Black	40	-	-	-	-
0.01Silver® HAF Black	-	40	-	-	-
0.02Silver® HAF Black	-	-	40	-	-
0.04Silver® HAF Black	-	-	-	40	-
0.08Silver® HAF Black	-	-	-	-	40
Naphthenic oil	7	7	7	7	7
Sulphur	2.5	2.5	2.5	2.5	2.5
CBS	0.7	0.7	0.7	0.7	0.7

3.2. Nanocomposites

3.2.1. Nanosilver based nanocomposites

Nanosilver dispersion was prepared through radiolytic reduction using gamma radiation. TEM image of stabilised nanosilver dispersion prepared by exposure to gamma radiation is shown in Fig. Chem. 2. It was observed that particle size of silver in the dispersion was in the range of 15-20 nm. Rubber-silver nanocomposites were prepared using nanosilver coated HAF black (0.01 to 0.08% on HAF) based on the

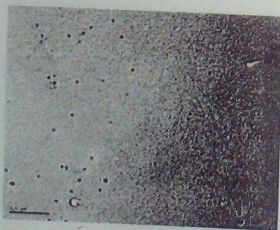


Fig.Chem. 2. TEM image of the citrate capped nano silver

formulation given in Table Chem. 5, through conventional mill mixing technique. The mechanical and dynamic properties of the composites were evaluated. The presence of nanosilver did not affect the ageing behaviour of the nanocomposites on ageing the rubber samples at 100 °C for 3 days (Fig. Chem. 3 and Fig. Chem. 4). Interestingly the nanocomposites exhibited lower tan delta and heat build-up even at very low percentage incorporation of nanosilver (Table Chem. 6).

Table Chem. 6. Dynamic properties of nanosilver composites

Sample	Tan delta at 0° C	Heat build-up, ΔT, °C
Control	0.07050	15
RSnC1	0.06436	14
RSnC2	0.05978	13
RSnC4	0.05304	09
RSnC8	0.05316	09

3.2.2 Layered clay nanocomposites

Latex blend nanocomposites were prepared by adding suitable nanoclay dispersion in carboxylated nitrile rubber (XNBR) latex and then blending the same

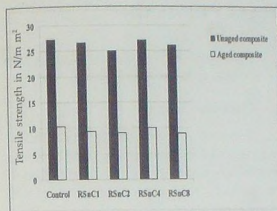


Fig. Chem. 3. Tensile strength of the nanocomposites

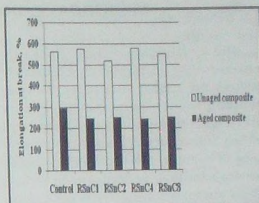


Fig. Chem. 4. Elongation at break, % of the nanocomposites

with radiation vulcanised NR latex. An improvement in tensile strength was observed with addition of layered clay. It is expected that due to polar nature of XNBR latex there can be better dispersion of modified clay. Out of the two nanoclays Cloisite 15 A showed better properties in comparison with Cloisite 93 A. The nanocomposite also showed a higher solvent resistance in comparison with pure RVNRL and RVNRL/XNBRL blend.

3.3. Peroxide vulcanisation

Sulphur/accelerator like scorch control could be achieved in peroxide vulcanisation using a stable free radical (TEMPO). Various co-agents were used to enhance the level of cross-linking. The co-agent N,N'-1,3-phenylene bismaleimide showed the highest efficiency in regaining the loss of cross-links due to scavenging of polymeric radical by the stable-free radical, when DCP was used as cross-linking agent.

3.4. Epoxidised natural rubber (ENR)

Documentation work was done for the

joint patent on "Development of silica filled tyre compounds based on ENR". Laboratory scale studies to assess the suitability of ENR compound in specialized tyre were completed.

4. Collaborative project

4.1. Development of footwear sole for physically handicapped, for Scheffliem Leprosy Research and Training Centre (SLR&TC), Karigiri

Formulations for two compounds for hard sole of the footwear for leprosy/diabetic patients were finalised and transferred the technology to (SLR&TC) for implementation. The tenure of the project is completed.

4.2 Development of Hurth coupling for Indian Railways

In connection with the development of Hurth coupling membrane for Chithranjan Locomotive Works (CLW), West Bengal, dispatched 10 more membranes for evaluation. Development of Spheri block is being attempted.

TECHNICAL CONSULTANCY DIVISION

The Division is primarily concentrating on technical consultancy services for rubber industries. Services include product development, quality control and certification, technical problem solving, training *etc.* Evaluation of different types of rubbers, fillers and other rubber chemicals for their performance in various rubber products is one of the major services rendered by the Division.

1. Product development

Based on the components supplied by the clients/requests received, the products were developed. The final formulation was optimized based on the specification requirements/ reverse engineering followed by trial mixes.

In the reporting period, technology know-how was transferred to 12 clients as per their requests.

Development works on the following product were completed.

- i) Room temperature curable rubber band: The new process is fast and less energy demanding compared to the conventional process, which in turn can reduce the cost of production.
- ii) Bonding gum using different grades of natural rubber: Conventional bonding gum - a tyre treading material - is prepared from highly pure grade of natural rubber. Partial replacement of the pure grade rubber with suitable additives may reduce the cost.
- iii) Transparent rubber band: Optimization of the production process for transparent rubber band was

continued with an eye to improve the colour retention and transparency.

2. Quality control

Technical support has been rendered to various rubber industries, mainly to the small-scale sector by testing their rubber compounds, rubber products, and by the evaluation of raw materials. For the reporting period 6241 parameters were tested for different types of samples (1309) received. Evaluation of nine rubber chemicals was completed.

3. Advisory services

Queries on technical matters from 156 units were addressed during the reporting period.

4. Other activities

- i) The Division is also involved in the training activities of the Rubber Training Institute by offering lecture classes on rubber mixing, manufacturing and also on product development. Hands-on training will be provided to beginners in the product manufacturing sector.
- ii) Offered assistance to the Dept. of P & PD in the grading of ISNR and inspection of centrifuged latex in connection with export. A total of 98.9 Mt of Cenex, 850Mt of ISNR and 200Mt of RSS were graded accordingly during the period under report.
- iii) Samples from 33 consignments were tested in the Division and detailed report was forwarded to the

Department of Revenue Intelligence, Govt. of India in connection with the import of rubber compounds.

iv) For giving awareness to the clients,

Division has participated in Exhibitions in connection with international conferences at different industrial centres in India.

ECONOMICS DIVISION

The five major thrust areas of research of the Division were; i) farm management, ii) primary processing and marketing of NR, iii) rubber products manufacturing industry and foreign trade, iv) inter-crops and by-products, and v) inter-divisional collaborative projects. Three projects were completed and reported during the period under review. The summaries of the results pertaining to these projects are given in the following sections.

1. Income inequality among beneficiaries under natural rubber Block Planting Scheme in Tripura: a preliminary assessment

The study was undertaken to; i) understand the pattern of distribution of household income under the seven selected mature Block Planting Units (BPUs), ii) to analyse the extent of inequalities in the income, and iii) to highlight major observations from a policy angle.

The analysis was based on primary data gathered in 2008 from 271 beneficiary households (tribals) attached to seven BPUs with mature rubber plantations in Tripura. For a comparative analysis on the level of income inequalities, data from 131 beneficiary households of BPUs with immature rubber plantation were also collected. The income profile, the extent of

inequality, and variability in the household income with immature plantations are expected to be functional proxy for household without income from rubber.

Three different patterns of income distribution were observed among the BPUs in Tripura, viz. i) equal distribution of income irrespective of the size of individual holdings, ii) distribution of income from rubber on the basis of area slabs, and iii) distribution of income from rubber based on actual area under the possession of individual holdings. The analysis revealed that despite of higher differences in the annual household income of mature and immature BPUs (Rs. 99167 vs Rs. 46824) the estimated inequalities were comparable. The income from rubber constituted the largest source of household income of mature BPUs and the observed inequalities among these growers were lower compared to the other two sources of income, namely, income from 'employment' and 'other sources'. Table Eco. 1 shows the BPU-wise and source-wise income inequalities.

The BPU-wise analysis indicated significant differences in the inequalities of income from rubber and they were primarily determined by the mode of distribution rather than distributional differences in the allocation of area under individual holdings. Though the combined

Table Eco. 1. Source-wise income inequalities among the beneficiaries of mature BPUs

Name of BPU	Rubber		Employment		Other		Total	
	Gini*	CV**	Gini	CV	Gini	CV	Gini	CV
PS Para	0.40	0.83	0.68	1.61	0.47	0.97	0.37	0.87
RS Para	0.34	0.63	0.61	1.26	0.77	2.16	0.29	0.51
Dariabagga	0.04	0.09	0.55	1.10	0.79	2.67	0.22	0.50
Kamalasagar	0.24	0.63	0.45	0.88	0.77	2.21	0.28	0.57
Laxmandepha	0.02	0.13	0.67	1.85	0.56	1.10	0.08	0.20
Kariyamura II	0.10	0.41	0.36	0.97	0.56	1.14	0.29	0.68
Rani	0.42	0.98	0.72	1.71	0.71	1.90	0.37	0.83
Total	0.43	0.91	0.61	1.36	0.68	1.65	0.33	0.71

* Gini co-efficient, ** Co-efficient of variation

measure of income inequality among the selected BPUs is lower (0.32) than the reported national average (0.37) (UNDP, 2009) the shift towards individualistic approaches to income distribution poses important policy challenges to the organisational framework of Block Planting Scheme (BPS) as the distribution of income is based on community-oriented priorities and strategies. However, the emerging trends in Tripura indicate a growing shift towards distribution of income from rubber based on actual area under possession as the tribal communities are increasingly integrated to the mainstream society.

2. The composition of natural damage and crop loss in rubber plantations: a case study of the estate sector in India

The major objectives of the study were; i) to assess the extent and composition of tree loss due to natural damage in rubber plantations in the estate sector, ii) to evaluate the variations in the damage due to clonal differences, iii) to investigate the regional differences in the natural damage, and iv) to examine the economic loss due to natural damage.

Though the estate sector covers only 10.4 per cent of the total area under NR in India the study is confined to the data related to the tree loss reported in the estate sector in the traditional rubber growing regions of Kerala and Tamil Nadu during 2006-07. The estate sector is chosen as reliable documented information are available only from this sector. The study covered 40 estates with a total area of 13,761 ha. At micro level, a total number of 671 fields of different age groups in the 40 estates were covered.

The extent of crop loss, the composition of natural damage, and the economic loss were estimated using life cycle approach. The results highlighted contradictions between popular perceptions and field level data on the composition of natural damage, which is highly caused by wind (66.44%) across different age groups and regions. The physical and economic loss of crop for the entire life cycle and for seven different phases were estimated, where each phase is spanning for a period of five years corresponding to panel duration. The estimated discounted value of life cycle economic loss per tree and per hectare were Rs. 1,874/- and Rs.1,57,393/- respectively

Table Eco. 2. Discounted value of economic loss

Period of loss	Yield/tree (kg)	No. of trees lost/ha	Discounted value of loss (Rs/tree)	Discounted value of loss per ha (Rs)
Immature phase	4.53	2.55	4428.30	11292.17
1-5 years of tapping	4.62	8.79	3640.87	32003.26
6-10	4.73	9.45	2360.47	22306.47
11-15	4.79	16.32	1426.72	23284.09
16-20	4.82	10.89	795.88	8667.12
21-25	4.76	16.83	367.66	6187.76
>25	4.70	19.17	96.15	1972.28
Life cycle	4.71	84.00	1873.72	157393.00

during the study period (Table Eco. 2). The dominance of weather-related sources of damage (79.01%) indicates limited role of policy interventions targeted towards preventive mechanisms. However, identification of phase-wise sources of damage during the entire life cycle of rubber plantations assumes significance since the generation of reliable annual database on the natural damage in rubber plantations is an essential pre-requisite for the adoption of policy initiatives for minimising loss through the coverage of comprehensive insurance schemes.

3. NR cultivation in the north-eastern region under the Block Planting Scheme: a socio-economic assessment of its impact on the beneficiaries in Tripura

The primary objectives of the study were; i) to understand the socio-economic profile of the households covered under the Block Planting System (BPS), ii) to estimate, analyse, and compare the sources of income and consumption expenditure of the beneficiary households possessing mature and immature Block Planting Units (BPUs) as well as growers covered under Group Processing Scheme (GPUs) which represent growers from the mainstream, iii) to

estimate the assets and liabilities of households under the three categories, and iv) to draw inferences for compatible policy initiatives from regional perspective.

The study considered all the 47 functional BPUs for the analysis. The sample households were selected from nine BPUs and two GPUs for the field survey. Data from 271 households with mature area, 131 households with immature area under the nine BPUs, and 78 households under two GPUs were gathered in 2008. The selection of sample households attached to BPUs and GPUs were based on the geographical distribution of area under NR cultivation and the beneficiaries.

The study showed that the average income of household with mature BPUs (Rs.99,168/-) was 112 per cent higher compared to immature BPUs (Rs. 46,824/-) during the year 2008. However, the average household income of GPUs was Rs.1,58,196/- during the study period. Table Eco. 3. shows the annual average household income from different sources.

The composition of income of both GPUs and mature BPUs showed growing dependence on income from NR (75% and 66% respectively). The income impact is reflected more in the differences in savings

Table Eco. 3. Sources of annual average household income (Rs.)

	BPU mature	BPU immature	GPU
Rubber	65136 -65.68	---	118308 -74.79
Employment	25824 -26.04	35844 -76.55	22560 -14.26
Others	8208 -8.28	10980 -23.45	17328 -10.95
Total	99168 -100	46824 -100	158196 -100

Figures in parentheses indicate respective shares (%)

and assets (72.87%) than the consumption expenditure (9.71%) between households with and without income from rubber under BPUs. The difference in annual consumption expenditure and savings and assets between households under GPUs and BPUs were around 150 per cent. It underlines the pivotal role of traditional customs and conventions of tribal communities in determining the pattern of their household consumption expenditure and the better awareness and access to the facilities of mainstream society by the non-tribal communities. In sharp contrast to the experience of Kerala, where the surplus

generated from the NR sector had been systematically channelised by the pioneering planters for their children's formal and professional education, investment in higher education in Tripura among the households under mature BPUs was negligible as only 3.95 per cent of the population in the age group of 18-24 after class XII was pursuing higher education. It also indicated the BPU's linkage effects on the regional economy were seriously constrained by the nature of human capital, the availability of infrastructural facilities, and access to the same. The two critical components required for the effective utilization of the income of the BPS are; imparting skills through technical/professional education and providing access to infrastructural facilities related to higher education, healthcare, communication and transport. An important impact of the NR-based rehabilitation programmes in the state is the growth of rubber area outside various government sponsored schemes (45%) and the share of smallholdings (82%) in the total area under the crop in the state.

LATEX HARVEST TECHNOLOGY DIVISION

The division continued research activities on various aspects of crop harvesting which has direct field application. The collaborative programme on popularising low frequency, d3, tapping among small holdings initiated in 10 regional offices of the Rubber Board during 2009-10 was extended to all the remaining regional offices during the current year. In all the regional stations where CUT

demonstration trials were initiated continued successfully. Evaluation of eco-friendly bio-degradable polythene was continued, but further modification is required to enhance the service period (period of effective rainguarding).

1. Low frequency tapping

The division had various experiments and onfarm trials on low frequency tapping

Table LHT. 1. Comparison of rubber yield under d7 frequency with global and population mean in different agroclimatic zones (A to F)

Location & region	Clone	Global mean	Population mean	Expt. result
Neria Estate (F)	GT 1	1008	936	1416
-do-	RRII 105	1315	936	> 1503
Thrithala (D)	RRII 105	1695	1432	> 2141
Chethackal (B)	RRII 105	1664	1873	>1997
Mundakayam (C)	RRII 105	1821	1431	> 1985
Kanthimathi (A)	RRII 105	1943	1486	> 2340

Global mean is the overall performance of a clone in a specific agroclimatic region. Population mean is the performance of all clones together in an agroclimatic region.

Table LHT. 2. Comparison of rubber yield under d4 frequency with global and population mean in different agroclimatic zones

Location & region	Clone	Global mean	Population mean	Expt. result
Apella Estate (F)*	GT 1	1296	1486	1763
Koney Estate (B)*	PB 217	1498	1508	2155
New Ambadi (A)	RRIM 600	1401	1486	1532
Ambanaad (B)	RRII 105	1664	1213	1543
Vennimala (C)*	RRII 105	1675	1273	2024
Haniharaputra (A)*	RRII 105	1904	1602	2147

*1 - 10 phase (tapping)

(d4 and d7) in the past under different agroclimatic conditions. These experiment results were compared with global mean (the overall performance of a clone in a specific agroclimatic region), and population mean (the overall performance of all clones together in an agroclimatic region). The observed results from the experiments were equal or better than global and population mean (Tables LHT 1 & 2).

In the trials leading to the recommendations on low frequency tapping (LFT) during 2001, tapping was conducted under 6d/7 i.e. with tapping rest on Sunday. However, there are estates and growers who do not give such regular weekly rest i.e. they do continuous tapping (7d/7) ensuring more days of tapping under any given frequency. Therefore, it is

essential to identify stimulation schedule for various tapping frequencies under 7d/7 practices as well. Such a study along with the existing recommended practice for d3, d4 and d7 frequencies and also in comparison with d2 frequency is essential. A comprehensive study was initiated at Koney estate of Harrison Malayalam Ltd to understand the performance of clone RRII 105 under diverse latex harvesting practices. It also covered the tolerance study of the lactiferous system under different tapping frequency and stimulation based on biochemical indicators. Results from large-scale field study with all frequency and stimulation can be utilised to finalise a comprehensive package of practices for LFT.

Estates under Rehabilitation Plantations Ltd., Punalur, have initiated

field level adoption trial of LFT in clone RRII 105 under the guidance of Division. They introduced comparative yield evaluation of d3 and d4 frequencies in two of their estates viz. Kulathupuzha (20 blocks) and Ayiranallur (12 blocks) from 2009-10. Details of yield are presented in Table LHT.3.

Table LHT. 3. Yield performance (clone RRII 105) under d3 and d4 frequency at estates of RPL (kg/400 trees)

Year of planting Year of opening	Kulathupuzha		Ayiranallur	
	2001		2001	
	2007		2007	
	d3	d4	d3	d4
2007-08 (Apr-Mar)	512	-	716	-
2008-09 (Apr-Mar)	1128	-	1360	-
2009-10 (Apr-Mar)	1764	2036	1900	1856
2010-11 (Apr-Mar)	1920	2120	2120	2160
TPD %	6.1	6.6	3.8	5.8

2. Demonstration of d3 frequency tapping in small holdings (collaborative programme)

The programme proceeded smoothly as per the original schedule i.e. to cover all regional offices in Kerala, Tamil Nadu and Karnataka in two phases (2009 Phase I - 10 regional offices and 2010 Phase II - 16 regional offices). Performance review of the first phase participants and awareness training on LFT, and yield stimulation for the second phase growers were successfully completed. In general, an average yield of over 5 kg/tree/year was realized by the Phase I growers and over 4kg/tree (due to delayed opening) by the Phase II growers. Among the participants, one-third of the growers are utilising family labour for tapping whereas two-third is still depending on hired labour. Moreover, when 30 per cent follow d3 frequency with Sunday as rest, 70 per cent follow d3

frequency without Sunday as rest leading to higher number of tapping days per year. According to this, stimulation rounds per year was also modified (3 or 2). Yet another information from this programme is that there is a quantum jump in crop during the first year on BO-2 panel, but the second year yield of BO-2 panel is only 60 per cent of first year for the clone RRII 105. Of the two growers, one tapped for 99/104 days (d3 with Sunday off) and the other for 121/121 days (without Sunday off) recorded a crop of 7.63 kg/tree and 6.1 kg/tree year respectively during 2010-11. They achieved nearly 100% tapping regularity by adopting delayed tapping (start around 8.30 - 9.00am) during June-July and leak preventing by fixing mini rainguard during North East monsoon. It is also observed that, cordial relation between owner and tapper resulted in good yield and vice versa. Irregularity in tapping not only leads to crop loss of the lost day/s but also leads to nearly 50 per cent crop reduction in the next 3 - 4 tapping after resuming regular tapping.

One of the major factors for the success of LFT is regular tapping under the given frequency throughout the year. Lower the frequency, more stringent should be the mode to ensure regularity in tapping. Though rain guarding is a practice, on many occasions its performance was observed to be poor. Extended and undue rains cause problems in tapping. During the north-east monsoon nearly 50 per cent of trees are left untapped due to rainwater entry through hairline cracks on the rain guard compound as well as on the tree leading to considerable yield loss (Table LHT.4). Though attempts were made to leak-proof it using extra coating with rainguard compound, latex etc., benefits

Table LHT. 4. Yield performance under d4 frequency without (A & D) and with (B & C) miniguard (September 2009)

Group	Task number	Crop (kg/month)
A	1	73
	2	73
	3	81
B	4	153
	5	186
	6	135
C	7	134
	8	131
	9	130
D	10	77
	11	79
	12	86

were short lived. Hence, an additional mini rainguard was attempted above the existing one during August-September. This was found to be very effective in protecting the tapping panel even during rain in the month of December.

The experiment on performance of low frequency tapping systems with stimulation in clone RR11 105 at Experiment Farm Unit, RIT, Pampady, was continued

during the reporting period. Yield under d2 frequency tapping was comparable to d3 and d6 frequencies of tapping. Renewed panel yield under d2, d3 and d4 frequencies of tapping were also comparable to that of d6 frequency of tapping (Table LHT.5).

Table LHT. 6. Monthly yield response of LFT (S/2 d6 6d/7) in clone RR11 105 at CES, Chethackal

Months	kg/400 trees	kg/tap	drc (%)
Apr' 10	144.7	28.9	37.4
May	89.4	22.3	37.2
June	127.3	31.8	39.1
July	192.5	38.5	36.1
Aug	204.5	51.1	33.1
Sep	265.0	53.0	36.6
Oct	225.0	56.2	42.2
Nov	161.3	40.3	39.5
Dec	170.5	34.1	41.5
Jan' 11	145.6	36.4	39.5
Feb	109.3	27.3	39.9
Mar	84.1	21.0	38.8
Total	1919	36.8	38.4
Kg/tree	4.8	(mean)	(mean)

Table LHT. 5. Yield performance of low frequency tapping systems with stimulation on clone RR11 105 at EFU, RIT, Pampady, during April 2010 – March 2011

Treatment	Yield kg/ha**	Tapping days	Panel
T0 – S/2(RG) d2 6d/7 (control)	1798 bc	148	BI-2(2)
T1 – S/2(RG) d3 6d/7. ET2.5% Pa1(1.5) 3/y*	1950 abc	100	BI-1(4)
T2 – S/2(RG) d3 6d/7. ET2.5% Pa1(1.5) 4/y*	2202 ab	101	BI-1(4)
T3 – S/2(RG) d3 6d/7. ET2.5% Pa1(1.5) 5/y*	2419 a	100	BI-1(4)
T4 – S/2(RG) d4 6d/7. ET2.5% Pa1(1.5) 5/y*	2105 abc	75	BI-1(2)
T5 – S/2(RG) d4 6d/7. ET2.5% Pa1(1.5) 7/y*	2075 abc	76	BI-1(2)
T6 – S/2(RG) d4 6d/7. ET2.5% Pa1(1.5) 9/y*	2081 abc	76	BI-1(2)
T7 – S/2(RG) d6 6d/7. ET2.5% Pa1(1.5) 10/y*	1918 abc	51	BO-2(7)
T8 – S/2(RG) d6 6d/7. ET2.5% Pa1(1.5) 12/y	1965 abc	51	BO-2(7)
T9 – S/2(RG) d6 6d/7. ET2.5% Pa1(1.5) 16/y*	1603 c	52	BO-2(7)

**Values followed by same letter/s are not critically different from each other

Table LHT. 7. Yield response of LFCUT with rain guard in clone RRII 105 at CES, Chethackal, during April 2010 - March 2011

Treatment	Yield** (kg/400 trees)	No. of taps
T1- S/4U d3 6d/7 ET5% 12/y	5082 ab	98
T2- S/2 d3 6d/7 ET2.5% Pa1(1.5) 12/y	5141 ab	98
T3- S/4U d4 6d/7 + stim. after every 5 th tapping (ET5% La)	6563 a	75
T4-S/4U d4 6d/7 + stim. after every 4 th tapping (ET5% La)	5087 ab	75
T5- S/2 d4 6d/7 ET2.5% Pa1(1.5) 6/y*	5413 ab	75
T6- 1/3SU d6 6d/7 + stim. after every 4 th tapping (ET5% Ga)	3590 b	51
T7- S/2 d/6 6d/7 + ET2.5% Pa1(1.5) 12/y	4147 b	51
T8- 1/3SU d6 6d/7 + stim. after every 2 nd tapping (ET5% Ga)	4157 b	51

* Values follow ** values followed by same letter/s are not critically different from each other

At CES, the demonstration plot under weekly tapping with monthly stimulation continued to give promising yield during 2010-11 (52 tapping days). It was 4.8 kg/tree/year and 36.8 kg (mean per tap). Incidence of tapping panel dryness is 5.6 % (Table LHT. 6).

In another experiment on comparative yield performance of low frequency tapping (d6 & d10) in clone RRII 105, weekly tapping with monthly stimulation gave good yield. But it was less under d10 tapping during the reporting period due to loss of five tapping. In the exploratory trial on LFT (d10) in clone RRII 105 at Central Experiment Station (CES), Chethackal, yield of 1799 kg/400 trees was obtained in the 9th year of tapping on BO-2 panel. Compared

to previous year, yield was lower due to the proximity of tapping panel to the bud union but the TPD incidence is very low (2.2 %).

3. Low frequency controlled upward tapping

In the evaluation trial of Low Frequency Controlled Upward Tapping (LFCUT) with rain guards in clone RRII 105 at CES during the sixth year yield under S/4U d3 with monthly stimulation was comparable with S/4U d4 and S/3U d6. Similarly yield of trees tapped in the basal panel under different frequencies was also comparable (Table LHT.7).

The experiment on LFCUT with rain guards in clone RRII 118 shows that yield

Table LHT. 8. Yield response of LFCUT with rain guard in clone RRII 118 at CES, Chethackal, during April 2010 - March 2011

Treatment	Yield*(kg/400 trees)	kg/tree	No. of taps
T1- S/4U d3 6d/7 ET5% - once in three weeks	2480 bc	6.2	101
T2- S/4U d4 6d/7 ET5% - once in three weeks	2605 bc	6.5	77
T3- S/3U d4 6d/7 ET5% - once in six weeks	2395 c	5.9	76
T4- S/3U d6 6d/7 ET5% - once in three weeks	3565 a	8.9	51
T5- S/3U d6 6d/7 ET5% - Monthly	3139 ab	7.8	51

*Values followed by same letter/s are not critically different from each other

Table LHT. 9. Effect of stimulation under d2 and d3 frequencies of tapping in clone RRH 105 without rainguarding on recovery of yield loss at EFU, RIT, during 2010-11

Treatment	Yield*	kg/tap/ 400 trees	kg/tree	g/t/t	drc (%)	No. of tsaps
	(kg/400trees)					
S/2(RG) d2 6d/7	2482 abc	18.3 d	6.2 a	45.9 d	37.6 c	135
S/2 d2 6d/7	2023 abc	27.1 abc	5.1 abc	67.7 abc	39.0 abc	75
S/2 d2 6d/7 ET2.5% Pa. 3/y	1756 bcd	21.9 bcd	4.4 bcd	54.9 bcd	38.7 bc	80
S/2 d2 6d/7 ET2.5% Pa. 5/y	1516 cd	19.2 cd	3.8 cd	48.0 cd	38.7 bc	79
S/2(RG) d3 6d/7 ET2.5% Pa.3/y	2119 ab	22.2 bcd	5.3 ab	55.4 bcd	37.7 c	96
S/2 d3 6d/7 ET 2.5% Pa (3/y)	1823 bcd	33.0 a	4.6 bcd	82.5 a	40.4 a	55
S/2 d3 6d/7 ET 2.5% Pa (5/y)	1413 d	25.7 abcd	3.5 d	64.3 abcd	39.2 abc	55
S/2 d3 6d/7 ET 2.5% Pa (7/y)	1532 cd	28.0 ab	3.8 cd	70.1 ab	39.5 ab	55
LSD (0.05)	568.30	8.15	1.42	20.37	1.69	-

*Values followed by same letter/s are not critically different from each other

under various systems with matching variation in yield stimulation frequencies were comparable. However S/3 d6 with once in three week's stimulation gave highest yield of 8.9 kg/tree (Table LHT.8).

4. Other experiments

The experiment on long-term evaluation of rain guard was continued. Significant yield variation was observed among various treatments. During the year 2010-11, the highest yield was observed under d2 frequency of tapping which was at par with d3 frequency of tapping, both with rain guarding. Crop loss in the non-rainguarded conditions could be partially compensated by stimulation (Table LHT.9).

5. Evaluation of "Mortex" as an yield stimulant in rubber

The experiment initiated at Kaliyar

Estate, Thodupuzha, and at EFU, RIT, Pampady, during 2009-10 to evaluate "Mortex" in comparison with ethephon was continued. No significant difference in yield or drc % could be observed between various treatments during the year 2010-11. (Table LHT.10).

Evaluation of bio-degradable polythene for rainguarding rubber trees

Evaluation of bio-degradable polythene along with conventional Low Density Poly Ethylene (300 gauge) for rainguarding of rubber trees was continued. Within 5 ½ months, the bio-degradable polythene started degrading *i.e.* faster than required period of 8-9 months. However, there was an improvement over the previous samples.

GENOME ANALYSIS LABORATORY

Major research activities of the Genome Analysis Laboratory encompass (I) the development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping (II) development of genetic markers for biotic and abiotic stress tolerance and understanding the stress adaptation processes through transcriptome analysis and (III) cloning and characterization of agronomically important genes.

1. Development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping

Different marker technologies have been adopted and successfully used in characterization of rubber genome. The details of the progress achieved so far are described.

1.1. Development of microsatellite markers and its application in the characterization of *Hevea* germplasm

Microsatellite marker development in *Hevea* was continued with the isolation and characterization of *Hevea* genomic clones containing microsatellite/ simple sequence repeats (SSR). So far 104 microsatellite markers were generated from a normal genomic library. Parameters affecting amplification of the markers were standardized.

An attempt was also made to construct an enriched genomic library for dinucleotide repeats for SSR marker development. Three biotinylated synthetic repeat oligos (AC)₂₀, (AC)₃₀ and (AT)₃₀ bound to streptavidin

coated magnetic beads, were used in hybridization with *Tsp*5091 digested genomic DNA, isolated from the clone GT 1. DNA fragments containing repeats, hybridized to synthetic oligos, were captured and PCR amplified. Amplified enriched DNA fragments were then digested with *Eco*RI, size - selected and cloned in pre-digested lambda ZAP II vector followed by packaging of the same. The primary library consists of ~ 6 x 10⁸ clones as revealed through plating and titrating of the packaged phage. Primary screening of the library for positive clones bearing simple repeat sequences is in progress.

DNA-based markers were used for accurate identification through genotyping of *H. brasiliensis* clones, essential for maintaining genetic purity of cultivated clones and to resolve any dispute regarding its identity. Earlier 27 clones were identified unambiguously using microsatellite markers. This was further extended to 38 popular clones originating from South East Asian rubber-growing countries including India. All these clones could successfully be identified using only three polymorphic microsatellites developed in our laboratory, singly or in combination, based on their power of discrimination. In order to identify the clones, microsatellites *hev-glu* (identified within the intron of β -1,3 glucanase gene), *lmct19* and *hnnac14* (generated from *Hevea brasiliensis* genomic library) were used sequentially in descending order of their power of discrimination.

1.2. Single nucleotide polymorphisms (SNPs) in *Hevea*

SNPs identified in wild accessions were used as marker for genotyping. Four representative of wild *Hevea* accessions from each of the three different provinces: Acre,

Mato Grosso and Rondonia were used in SNP detection. Allele-specific primers for SNPs in glutathione peroxidase gene were tested for amplification in wild accessions using latex plastidic aldolase gene and β -actin gene as PCR reaction control in SNP validation analysis. SNP genotyping of individual plant was carried out by employing allele-specific primers for two SNPs in 60 wild accessions of which 24 were from Acre, 18 from Mato Grosso and 18 from Rondonia.

1.3. Single nucleotide polymorphisms (SNPs) and haplotype structuring in the latex biosynthesis genes

Full-length genomic sequence of hydroxymethylglutaryl-CoA reductase (*HMGR*) and cDNA sequence of hydroxymethylglutaryl-CoA synthase (*HMGS*) were obtained from NCBI database. Primers were designed for overlapping fragments of approximately 650 bp sizes covering the entire genomic region of *HMGR*, whereas primers for *HMGS* were designed based on the available cDNA sequence. The genomic region of the above genes was amplified by PCR from five *Hevea* genotypes: RR11 105, RR11 118, RRIM 600, RRIC 52 and GT 1. The PCR products obtained from *HMGR* and *HMGS* were gel purified and sequenced to identify the SNPs. Sequences obtained were aligned using the multiple sequence alignment option in DNASIS sequence analysis software.

HMGR gene contains four exons and three introns in its entire genomic region of 2.33 kb. Four overlapping fragments consisting of 547 bp, 761 bp, 657 bp and 592 bp were amplified from five *Hevea* clones using four primer-pairs, designed based on genomic sequences available in the GenBank. Four SNPs were identified from the first exon, one from the second exon and six from the second intron including an indel. All the five SNPs within the exonic

region of *HMGR* were found to be resulting in degenerate codons coding for the same amino acid. No SNPs were identified from the 3' end. SNP analysis revealed the existence of two distinct haplotypes: GGCAAGTA-TG and CCTTCTATTCT. The clone RRIC 52 could clearly be discriminated from RR11 118 and RR11 105 based on their haplotypes, as RR11 118 and RR11 105 contained similar haplotypes (GGCAAGTA-TG), while RRIC 52 had different haplotype (CCTTCTATTCT). All the SNPs were in homozygous state in above three clones, whereas RRIM 600 and GT 1 showed heterozygous conditions. SNP information of *HMGR* gene of GT 1 was incomplete due to the poor sequence quality of certain regions.

In the case of *HMGS* gene, three overlapping fragments, approximately 1.6 kb, 1.4 kb and 1.0 kb were amplified using three primer-pairs based on cDNA sequences. *HMGS* amplicons were larger than the size of the cDNA, indicating the presence of large introns in the genomic sequences. The fragments were purified and two of them (2nd and 3rd) were sequenced from both directions. Sequence data was available only for the middle and 3' end of the region with gaps in between. A total of 16 SNPs were identified from the analyzed region. Interestingly in RRIC 52, two SNPs existing in the coding region showed heterozygous conditions with the mutant allele coding for a different amino acid. The alleles were 'C' and 'T' with 'C' as the major allele existing in homozygous condition in other genotypes, while 'T' appeared to be the mutant one. In the first locus, 'ACT' is the major codon, coding for threonine (Thr), whereas the minor one 'ATT' codes for isoleucine (Ile). Similarly, at another locus, the dominant codon seems to be 'CCC' coding for proline (Pro) while the other one is 'CTC' coding for leucine (Leu).

1.4. Retrotransposons and retro-based markers in rubber

Abundance of retrotransposons in *Hevea* genome was reported earlier. Genomic clones bearing retro-sequences from a genomic library were identified through plaque hybridization using 'reverse transcriptase' (RT) gene as the probe. Efforts were made to develop retrotransposons – based suitable marker system for differentiation of *Hevea* clones and also their evaluation as useful complements to SSR and AFLP markers. Universal primers for retro-sequences were synthesized and used in amplification to develop S-SAP markers. Work is in progress in developing retro-based marker system in rubber.

1.5. Genetic linkage map in rubber

In linkage analysis work, marker segregation data is being continuously integrated into the mapping data to populate the linkage map of rubber. A new set of 80 SSR primer-pairs including 13 M-series SSRs (used by French group), 58 Hv-series and 9 EST-derived SSRs were tested for polymorphisms between the parents: RRII 105 and RRII 118. M-series microsatellite primer-pairs were synthesized based on the markers for different linkage group of rubber from the literature published by CIRAD group and tested for polymorphism in RRII 105 and RRII 118. This is to generate synonymous linkage groups in the mapping study using those markers as anchor points. RAPD and AFLP marker analysis was also performed and the segregation data were recorded. Marker segregation among the progenies was found to be perfect as segregation distortion was noticed only with small number of AFLP markers.

Ninety-six RAPDs (36 RAPDs - RRII 105 specific, 39 RAPDs - RRII 118 specific and 21

common RAPDs - present in both the parents) were analyzed for segregation in progeny population. Segregation data of 74 AFLP markers (32 AFLPs - RRII 105 specific, 26 AFLPs - RRII 118 specific and 16 common AFLPs) generated with 17 primer combinations were also incorporated in linkage map. Besides RAPD and AFLP markers, 43 polymorphic SSR markers (18 genomic and 25 EST-derived SSRs) and two SNP markers (identified in ubiquitin precursor and mevalonate kinase) were used to genotype the progeny population and segregation data was recorded for linkage map construction.

In total 215 marker loci were used to genotype 60 progenies along with their parents RRII 105 and RRII 118. Linkage analysis and map calculations were performed using the program JoinMap v. 3.0. Two parental maps were generated. In RRII 105, 72 loci were distributed in 18 linkage groups and 74 remained unlinked. Whereas 20 linkage groups were formed with 65 loci in RRII 118 and 72 loci were unlinked.

2. Development of genetic markers for biotic and abiotic stress tolerance and understanding the stress adaptation process through transcriptome analysis

2.1. Development of molecular marker(s) linked to the locus conferring resistance to fungal diseases in *Hevea*

2.1.1. Resistance gene analogue (RGA) in rubber

RGA approach was adopted to identify disease resistant gene in *Hevea*. Isolation and characterization of 48 genomic RGAs as well as 32 functional RT-RGAs in rubber

were reported last year. Analysis of RT-RGA13, one of the functional resistance gene analogue identified in rubber, showed significant over-expression in *Corynespora* infected rubber clones and therefore an attempt was made to clone full-length of the gene. A ~2.0 kb 3' RACE product of the RT-RGA13 was cloned and sequenced partially. Sequence analysis showed significant homology with disease resistance gene from several plant species. But, generation of 5'RACE of the same from the first-strand cDNA product did not succeed. Therefore, same gene-specific 5'RACE primers were used for genome walking using restricted and adapter ligated genomic DNA (genome walker library) and a 1.3 kb *Dra*I fragment was amplified and cloned successfully.

2.1.2. Genes involved in host tolerance to *Corynespora* leaf disease

Differentially expressed 120 transcripts/bands induced by challenge inoculation of rubber plants with *C. cassiicola* were identified; 60 of them were cloned and characterized to get an idea about the genes induced during interaction of the pathogen with RR11105, a susceptible clone, while developing disease symptoms. Earlier identified 63 differentially expressed clones were subjected to reverse northern analysis using total RNA from infected (24 h, 48 h and 96 h after inoculation with the pathogen) and non-infected (control) RR11105 and GT 1 as probes to identify the cDNA clones showing differential pattern of expressions in susceptible and in tolerant clones respectively. Twenty-six cDNA clones were found to be over-expressed compared to control within 24 h of infection in tolerant clone GT 1. Among the over-expressed clones, nine clones showed almost similar response (up-regulation) in susceptible

clone RR11105 and four clones: CT34, CT54, CT72 and CT73 showed opposite trends in expression levels between susceptible and tolerant clones. These four clones down regulated in RR11105, showed considerable levels of up-regulation in GT 1 at 24 h of infection. Out of 26 over-expressed clones at 24 h of infection in GT 1, 18 of them showed reduced levels of expression at 48h. Thirty-one cDNA clones didn't show any response to challenged inoculation and one clone CT74 showed down regulation in GT 1. Attempts are being made to confirm these preliminary results.

Efforts to clone full-length genes encoding anthocyanidin 3-O-glucosyltransferase and transcriptional regulators of the GRAS family through RACE resulted in amplification of desired 5'cDNA end in anthocyanidin 3-O-glucosyltransferase (1.2 kb) and a truncated 5' RACE product for GRAS gene from GT 1. Members of the GRAS gene family encode transcriptional regulators that have diverse functions in plant growth and development and span over 2100 bp (688 amino acids encoded in *Ricinus communis*). To amplify initial 900 bp coding sequence at the 5' end RAGE technique was adopted with gene-specific primer from the truncated 5' sequence. Consecutively four fragments were amplified from adapter ligated genomic DNA restricted with *Dra*I, *Eco*RV, *Pvu*II and *Stu*I and cloned for sequencing.

Sequencing of 72 subtracted cDNA clones (Sc-CT), derived from *Corynespora* challenged RR11105, was carried out. Surprisingly all the sequences were grouped into six contigs. Therefore, an effort was made to construct a subtraction library with the *Corynespora* challenged RNA samples.

2.2. Characterization of stress-tolerant clones of *Hevea* using molecular markers and gene regulation under abiotic stresses

Transcript profiling of both tolerant and sensitive responses under different abiotic stresses was continued to understand stress adaptation processes in rubber growing under extreme climatic conditions. This will provide clues for identification of genes, which are useful for improvement of abiotic stress tolerance in rubber.

2.2.1. Cold tolerance in rubber

Transcript profiling in two stress tolerant *Hevea* clones PR 261 and RRII 208 in relation to cold stress was continued. Characterization of 131 differentially expressed cDNA sequences were reported. One hundred and ten unique sequences comprised of 13 clusters/contigs and 97 singletons were identified. More number of differentially expressed transcripts (~100) was generated through DD-RT PCR for characterization.

2.3. Rubber EST Project

A second plasmid library, containing more than 600 subtracted cDNA clones derived from cold stressed leaf samples of PR 261, was constructed to identify cold responsive genes. Sequence analysis was carried out with 59 subtracted cDNA clones (ScDNA).

A bark cDNA library was constructed in lambda ZAP Express vector using mRNA from the clone RRII 118. Efficiency of the library was found to be 9.6×10^4 pfu. This library is the source of genes, actively expressing in bark tissue. Mass *in-vivo* excision of the bark cDNA lambda phage library to phagemid library was carried out

and excised phagemid clones containing cDNA inserts were grown individually in microtiter plates. In total 560 clones were grown and maintained. One hundred and twenty-six cDNA inserts were amplified from the clones of a bark cDNA library of RRII 118 using vector directed primer-pairs and sequenced. Approximately 54,500 bp bark transcriptome sequence data was generated. High quality sequences of 108 bark cDNA clones (bcDNA) were subjected to 'contig analysis' to assemble similar sequences in groups. Ten contigs containing 22 clones (2 to 4 clones per contig) and 86 singletons (single sequences) were identified. All sequences were subjected to BLASTX search to know about the homology with the gene sequences existing in GenBank from rubber or other plant species. Sequence annotation was performed.

2.4. Methylation dynamics of *H. brasiliensis* genome

Understanding the methylation pattern of *Hevea* clones will provide an explanation for the differential expression of characters by the same clone under different stressed conditions and how the plant is getting adapted to a particular stressed condition by controlling the expression rate of several genes.

Growth chamber experiment was designed to study the epigenetic modifications in the genome of *Hevea* clones under cold stress and to validate the results obtained from the preliminary studies. Two polybag plants of clones RRII 105, RRII 600 and PB 260 were subjected to cold stress in a growth chamber. The DNA samples collected from these plants before and after the cold treatment were bisulfite treated. Promoter regions of *hmgr 1*, Cronatine-

insensitive 1 and *ref* gene were amplified using bisulfite primers, purified and cloned for sequencing. RNA samples were also isolated from these plants before and during the treatment period for gene expression studies.

To study epigenetic modifications in *Hevea* genome under cold stress, a simple RAPD - based technique was adopted. DNA was isolated from a single plant of RR11 105 before and after cold treatment for digestion with methylation sensitive/insensitive isochizomers - *HpaII* X *MspI*. Twenty RAPD primers were screened to find out polymorphic bands due to methylation. Methylation sensitive bands were observed in the profiles of OPA10 & OPAB 07 primers. Initial results of these two primers indicate that cold stress is inducing methylation at certain regions of the genome.

In another experiment, genomic DNA of all the three clones (from growth chamber experiment) was digested with *McrBC* enzyme (an endonuclease which recognizes methylated cytosines and cleaves there) to analyze the extent of DNA methylation polymorphisms among them. Smearing was observed when *McrBC* digested genomic DNA was checked on agarose gel indicating methylation in *Hevea* genome. *McrBC* - RAPD of the DNA samples derived from three clones prior to and after cold treatment using the above two primers OPA-10 and OPAB-07 could not show any difference in banding patterns.

An attempt was made to amplify the *Hevea* methyltransferase gene *CMT3* (responsible for site-specific *de novo* methylation) from *Hevea* genome using degenerate primers. A 600 bp fragment was amplified and cloned for sequencing to confirm the identity.

3. Cloning and characterization of agronomically important genes

3.1. Cloning and characterization of lignin biosynthesis gene(s) in *Hevea* for their over-expression in timber clones

3.1.1. Cinnamyl alcohol dehydrogenase (CAD) gene from rubber

For improving wood quality through enhanced lignin biosynthesis in rubber a substantial effort has been put on the functional characterization of genes involved in lignin production. Successful cloning and characterization of cDNA encoding Cinnamyl alcohol dehydrogenase (HbCAD). Southern hybridization with full-length cDNA probes of *HbCAD* clearly indicated the presence of minimum two forms of the respective genes in rubber. Sequence of genomic *HbCAD* (2091 bp) was also reported.

3.1.1.1. HbCAD gene expression in selected wild accessions and Wickham clones

Attempts were made to establish a correlation between CAD gene expression and lignin content in selected wild accessions and Wickham clones using real-time quantitative PCR. cDNA derived from the bark RNA was analyzed to quantify the level of CAD transcripts in different accessions. No significant variation in CAD gene expression was detected among the wild accessions studied, which had estimated lignin content in the range of 21 to 27%.

3.1.2. Cinnamoyl-CoA reductase (HbCCR) gene

A full-length cDNA of CCR, involved in lignification, was cloned successfully from rubber and designated as *HbCCR*. Sequencing of the putative *HbCCR* clones

followed by homology search confirmed its gene designation, which is of 1114 bp in length. *HbCCR* cDNA clone showed maximum homology with *Ricinus communis* CCR gene sequences (E value: $1e^{-479}$).

To determine the genomic organization of *HbCCR* gene, an effort was made to isolate a genomic clone of CCR gene following "expand long template PCR" method. Primer walking strategy was adopted to generate information about the full-length sequence of the gene (~3.2 kb).

Southern hybridization was performed to know the number of *HbCCR* genes involved in lignification in rubber. Genomic DNA of GT 1 was digested with *Bam*HI, *Eco*RI, *Hind*III, *Sac*I, *Pst*I and *Xba*I and transferred to nylon membrane for hybridization. Hybridization with full-length cDNA probes of *Hev*-CCR clearly indicated the presence of more than one form of the respective gene in rubber.

Semi-quantitative RT-PCR was carried out for expression analysis of *HbCCR*. Gene expression was comparatively high in leaf than in bark of RRII 105. *HbCCR* activity was also found to be highly variable in bark tissues of different *Hevea* species. In RRII 105 (*H. brasiliensis*), CCR expression was higher than other two species, *H. benthamiana* and *H. spruceana* as noticed with the *HbCAD*.

3.1.2.1. Bacterial expression of *HbCCR* cDNA

An expression cassette for *HbCCR* gene was constructed by cloning the CCR coding sequence in frame with the T7 promoter of pRSET-A vector. The construct was used to transform BL21 (DE3) pLysS cells. Positive

colonies were grown in LB and cells were collected prior to and after IPTG induction for CCR gene expression. Crude extract of the collected cells was loaded on SDS PAGE to detect the recombinant protein. A new protein band (42 kD) was detected. Further experimentation is in progress.

3.1.2.2. Relative quantification of *HbCAD* and *HbCCR* transcripts in the bark tissues of three species: *H. brasiliensis*, *H. benthamiana* and *H. spruceana* using qPCR

Significant variation was observed in the expression levels of both CAD and CCR genes among the three *Hevea* species studied through qPCR. *H. brasiliensis* clone RRII 105 have high CAD and CCR activity compared to *H. benthamiana* and *H. spruceana*. Comparatively low levels of CAD and CCR gene expression was noticed in bark samples of *H. spruceana*, which was supported by the earlier northern analysis results.

3.2. Cloning and characterization of stress responsive gene (s)

MT gene codes for a low molecular weight protein that acts as a reactive oxygen species (ROS) scavenger in the cells to protect plants from oxidative stress. *Hevea* metallothionein gene was cloned successfully, and the same was identified as the *MT-3a* isoform based on sequence homology, whose expression was prevalent in leaf tissues. Another class of metallothionein gene, *MT-2* was isolated from bark cDNA library of RRII 118.

CENTRAL EXPERIMENT STATION, CHETHACKAL

The Central Experiment Station, Chethackal, located near Ranni at a distance of about 56 km from Kottayam was established in 1966 to cater research needs of the different Divisions of the RRII. The Station has a total land area of 254.8 ha which is planted for different research projects.

The Station meets the needs of the scientists of various disciplines of Crop Improvement, Crop Management, Crop Protection, Crop Physiology and Latex Harvest Technology. The station has two divisions A and B of almost equal area. Apart from clone trials and budwood nursery of pipeline clones, trials on low frequency tapping, CUT, Germplasm accessions, disease management and fertilizer dosages make up bulk of the

experimental areas. Specialised trials like gas-based tapping (G-Flex), intercropping and immaturity reduction *etc.* also make part of the experimental area. A three-part tree crown-budded area with canopy from F, 516 is laid to study disease resistance mechanisms. An Eddy covariance tower gives micro-environmental data. Trainees of Rubber Training Centre and various Sastradarsan teams visit the station to gather first hand information.

During the reporting period, the total crop realised was 126698 kg. A total of 299 tapping days was possible in the year and 42 tappers (per day) were engaged for tapping. The CES Dispensary attends to the medical needs of the workers and the total number of visits of patients during the period under report was 4640.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

The priority areas of research of the station included crop improvement, management, protection and latex harvest technology.

1. Crop improvement

1.1. Large-scale trial for selection of location-specific clones:

Field planting of 720 polybag plants of 15 promising clones (*viz.* RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, RRIM 600, RRII 203, RRII 208, SCATC 88/13, IRCA 109, IRCA 111, IRCA 130, PB 280, PB 312 and PB 314) was completed at RRTC, Hahara in Assam for identifying location specific, high-yielding clones.

1.2. Large-scale trial for evaluation of potential primary clones

Field planting of 576 polybag plants of 10 potential primary clones from Sarutari Research Farm (*viz.* Gh1, Gh 2, Gh 3, Gh 4, Gh 5, Gh 6, Gh 7, Gh 8, Gh 9 and Gh 10) along with two control clones (RRIM 600 and SCATC 88/13) was completed in large-scale trial at RRTC, Hahara, Assam.

1.3. On-farm evaluation of selected clones of *Hevea* in Assam

Field planting of 480 polybag plants of six promising clones (*viz.* RRII 417, RRII 422, RRII 429, RRII 430, RRIM 600 and SCATC 88/13) was completed in farmers field at

Table Ghy. 1. Girth of plants

Treatment	Diameter ¹ (mm)		Girth ² (cm)		
	Dec.08	June-09	Dec.09	June 10	Dec.10
Control	16.98	20.63	7.27	7.53	10.49
Standard practice	16.77	20.00	7.69	7.99	11.45
25%N & P + BF *	16.86	20.22	7.44	7.75	10.22
50% N&P + BF *	17.33	21.41	8.12	8.82	12.27
75% N&P +BF *	17.11	20.34	7.73	7.95	11.02
Standard practice +BF	16.28	19.87	7.66	8.18	11.94
BF alone	17.51	21.83	8.00	8.51	11.69
SE	0.79	0.97	0.33	0.43	0.54
CD(P = 0.05)	NS	NS	NS	NS	NS

N.B * Full dose of K 1. Diameter at 25 cm height 2. Girth at 150 cm height

three locations (Umsiang, Byrnihat and Bhakuagoog).

2. Crop management

2.1. Development of an Integrated Nutrient Management system for young rubber

Continued the experiment for development of integrated nutrient management system for young rubber with cover crop initiated in 2008. The treatments involved combination of different doses of inorganic fertilizers with and without biofertilisers and biofertiliser alone. The biofertilisers applied are *Azotobacter*, *Pseudomonas*, *Phosphobacteria* and *AMF*. The girth data was found to be non-significant (Table Ghy.1).

2.2. Evaluation of different biological bunds for soil and water conservation in rubber

Completed the establishment of rubber, platform making and terrace widening with trenches to collect eroded soil. Establishment of vegetative bunds to check soil erosion was under progress.

2.3. Effect of crop intensification with intercrops on establishment and growth of rubber in rubber plantation

In the on-farm trial, carried out

planting of main crop rubber and cultivation of first cycle of intercrops.

2.4. Comparative study of zero tillage and normal planting technique for rubber

Completed the establishment of rubber as per treatments under zero tillage and normal planting.

2.5. Development of locally viable root trainer technique for propagation of rubber

The feasibility of root trainer cups made of earthen pots and bamboo is being tested for raising planting materials.

3. Crop protection

3.1. Survey on pests and diseases of rubber

Survey on pests and diseases of rubber was carried out in 19 pockets covering 11 locations in Assam and Meghalaya during 2010-11. Powdery mildew disease was noticed in all the locations and the severity of the disease was more on the lower branches of the affected trees. Incidence of *Colletotrichum* leaf disease (10%) was noticed in the nursery on tender leaves during August/September and caused defoliation of affected leaves. *Periconia* leaf

blight disease was noticed in nursery at Umling (30%) in Meghalaya and also noticed in other locations (10%). Minor incidence of *Corynespora* leaf spot (below 5%) and *Colletotrichum* leaf spot (10%) was noticed in nursery in most of the locations. Occurrence of *Colletotrichum* leaf spot was higher in SCATC 88-13 and Haiken 1 than other clones. Fresh incidence of purple root disease was noticed on five plants (6-year-old) in untreated block at RRTC, Hahara and the root systems of purple root disease-affected plants in treated/untreated (20 nos. each) block are still healthy. Girth of disease free rubber plants (healthy block) and purple root disease affected plants (treated and untreated block) was recorded. Girth increment was found higher (5.2 cm) in healthy block as compared to untreated block (4.1 cm). The purple root disease is found under control by application of tridemorph (Calixin 6.25 ml/litre water). Incidence of thread blight disease (40%) on mature trees (25-year-old) was observed only at Umling in Meghalaya. Infestation of scale insect (10%) was noticed in nursery which caused a total loss of the affected plants in the seedling nursery.

3.2. Isolation and identification of fungal pathogens of rubber

The growth of *Colletotrichum gloeosporioides* causing *Colletotrichum* Leaf disease of rubber was studied in laboratory at 20 °C to 40 °C at an interval 5 °C and the optimum growth of *C. gloeosporioides* in terms of colony diameter was found at 25 °C (9 cm). The growth of *Corynespora cassicola* was also studied in different temperatures and the optimum growth was noticed in oat meal agar medium at 35 °C (8.8 cm) indicating that the pathogen prefers high temperature for fast multiplication.

3.3. Evaluation of wild germplasm against tolerance to powdery mildew disease

Screening of nine short-listed accessions of wild *Hevea* germplasm was carried out in field by staggered cutting method. Three out of nine short-listed accessions of wild germplasm seems to be tolerant to powdery mildew disease.

3.4. Relevance of inocula of AM fungi and growth responses of rubber in P deficient soils

Mycorrhizal endogone spores of three dominant species and other beneficial organisms have been isolated from the rhizosphere soil of rubber. From these

Table Ghv. 2. Girth of rubber seedlings in polybags (after 15 months)

Treatment	Girth (cm)	Height (cm)
Control	4.19 ^a	78.66 ^a
<i>G. margarita</i>	4.38 ^a	80.13 ^a
<i>G. mosseae</i>	5.70 ^b	108.66 ^b
<i>G. fasciculatam</i>	6.19 ^{ab}	113.99 ^{bc}
<i>G. fasciculatam</i> + <i>G. margarita</i>	6.43 ^{ab}	125.25 ^b
<i>G. fasciculatam</i> + <i>G. mosseae</i>	7.40 ^a	142.18 ^a
Sig.	**	**

Figures with same alphabets do not differ significantly at 1 % level

Table Ghv. 3. Girth increments of rubber seedlings in nursery (after 15 months)

Treatment	Girth (cm)	Height (cm)
Control	4.56 ^a	76.39 ^a
AMF	6.19 ^{bc}	88.15 ^{bc}
Phosphobacteria	5.02 ^a	82.19 ^a
<i>Azotobacter</i> sp.	4.72 ^a	80.00 ^a
AMF+ <i>Azotobacter</i> sp.	6.49 ^{bc}	93.78 ^{bc}
AMF+PSM	7.50 ^a	118.25 ^a
Sig.	**	**

Figures with same alphabets do not differ significantly at 1 % level

Table Ghy. 4. Total biomass of rubber seedlings in polybag and nursery (after 15 months)

Treatment (Polybag plants)	Dry weight (g)	Treatment (seedlings)	Dry weight (g)
<i>G.fasciculatum</i>	165.4	AMF	192.9
<i>G.mosseae</i>	136.2	PSM	118.4
<i>G.margarita</i>	100.0	Azotobacter	110.6
<i>G.fasciculatum</i> + <i>G.mosseae</i>	182.0	AMF+PSM	233.7
<i>G.fasciculatum</i> + <i>G.margarita</i>	171.0	AMF+Azotobacter	217.0
Control	64.6	Control	91.2

organisms, *Glomus fasciculatum*, *G. mosseae*, *Gigaspora margarita*, *Azotobacter* and phosphobacteria were inoculated on the rubber seedlings and assessed the growth performance. The rubber seedlings in polybag treated with *G. fasciculatum* alone attained the highest growth followed by the plants treated with *G. mosseae* and *Gigaspora margarita*, respectively. The growth has been further stimulated in the mixed inocula with *G. fasciculatum* and *G. mosseae*. The girth and height of the dual inocula treated plants in polybag were significantly higher while the singly inoculated plants were on par in their growth performances. No differences were observed in whorl numbers of the seedlings in both inoculated and control plants. The highest spore numbers were observed in the mixed inocula of *G. fasciculatum* and *G. mosseae* treated plants. In single inoculum, *G. fasciculatum* had the highest spore number followed by *G. mosseae* and *G. margarita*, respectively. The percentage root colonization also increased with age of the treated plants with AMF. The dual inoculated seedlings with AMF and phosphobacteria attained the highest girth and height. (Table Ghy. 2, 3 & 4).

3.6. Microbial activity, disintegration of leaf litter and nutrient release under a mature rubber and natural forest covers

The rate of weight loss of each type of litter (viz. sal, teak, bamboo, rubber) from

the litter bag was faster under the forest cover than those placed under the rubber plantation. The weight loss of rubber (62.5%), bamboo (63.0%), Sal (47.0%) and teak (49.0%) litters were rapid under the forest system while under the rubber plantation the rate of disintegration was rather slow.

The total microbial population was also quantitatively associated with the litters placed under the forest cover.

Saprophytic soil fungi of the genera *Penicillium*, *Aspergillus*, *Trichoderma*, *Fusarium* and a group of filamentous yeasts were found to be the most dominant decomposers. The rate of disintegration of hemicellulose was rapid both under forest and rubber followed by cellulose and lignin.

3.7. Development of Integrated Nutrient Management System from non-traditional region- assessment of microbial population

A consortium of beneficial microorganisms including *Azotobacter*, *Pseudomonas*, phosphobacteria and inorganic fertilizer in different doses were applied to the immature rubber plants and assessed the growth of plants and monitored the changes in the microbial populations and their survival pattern. It was observed that there was an increase in the population of microorganisms in the soil after three months of treatment.

Quantitatively the bacterial population was high in all the treatments, but differences were on par among the population of actinomycetes, *Pseudomonas*, *Azotobacter* and phosphobacteria.

4. Crop physiology

4.1. Shallow tapping – an option for stress alleviation in *Hevea* plantation during winter season in NE regions

The experiment was initiated in January 2010 with an objective of evaluating the impact of shallow tapping during winter (January to March) with normal tapping (April to December) on yield, DRC and TPD in RRIM 600 and RR11 105 clone at Sarutari Research Farm in Assam. Another set of experiment under normal tapping from January to December without rest and with rest from January to March was also conducted in RRIM 600 and RR11 105 for comparison. Yield (g/t/t), DRC (%) and volume of latex (ml/t/t) were recorded from three different blocks of each clone (50 trees in each block). Observations on TPD and soil moisture were also carried out in each block.

RRIM 600 showed the highest mean yield (39.1 g/t/t) under normal tapping system without rest followed by shallow tapping in winter and normal tapping from April to December (33.3 g/t/t) and the lowest

in normal tapping with rest during winter (30.6 g/t/t) while RR11 105 showed maximum (32.6 g/t/t) under normal tapping with rest in winter followed by normal tapping without rest (31.8 g/t/t) and the minimum was under shallow tapping in winter and normal tapping in rest of the period (27.7 g). Both RRIM 600 and RR11 105 showed the highest DRC (32.5% and 35% respectively) under normal tapping with rest while the highest volume of latex was recorded in both RRIM 600 and RR11 105 under normal tapping without rest. (Table Ghy.5)

5. Latex harvest technology

5.1. Controlled upward tapping (CUT)

The experiment on CUT was continued. RRIM 600 clone has been selected with four treatments (T1= S/4U d2 (5% ET) 21 days interval-S/2 d2 (2.5% ET) twice in the tapping period, T2= S/4U d2 (5% ET) Monthly interval-S/2 d2 (2.5% ET) twice in the tapping period, T3= S/3U d2 (5% ET) Monthly interval-S/2 d2 (2.5% ET) twice in the tapping period, T4= S/3U d2 (5% ET) 45 days interval-S/2 d2 (2.5% ET) twice in the tapping period), six replications and eight trees/plot. The results indicated that T1 showed maximum yield followed by T2, T3 and T4.

Table Ghy. 5. Mean yield, DRC and volume of latex in RRIM 600 and RR11 105 under different tapping system
Parameters Annual mean of yield, DRC and volume of latex

Parameters	RRIM 600			RR11 105		
	ST	NT	NT*	ST	NT	NT*
Yield (g/t/t)	33.3	39.1	30.6	27.7	31.8	32.6
DRC (%)	29.5	29.9	32.5	33.2	32.6	35.0
Volume of latex (ml/t/t)	101.1	111.2	97.1	94.5	113.1	103.8

ST = Shallow tapping in winter and normal tapping from April to December; NT = Normal tapping from January to December without rest and NT* = Normal tapping from April to December with rest in winter.

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The station continued its research activities on cropping system models, nutritional requirement, crop improvement, plant pathological studies and latex harvesting. The other aspects of investigations were socio-economic survey of rubber growers and advisory services to rubber growers.

1. Crop improvement

1.1. Development of clones

In recombination breeding, 391 hybrid progenies are being evaluated in four seedling nursery trials and 19 selected hybrids are being evaluated in two clonal nurseries. Seedling nursery evaluation of 1023 half-sib progenies is in progress in two trials. Eleven ortets are being evaluated in SST (2000), ortet 114 (41.3 g/t/t) exhibited the highest mean yield over two years followed by ortet 98 (36.7 g/t/t) and ortet 315 (34.6 g/t/t).

Thirteen ortet selections from four Regional Research Stations in North Eastern region and 12 ortets from traditional area are being evaluated in clonal nurseries.

1.2. Evaluation of clones

In the large-scale trial (LST) planted in 1995, mean yield over six years revealed that PB 311 (47.6 g/t/t) was the best yielder among the 10 clones, followed by PB 260 (46 g/t/t) and PB 235 (45.2 g/t/t). In the 1996 LST (GxE trial), 13 clones are under evaluation. Mean yield over eight years revealed that clone RRII 422 (48.7 g/t/t) and RRII 429 (46.1 g/t/t) recorded higher yield compared to the local check clone RRIM 600 (39.2 g/t/t).

Potential clones are being evaluated in four on-farm trials. In Killamura block plantation (1998), West Tripura, PB 235 (1584 kg/ha/yr) was the highest yielder in the fifth year of tapping. At TFDPC plantation (2000), Bagafa, South Tripura, RRIM 600 (845 kg/ha/yr) gave the highest mean yield in the first two years of tapping. Growth data from TRPC plantation (2005), Pathalia revealed that among the RRII 400 series clones, girth was the highest for RRII 429 (29.4 cm) followed by RRII 417 (28.8 cm). Initial establishment was observed to be poor for RRII 422, which showed partial to complete drying of 36% and 33 % plants during first and second year of planting respectively in on-farm trial (2009) at Hirapur.

Three clonal nursery evaluations involving 12 pipeline clones from traditional area, 11 popular clones and 19 potential clones are in progress.

2. Crop management

In the nutrition study, clone RRII 429 attained the highest girth (55.9 cm) and tappareability (80%) in the seventh year compared to other clones viz. RRII 417, RRII 430 and RRIM 600 with one and half times recommended dose of fertilizer.

The integrated nutrient management experiment with clone RRIM 600 is being continued with graded dose of inorganic fertilizers with and without bio-inoculum (*Azotobacter*, *Phosphobacteria*, *Pseudomonas* and VAM). It was observed that the growth of plants increased with combined application of 50 per cent inorganic fertilizer together with bio-inoculum in the second

year. In another experiment, the growth of RRIM 600 during third year (23.7cm) was higher with application of 20 kg FYM with 50 per cent recommended dose of fertilizer.

In the cropping system models, two annual crops viz. cowpea (*Vigna unguiculata*) and maize (*Zea mays*) were cultivated in between rubber rows during third year of the experiment. Cowpea yielded @ 1.62 t/ha and maize yielded @ 1.9 and 1.73 t/ha in Model I and II respectively. Pineapple (*Ananas comosus*) yielded @ 10, 383 and 9615 nos in model I and II respectively. The yield of banana was 2.56 t/ha in Model I.

Top soil with cowdung in 8:2 ratio is suitable as on potting media for root trainer and showed higher growth of plant and this combination can be used as an alternative to coirpith for this region.

In tea intercropping trial, average rubber yield was 1749 kg per ha in the sixth year of tapping. Tea green leaf yield was 309 kg/ha and the yield was low due to increase of shade.

In the fodder trial, after two cuttings Guinea grass (*Panicum maximum*) showed maximum yielding potential than rest of the fodder grasses and yielded 12.37 t/ha in the first cut and 11.8 t/ha in the second cut. Stylo (*Stylosanthes guianensis*) and Signal mixture (*Brachiaria decumbens*) yielded 11.65 t/ha in the first cut and 11.0 t/ha in the second. Annual fodder crops cowpea (*Vigna unguiculata*) and fodder maize yielded 8.85 t/ha and 6.30 t/ha respectively.

3.1. Latex harvest technology

In different systems of tapping experiment, clone PB 235 continued to give the highest yield in S/2 d3 system of tapping compared to S/2 d4 and S/2 d6 systems. In another experiment, S/2 d3 system of

tapping showed the highest yield in clone RRIM 600 compared to S/2 d2 and S/2 d4 system of tapping.

In controlled upward tapping (CUT) of the high panel (HO-1) in combination with short cut (S/4 U d2 d6d7) and stimulation ET 5% La 12Y, clone RRIM 600 continued to give the highest yield compared to S/2 d2 system of downward tapping in BI panel.

3.2. A project on "Identification of potential areas for rubber cultivation using remote sensing, GIS and pedo-climatic variables for Tripura" has been initiated in collaboration with Regional Remote Sensing Center-South (RRSC-S) of Indian Space Research Organization (ISRO) and Indian Institute of Technology, Kharagpur.

3.3. Survey of quality planting materials in Tripura

A survey was conducted to estimate the availability of quality planting material (budwood) in West and Southern districts of Tripura state. It was found that approximately 51 per cent of the total budwoods in the west Tripura district is contributed by the state run agencies like TFDPC and TRPC and about 44 per cent is the contribution of private nurseries. Though a total of 5,82,087 meter budwood is available in the West Tripura, only 74 per cent of the total budwood can be considered as useful quality planting material. Survey results also suggest that there is no shortage of budwood in the state, however, quality is not maintained as per Rubber Board's specifications. In South Tripura, survey was conducted in 41 private nurseries and found that a total of 24,016 bud-wood points were available having 2, 37, 192 meter of budwood. Bud-wood produced in South Tripura is not sufficient to fully cover the actual planting area. The available

budwood can produce around 6,60,000 bud-grafted plants which is much lesser than what is actually required.

4. Crop protection

Survey of diseases and pests of rubber was carried out in nine locations covering 29 sites of West and South Tripura districts. The geographic coordinates of the survey sites were also recorded with hand-held GPS device. Low to moderate incidence of powdery mildew disease was recorded in young nursery plants at NC Nagar, Kariamura, Bagma and Taranagar area, recording average 30.5 per cent disease index (PDI). During March, 2011, moderate to severe incidence of powdery mildew disease was observed in 4-6-year-old plantations at Jashmura, Borpathari and Kashari, South Tripura recording average 55.6 PDI. A few germplasm accessions, viz. RO 3794, RO 5055, RO 5365 and MT 4859 were observed moderately tolerant to powdery mildew disease. Moderate to severe incidence of Periconia leaf blight, Colletotrichum and Corynespora leaf disease was observed in young nursery plants at Taranagar farm. Pink disease (12.2%) was observed at Kolachara region of Sabrum, South Tripura. Low incidence of brown root disease (<3%) was observed in few places. The growth of pathogen, *Phellinus noxius* was observed *in vitro* at different temperatures. No growth was observed at 10 °C after seven days of incubation. The mean linear growth was 0 mm at 10 °C, 7.75 mm at 14 °C, 35.5 mm at 18 °C, 39.3 mm at 22 °C and 45.3 mm at 26 °C after seven days of incubation. No *Phytophthora* incidence was observed. In *Phytophthora* soil baiting study, very few infection propagules of *Phytophthora* were observed. Isolates were observed to be very

slow growers and less virulent compared to those of Kerala. Among pest incidence, mild infestation by scale insect, mealy bug, termites, borer and mooply beetle was observed at different places.

Three rounds of sulphur dusting @ 12 kg/ha/round at 10 days interval was observed to reduce powdery mildew disease by 35% in RR11 105 and 25% in RRIM 600 of 22-year-old plantation over undusted control. The yield loss in undusted blocks of RRIM 600 and RR11 105 was estimated to be 11.7 and 13 per cent respectively. Two rounds of foliar spray with 1.0% monopotassium phosphate were observed to reduce powdery mildew disease in polybag plants of RRIM 600 and RR11 105 clone by 15-22% compared to control. Spraying with carbendazim 0.05% was observed best in controlling powdery mildew disease. Efficacy of three antagonistic phylloplane bacteria was tested against *Colletotrichum* leaf disease in young polybag plants of RRIM 600 clone. *In vivo* efficacy of the antagonists was observed low recording average 15 per cent disease suppression over control.

5. Processing technology

A study on the effect of temperature on the coagulation of latex showed that 280 ml of 1 % formic acid gave best and complete coagulation of latex in winter season against recommended dose of 300 ml of 0.5 % formic acid. In order to improve the colour of the sheets, different types of smoke filters like filter with simple projections, filter with partitions, filter with brick chips, filter with wire mesh, filter with flower-like partitions etc. were put for evaluation. The results showed that lot of carbon can be removed by these filters and the colour of the sheets can be improved.

Another study to enhance the durability of the mud-wall using rubber as a reinforcing material is in progress. Different treatments with pre-vulcanized latex, post-vulcanized latex and latex mixed clay were done and are under evaluation. Initial results showed that mud wall with latex-mixed clay is much stronger than ordinary mud walls.

6. Economics

The study on the impact of Block Planting Scheme on Socio-economic status of beneficiaries was completed. The sample households were selected from nine BPUs and two Group Processing Units (GPUs) from the West and South Tripura districts for the field survey. Primary data from 271 households with mature area, 131 households with immature area under the nine BPUs and 78 households under two GPUs were collected.

The study showed the pivotal role of traditional customs and conventions of tribal communities in determining the pattern of household consumption, expenditure and better awareness and access to facilities by the non-tribal communities integrated more into the mainstream of the society. In sharp contrast to the experience of Kerala where the surplus generated from the NR sector had been systematically channelised by the pioneering planters for the formal and professional education of their children, investment in higher education in Tripura among the households under mature BPUs was negligible as only 3.95 per cent of the population in the age group of 18 - 24 after class twelve was pursuing higher education. It also indicated the BPU's linkage effects on the regional economy were seriously constrained by the nature of

human capital, the availability of infrastructural facilities and access to the same.

The two critical components required for the effective utilization of the income impact of the BPS are imparting skills through technical/ professional education and providing access to infrastructural facilities related to higher education, healthcare, communication and transport. An important impact of the NR-based rehabilitation programmes in the state is the growth of rubber area outside various government sponsored schemes (45 %) and the share of smallholdings (82 %) in the total area under the crop in the state. The contiguous land under majority of the blocks is not clearly demarcated and the income from NR is also shared either on the basis of area under possession or on an equal basis irrespective of the production from individual holdings. In the long-run, the concerned agencies of the state government may initiate skill-oriented programmes for empowering the unemployed/ underemployed youth along with improvements in the infrastructural facilities. It will ensure the diversification of the sources of income, sustenance of rubber cultivation and linkage effects on the regional economy. In the short-run, close monitoring and supervision by the Rubber Board are essential because the beneficiary households are yet to fully assimilate the agro-management practices and the RFSs attached to mature BPUs require the inputs and guidance of the Board.

7. Advisory work

Seven demonstration programmes were conducted. Discriminatory fertilizer recommendations based on soil and leaf analysis were offered to 291 rubber growers

of this region. A total of 1072 latex samples were analysed for DRC and other latex parameters. 2715 m of budwood of high yielding clones were supplied to Rubber Production Department for distribution to

growers. As part of advisory services, field visits conducted to 16 rubber plantations for inspection and identified disease and pest problem and remedial measures were advised.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

The Regional Research Station, Tura continued its research activities on evaluation of clones, polyclonal seedling evaluation evolving suitable exploitation system and crop management.

1. Crop improvement

1.1. Polycross progeny evaluation

The population attained an average girth of 8.7 cm during the year. On the basis of test-tapping yield and girth of the progenies, top 20 per cent of the population has been selected for further evaluation in clonal nursery trials. Selected seedlings have been cut back to generate budwood for cloning of the selections. In 2010, a new nursery population of 880 polycross progenies was established and 291 progenies survived up to March 2011.

1.2. Clonal nursery evaluation

A clonal nursery was established with three selections from Tura, four each from Agartala and Guwahati with RRIM 600 as the check (RRST 24, RRST 37 and RRST 39 – Tura, X1, X2, X3 and X9 – Guwahati and RRSA 121, RRSA 315, RRSA 461 and RRSA 585 – Agartala). The experiment was laid out on randomized block design with 12 clones and three replications and the plot size is 6.

1.3. On-farm evaluation of select clones

Three on-farm trials have been laid out in the East & West Garo Hills of Meghalaya. 600 plants each of six clones viz. RRII 417, RRII 422, RRII 429, PB 235, RRII 203 and RRIM 600 were planted in two locations in the East Garo Hills and 400 plants of four clones, viz. RRII 417, RRII 422, RRII 429 and RRIM 600 clones were planted in the West Garo Hills of Meghalaya (100 plants were used per clone). In East Garo Hills, highest girth (125cm from bud union) was recorded in RRII 429 (9.5 cm) followed by RRIM 600 (9.4 cm), RRII 203 (8.8 cm), PB 235 (8.6 cm), RRII 417 (8.55 cm) and lowest was in RRII 422 (8.1 cm). Maximum height was recorded in PB 235 (4.35 m) followed by RRIM 600 (4.1 m) and RRII 429 (4.0 m). Maximum number of branches was recorded in RRII 203 (16.7) followed by PB 235 (11.8) and RRIM 600 (10.8). In West Garo Hills the highest was recorded in RRIM 600 (110 cm), while RRII 417 recorded the highest girth (cm), number of leaves and number of whorls.

1.5. Half-sib progeny evaluation

From 2008 selections on the basis of test-tapping yield and girth of the progenies, top 20% of the population has been selected for further evaluation in clonal

nursery trials. Selected seedlings have been cut back to generate bud-woods for cloning of the selections. From 2009 selections, growth parameters were recorded and maximum height was observed in progeny of RRII 203 (136.3 cm) and the highest girth was in that of Gl-1 (4.8 cm) while the lowest height and girth was recorded in progeny of PB 260.

1.6. Development of polyclonal seed garden

Planting materials elonging to clones RRII 118, RRII 203, RRII 208, RRII 422, RRII 429, Haiken 1, SCATC 88/13, PB 280 and RRII 600 were raised in poly-bags for large-scale planting season at Hahara in Guwahati.

1.7. Evaluation of polycross progenies from four stations of NE region

Assorted seeds were collected from four locations in the NE region and planted

in RBD with five replications (360 progenies). Preliminary results show no regional variations among the seedlings.

2. Crop physiology and latex harvest technology

Low temperature (below 10 °C) during winter period is one of the main factors for depression of yield and dry rubber content in *Hevea* in the agroclimatic condition of Garo Hill. Yield and yield components were recorded and results indicated that average total volume of latex was 233 ml/t/t, yield was 79.9 g/t/t and DRC was 32.65%. Complete defoliation occurred in second week of February and refoliation in first week of March, while flowering was noted in third week of March.

Under the controlled upward tapping system (CUT) experiments, treatmentwise monthly yield was recorded and the result showed that the highest yield was recorded

Table Tura. 1. Annual yield (g/t/t) under different treatments of ethephon application in controlled upward tapping system

Month/ Treatment	T1	T2	T3	T4	LSD (5%)
April 2010	63.00	58.00	59.16	51.66	16.50
May 2010	57.07	55.01	54.62	52.46	
June 2010	69.85	70.33	60.41	61.03	
July 2010	69.94	68.30	59.60	58.95	
August 2010	103.58	108.75	95.84	87.150	
September 2010	87.40	85.85	78.30	70.78	
October 2010	113.46	106.91	98.36	89.43	
November 2010	131.89	128.98	125.81	116.43	
December 2010	122.46	110.80	109.50	106.68	
January 2011	83.63	75.44	74.55	69.79	
February 2011	79.42	81.15	77.25	71.63	
March 2011	60.63	70.82	70.17	60.97	
Mean	87.69	85.03	80.30	75.15	4.76

T1- S/4U d2 (5% ET) 21 days interval- S/2 d/2 (2.5 % ET) twice in the tapping period

T2- S/4U d2 (5% ET) Monthly interval- S/2 d/2 (2.5 % ET) twice in the tapping period

T3- S/3U d2 (5% ET) Monthly interval- S/2 d/2 (2.5 % ET) twice in the tapping period

T4- S/3U d2 (5% ET) 45 days interval- S/2 d/2 (2.5 % ET) twice in the tapping period

in T1 (87.69 g/t/t) followed by T2 (85.03 g/t/t), T3 (80.30 g/t/t) and the lowest was in T4 (75.15 g/t/t) (Table Tura. 1).

Comparison of yield and yield parameter between normal and shallow tapping was initiated with RRIM 600 clone and 100 plants selected for normal and shallow tapping system. Latex volume and DRC were recorded. Results showed that maximum volume of latex (143.1 ml/t/t) was recorded in normal tapping and minimum shallow tapping (103.22 ml/t/t) while higher DRC (29.01%) was recorded in shallow tapping and minimum in normal tapping (27.51%).

An experiment on location-specific stimulant application of ethylene-induced stress responses in the tapping panel of the *Hevea* trees has been initiated with the objective of reducing the ethylene-mediated

stress responses in tissues in the tapping panel by applying the ethylene compounds away from the tapping area without compromising the latex yield under the agro-climatic conditions of Garo Hills. RRIM 600 clone has been selected for the study with eight treatments and with bark applications of five per cent ethephon (three times per year). Initial result indicated that bark application of five per cent ethephon (above 125 cm from bud union and on the bud union) showed maximum volume of latex while the lowest in without application of ethephon.

3. Crop management

3.1. Nutritional studies (On-farm trial at Borgang, Assam)

Treatmentwise soil samples were collected and analysed for available

Table Tura. 2. Effect of N, P and K combinations on growth, yield, total mean volume and DRC in the Central Brahmaputra Valley zone (CBVZ) of Assam during mature phase (2010-11)

Treatment combination (N:P:K) kg/ha	Girth (cm)	Girth increment (cm)	Yield (g/t/t)	DRC (%)	Total volume of latex (ml/t/t)
T1 (0:0:0)	69.61	0.87	21.82	32.72	68.0
T2 (0:0:15)	70.74	1.05	23.72	32.89	73.6
T3 (0:15:0)	65.45	1.22	25.6	33.04	79.0
T4 (15:0:0)	71.61	1.36	29.17	33.22	89.6
T5 (0:0:30)	70.81	1.43	32.21	33.51	98.0
T6 (0:30:0)	73.92	1.63	35.08	33.65	106.2
T7 (30:0:0)	75.93	1.66	37.60	33.85	113.2
T8 (15:15:15)	74.60	1.84	40.68	33.96	122.0
T9 (30:15:30)	78.98	2.08	45.05	34.20	134.6
T10 (30:30:30)	79.46	2.08	50.05	34.39	149.0
T11 (45:15:30)	81.65	2.18	52.70	34.56	155.8
T12 (45:30:45)	81.43	2.37	58.27	34.90	170.8
T13 (60:15:30)	81.76	2.46	62.88	35.13	183.0
T14 (60:30:30)	82.62	2.62	67.59	35.43	195.2
T15 (60:30:45)	87.50	2.82	72.75	35.76	208.6
SE	2.35	0.046	0.67	0.047	2.069
CD (P = 0.05)	6.82	0.13	1.94	0.136	5.99

nutrients. Monthly recording of yield (g/t), DRC (%) and total volume (ml/t) were undertaken. Results indicated that maximum girth (87.50 cm), girth increment (2.82 cm), yield (72.75 g/t), DRC (35.76%) and latex volume (208.6 ml/t) were recorded under the treatment combination of $N_{60}P_{30}K_{45}$ kg/ha and minimum was $N_{10}P_{10}K_{10}$ (Table Tura. 2). Improvement of fertility status of soil with application of NPK fertilizers was observed.

3.2. Soil moisture retention characteristics in the rubber growing area of Meghalaya

Soil samples were collected each month at 0-15, 15-30 and 30-60 cm for soil moisture

study. Soil moisture content showed increasing trend with increasing the depth of soil in all the months. Soil moisture content was maximum during October and minimum in February (Table Tura. 3).

3.3. Analytical/Advisory work for fertilizer recommendation

Forty six soil samples were collected from the rubber growing areas and were analyzed. Results indicated that in the surface soil (0-30cm) OC content was in the medium range (1.34- 1.47 %), available phosphorus in the low range (0.28-0.42 mg/100g) and available K in the medium range (7.3-8.0 mg/100g). The soil is acidic in nature with pH ranging from 4.52- 5.57. Based on these, fertilizer recommendations were given to the growers.

For obtaining the distribution of soil at Garo Hills of Meghalaya, 302 soil-samples were collected from the rubber growing area of Meghalaya pH. Soil pH varied from 3.97 to 5.41 with a mean value of 4.94. About 5.3 per cent samples were extremely acidic (soil pH 4.0 - 4.5), 63.6 per cent very strongly acidic (soil pH 4.5-5.0), 28.8 per cent strongly acidic (soil pH 5.1-5.5) and 2.3 % soils medium acidic (soil pH 5.6-6.0) (Table Tura. 4).

Table Tura. 3. Monthly variations in soil moisture content (%) at RRS, Ganolgre farm

Month	Soil moisture content (%)		
	0-15 cm	15-30 cm	30-60 cm
	depth	depth	depth
April, 2010	18.38	19.37	19.80
May	24.92	26.04	26.72
June	26.10	26.96	27.70
July	27.67	27.96	29.41
August	26.89	27.31	27.97
September	29.39	29.81	30.90
October	29.63	30.49	30.78
November	24.56	25.17	26.24
December	22.79	23.24	24.26
January, 2011	18.25	18.33	19.35
February	17.15	17.91	18.14
March	19.85	20.86	21.79
Mean	23.79	24.45	25.25
SE	1.29	1.30	1.32
SD	4.31	4.32	4.38
CV (%)	18.11	17.66	17.34

Table Tura. 4. Distribution of soil pH (0-60 cm depth) in rubber growing soils of Meghalaya

Soil acidity category	pH range of soil	No. of soil samples	% of soil samples
Extremely acidic	> 4.0	2	0.67
	4.1 - 4.5	14	4.63
Very strongly acidic	4.5 - 5.0	192	63.6
Strongly acidic	5.1 - 5.5	87	28.8
Medium acidic	5.6 - 6.0	7	2.31
Total		302	

REGIONAL EXPERIMENT STATION NAGRAKATTA, WEST BENGAL

1. Crop improvement

1.1. Evaluation of clones

Multidisciplinary clone evaluation trials were initiated at RES, Nagrakatta, Jalpaiguri, West Bengal to screen the clones for growth and yield performance. Data was recorded in four trials and the results on growth during 2010-11 are shown in Table Nag. 1. In trial I, the girth was the highest in SCATC 93/114 followed by RRII

118 and Haiken 1 compared to rest of the clones. Similarly, RRII 612 showed significantly higher girth in trial II than the check clone RRII 105 followed by RRII 605. In trial III, SCATC 93/114 scored significant higher girth than the check clone RRII 600 and in trial IV, compared to the check clone RRII 600, none of the clones was superior.

In terms of average yield (Table Nag. 2), the PB 311, SCATC 93/114 and SCATC

Table Nag. 1. Girth in different clone trials

Trial I	Girth (cm)	Trial II	Girth (cm)	Trial III	Girth (cm)	Trial IV	Girth (cm)
PB 5/51	66.78	RRII 105	64.02	PB 260	63.02	RRIC 104	60.04
RRII 203	67.36	GL 1	64.60	RRII 208	64.11	RRII 308	60.68
PB 311	68.32	PR 107	70.10	PB 310	66.24	RRII 105	62.17
GT 1	70.47	PB 86	70.51	PB 86	66.74	RRII 300	63.74
SCATC 88/13	70.98	RRII 208	70.91	PR 107	66.88	RRII 208	64.17
RRII 300	71.96	RRIM 605	75.98	PB 235	67.31	PR 261	64.56
RRIM 703	71.96	RRIM 612	86.77	RRIM 600	67.74	PB 235	65.61
PB 235	72.43			RRIC 102	69.89	RRIM 600	66.71
Haiken 1	77.26			HK 1	70.01	Haiken1	68.11
RRII 118	78.96			RRIM 612	71.88	PB 280	69.20
SCATC 93/114	79.54			SCATC 93/114	75.20	SCATC 93/114	70.69
CD (P = 0.05)	4.43	CD (P = 0.05)	9.78	CD (P = 0.05)	3.46	CD (P = 0.05)	3.40

Table Nag. 2. Pattern of yield in different clone trials

Trial I	Yield (g/t/t)	Trial II	Yield (g/t/t)	Trial III	Yield (g/t/t)	Trial IV	Yield (g/t/t)
PB 5/51	27.67	GL 1	31.33	PB 260	26.98	RRIC 104	32.72
Haiken1	33.79	PR 107	36.39	PR 107	28.99	SCATC 93/114	32.87
RRII 300	34.38	RRIM 612	39.92	SCATC 93/114	29.14	RRII 300	40.95
RRII 203	37.46	RRII 105	41.49	RRIM 612	29.26	RRIM 600	43.10
RRII 118	43.36	PB 86	42.71	RRIC 102	29.46	RRII 308	44.10
RRIM 703	43.42	RRIM 605	50.99	PB 86	31.58	PB 235	44.76
GT 1	44.44	RRII 208	55.21	Haiken1	32.53	PR 261	44.79
SCATC 88/13	44.81			PB 235	37.83	RRII 105	49.72
SCATC 93/114	47.15			RRIM 600	39.54	Haiken1	52.29
PB 311	47.20			PB 310	41.53	PB 280	53.40
PB 235	47.73			RRII 208	41.54	RRII 208	59.40
CD (P = 0.05)	6.65	CD (P = 0.05)	11.60	CD (P = 0.05)	5.50	CD (P = 0.05)	8.14

Table Nag. 3. Growth and yield in different accession of *Hesoa*

Accession	Girth (cm)	Yield (g/t/t)	Accession	Girth (cm)	Yield (g/t/t)
AC 1950	61.16	18.52	RO 2629	63.97	11.35
AC 607	36.80	7.26	RO 2635	70.14	3.96
AC 619	62.76	6.08	RO 2890	74.99	9.63
AC 623	46.32	3.00	RO 3172	76.09	4.36
AC 68	61.68	4.83	RO 5329	57.28	17.12
AC 763	53.40	26.18	RO 5348	72.86	9.61
MT 196	64.12	26.42	RO 5363	63.31	57.64
MT 2229	67.40	12.88	RO 5408	57.29	7.98
MT 2594	52.94	8.87	RO 5430	66.20	15.21
MT 44	66.32	10.94	RO 5557	69.29	12.65
RR11 105	60.12	53.74	RO 6139	53.68	6.83
		CD ($P = 0.05$)			1.28
					8.38

88/14 scored high rank in trial I. In trial II, significantly higher yield was shown by RR11 208 compared to check clone RR11 105. The clone RR11 208 and PB 310 showed higher yield in trial III and in trial IV, RR11 208, PB 208 and Haiken 1 showed significantly higher yield than the check clone RR11 600, none of the clones was superior.

1.2. Evaluation of germplasm

Evaluation of germplasm for cold

tolerance with 21 wild accessions was continued. Maximum girth (Table Nag. 3) was found in RO 3172 followed by RO 2890 while the control clones RR11 105 showed comparable girth. In terms of yield, among the accessions, RO 5363 showed the highest yield followed by AC 763 and MT 196.

1.3. Performance of polyclonal seedlings

Since 1990, polycross seedling trees are being evaluated for their performance. Mean girth during the reporting period was

Table Nag. 4. Effect of N, P and K on girth (cm) and yield (g/t/t)

Combination	Girth	Yield	Combination	Girth	Yield	Combination	Girth	Yield
N0P0K0	70.84	39.46	N1P1K0	66.17	39.34	N2P2K0	73.72	55.26
N0P0K1	69.25	42.11	N1P1K1	74.73	42.98	N2P2K1	69.61	35.86
N0P0K2	70.12	49.49	N1P1K2	70.61	41.33	N2P2K2	70.98	37.33
N0P1K0	74.33	52.35	N1P2K0	69.43	41.58	N3P0K0	68.86	44.22
N0P1K1	70.12	42.63	N1P2K1	71.14	37.18	N3P0K1	75.19	44.11
N0P1K2	69.81	45.41	N1P2K2	71.82	38.48	N3P0K2	72.19	46.99
N0P2K0	71.95	44.35	N2P0K0	69.91	43.49	N3P1K0	72.44	41.93
N0P2K1	68.89	38.33	N2P0K1	65.89	40.53	N3P1K1	68.82	44.66
N0P2K2	71.38	42.84	N2P0K2	71.57	43.24	N3P1K2	72.78	55.72
N1P0K0	65.19	35.58	N2P1K0	70.84	39.39	N3P2K0	74.52	47.74
N1P0K1	69.49	45.41	N2P1K1	71.18	40.12	N3P2K1	74.37	41.55
N1P0K2	70.72	47.58	N2P1K2	68.99	40.98	N3P2K2	68.67	38.79
		CD ($P = 0.05$)			1.07			2.84

Table Nag. 5. Growth and yield of crops

Treatments	Spacing	Tea yield (kg)	Rubber girth (cm)	Rubber yield (kg/tree/yr)
T1 (Pure Rubber)	Rubber - 5 x 5m		62.8	1.42
T2 (Rubber + Tea)	Rubber - 10 x 2.5m Tea - 10x(1.0 x 0.6)m	2.65	69.2	1.03
T3 (Rubber + Tea)	Rubber - 12 x 2.5m Tea - 12x(1.0 x 0.6)m	3.32	57.4	0.895
T4 (Rubber + Tea) (paired row)	Rubber - 18x(3x3)m; 2rows Tea - 18x(1.0 x 0.6)m	5.15	57.6	1.00
T5 (Rubber + Tea)	Rubber - 10x 5.0m Tea - 10x(1.0 x 0.6)m	3.96	61.0	0.36
T6 (Pure Tea)	Tea - 1.0 x 0.6m	13.7		

65.98 cm with average block yield of 4.1 kg/tree/year. Among the existing 178 plants, 59% plants showed above average yield.

2. Crop management

2.1. Nutritional trial

Experiment is being continued to find out the optimum requirement of fertilizer doses for *Hevea* at RES, Nagrakata. The doses of nitrogen were 0, 15, 30 and 45 kg/ha. The doses of phosphorus and potassium were 0, 20 and 40 kg/ha/year. Significantly higher girth was found in the N45 P20 K40 kg/ha combination (Table Nag. 4). In terms of yield, N45 P20 K40 kg/ha showed positive effect on yield followed by N30 P40 K0 kg/ha combination.

2.2. Cropping system

With an aim to introduce rubber as shade tree inside tea garden, rubber trees were interplanted in tea blocks in the Dooars area of West Bengal. The data showed that green tea leaf yield of pure tea plot was significantly higher than the rest of the combinations. Lower green tea leaf yield in rubber tea combination treatment was

mainly due to more shade imposed by mature rubber tree canopy that attracts pests *viz.*, *Helopeltis*, red-spider etc. The rubber girth (Table Nag. 5) in T2 was higher than pure rubber plot. The yield of rubber was higher in T1 followed by T2 and T4 whereas in T5 it was very low.

3. Crop physiology

3.1. Performance of polycross progeny raised from seeds of locally adapted mature rubber plantation

Polycross rubber seeds procured from NE India (Nagrakata, Jalpaiguri; Sarutari, Assam; Genoligiri, Tura) and Kerala (HBSS, Kanyakumari) were raised in the non-traditional environment of Dooars area of

Table Nag. 6. Growth characteristics of *Hevea* seedlings raised from seeds developed in different climatic conditions

Source of seeds	Girth (cm)	Girth increment (cm/yr)	Height (cm)
Nagrakata	14.0	12.2	185
Kanyakumari	18.3	15.7	276
Guwahati	13.5	12.1	145
Tura	14.8	13.0	193

West Bengal in 2008 (Table Nag. 6). The data showed that the average girth and girth increment of seedlings from Kanyakumari stock was higher compared to that of Jalpaiguri and Tura. Data on height also followed the same order.

3.2. Performance of rubber clones in abandoned tea growing areas of Dooars belt of North Bengal

An attempt was made to introduce rubber in abandoned tea growing areas of

sub-Himalayan, West Bengal where the soil pH is high (above 8.0). Four clones, viz. RRIM 600, RR11 208, RR11 105 and RR11 429 were planted in blocks, soil having pH of 8.3. A block of hundred plants from each clone per hectare (total of 400 plants) were planted. However, 90% of the plants were fully damaged by the wild elephants as the area was the passageway of the animals. The experiment in this area was discontinued as existing plant number was very low (below 10%).

REGIONAL RESEARCH STATION DAPCHARI, MAHARSHTRA

The thrust area of the station is to develop suitable clones and location-specific agrotechnology for drought-prone areas. The experiments on crop improvement include screening of wild *Hevea* accessions, evaluation of clones, different genotypes such as polyclone, pipeline clones, selected ortets and wild *Hevea* accessions for growth and yield performance. Studies on environmental physiology (irrigation requirement and irrigation methods, drought studies) and crop management (practices to mitigate the drought like soil moisture conservation) are also being carried out.

1. Environmental physiology

1.1. Drip and basin method of irrigation

In order to identify clones having physiological tolerance to water stress and high temperature, two experiments based on irrigation scheduling and methods of irrigation are in progress.

An irrigation experiment was started during 1987 for ETC-based irrigation scheduling in two methods of irrigation (basin and drip) with the objective to standardize and evaluate the advantages of drip irrigation over basin irrigation in terms of water saving and total economy in the quantity of water. The treatments comprises of 1.00, 0.75 and 0.50 ETC in basin

Table Dap. 1. Effect of different irrigation methods and irrigation scheduling on girth and yield of rubber

Treatment	Girth (cm)	Yield (g/t/0)
Control (Rainfed)	64.10	40.56
Basin 1.00 ETC	74.26	52.13
0.25 ETC ^a	69.88	45.86
0.50 ETC	70.59	40.04
Drip 0.75 ETC	69.94	46.56
0.25 ETC ^{a*}	69.11	41.17
0.25 ETC	66.79	36.31
SE ^a	1.78	1.87
CD (P = 0.05)	3.89	4.07

^achanged from 0.75 ETC to 0.25 ETC; ^{a*} changed from 0.50 ETC to 0.25 ETC

and 0.75, 0.50 and 0.25 ETc in drip method of irrigation. From February 2000 onwards, the 0.75 ETc in the basin and 0.50 ETc in the drip were reduced to 0.25 ET in order to find out whether irrigation requirement can be further reduced. The results indicated that, irrigation scheduling at 1.00 ETc with basin irrigation method recorded better growth and yield (74.26 cm, 52.13 g/t/t) than other treatments but was on par with irrigation scheduling at 0.50 ETc (70.59 cm girth, 40.04 g/t/t) under the same method of irrigation (Table Dap.1). Trees under different levels of basin irrigation showed higher girth than drip system. The same trend was noticed in yield also.

The cost evaluation was done to find out the expenses incurred towards various inputs, farm practices and irrigation. The irrigated trees were divided into two parts according to soil depth. Among them, one being maintained under reduced irrigation of 1/5th ETc (deep soil) and another under 1.0 ETc (shallow soil) level of irrigation. Recorded block yield, total latex volume and

DRC. The reduced level of irrigation was further reduced from 1/4th to 1/5th to find out the optimum irrigation requirement for mature trees in good soil depth area. The results indicated (Table Dap. 2) that the trees under the reduced level of irrigation in deep soil shows better growth and yield than the rainfed ($A_v = 23.05$ g/t/t) and with higher level of irrigation (1.0 ETc).

2. Latex harvest technology

One demonstration trial was initiated on CUT during 2009 in 1983 planted RRII 105 with current tapping on BI 1 panel in randomized block design. The objective is to identify CUT practice suitable to this region along with the enhancement of yield during low yielding phase (regenerated bark) of clone RRII 105, and to optimize the stimulation schedule for further reduction of cost of production and to better economic life without stress to the trees. Treatments T1 – S/4U d3 6d/7+ ET 5% La + stimulation once in 3 weeks, T2 – S/3U d3 6d/7+ ET 5% La + stimulation three weeks interval, T3 – S/4U d3 6d/7+ ET 5% La + stimulation monthly interval, and T4 – S/3U d3 + d3 6d/7+ ET 5% La + stimulation monthly interval. Normal tapping was the practice during rainy months. Observation on yield recorded through cup lump of all tapping days) and monthly DRC% (from one week before stimulation), annual TPD % and girth increment was measured.

Results show that, treatment T2 - S/3U d3 6d/7+ ET 5% La with application of ethephon once in three weeks recorded significantly higher yield of 74.59 g/t/t (1999 kg/ha) than the other treatments. Lower yield was recorded under T3 – S/4U d3 system with stimulation of once in a month (59.98 g/t/t). Results revealed that, following

Table Dap. 2. Effect of depth and irrigation on yield of rubber (g/t/t)

Month	Yield (g/t/t)		
	Group A (Rainfed)	Group B 1/5 ETc	Group C 1.0 ETc
April	8.75	43.63	29.59
May	6.11	31.24	22.51
June	5.78	29.61	22.35
July	11.99	39.16	29.59
August	13.48	45.51	28.67
September	20.33	53.52	40.35
October	24.87	63.39	47.86
November	30.74	92.34	69.20
December	41.29	107.29	69.41
January	53.43	103.31	66.50
February	32.12	18.24	12.76
March	27.74	28.19	17.43

Table Dap. 3. Yield response of Low Frequency Controlled Upward Tapping and basal tapping in clone RR11 105

Treatment	CUT (April-June 2010 and November 2010-March 2011)				Basal Panel (July 2010 to October 2010)			
	Tapping days	g/t/t	kg/ha	kg/tree	Tapping days	g/t/t	kg/ha	kg/tree
T1 - S/4U d3 6d/7+ET 5% La + (3 w)	67	62.62	1678	4.20	34	43.20	587	1.46
T2 - S/3U d3 6d/7+ ET 5% La + (3 w)	67	74.59	1999	5.00	34	47.54	647	1.61
T3 - S/4U d3 6d/7+ ET 5% La + (m)	67	59.98	1607	4.00	34	39.51	537	1.34
T4 - S/3U d3 + d36d/7+ET 5% La + (m)67		60.55	1622	4.05	34	44.88	610	1.52
SE +		6.47				4.83		
CD ($P = 0.05$)		13.75				10.3		

S/3U d3 tapping system with application of ethephon once in three weeks proved promising (Table Dap. 3). In general, CUT recorded high yield in all the treatments tested than the normal basal panel tapping (S/2 d3 6d/7+ ET 2.5% La + (m)).

208, RR11 308, RR11 605, PB 260, PB 310, PB 311, RR1C 100, RR1C 102, RR1C 105, PR 255, PR 261 and RR11 105) and to select suitable high yielding and drought tolerant clone for North Konkan region was in progress. All the clones under this trial were maintained under restricted irrigation since 1985.

3. Crop improvement

Development of drought tolerant clones and screening of wild *Hevea* accessions for drought tolerance and future evaluations of modern clones, ortet selection, clones from half-sib progeny of prepotent clones along with 400 series clones continued to be the thrust areas of research. A total of 10 experiments are being conducted with these objectives. The experiment on identification of reliable juvenile and mature characteristics for clone identification (50 divergent clones) in *Hevea*: standardization of distinctiveness, uniformity and stability (DUS) testing norms for evolving specific guidelines for varietal registration in rubber was also initiated.

3.1. Clone evaluation trial

The clone evaluation trial started in 1985 to evaluate growth and yield performance of 15 clones (RR11 5, RR11 6, RR11

Table Dap. 4. Growth and yield of *Hevea* clones

Clones	Girth (cm)	Yield (g/t/t)	Projected Yield (Kg/ha)
RR11 5	63.2	36.4	1203
RR11 6	67.0	41.8	1382
RR11 105	59.8	35.0	1157
RR11 208	68.4	46.5	1537
RR11 308	60.5	20.5	678
RR11 605	62.7	26.0	859
PB 260	64.3	27.7	916
PB 310	64.7	30.5	1008
PB 311	62.6	31.9	1055
RR1C 52	69.2	20.5	678
RR1C 100	62.4	34.0	1124
RR1C 102	63.5	32.8	1084
RR1C 105	60.5	20.9	691
PR 255	62.8	32.2	1065
PR 261	60.1	24.8	820
SE	1.96	1.03	
CD ($P = 0.05$)	4.03	3.25	

Monthly girth, DRC and fortnight yield was recorded. Results indicated that, RRIC 52 recorded higher girth of 69.2 cm than RRII 105. RRII 208 recorded higher yield of 46.5 g/t/t which was followed by RRII 6 and RRII 5 (Table Dap. 3).

3.2. Ortel selection

The large-scale evaluation of 14 ortets of Dapchhari along with control clones (RRII 105, RRII 208, RRII 430 and RRIM 600) was started during 2008 to evaluate the growth and yield performance of ortets selected from polycross seedling planted at this station. OS 173 recorded significantly higher girth and height than clone RRII 105 and RRII 430. The highest number of whorls (3.5) was recorded in OS 42 and OS 111 (Table Dap. 4).

Table Dap. 4. Growth of different ortets under Dapchhari

Ortet	Girth (cm)	Height (cm)	No. of whorls
OS 1	9.5	333.8	2.7
OS 8	8.1	306.3	2.7
OS 34	11.1	378.2	2.9
OS 35	10.4	369.2	3.0
OS 36	9.2	304.1	3.2
OS 37	9.5	364.4	2.9
OS 42	9.1	304.5	3.5
OS 111	10.3	367.0	3.5
OS 135	8.6	306.8	3.0
OS 136	7.1	300.4	2.2
OS 173	11.6	356.7	3.0
OS 216	10.3	358.5	3.1
OS 236	9.9	338.3	3.1
OS 317	10.6	367.9	2.8
RRII 105	8.2	279.4	2.3
RRII 208	9.5	329.0	3.4
RRII 430	8.5	279.6	2.6
RRIM 600	10.1	411.5	3.3
SE	1.07	42.33	0.33
CD (P = 0.05)	2.18	NS	0.67

3.3. Germplasm screening

Wild *Hevea* accessions (130) and RRII 105, RRIM 600 and Tjir 1 as a check clones were planted for screening against drought tolerance. The observations on pre and post drought growth and RWC (%) were recorded. The accessions showed wide variability in all characters studied. It was found that Mato Grosso accessions were superior for all the growth characters studied than those from Rondonia and Acre provenances. Among the control clones, RRIM 600 and RRII 208 were superior to RRII 105. Twenty five potential drought-tolerant accessions were identified based on 3-4 years observations and further detailed studies are in progress.

3.4. Further evaluation of selected wild *Hevea* accessions

A total of three experiments comprising of 25, 47, 11 accessions are being conducted. Field evaluation of 25 wild *Hevea* clones along with five HP clones and RRII 105, RRIM 600, Tjir 1, RRII 430, RRII 208 as a check for drought tolerance (2007) was under progress. Observations showed wide variability for all characters studied.

Two small-scale field evaluation trials of selected wild accessions (47 and 11) for drought tolerance planted in 2010 are also under observation.

3.5. Evaluation half-sib progeny, polycross, half-sib progeny of prepotent clones, pipeline clones

A total of four clonal nursery evaluation trials are in progress.

4. Crop management

Project on effect of certain moisture conservation practices on rubber plantation under su-humid region of Maharashtra has

been started during 2008 with the view to find out the effect of vertical mulching and Kaoline spray (6%) on growth and yield of rubber. The treatment comprises soil moisture conservation practices (vertical

mulching and Kaoline spray 6%) and control. The results showed that, there was no significant difference between the treatments in terms of girth and plant height.

REGIONAL RESEARCH STATION DHENKANAL, ORISSA

The Regional Research Station situated in Dhenkanal district of Orissa state represents dry sub-humid climate. The station continued its research activities on crop improvement with the particular objective of identifying clones suited to prevailing drought conditions of this region.

1. Crop improvement

Crop improvement includes five ongoing trials mainly on evaluation of clones and polyclonal population. The trials were laid out to screen most adapted and high yielding clones under the dry-sub humid climate.

1.1 Clone evaluation

In the 1987 experiment, both GT 1 (75.2 cm) and RRIM 600 (71.9 cm) recorded significantly higher girth over RR11 105 (67.9 cm). RRIM 600 has recorded mean yield of

33.8 g/t/t while GT 1 recorded (33.1 g/t/t) the lowest yield (Table Ori. 1).

In the clone trial 1990, the highest girth was recorded in SCATC 93/14 (86.8 cm), RRIM 600 (78.7 cm) and RR11 208 (78.3 cm). The highest yield of 61.1 g/t/t was noted in RR11 208 followed by SCATC 88-113 (60.0 g/t/t) and RRIM 600 (47.6 g/t/t), while the SCATC 93-114 (32.6 g/t/t) and RR11 300 (41.9 g/t/t) recorded the most poor yield performance.

In another clone trial (1991), the performance of *Hevea* clones with polyclonal seedlings was compared. Clone GT 1 (86.8 cm), RR11 208 (81.4 cm) and RRIC 102 (79.7

Table Ori.1. Mean girth and yield of elite clones

Clone	Girth (cm)	Yield (g/t/t)
	March 2011	
RR11 105	67.9	35.7
RRIM 600	71.9	33.8
GT 1	75.2	33.1
Mean	71.7	34.2
SE	0.96	2.08

Table Ori. 2. Performance of various clones

Clone	Girth (cm)	Mean yield (g/t/t)
	March 2011	
RR11 5	77.4	42.8
RR11 105	75.9	51.9
RR11 208	81.4	68.9
RR11 300	79.9	44.5
RRIC 102	79.7	62.5
RRIM 600	73.4	45.3
GT 1	86.8	48.9
PR 255	80.8	40.9
PR 261	75.5	48.9
Polyclonal	97.4	42.9
Mean	80.8	49.7
SE	1.83	3.65

cm) showed superior growth. However, polyclonal seedlings with a mean girth of 97.4 cm exhibited better growth and adaptability as compared to other clones. RRII 208 recorded higher mean yield (68.9 g/t/t) followed by RRIC 102 (62.5 g/t/t). In this trial also, RRII 208 had higher growth, yield and adaptability in the region. RRII 5 showed lowest yield of 42.8 g/t/t among the clones (Table Ori. 2).

Table Ori. 3. Performance of various clones

Clone	Girth (cm) March 2011	Mean yield (g/t/t)
RRII 5	40.3	21.3
RRII 105	46.3	25.7
RRII 208	49.8	18.2
RRII 300	53.3	23.4
RRII 351	45.5	22.8
RRII 352	47.3	21.7
RRII 357	46.1	19.3
RRII 28/59	48.9	20.9
RRIM 600	49.0	27.8
IRCA 109	38.5	26.9
IRCA 111	50.7	22.5
Mean	46.8	22.7
SE	1.45	0.98

In the clone trial 1999 for evaluation of modern clones, the highest mean girth was recorded in RRII 300 (53.3 cm), RRII 208 (49.8 cm) RRIM 600 (49.0 cm) followed by and RRII 352. The lowest girth was recorded in RRII 5 (40.3 cm). The Highest initial mean yield for few months was observed in RRIM 600 (27.8 g/t/t) followed by IRCA 109 (26.9 g/t/t) and RRII 105 (25.7 g/t/t) (Table Ori. 3).

1.2. Polyclonal seedling evaluation

In a polyclonal seedlings trial 1989, trees are under evaluation for growth, yield performance and adaptability in Orissa conditions. The highest mean girth was recorded in OR 4 (126.5 cm), followed by OR 5 (116.5 cm). The highest annual mean yield was recorded in OR1 (98.1 g/t/t) followed by OR 2 (86.5 g/t/t). Ten elite polyclonal trees have been selected and multiplied for further field evaluation.

1.3. Ortets evaluation

In an ortets evaluation trial planted during 2008, ten ortets with few modern clones were under observation. Ortets OR7 (15.9 cm), OR8 (15.9 cm) and the chinese clone SCATC 93/114 (13.8 cm) showed better growth in juvenile phase.

REGIONAL RESEARCH STATION PADIYOOR, KANNUR

The station continued with the research programs for identification of clones suited to the region and evaluation of clonal tolerance to drought and disease incidence. Field trials on agromanagement practices for reduction of the gestation period in rubber are also in progress.

1. Crop management

1.2. Water requirement studies

The experiment on irrigation in immature rubber with irrigation levels at IW/CPE ratio of 0.3, 0.6, 0.9, 1.2 and an unirrigated control indicated significantly

Table Pad. 1. Effect of irrigation on growth

Treatment (IW/CPE)	Girth (cm)	Yield (g/t)
	(tenth year)	Jan-May
1.2	59.0	36.2
0.9	57.8	39.6
0.6	60.8	45.0
0.3	56.8	41.4
Control	54.2	38.0
CD (P = 0.05)	3.9	NS

higher girth of the irrigated plants over the control plants with the highest girth recorded at IW/CPE of 0.6. Observations recorded during the first year of tapping showed no significant difference with respect to per tree yield (Table Pad. 1)

1.3. Response to applied fertilizers in high yielding clones

The experiment laid out with three clones (RRII 105, RRII 414, RRII 429) and four fertilizer levels (30:30:20, 60:30:20, 90:60:40

Table Pad. 2. Effect of fertilizer on growth

Treatment	Girth (cm)		
	RRII 105	RRII 429	RRII 414
30:30:20	50.2	42.0	49.5
60:30:20	45.2	46.9	51.5
90:60:40	43.7	42.0	52.5
120:60:40	47.7	44.5	51.2
CD (P = 0.05)	NS		

and 120:60:40 kg/ha of N, P₂O₅ and K₂O) indicated no significant response in growth of the different clones with varying levels of fertilizer applied (Table Pad. 2).

2. Crop improvement

2.1. Large-scale evaluation of clones

In the 1996 planted clone trial with 11 clones significant growth differences were observed with PB 330 and IRCA 130 being significantly superior to RRII 105 with respect to girth while IRCA 130 showed

Table Pad. 3. Growth performance of modern Hevea clones

Clone	Girth (cm)	Yield (g/t)	Summer yield (g/t)
RRII 105	60.1	56.4	22.9
PB 314	61.7	45.1	23.5
IRCA 130	65.3	74.3	53.8
PB 28/59	59.7	50.2	27.1
IRCA 109	58.4	36.4	23.0
PB 330	65.6	49.7	23.3
IRCA 18	64.2	42.9	17.0
RRIM 703	53.7	37.7	20.9
IRCA 111	58.4	36.8	21.6
PB 255	60.4	63.5	42.2
IRCA 230	61.4	42.9	22.4
CD (P = 0.05)	4.5	12.3	11.0

superiority over RRII 105 with respect to average annual yield and summer yield (Table Pad. 3). The summer yield of PB 255 was also found to be significantly higher than that of RRII 105.

2.2. Evaluation of rubber clones/selections at high altitude situations

The trial field planted in 1996 under high altitude conditions (974 m MSL) with

Table Pad. 4. Growth and yield in high altitude area

Clone	Girth	Yield (g/t)
		May-Aug
P 296	53.35	23.68
RRII 105	45.66	29.87
RRII 203	63.29	54.08
P 90	48.63	19.11
P 270	59.9	37.38
P 280	51.29	12.61
P 2	44.94	16.01
RRIC 102	49.02	17.42
RRIC 101	51.24	43.42
PB 86	62.05	44.51
RRIC 100	45.43	13.88
P 121	57.19	29.51
P 213	53.58	25.48
P 1	44.38	17.39
P 155	55.79	31.25
Intity		
CD (P = 0.05)	11.03	6.9

10 selections and 5 clones indicated the significant superiority of the clones RRII 203 and RRIC 100 and the selections P 270 and P 213 over that of RRII 105 with respect to girth. (Table Pad.4). Yield of RRII 203 was found to be significantly superior to other

clones and selections. Yield of RRIC 100 and PB 86 were on par. The selections P 270 and Iritty gave significantly higher yields than the other ortets tested. The yield of P 270 was significantly higher than that of RRII 105 and was on par with that of Iritty.

HEVEA BREEDING SUB-STATION KADABA, KARNATAKA

The major thrust areas of research in the station are to evaluate clones under different biotic and abiotic stress factors and to identify clones suitable for commercial cultivation.

1. Crop improvement

1.1. Small-scale trials of selected ortet clones

Three trials (1988A, 1988B and 1988C) are in progress. All the trials were planted in 1988 with the objective to evaluate selected ortet clones along with popular clones as controls. In the first trial (1988A), 15 ortet clones and three control clones are under evaluation. The ortets are: T 2, C 1/2, C 42, O 17, C 70, O 15, O 41, O 34, O 47, O 44, O 46, C 7/2, O 50, O 45 and O 19 and the control clones are: GT 1, RRIM 600 and RRII 105. Tapping in the trial was started in 2002. Mean yield over nine years of tapping indicated T2 to be the highest yielder with 73.7 g/t/t closely followed by the ortets namely O 17 (69.1 g/t/t) O 15 (66.1 g/t/t). Yield of control clones GT 1, RRII 105 and RRIM 600 was 65.9, 49.5 and 47.9 g/t/t respectively. Sixteen ortet clones and three control clones namely RRII 105, RRIM 600 and GT 1 are

under evaluation in the second trial (1988 B). After nine years of tapping high yielding clones were T1 (70.0 g/t/t) followed by GT 1 (70.0 g/t/t). The third trial (1988C) consists of 14 ortet clones and three control clones namely RRII 105, RRIM 600 and GT 1. Nine years of tapping indicated maximum yield in GT 1 (85.1 g/t/t) followed by ortet C 140 (77.1 g/t/t), RRII 105 (68.6 g/t/t). The other control clone RRIM 600 yielded 31.6 g/t/t. Other ortets with notable yield were O 49 (67.7 g/t/t), O 26 (67.0 g/t/t) and O 55 (65.2 g/t/t).

1.2. Large-scale clone trial

This trial planted in 1989, has 14 clones under evaluation. The clones are RRII 203, KRS 25, KRS 163, KRS 128, RRII 105, PB 255, SCATC 88-13, RRII 308, PR 255, Haiken 1, PR 261, RRIM 600, RRII 300 and SCATC 93-114. After nine years of tapping, maximum yield was in clone RRII 203 (67.5 g/t/t) followed by KRS 25 (57.6 g/t/t). Check clones RRII 105 and RRIM 600 respectively yielded 47.4 and 33.8 g/t/t.

1.3. Large-scale clone trial

In this experiment planted in 1990, 15 clones are under evaluation. The trial

plants are 21 years old and have completed seven years of tapping. Maximum yield was recorded for clone PB 260 (62.0 g/t/t) closely followed by PB 235 (59.9 g/t/t) and HP 372 (57.1 g/t/t). Tjir 1 was the lowest yielder (19.4 g/t/t).

1.4. Estimation of genetic parameters

This trial planted in 1990 has the aim to evaluate parents and their progenies for estimating genetic parameters that are essential for planning plant breeding activities. Twelve clones and their progenies are under evaluation. On completion of eight years of tapping parent clones PB 235, RR11 203 and RR11 105 were the leading in yield with a yield of 77.6, 54.9 and 50.8 g/t/t, respectively. Among the progenies, half-sibs of RR11 203, PB 235 and GT 1 recorded a yield of 45.1, 41.7 and 41.1 g/t/t, respectively. Progenies of PB 86, PB 213 and Tjir 1 were low yielding with a yield of 28.7, 23.9 and 21.5 g/t/t, respectively.

1.5. Small-scale clone trials of popular clones (1991A, 1991B and 1991C)

Three small-scale trials of popular clones were initiated in 1991. A total of 54 trial clones and three control clones are under evaluation in three trials to compare the growth and yield of trial clones vis-à-vis control clones namely, RR11 105, GT 1 and RR11 600. The trials have completed seven years of tapping.

The trial, 1991A contains 36 clones, both indigenous and exotic, are under evaluation. Maximum yield was noted in clones PB 235 (80.7 g/t/t), PB 314 (79.0 g/t/t),

PB 280 (77.0 g/t/t), PB 312 (73.3 g/t/t) and PB 311 (67.8 g/t/t). The lowest yielding clones were SCATC 93-114 (21.0 g/t/t), AVROS 352 (16.6 g/t/t) and CH 4 (14.1 g/t/t). Thirteen clones are under evaluation in the second trial. Clone RR11 5 gave maximum yield of 66.21 g/t/t followed by RR11 3 (59.2 g/t/t). In the third trial also 13 clones are under evaluation. The highest yielding clone was HP 83/224 (61.2 g/t/t) followed by PB 28/59 (54.3 g/t/t) after six years of tapping.

1.6. Large-scale clone trial

The trial was initiated in 2000, eight clones under evaluation where most of the clones are RR11 400 series clones and are the latest clones developed by the Rubber Research Institute of India. The trial has completed 11 years of growth and fourteen months of tapping. Maximum yield was noted for clone RR11 414 (62.8 g/t/t) followed by RR11 430 (52.0 g/t/t) and RR11 422 (51.0 g/t/t). Parent clones RR11 105 and RR11 100 yielded 32.9 and 31.4 g/t/t, respectively.

1.7. Visual scoring of reaction of Hevea clones and genotypes to *Phytophthora* leaf disease

Response of various clones and genotypes was visually scored on a scale of 1 to 4, with 1-for no tolerance, 2-for mild tolerance, 3-for good tolerance and 4- for very good tolerance. This work was done totally under unsprayed conditions. From the four year results, it has been found that a total of 104 clones/genotypes having good tolerance to *Phytophthora* leaf disease.

HEVEA BREEDING SUBSTATION PARALIAR, TAMIL NADU

The major research activities of the station could be categorized under four projects, *viz.*, crop improvement, root trainer planting technique for rubber, participatory clone evaluation and solar radiations & TPD.

1. Crop improvement

This project consisted three sub-projects and the work carried out under each sub-projects is furnished below,

1.1. Clone evaluation

Nine large-scale clone evaluation experiments are being pursued under this sub-project. In the large-scale clone evaluation experiment initiated at Keeriparai (1994) tapping was completed for nine years and PB 255 (84.94 g/t/t) continued to occupy the first position with respect to the pooled data for nine years of tapping (Table Par. 1), followed by IRCA 109 (77.76 g/t/t). The mean yield obtained

from IRCA 111 (75.91 g/t/t) was also found to be promising, but the yield performance of the other two new clones namely IRCA 230 (62.05 g/t/t) and IRCA 18 (63.97 g/t/t) were found to be on par with the control clone RRII 105 (61.68 g/t/t). In the block evaluation experiment, initiated with the financial aid from the World Bank during 1994, RRII 105 (67.33 g/t/t) presented the maximum yield up to the ninth year of tapping and the gap between this clone and the second best PB 28/59 (60.90 g/t/t) was noticed to widen year after year (Table Par.

Table Par. 2. Mean yield on the block trial (1994)

Clone	Yield (g/t/t)	
	2010-11	9 years
RRII 5	59.19	45.93
RRII 50	55.14	46.35
RRII 51	60.10	40.72
RRII 105	118.90	67.33
RRII 176	59.01	45.01
RRIC 102	63.85	53.76
PB 217	56.58	49.40
PB 235	51.18	55.13
PB 260	72.29	52.99
PB 311	64.26	59.41
PB 28/59	71.71	60.90
PR 255	74.69	52.36
PR 261	64.84	49.49
Mean	66.99	52.21
SE	4.03	3.11

Table Par. 1. Mean yield on LSCT (1994)

Clone	Mean yield (g/t/t)	
	During 2010-11	Pooled (9 years)
RRII 105	62.08	61.68
PB 314	94.34	76.61
IRCA 130	79.65	70.79
PB 28/59	63.87	58.84
IRCA 109	88.73	77.76
PB 330	88.75	60.48
IRCA 18	77.45	63.97
RRIM 703	47.17	64.40
IRCA 111	81.46	75.91
PB 255	90.32	84.94
IRCA 230	70.02	62.05
Mean	76.71	68.85
CD (P=0.05)	16.03	22.47

2). PB 311 (58.9 g/t/t) also presented promising yield trend, but the clone was noticed to be highly susceptible to wind damage. Maximum TPD incidence was observed in PB 235 (41.74%), followed by RRII 105 (40.75%).

In the multi-location clone trial entitled *GxE Interaction of selected Hevea clones* (1996) RR11 203 (59.81 g/t/t) presented numerically better yield than the control clone RR11 105 (58.30 g/t/t) up to the eighth year of tapping (Table Par. 3). Among the hybrid clones belonging to the 400 series RR11 430 (54.07

opened for regular tapping during 2010. Juvenile growth of the remaining three block evaluation experiments initiated at Bethany estate (2006), Palazhi estate (2006) and Tholicode estate (2007) are being closely monitored at regular intervals.

1.2. Hybridization and clonal selection

By constant pruning and pollarding of branches, canopy of all the trees in the breeding orchard was maintained at a low profile, so that hand pollination could be attempted conveniently by standing in the ground. Hand pollinations were attempted at various parental combinations during 2010-11 also and the hybrids obtained from HP carried out during 2010 were raised in a nursery for preliminary evaluation. Potential high yielders (32 no.) selected in previous years were multiplied for conducting small-scale clone evaluation experiments. The new experiment entitled *Progeny evaluation for early selection in Hevea* was also in progress and hand pollinations were attempted.

2. New generation polyclonal seed garden

The polyclonal seed garden (2000) established at New Ambadi estate, Maniankuzhy was well maintained by carrying out all the cultural operations like dusting/spraying, manuring *etc.* in time. Polycross seeds collected during 2010 were raised at Nagamalai estate, Punalur for field planting as improved planting material during the planting season in 2011. These plants evolved from polycross seeds would be screened for the presence of unusual yield and promising secondary characters. A total of 1500 plants originated from polycross seeds were test tapped during 2010 at HBSS, Paraliar and 22 potential high

Table Par. 3. Mean yield on the trial GxE interaction (1996)

Clone	Yield (g/t/t)	
	2009-10	7 years
RR11 414	59.91	46.83
RR11 417	73.45	51.26
RR11 422	75.88	51.72
RR11 429	57.18	41.76
RR11 430	74.96	54.07
RR11 51	59.52	38.27
RR11 176	75.16	44.40
RR11 203	88.17	59.81
RR1C 100	61.06	45.22
PB 217	65.80	38.77
RR1M 600	75.27	44.59
RR11 105	78.93	58.30
Mean	70.44	47.91
CD (P = 0.05)	4.26	2.09

g/t/t) continued to present maximum yield, followed by RR11 422 (51.72 g/t/t) and RR11 417 (51.26 g/t/t). RR11 414 (46.83 g/t/t) and RR11 429 (41.76 g/t/t) presented feeble performance under the particular agro-climate of Kanyakumari region. In the observational trial at Vaikundam estate (2000) RR11 422 (61.35 g/t/t) presented the maximum yield followed by RR11 429 (60.72 g/t/t). RR11 414 (52.25 g/t/t) presented more or less equal yield to the control clone RR11 105 (52.66 g/t/t).

The on-farm trials on the 400 series clones initiated at New Ambadi Estate (2003) and Velimalai estate (2003) were

yielders were selected for further evaluation in a small-scale trial.

Action was also taken to collect polycross seeds from selected mother trees in connection with an approved project initiated at RRII, Kottayam. The remaining seeds would be collected and raised at HBSS, Paraliar and Nagamalai estate, Punalur (Harrison Malayalam Ltd.) for another cycle of selection process.

3. Root trainer planting technique

In the field trial initiated at Churulacode (2002), root trainer plants continued to exhibit numerically better growth and yield (51.07 g/t/t) than polybag plants (47.3 g/t/t). The experiment entitled '*In situ* young budding on stocks raised in root trainers' was concluded and data were tabulated (Table Par. 4). *In situ* young budding in root trainers was found to have several advantages over stump planting. They presented more budding success and scion establishment compared to green budding. Plants raised by *in situ* young budding in root trainers exhibited more lateral root formation (47.7 laterals) than stump planting in polybags (7.8) and root

trainers (14.4). Field planting of a new experiment entitled 'Comparative evaluation of advanced planting materials produced by different propagation techniques' was carried out at Bethany estate during 2010. Plots were demarcated and observation on initial establishment success was recorded. Advanced planting materials of 40 clones were raised in root trainers for a study on the comparative drought tolerance of these clones. Action was also taken to initiate a new experiment entitled 'Comparative evaluation of labour requirement and cost effectiveness of root trainer and polybag planting techniques' during the year 2011. Training was also imparted on root trainer planting technique.

4. Participatory clone evaluation experiment

Observations on juvenile growth were recorded in the on-farm trial of pipeline clones (2008) initiated at Tharuvaiyar and a disease survey was conducted made by the Scientist from RRII, Kottayam. Field planting of two more experiments under the project was carried out at Bethany

Table Par. 4. Mean values of budding success, scion establishment, growth and root parameters

Treatment lateral	Budding success (%)	Scion establishment (%)	Height (cm)	Growth parameters (mean values)				Plants (%)	
				Diameter (mm)	Sturdiness	No. of		tap root	No. of roots with collod
						Whorls	Leaves		
T1 (RT)	95.6	91.8	59.6	8.66	68.12	1.68	11.4	—	47.7
T2 (PB)	96.1	92.3	68.1	7.93	85.75	1.86	13.0	89.1	33.8
T3 (RT)	86.6	90.0	62.9	8.49	73.02	1.69	11.8	—	58.6
T4 (PB)	88.3	92.1	71.0	8.31	85.83	1.74	12.1	77.7	30.7
T5 (RT)	89.1	82.7	62.2	8.11	64.11	1.66	11.6	—	14.4
T6 (Control)	90.3	86.6	67.9	8.95	77.09	1.81	12.6	73.2	7.8
G. mean	91.0	89.3	65.5	8.41	75.65	1.72	12.1	—	32.2
CD (P = 0.05)	6.3	4.9	8.3	0.91	—	0.27	1.6	—	30.6

estate during the month of July 2010. Plants were paint marked and plots were demarcated with plot boards. Collected soil samples for fertilizer application. Enumerated vacancies and action was taken for gap filling during the month of June 2011.

5. Solar radiations and TPD

Observations on juvenile growth were recorded on the large-scale clone trial (Ponmanai 2008) and the block trial initiated at Bethany estate (2008). The bark protection device was replaced with four fold waste newspaper.

LIBRARY AND DOCUMENTATION CENTRE

During the year, 163 books were added to the stock of the library. The library subscribed 40 foreign journals and 75 Indian journals. About 31 other journals were also received as gift/exchange. Literature searches from AGRIS and RAPRA CDs were carried out.

Three issues of Documentation List, two issues of Rubber Alerts and 28 issues of Current Content Bulletins and one issue of New Additions List 2010 were compiled and distributed. Databases were updated by

adding 149 books, 516 journal articles and 335 theses/dissertations. Arranged the distribution of 614 numbers of Press Clippings and 270 numbers of other SDI bulletins.

Organized the distribution of 48 numbers of Annual Report 2007-08. Arranged the sale and distribution of 235 numbers of RRII publications. Photocopies of about 58,272 numbers of information materials were provided during this period.

AGROMETEOROLOGY

1. Climate resource characteristics of rubber growing tracts

Trend analysis of the rainfall derivative parameters like Standardized Precipitation Index and Precipitation Concentration Index of the traditional and non-traditional regions were carried out.

1.1. Standardized Precipitation Index (SPI)

Seasonal and annual SPI of 26 rain gauge stations in Kerala were analyzed for

the period from 1901 to 1948 (1st half) and 1949 to 1996 (2nd half). The same has been carried out for 14 stations in the North East India for datasets ranging from 40 to 60 years. Most locations in Kerala showed a shift from positive SPI (during 1901-1948) to negative SPI (during 1949-1996). SPI showed a declining trend during winter in Central Kerala (Table Agromet. 1a). Pathanamthitta showed a positive trend during winter. Out of 26 stations analyzed

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T5 (RT)	89.1	82.7	62.2	8.11	64.11	1.66	11.6	—	14.4
T6 (Control)	90.3	86.6	67.9	8.95	77.09	1.81	12.6	73.2	7.8
G. mean	91.0	89.3	65.5	8.41	75.65	1.72	12.1	—	32.2
CD (P = 0.05)	6.3	4.9	8.3	0.91	—	0.27	1.6	—	30.6

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Table Agromet 1a. Trends in SPI for the winter and pre-monsoon seasons for first and second half analysis periods

Place	Winter season SPI (January-February)				Pre-monsoon season SPI (March-May)			
	(1901-48)		(1949-96)		(1901-48)		(1949-96)	
	Slope	R ²	Slope	R ²	Slope	R ²	Slope	R ²
Alleppey	-0.008	0.02	-0.007	0.02	0.016	0.11	-0.020	0.13
Chengannur	-0.005	0.02	-0.012	0.11	0.008	0.04	-0.012	0.11
Thiruvalla	-0.005	0.02	-0.015	0.14	0.010	0.07	-0.026	0.24
Irikur	0.003	0.02	-0.001	0.04	0.004	0.02	-0.010	0.06
Kasaragod	0.004	0.02	0.000	0.01	0.011	0.08	-0.008	0.06
Muvattupuzha	-0.003	0.01	-0.009	0.10	0.013	0.11	-0.012	0.05
Perumbavoor	0.000	0.00	-0.007	0.12	0.004	0.01	0.018	0.22
Devikulam	-0.005	0.03	-0.013	0.21	-0.005	0.03	-0.013	0.21
Ethomanur	-0.004	0.01	-0.005	0.03	0.014	0.11	-0.004	0.01
Kottayam	0.004	0.01	-0.012	0.15	0.005	0.02	-0.018	0.16
Kumily	0.004	0.01	-0.017	0.07	0.005	0.01	-0.032	0.21
Munnar	-0.001	0.00	-0.003	0.01	0.011	0.07	-0.013	0.08
Vaikom	0.000	0.00	0.002	0.00	0.014	0.11	-0.014	0.10
Vadagara	0.001	0.00	0.000	0.00	0.006	0.03	-0.008	0.06
Kuttiadi	0.001	0.00	-0.004	0.05	0.009	0.06	-0.011	0.07
Vayittiri	-0.007	0.06	-0.009	0.13	0.008	0.05	-0.025	0.34
Nilambur	0.000	0.00	-0.003	0.06	0.009	0.06	-0.005	0.03
Ottapalam	0.001	0.00	-0.004	0.07	0.002	0.01	-0.014	0.14
Adoor	0.002	0.01	-0.005	0.02	0.000	0.00	-0.019	0.16
Trichur	0.002	0.02	-0.005	0.11	0.008	0.06	-0.020	0.25
Nedumangad	0.002	0.00	-0.006	0.03	0.002	0.00	-0.016	0.11
Neyyattinkara	0.000	0.00	-0.002	0.00	0.000	0.00	-0.012	0.09
Trivandrum	0.003	0.01	-0.005	0.01	0.001	0.00	-0.008	0.06
Konni	-0.004	0.01	-0.006	0.02	0.004	0.01	-0.011	0.04
Pathanamthitta	0.000	0.00	0.074	0.15	0.006	-0.00	0.000	0.00
Kollam	0.001	0.00	0.000	0.05	0.009	0.06	0.011	0.07

13 stations showed significant negative trends during the pre-monsoon season. Thiruvalla, Vaikom and Vythiri showed a negative trend during monsoon (Table Agromet 1b). Places in Central Kerala showed negative trends during the post-monsoon season. Perumbavoor, Kottayam, Kumily, Vythiri, Nedumangad and Pathanamthitta showed a decline in the annual SPI values. A decrease in annual SPI values of 0.5 to 1.6 per 100 years were noted for these places.

In the NE region, increasing trends in SPI were noted for Agartala during the

winter and monsoon seasons. Increase was seen in Sabroom in all seasons except winter. Imphal and Amarapur showed increasing trends in SPI values during pre-monsoon, Tura during the monsoon and post-monsoon and Udaipur during the winter and post-monsoon. Annual increasing trends in SPI for Tura, Udaipur and Sabroom ranged from 1.6 to 1.8 per 100 years.

1.2. Precipitation Concentration Index (PCI)

Monthly rainfall dataset was obtained from the India Meteorological Department

Table Agromet 1b Trends in SPI for the monsoon and post-monsoon seasons for first and second half analysis periods

Place	Monsoon season SPI (June-September) (1901-48)		Post-monsoon season SPI (October-December) (1949-96)		Monsoon season SPI (June-September) (1901-48)		Post-monsoon season SPI (October-December) (1949-96)	
	Slope	R ²	Slope	R ²	Slope	R ²	Slope	R ²
Alleppey	0.002	0.00	-0.036	0.01	0.009	0.04	0.000	0.00
Chengannur	0.003	0.01	-0.001	0.00	0.004	0.02	-0.005	0.02
Thiruvalla	0.000	0.00	-0.019	0.15	0.000	0.00	-0.019	0.15
Inkurr	-0.001	0.00	-0.008	0.02	-0.001	0.00	-0.008	0.02
Kasargod	0.002	0.00	0.000	0.00	0.009	0.05	0.001	0.00
Muvattupuzha	0.003	0.01	-0.013	0.03	0.007	0.06	-0.017	0.08
Perumbavur	0.000	0.00	-0.147	0.00	0.002	0.00	-0.012	0.09
Devikulam	0.004	0.01	-0.013	0.02	0.010	0.09	-0.014	0.05
Ettumanur	0.005	0.02	0.008	0.01	0.004	0.02	-0.014	0.05
Kottayam	-0.002	0.01	0.001	0.00	-0.002	0.01	-0.008	0.05
Kumily	-0.004	0.01	-0.018	0.05	0.003	0.01	-0.009	0.01
Munnar	-0.006	0.03	0.000	0.00	0.004	0.01	0.006	0.02
Vaikom	0.014	0.11	-0.014	0.10	0.006	0.01	-0.002	0.00
Vadagara	-0.002	0.00	-0.002	0.00	-0.002	0.00	-0.002	0.00
Kuttiadi	0.000	0.00	0.016	0.04	-0.002	0.00	-0.007	0.03
Vayittiri	0.000	0.00	-0.015	0.13	0.002	0.01	-0.006	0.04
Nilambur	-0.004	0.01	-0.002	0.00	-0.002	0.01	-0.002	0.01
Onappalam	0.002	0.00	0.003	0.00	0.003	0.01	-0.005	0.02
Adoor	-0.002	0.01	-0.011	0.08	-0.002	0.01	-0.003	0.01
Trichur	-0.001	0.00	0.000	0.00	0.002	0.01	-0.003	0.01
Nedumangad	0.001	0.00	-0.012	0.07	0.006	0.03	-0.007	0.03
Neyyattinkara	0.004	0.01	-0.004	0.02	0.007	0.02	0.002	0.00
Trivandrum	0.001	0.00	-0.001	0.00	0.005	0.02	0.003	0.01
Konni	0.000	0.00	-0.001	0.00	-0.004	0.01	-0.004	0.01
Pathanamthitta	-0.004	0.01	0.004	0.01	0.001	0.00	-0.032	0.27
Kollam	0.000	0.00	0.016	0.04	-0.002	0.00	-0.007	0.03

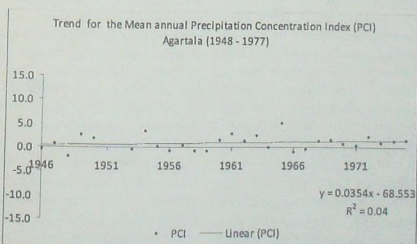


Fig. Agromet 1a. Increasing trend of mean annual PCI in Agartala for the first half period 1948-1977

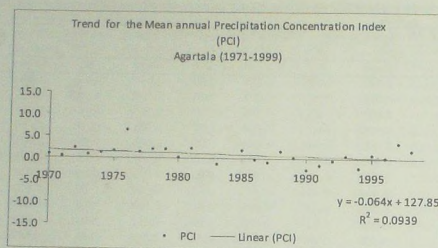


Fig. Agromet 1b. Decreasing trend of mean annual PCI in Agartala for the second half period 1971-1999

(IMD). Annual PCI of 41 rain gauge stations in Kerala was analyzed for two periods from 1901 to 1948 (1st half) and 1949 to 1996 (2nd half). The same has been carried out for 21 stations in the North East India for datasets ranging from 40 to 60 years. Out of 41 stations analyzed for PCI, 11 stations mainly in Central and North Kerala showed positive trends. PCI values ranged from 6.0 to 31.1 per 100 years. This denotes that strong seasonality of rainfall can be expected in most places in Kerala. A total of 7 stations out of 21 stations mainly spread over Assam and Tripura, showed significant negative trends in PCI denoting future uniformity of rainfall distribution compared to that of the present. In Agartala, the long-term trend showed a change from a positive trend in the early half to a negative one during the second half (Fig. Agromet. 1a and 1b). The study showed that there is an indication of decreasing amount of wetness in the traditional regions with an increase in the NE region. The same

is the case with the uniform distribution of rainfall.

2. Forewarning of pests and diseases

The onset of diseases, abnormal leaf fall (ALF), powdery mildew and corynespora leaf fall (CLF) in the traditional rubber growing regions were related with weather and analysed. ALF and powdery mildew were mild in the Kanniyakumari area. Moderate incidence of the disease was observed in the Central and North Kerala. In Padiyoor in the North Kerala, the peak leaf fall due to powdery mildew occurred on 16th April, 2010 (Fig. Agromet. 2). A decrease in sunshine hours and increase in relative humidity were the precursors to the disease trigger. Progress of ALF in Padiyoor during July-August period is shown in Fig. Agromet 3. RRIM 600 registered the highest leaf fall compared to other high yielding clones.

Corynespora leaf disease severity was observed in North Kerala and South

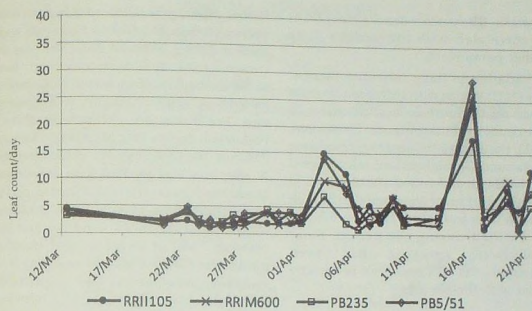


Fig. Agromet 2. Oldium leaf fall during March-April in Padiyoor during 2010

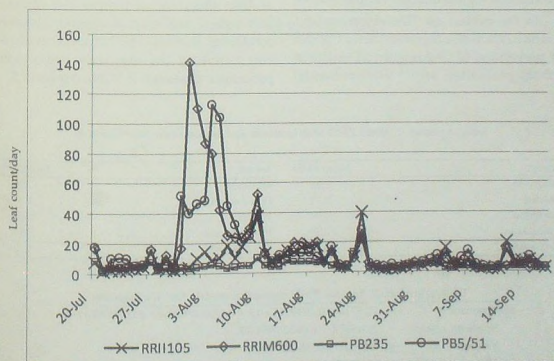


Fig. Agromet 3. Abnormal leaf fall during July-August 2010 in Padiyoor

Karnataka. The percent disease index (PDI) was correlated with the various daily weather parameters.

Variation in daily humidity thermal index (HTI) values with corresponding leaf fall was related for three locations and with spore count for Pinavoorkudi, Ernakulam. In 2011, the disease incidence was moderate in Adur and Guthighar. Leaf fall occurred in early March and ended after 15 days. In Guthighar leaf spots were found to be high but with little leaf fall. HTI above 5.5 was also verified during the period. Since prophylactic spraying had been undertaken, the HTI could not be directly related with the severity.

3. Agromet Database Management

All research centers, stations in the private estates and several field stations are being monitored for accumulating agrometeorological data for various studies

In the project on "Development and demonstration of Integrated Vector Control for prevention of Chikungunya/Dengue in Rubber plantation area" environmental

factors affecting the build-up of high density vectors of *Aedes albopictus* in Kottayam were studied during 2008 and 2009. The seasons chosen for the study were monsoon (June to September), post-monsoon (October to December), Winter (January to February) and Pre-monsoon (March to May). Population density (Per Man Hour Density-PMD) of the Chikungunya causing vector *A. albopictus* was studied with antecedent atmospheric parameters and the lag period correlation of up to 14 days were calculated. Individual correlations were worked out between PMD of locations and weather parameters and PMD of seasons and weather parameters. No significant difference was noted in PMD between Malankara and Chethackal, the monsoon and post-monsoon differences were insignificant. The maximum PMD occurred during the pre-monsoon period. Conditions affecting the build-up of population density of *Aedes albopictus* are mainly concentrated within the 7 to 12 days antecedent weather conditions. Seasonal PMD models have been computed by the stepwise regression procedure as shown in Table Agromet 3.

Table. Agromet. 2. Mean PMD over locations and seasons over four locations

Location	Mean PMD	Season	Mean PMD
Malankara	2.04	Monsoon	1.80
Chetkal	2.15	Post-monsoon	1.53
Ainkombu	0.99	Winter	0.52
Kumarakom	0.30	Pre-monsoon	3.93
CD (P=0.05)	0.87		1.08

Table. Agromet. 3. Seasonal PMD regression models for *A. albopictus*

Ymonsoon	= -12.9713 + 0.303556Tx-13 + 0.033327Rh2-8+ 0.075088Tav-12
Ypost-monsoon	= -7.049674 - 0.06506Rh1-13
Ywinter	= -1.56542 + 0.030221 AvRh-10
Ypre-monsoon	= 0.362127 + 0.00193R-9 + 10Rday

ANNUAL EXPENDITURE
Expenditure at a glance (2010-11)

Head of Account	Expenditure (Rs. In lakhs)
Non-Plan	
General charges	798.75
Projects (CES)	
Plan	
General charges	1,474.96
NERDS Research Component	302.76
Total	
Grand Total	2,576.47

*Non plan expense includes non plan projects (CES)

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RUBBER RESEARCH INSTITUTE OF INDIA

Rubber Board, Kottayam- 686 009, Kerala, India

Phone- 91 481 2353311-20, 2352770-71, 2352773-79 (20 lines) Fax: 91 481 2353327

E mail: rrii@rubberboard.org.in Website: www. rubberbord.org.in

REGIONAL RESEARCH STATIONS

Central Experiment Station

Rubber Board
Chethackal, Thompikandom. P.O.
Ranni- 689 676, Kerala
Phone: 91 4735 261500, 261176

Regional Research Station

Rubber Board, Padiyoor. P.O
Kannur- 670 703, Kerala
Phone: 91 4982 273003

Regional Research Station

Rubber Board
Dapchari- 401 610, Thane, Maharashtra
Phone: 91 2528 202042

Regional Research Station

Rubber Board, PWD Road
Near District Employment Exchange
Dhenkanal- 759 001, Orissa
Phone: 91 6762 224946

Hevea Breeding Sub-Station

Rubber Board, Subrahmanya Road
Kadaba, Puttur Taluk- 574 221
D.K.Dt., Karnataka
Phone: 91 8251 262336

Regional Research Station

Rubber Board, Grassmore
Nagrakata, Jalpaiguri- 735 225, West Bengal
Phone: 91 3565 270016

Research Complex (N.E. Region)

Rubber Board
Beltola - Basista Road, Housefed Complex
Dispur, Guwahati-781 006, Assam
Phone: 91 3612 228220

Regional Research Station

Rubber Board, Baluakiattila
Kunjaban- 799 006, Agartala, Tripura
Phone: 91 381 2355143

Regional Research Station

Rubber Board, Near AIR Quarters
Dakobgre, PB No. 26, Tura- 794 001
West Garo Hills, Meghalaya
Phone: 91 3651 232413

Hevea Breeding Sub-Station

Rubber Board, Thadikarankonam. P.O
Kanyakumari-629 851, Tamil Nadu
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REGIONAL SOIL TESTING LABORATORIES IN KERALA

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Thrissur- 680 545

Regional Laboratory

Mary Matha square
Arakuzha Road
Moovattupuzha- 686 661

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Regional Laboratory

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1. Honouring Karshakashree, Sri. K. C. Kuriakose, Cheerakuzhi Nursery and T. G. Vidyasagar, Founder President of Illithode RPS in the Rubber Gowers Seminar, 25th June 2010, Rubber Research Institute of India, Kottayam.
2. Inaugural function of International workshop on climate change and Rubber Cultivation: R & D Priorities, 28-30 July 2010 Rubber Research Institute of India, Kottayam.
3. Inaugural function of PLACROSYM XIX, 7-10, December 2010, Rubber Research Institute of India, Kottayam

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Research divisions and functions

The major research divisions are Agronomy/ Soils, Biotechnology, Botany, Germplasm, Plant Pathology, Plant Physiology, Latex Harvest Technology, Rubber Technology and Economics. Studies on Clone Evaluation, Genome Analysis and DRIS Fertilisation are dealt separately.

The thrust areas of research of Agronomy/ Soils Division are investigations on the nutritional requirements of rubber, irrigation, intercropping, cover crop management, weed control and the study of the rubber growing soils. Development of tissue culture and anther culture systems for propagation and crop improvement of *Hevea* are the important areas in which the Biotechnology Division is engaged. The important fields of research of the Botany Division are breeding, evaluation and selection of new clones, propagation techniques, planting methods, anatomical studies and cytogenetic investigations. The Germplasm Division is concentrating on the introduction, conservation and evaluation of *Hevea* germplasm. The Plant Pathology Division is engaged in investigations on the diseases and pests of rubber and associated cover crops and their control. The Plant Physiology Division conducts studies on both fundamental and applied aspects of *Hevea* tree physiology. The Exploitation Technology Division is concentrating on all applied aspects of crop harvesting in rubber. The Rubber Technology Division concentrates on improvement in primary processing of rubber, its chemical modification, rubber product manufacture and quality control of processed rubber. The Economics Division undertakes studies on economic aspects related to rubber plantations.

The research supporting sections includes Library and Documentation, Instrumentation, Statistics, Computer and Maintenance Wing. There is also a small experimental farm of 33 ha. at the headquarters of RRII.

Central Experiment Station

The 255 ha. Central Experiment Station at Chethackal (Ranni), 50 km away from Kottayam, was started in 1966. Field trials laid out by the research divisions cover almost the entire area.

Regional Research Stations

RRII has established a North-Eastern Research Complex with headquarters at Agartala having

regional research stations at Agartala in Tripura, Guwahati in Assam and Tura in Meghalaya. The RRII has also set up regional research establishments at Dapchari (Maharashtra), Dhenkanal (Orissa), Nagrakata (West Bengal), Paraliar (Tamil Nadu), Nettana (Karnataka) and Padiyoor (Kerala).

Regional soil testing laboratories have been established at Taliparamba, Kozhikode, Thrissur, Muvattupuzha, Pala, Kanjirappally, Adoor and Nedumangad. Mobile units for soil and leaf analysis are available at Kozhikode laboratory, apart from that at the headquarters

National/International collaboration

RRII is a member of the International Rubber Research and Development Board (IRRDDB), an association of national organizations devoted to research and development on natural rubber. Rubber Board is a member of the Association of Natural Rubber Producing Countries (ANRPC) and International Rubber Study Group (IRSG).

The RRII has research/ academic linkages with the Banaras Hindu University (Varanasi), Kerala Agricultural University (Thrissur), Kerala University (Thiruvananthapuram), Mahatma Gandhi University (Kottayam), Cochin University of Science and Technology (Kochi), Indian Agricultural Research Institute (New Delhi), Indian Institute of Sciences (Bangalore), Indian Institute of Technology (Kharagpur), National Chemical Laboratory (Pune), Sree Chitra Tirunal Institute of Medical Sciences and Technology (Thiruvananthapuram), Tamil Nadu Agricultural University (Coimbatore), University of Agricultural Sciences (Bangalore) and University of Goa (Goa).

Correspondence

The Director of Research
Rubber Research Institute of India
Kottayam - 686 009, Kerala, India

Phone 91 481 2353311-20

91 481 2352770-71

91 481 2352773-79 (20 lines)

Fax 91 481 2353327

Email rrii@rubberboard.org.in

Website www.rubberboard.org.in