RUBBER RESEARCH INSTITUTE OF INDIA

ANNUAL REPORT 2008-2009

ISSN 0970-2490. CODEN ARRIEU

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 major International Airports, one at Thiruvananthapuram, 160 km south and the other at Nedumbassery, 95 km north to 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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ANNUAL REPORT
2008-2009

RUBBER RESEARCH INSTITUTE OF INDIA
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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 19 April 1947. This Act was amended in 1954, 1960 and 1982. The Act was again amended by the Rubber (Amendment) Act, 1994 (Act 33 of 1994), which is now in force.

Organization

The Chairman is the principal executive officer and exercises control over all departments of the Rubber Board. The Rubber Research Institute of India (RRII) works under the administrative control of the Board, the Director being the head of the institution. Besides RRII, there are eight departments under the Board *viz.* Administration, Rubber Production, Processing & Product Development, Finance & Accounts, Training, Licensing & Excise Duty, Statistics & Planning and Marketing Promotion.

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DIRECTOR'S REVIEW

Declining prices and growing market uncertainties of primary commodities have been important characteristics of the post-reforms phase since the second half of the 1990s. With the advent of the new economic system of market integration, there occurred close association between international prices and domestic prices and hence, the financial crisis that has engulfed the global economies during the second half of 2008 had a direct impact on natural rubber (NR) prices as the NR sector was highly sensitive to changes in the global economic activities. Though the adverse impact of the crisis was felt in India through a downturn in industry and agriculture, India could reasonably manage the global financial crisis on account of its strong fundamentals and well regulated banking system and India's domestic rubber industry as a whole also was able to withstand the onslaught of the financial crisis to some extend as compared to its counterparts elsewhere. But, since the comfort of protected market is no more available, global competitiveness has become imperative for the survival of the primary commodities including the NR where cost and quality are the only passwords for survival. As such, the Indian NR plantation industry has to reorient its priority areas by paying serious attention to the sustainability of NR cultivation, for which strong R&D support is indispensable. The Rubber Research Institute of India has been taking up research programmes keeping this perspective in view.

The various research programmes of the Agronomy Division were aimed at development and periodic refinement of agro-management practices to improve growth and yield of rubber, reduce cost of cultivation and sustain soil quality. Research programmes were also undertaken to address site-specific agronomic problems. Experiments were initiated in immature rubber to reduce the doses of chemical fertilizers integrating with organic and bio-fertilizers. Experiments on nutrient management in nurseries and mature rubber were also in progress. The various experiments on cropping systems and mixed planting with timber trees were continued. A study was undertaken in central Kerala to find out how far unscientific pineapple intercropping has affected growth of rubber and soil properties in smallholdings. Experiments to develop an agronomic package for reducing the gestation period of rubber are in progress. To monitor the sustainability of rubber growing soils, the soil organic carbon and pH data of estates and smallholdings over the years were compiled and analysed. A comparison was also made between the soil chemical properties of forest and rubber after first, second and third cycles using x-ray fluorescence technique. Development of the rubber information system using remote sensing and GIS was continued. Geo-referencing and extracting themes from toposheets and soil maps were initiated. Estimation of total area under rubber and rubber area distribution in relation to soil

and landscape attributes are in progress. The Division also functions as a centre for dissemination of knowledge on various soil and crop management techniques.

Under the crop improvement programmes, development and release of improved clones was the prime mandate. Research activities of the Botany Division were directed towards studying the long-term performance of newer series of indigenous hybrids as well as exotic clones. Forty three promising hybrid clones, resultant of the 1986 hybridization programme, were evaluated in four Small Scale Trials laid out in Central Experiment Station, Chethackal. In the 11th year of tapping, 37 hybrid clones maintained higher yield than the check clone RRII 105. The results of large scale trials were compiled in a technical report along with the evaluation of these clones in other locations for upgradation of PB 314, PB 255, PB 280 and PB 312 from Category 3 to Category 2 of the Planting Recommendations for the traditional region. Evaluation of clones for latex and timber yield, drought and disease tolerance, need based investigations on propagation aspects and studies on the anatomy of bark and wood were also carried out. Multi-clonal on-farm and large –scale evaluation of the next series of promising pipeline clones were initiated under the participatory clone evaluation project. Under the participatory evaluation of rubber clones, 20 pipeline clones and three check clones were field planted in various locations.

The Germplasm Division continued the maintenance of the domesticated gene pool collection, introduction and conservation of remaining *Hevea* species, conservation of wild germplasm, its agronomic evaluation, screening for diseases, drought and cold stress resistance, timber latex traits and molecular characterization. The three *Hevea* gene pools maintained at RRII, comprising the domesticated gene pool with clones derived from the original Wickham collection of 1876 and the wild germplasm belonging to the 1981 IRRDB collection, form an important source of variability required for genetic improvement of natural rubber.

Major research programmes in the Biotechnology Division were aimed at elucidating the molecular biology of the crop and genetic improvement of *Hevea brasiliensis* using modern biotechnological tools. The studies included: development of *in vitro* propagation methods for elite *Hevea* clones; development of transgenic *Hevea* plants for better adaptation to abiotic stress and increased rubber biosynthesis; development of *in vitro* fertilization techniques and embryo rescue to compliment conventional breeding programmes; study of molecular mechanism controlling tolerance to diseases, abiotic stress, tapping panel dryness and latex biosynthesis as well as characterization of related genes and; characterization of laticifer cell-specific promoters and gene expression. In the genetic transformation experiments with osmotin gene, preliminary results showed that slow drying of embryos in an unsealed, but closed Petri dish for 36 h improved germination. Five transgenic embryos could be germinated and plantlets were kept for hardening.

The major activities of the Genome Analysis Laboratory were development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping; development of genetic markers for biotic and abiotic stress tolerance and cloning and characterization of agronomically important genes. Microsatellite markers were used for characterisation of 40 popular clones and 60 wild *Hevea* germplasm accessions. SNP markers were utilised in clear discrimination

of the Amazonian germplasm accessions indicating geographic distinctiveness of accessions from different provenances. Thirty seven polymorphic SSR markers were used to genotype the progeny population and segregation data was recorded for linkage map construction.

Monitoring the occurrence of diseases and pests and their management by chemical and biological methods, characterization of pathogens *etc.* were the main research areas of the Plant Pathology Division. The field trials to evaluate the usefulness of biodegradable oil for abnormal leaf fall (ALF) disease management proved the efficacy of the oil. Leaf retention assessment in RRII 400 series clones indicated that all the clones were on par with RRII 105. DNA barcoding and barcode fingerprinting of *Phytophthora* spp. were initiated for presenting a genus-wide phylogeny of the species affecting rubber. Colonisation of transformed bacteria in tissues when applied to the root and leaves of the rubber seedlings was confirmed by scanning through confocal microscope. The germplasm accessions showing tolerance to *Corynespora* were short-listed. The large-scale survey in six locations in Kerala on TPD incidence indicated an increase in TPD incidence in all locations as tapping progressed from BO-1 to BO-2 panel. Out of the 80 bacterial isolates including rhizobacteria and endophytes selected during the previous year, 12 isolates showed improved growth of rubber seedlings. A Project Design Document for the bundled methane recovery from sheet processing effluent and bundled project for replacing fuel oil and electricity with biomass gasifier for heat generation were developed and submitted under the Clean Development Mechanism of the Kyoto Protocol.

The Physiology Division continued the research programmes in areas of environmental physiology, physiology of growth and yield, stock-scion interaction, tapping panel dryness, nutrition physiology, gene expression studies, secondary metabolites and ecological impact of NR cultivation. During, the reporting year, the Eddy Covariance System to monitor the ecosystem level CO_2 and water flux in rubber was installed at CES, Chethackal and net CO_2 assimilation and total water evaporation rate were recorded. In another study, up and down regulated gene transcripts associated with drought tolerance were identified. Biochemical analysis to find out the effect of stimulation in laticiferous tissues showed the development of stress related changes in the bark tissue due to stimulant application. Investigations on the molecular physiology of tapping panel dryness (TPD) and cloning and characterization of TPD associated genes by subtractive hybridization are in progress.

A method for quick determination of the dry rubber content for ammoniated latex was tested and confirmed by the Rubber Technology Division. Field latex preserved at low temperature at 4-5 °C could be centrifuged and the properties were comparable with that of LATZ latex. The Division evaluated the properties of tread rubber prepared using polymetric filler, in collaboration with a leading tyre tread manufacturing unit. Nano-silver with secondary capping agents such as polyvinyl alcohol (PVA), polyethylene oxide condensate and carboxy methyl cellulose were prepared and a protocol for pilot plant scale production of ENR 25 and ENR 50 with lower Mooney viscosity and reduced storage hardening behaviour was developed.

The Technical Consultancy Division mainly focused on assisting rubber based industries, product development, quality control, advisory services, training programmes and schemes on diversified uses of NR.

A study on TSR processing industry in India by the Economics Division found that the capacity utilisation of the industry was only 54 per cent and the five year average cost of processing of the industry was Rs.5.52 per kg. Another study on the impact of price volatility on non-tyre rubber products industry in Kerala found that latex based units have either temporarily closed down or cut down their production. A study on commercial yield performance of rubber clones in the estate sector found that the share of area under the yield declining phase is on the increase. During the 1-10 year phase of tapping PB 260 recorded the highest yield (1741 kg/ha) followed by RRII 105 (1732 kg/ha) in the estate sector.

The Exploitation Technology Division initiated a collaborative programme with the Rubber Production Department of the Rubber Board for popularizing d/3 frequency tapping among the smallholders. The Division also started evaluation of G-Flex technology for controlled upward tapping (CUT). For the large scale implementation of CUT in smallholdings training of trainers was organized. A CUT demonstration trial was initiated at Taranagar farm in Tripura.

In addition to meeting the needs of the scientists of various disciplines, the Central Experiment Station, Chethackal, realized a total crop of 140222 kg. during the reporting year. The Regional Research Stations continued their research activities in their respective priority areas to cater to the requirements of the region. Rubber Research Institute of India published 40 research articles, one book, one monograph, four book chapters, one report and 46 popular articles. In addition 51 papers were presented by our scientists in national and international conferences.

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, reduce cost of cultivation and sustain soil quality. Research programmes are also undertaken to address specific agronomic problems. Experiments were initiated in immature rubber to reduce the application of chemical fertilizers, integrating it with organic and biofertilizers. A study was undertaken in Central Kerala to find out the effect of unscientific pineapple intercropping on growth of rubber and soil properties in smallholdings. A comparison was made between the soil chemical properties of forest and rubber after first, second and third cycles using X-ray fluorescence technique. Georeferencing and extracting themes from toposheets and soil maps were initiated. Estimation of total area under rubber and rubber area distribution in relation to soil and landscape attributes is in progress. The Division also functions as a centre for dissemination of knowledge on various soil and crop management techniques.

1. Nutrient management

1.1 Nursery

The experiment to compare different nutrient sources indicated that growth of polybag plants was significantly superior when the source was water-soluble fertilizers (Table Ag. 1).

Comparison of polybag plants raised by direct seeding and planting budded stumps indicated superior growth of direct seeded plants (Table Ag. 2).

Table Ag. 2. **Growth of direct seeded polybag plants and budded stumps raised in polybags**

Growth parameter	Direct seeded polybag plants	Budded stumps raised in polybags
Height (cm)	39.26	24.96 **
Diameter (mm)	6.61	4.92**

** Significant at P < 0.01

In the experiment to study the effect of split application of fertilizers in seedling nurseries, the results were not consistent. Experiments were also conducted at CES, Chethackal and Central Nursery, Karikkattoor to explore the possibility of

Table Ag. 1. **Effect of nutrient sources on growth of plants**

Treatment	Direct seeded polybag		Budded stump polybag	
	Diameter (mm)	Height (cm)	Diameter (mm)	Height (cm)
T_1 - NPK@ 6-6-2.4 g/plant (N&P as urea and RP)	6.22	39.02	4.96	24.63
T_2 - T_1 + Cow dung slurry	6.24	38.53	5.09	26.51
T_3 - T_2 + Groundnut cake	6.39	39.18	4.86	25.16
T_4 - Standard practice (N&P as ammophos)	6.84	40.38	5.08	25.24
T_5 - T_4 + Cow dung slurry	7.06	41.25	5.06	25.60
T_6 - T_5+ Groundnut cake	6.99	42.35	5.23	24.21
T_7 - T_4+ Phytonol	6.83	39.83	4.84	24.45
T_8 - 50% of T_4+ PGPR	6.35	39.87	4.65	22.74
SE	0.16	1.16	0.09	0.66
CD (P = 0.05)	0.47	NS	0.30	1.99

reducing soil application of urea in seedling nurseries. Instead of the second dose of urea application at the rate of 250 kg/ha, foliar urea spray was given at varying reduced levels. Diameter and dry matter accumulation of plants were not significantly different between foliar applied and soil applied control plants.

1.2. Immature rubber

To develop an integrated nutrient management package for rubber, three experiments were initiated in the traditional rubber growing region and two in the north-east region. The experiments also aim to monitor the microbial population and study the survival pattern of inoculated microbes in the rhizosphere. In the traditional region, field experiments were initiated at CES, Chethackal in the rubber + cover crop (*Pueraria phaseoloides*) and rubber + banana (Nendran) intercropping systems and at Puthukad Estate, Trichur in the rubber + cover crop (*Pueraria phaseoloides*) system. The treatments indicated control without any fertilizers, inorganic fertilizers alone, biofertilizers integrated with 25, 50, 75 and 100 per cent inorganic fertilizers and biofertilizers alone. A consortium of *Azotobacter*, phosphobacteria, *Pseudomonas* and VAM was applied as the biofertilizer.

In the rubber + cover crop experiment at CES, Chethackal, diameter of plants showed significant difference between control (no fertilizer) and the treatments having combinations of inorganic N and P and biofertilizer (BF) and biofertilizer alone applied plants indicating positive response (Table Ag. 3). Significant differences between treatments with respect to growth were not indicated in the rubber + banana system at CES, Chethackal and rubber + cover crop system at Puthukad Estate. The microbial population was higher in the biofertilizer applied treatments after three months of application in all the experiments.

Table Ag. 3. **Effect of biofertilizer application on diameter of plants (mm) in rubber with cover crop at CES, Chethackal**

Treatment	Diameter (mm)
Control	9.53
Standard practice	10.69
25%N & P + BF	11.64
50% N&P + BF	11.29
75% N&P + BF	11.54
Standard practice +BF	10.97
BF alone	11.79
SE	0.47
CD (P = 0.05)	1.45

A field experiment was initiated at Pottamkulam Estate, Yendayar with the objective of improving carbon quality, microbial activity and fertility of rubber growing soils through the addition of biofertilizers/green manures. The treatments included different sources of green manure, *viz. Gliricidia*, *Mucuna* and natural cover integrated with 50 per cent chemical fertilizers and biofertilizers (consortium of *Azotobacter*, Posphobacteria, *Pseudomonas* and K-solubilizing bacteria supplied by the microbiology unit of RRII). No significant difference was observed among the treatments with respect to girth and soil microbial population three months after biofertilizer application.

The field experiment initiated in 2001 to study the effect of integrating chemical fertilizers with organic manures on growth of rubber and soil physico-chemical properties was continued. The treatment *viz.* 25 per cent chemical fertilizer + 75 per cent farm yard manure, recorded significantly higher girth and cumulative girth increment compared to control (Table Ag. 4).

The field experiment to study the effect of coir pith organic manure (C-POM) on early establishment and growth of rubber plants in sandy soil was continued at Thanneermukkom, Cherthala. Diameter of plants recorded after one year of planting showed no significant

Table Ag. 4. **Influence of chemical and organic manures on growth of rubber**

Treatment	Girth (cm)	Girth increment (cm)
	2009	2002-2009
No fertilizer/no manure (control)	43.98	40.56
Farm yard manure (FYM) alone	48.12	44.81
Fertilizers (standard recommendation)	47.27	43.81
25% fertilizers + 75% FYM	50.01	46.54
50% Fertilizer + 50% FYM	48.39	44.73
75% Fertilizer + 25% FYM	46.96	43.46
SE	1.07	1.48
CD (P = 0.05)	3.22	3.16

difference between treatments. Good establishment and satisfactory initial growth of rubber were observed in the sandy soil of the experiment area.

Field experiment at Malankara Estate, Thodupuzha to study the effect of zinc application on the growth of young rubber plants and on the availability of zinc in the soil was continued. The effect of zinc application on growth of plants was not significant.

Hydroponic experiment was conducted in the laboratory to study the acidity and Al tolerance of rubber seedlings. Different levels of Al in the nutrient solution *viz.* 0, 5, 10, 15 and 20 ppm were tried. The root growth was arrested and phytotoxic symptoms were noticed at 10 ppm concentration.

1.3. Mature rubber

The experiment on sequential skipping of fertilizer application in mature rubber at Kodumon Estate, Adoor is in progress. Growth and yield of rubber trees were not significantly influenced by skipping of fertilizers up to a period of seven years. Soil and leaf nutrient status among the treatments was not significantly influenced by skipping of fertilizers (Table Ag. 5).

The study on partial substitution of potassium with sodium in mature rubber at Malankara Estate, Thodupuzha was continued and no significant difference in yield was observed for substitution up to 50 per cent.

Biomass estimations of 400 series clones *viz.* RRII 414, 430, 429, 417 and 422 indicated

Table Ag. 5. **Effect of skipping of fertilizers on growth and yield of rubber**

Treatment	Girth increment (cm) 2002-2009	Yield (g/t/t) 2008-09
Standard practice	11.11	64.85
Application of full dose every year (pre-monsoon)	12.33	59.63
Skipping pre-monsoon fertilizer application (Application of half the dose as post-monsoon)	12.25	61.28
Skipping post-monsoon fertilizer application (Application of half the dose as pre-monsoon)	12.05	70.28
Skipping for one year	11.36	60.12
Skipping for two years	13.09	75.05
Continuous no manuring	12.88	68.84
SE	0.88	6.71
CD (P = 0.05)	NS	NS

that the clones differ in biomass accumulation. The biomass was more in RRII 414, 429 and 417 than 430 and 422.

2. Intercropping and cropping systems

Experiment to find out the feasibility of growing perennial intercrops in rubber was continued. Perennial intercrops *viz.* coffee, vanilla, *Garcinia* and nutmeg are being evaluated in the normal system of planting and paired-row system of planting of rubber. In the normal planting system, availability of photosynthetically active radiation at the centre of inter-row area was 3.2 per cent of the open, where as in the paired-row system of planting a strip of width 2.6 m was available at the centre of wide inter-row area without shade for intercropping. In both experiments, girth and girth increment of rubber were higher in the intercropped plots. (Table Ag. 6).

In the normal system of planting, *Garcinia* yield declined due to the intense shade effect and was less than that of monoculture. Vanilla and coffee yields were higher under intercropping. Nutmeg started flowering in both the experiments.

Experiment on interaction between rubber and wild jack was continued. Distance of wild jack from the rubber had significant effect on the growth and yield of rubber at both the locations, *viz.* Pala and Ponthenpuzha. At Pala, growth and yield of rubber without wild jack were significantly higher compared to those along with wild jack. Average rubber yield (g/t/t) showed significant negative relation with basal area (BSA) of wild jack. At Ponthenpuzha, rubber yield was higher when the distance between rubber and wild jack trees was more than 2 m compared to cases where the distance was less (1.5 m).

Experiment on inter-planting of rubber with teak, wild jack and mahogany at CES Chethackal was continued. Girth and girth increment of rubber were not significantly influenced by row spacing, type of timber inter-crop and their interaction.

Regression equations were developed to relate girth with total nutrients accumulated in the different tree species studied. Regression equation showed either exponential or power form and showed very good prediction (R^2 = 0.8 - 0.9). From these equations, the nutrients locked in the standing trees of different treatments were estimated. Among the different treatments, total nutrients locked in rubber and timber during fifth year was more in rubber mixed planted with wild jack.

Experiment on effect of density of timber inter-planting on growth of rubber was continued at CES Chethackal. Girth of rubber was not found to be significantly influenced by density of timber crops.

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

Treatment	Paired-row system of planting		Normal system of planting	
	Girth 2008	Girth increment (2003-08)	Girth 2008	Girth increment (2003-08)
Rubber alone	45.55	33.38	44.74	32.78
Rubber + *Garcinia*	47.47	34.74	48.38	35.95
Rubber + coffee	48.30	36.69	47.21	35.59
Rubber + vanilla	46.19	33.92	47.88	36.74
Rubber + nutmeg	45.42	33.75	46.26	35.10
SE	0.53	0.51	0.73	0.62
CD (P = 0.05)	1.65	1.58	2.26	1.92

Growth of teak was significantly better at the highest density. Growth of mahagony was not influenced by density.

2.1. Impact of unscientific pineapple intercropping on growth of rubber and soil properties

A survey was conducted among rubber smallholdings in Thodupuzha, Muvattupuzha and Kothamangalam regions in collaboration with the Microbiology group of Plant Pathology Division and RP Department to study the effect of unscientific pineapple intercropping on growth of rubber and soil properties. Smallholdings planted with clone RRII 105 and intercropped with pineapple during 2004 were selected for the study. Holdings were classified in to scientifically and unscientifically intercropped categories based on the distance of pineapple plants from rubber plants. Those with a distance of 1.2 m or more were categorized as scientifically intercropped and others as unscientifically intercropped. Out of the total 41 holdings surveyed, 37 were intercropped with pineapple and four with cover crop in the inter-row area. Pineapple intercropping in all the holdings was carried out on contract basis. Among the holdings surveyed, 65 per cent were scientifically intercropped. Growth of rubber was significantly lower when pineapple intercropping was unscientific. (Table Ag. 7).

Table Ag. 7. **Influence of pineapple intercropping on growth of rubber 4 years after planting**

Location	Girth (cm)	
	Scientific pineapple intercropping	Unscientific pineapple intercropping
Muvattupuzha	33.08 (n = 148)	31.29 (n = 145)
Thodupuzha	29.56 (n = 58)	25.86 (n = 91)

Available P status was higher in both the categories of pineapple intercropped fields. Soil organic carbon and other available nutrients were not influenced by both types of pineapple intercropping. Soil microbial population varied widely between holdings, and there was no definite trend in microbial population among the systems studied.

3. Reduction in gestation period

The field experiment initiated to develop an agronomic package to reduce the immaturity period of rubber was continued. The girth of the plants under the integrated management was significantly superior to all other treatments (Table Ag. 8).

Table Ag. 8. **Effect of agromanagement practices on growth of rubber**

Treatment	Girth (cm) 2009
Standard practice	27.56
Selective manuring	31.16
Enhanced nutrient application	30.54
Conservation-oriented tillage	31.37
Irrigation	32.57
Irrigation + Enhanced nutrient application	33.91
Integrated management	37.28
SE	0.79
CD (P = 0.05)	2.43

Another experiment was initiated at CES, Chethackal with the objective to reduce the immaturity period. Effect of planting materials *viz*. direct seeded green budded plants and green budded stumps raised in polybags coupled with standard practice and integrated management on growth is being compared. Planting materials were raised and field planting was carried out. The girth of both types of planting materials with integrated management was found to be superior compared to respective type of planting material with standard practice.

Another field experiment was initiated at CES, Chethackal during 2008 to study the effect of different types of planting materials (polybag plants with one whorl, two whorls and three whorls and root trainer plants with one whorl, two whorls and three whorls) on growth of rubber.

4. Planting systems

The experiment initiated at B.C. Cheruvally Estate during 2007, to study the effect of different planting geometries on canopy development, growth and yield of rubber is in progress. Growth of rubber was not influenced by the different treatments. The canopies in triangular planting and twin planting systems exhibited asymmetrical pattern of growth.

The study on effect of planting density on growth and yield of rubber at CES, Chethackal, was continued. Plants in the lowest density of 420 trees/ha recorded significantly higher girth (Table Ag. 9) and yield (g/t/t) (Table Ag.10) compared to all other treatments. No significant difference was recorded in the annual yield on per hectare basis.

5. Pit size for planting

The field experiment on effect of pit size on growth of rubber initiated at CES, Chethackal during 2002 was concluded. The soil depth in the experiment area was around 1 m. The study revealed that pits or pit sizes did not influence root development (Table Ag. 11) and growth of rubber.

The growth of tap root system in the treatments with no pitting and the biggest pit size showed that when the soil is deep, the length of the tap root was 2.2 m for the former and 2.3 m for the latter. In both treatments presence of hard pans 90 cm below the soil layers restricted the growth of tap roots to deeper soil layers.

Table Ag. 9. **Girth of trees during January 2009**

Treatment	Girth (cm)		
	M1	M2	Mean
D_1 – (420 trees/ha)	69.12	70.18	69.65
D_2 – (479 trees/ha)	64.10	62.52	63.31
D_3 – (549 trees/ha)	66.74	62.49	64.62
D_4 – (638 trees/ha)	62.10	61.30	61.70
D_5 – (749 trees/ha)	60.97	58.87	59.92
Mean	64.60	63.07	
Main plot treatment SE - 1.09 CD (P = 0.05) - 2.39		Sub-plot treatment SE - 1.43 CD - NS	

M1 - Manure on area basis
M2 - Manure on per plant basis

Table Ag. 10. **Yield of trees (April 2008 – March 2009)**

Treatment	Yield (g/t/t)			Yield (kg/ha/year)		
	M1	M2	Mean	M1	M2	Mean
D_1 – (420 trees/ha)	70.56	67.52	69.04	1672	1216	1444
D_2 – (479 trees/ha)	51.69	47.67	49.68	1677	1655	1666
D_3 – (549 trees/ha)	49.98	37.21	43.60	1607	1376	1492
D_4 – (638 trees/ha)	41.43	36.49	38.90	1721	1522	1622
D_5 – (749 trees/ha)	42.23	39.02	40.62	1975	1536	1756
Mean	51.18	45.58		1730	1461	
Main plot SE - 4.36 CD (P = 0.05) - 9.50				Main plot SE - 198.12 CD (P = 0.05) - NS		
Sub-plot SE - 3.61 CD - NS				Sub-plot SE - 118.46 CD (P = 0.05) - 252.49		
Interaction - NS				Interaction - NS		

M1- Manure on area basis M2 – Manure on per plant basis

Table Ag.11. **Effect of pit size on lateral root growth (6th year)**

Pit size	Root area (cm^2/100 cc of soil)	Root length (cm/100 cc of soi)
	2.3 m from plant	2.3 m from plant
45 x 45 x 45 cm	2.08	16.65
60 x 60 x 60 cm	2.65	23.74
75 x 75 x 75 cm	3.38	17.99
90 x 90 x 90 cm	2.40	20.58
60 x 60 x 90 cm	3.33	19.55
90 x 90 x 60 cm	3.46	25.75
No pitting and planting polybag plants in holes	3.36	16.73
SE	0.44	3.75
CD (P = 0.05)	NS	NS

6. Soil and water conservation

An observational trial was initiated at Puthukkad Estate, Trichur during 2008 to study the effect of restricted tillage in the plant basin after monsoon rain in October in conserving residual soil moisture. Tilling the plant basin (1 m around the plant) to a depth of 15 cm conserved soil moisture and reduced casualty compared to that in control plots. Diameter of plants was higher in tilled plots and was comparable to that of plants which received life saving irrigation twice weekly at the end of the rainy season.

7. Soil health/quality

A study was undertaken to compare the soil nutrient status and to study the fertility changes over years in different seedling nurseries. High available P and low Zn status were observed in all the nurseries (Table Ag. 12) and a decline in OC status and pH was observed during a period of 22 years.

Studies on seasonal variation of soil nutrient availability in rubber soils showed that the soil nutrient availability varied over seasons and nutrient availability was higher during October - December period compared to February- April period.

Soil nutrient data generated for offering fertilizer recommendations in estates were utilized to assess the changes in soil organic carbon and pH over years. The nutrient data of samples collected during 1975-76 and 2005-08 from the same fields of estates were compared. A significant decline in OC was observed in majority of the fields (Table Ag. 13). Currently, 63 per cent of the fields investigated have 1 to 2 per cent of OC status.

A similar data investigation was carried out for soil nutrient values of samples collected from smallholdings for fertilizer recommendation purpose from the northern, central and southern regions of Kerala. The samples collected during 1979-80 and 2006-08 were compared. The number of holdings with lower organic carbon status and lower pH were significantly higher during 2006-08.

Table Ag. 12. **Fertility status of seedling nurseries (0-30 cm)**

Name of nursery	OC (%)	pH	Av. nutrients (mg/100 g soil)				Micronutrient status (ppm)			
			P	K	Ca	Mg	Fe	Mn	Cu	Zn
Karikattoor	1.20	4.10	19.10	4.80	3.91	0.49	26.08	0.58	1.61	0.29
Kadackamon	1.31	3.70	8.10	10.90	7.73	1.98	32.76	1.39	0.42	0.59
Kanhikulam	0.72	4.60	7.25	7.00	20.91	3.84	30.78	39.70	1.43	0.57
Manjeri	0.57	4.30	6.55	10.10	17.57	3.00	15.21	6.46	1.84	0.38
Ulickel	1.58	4.20	9.25	5.10	5.15	1.01	31.20	3.57	1.12	0.33
Alakode	1.43	4.50	3.88	12.00	21.22	2.28	12.52	5.58	1.69	0.28
Kidangoor	0.84	4.50	2.32	6.00	11.80	1.70	23.78	4.28	1.79	0.65
Mundakkayam	2.14	5.00	2.06	8.80	60.62	6.22	49.46	5.45	17.20	1.39

Table Ag. 13. **Change in soil organic carbon status during 30 years of rubber cultivation**

Name of estate	Organic carbon (%) 0-30 cm		t-test	Organic carbon (%) 30-60 cm		t -test
	1975-76	2005-08		1975-76	2005-08	
Kinalur	1.89	1.23	**	1.32	0.95	NS
Cheruvally	2.33	1.70	**	1.63	1.45	**
Moopiy	1.41	1.41	NS	1.12	1.15	NS
Kundai	1.48	1.27	**	1.17	0.98	**
Mundakkayam	1.65	1.25	**	1.40	1.13	**
Kulathupuzha	2.15	1.30	**	1.54	1.00	**
Lahai	1.56	1.10	**	1.13	0.85	**
Ayiranallur	2.17	1.40	**	1.48	1.20	**
T.N. Forest Dept	1.77	1.39	**	1.72	1.48	NS
Arasu	1.38	1.31	NS	0.83	1.30	*
Kodayar	2.37	1.58	**	1.68	1.17	**
Mylar	1.88	1.42	NS	1.67	1.09	**

** Significant at P = 0.01 * Significant at P = 0.05

The study on comparison of different soil ecosystems, *viz.* rubber-mature, rubber immature with *Mucuna bracteata*, rubber-immature with pineapple, cassava, teak and forest with special reference to soil organic matter at Travancore Rubber Estate, Mukkoottuthara and Kanamala forest range in Kottayam region was continued. Water soluble organic carbon (WSOC), hot water extractable carbon (HWEC), mineral – N (Min N), total N (TN) and organic carbon (OC) in soil samples were estimated. OC estimations were carried out by modified Walkley-Black method with application of external heat. WSOC, HWEC and OC were found to be significantly higher in forest soils than in other systems (Table Ag. 14). OC was higher in *M bracteata* soil compared to teak soil, but its WSOC was lower. Significantly lower HWEC and OC contents were noticed in soils under pineapple and cassava systems. HWEC, OC, TN and Min N were significantly higher in *M.bracteata* cover cropped soils compared to pineapple intercropped soils. The results clearly indicate the decline in soil quality upon cultivation compared to virgin forest soils. The soil quality indices in rubber - *M. bracteata* system was superior to rubber pineapple system.

Table Ag. 14. **Water soluble carbon, Organic Carbon, Total N and Mineral N in soils under different systems**

Systems	WSOC (ppm)	HWEC (ppm)	OC (%)	TN (%)	Min N (ppm)
Rubber	11.36	110.25	2.73	0.08	32.46
M.bracteata	18.75	180.75	3.29	0.19	25.96
Pineapple	17.06	49.50	2.13	0.11	6.04
Cassava	19.19	23.25	1.66	0.11	4.68
Forest	67.53	206.25	5.40	0.35	44.82
Teak	57.58	149.25	2.89	0.24	11.17
CD (P=0.05)	14.90	25.56	0.36	0.10	6.68

Soil nutrient status after first, second and third cycles of rubber cultivation at TR & T Estate, Mundakkayam was compared with that of nearby virgin forests (Table Ag. 15). Relatively higher cation exchange capacity (CEC) and pH were observed in forest soil compared to rubber soils. Organic carbon (OC) percentage was significantly lower in all cycles of rubber compared to forest. Total nutrients were estimated by X-ray fluorescence technique. Total P content was higher in rubber soil. Total K, total Ca and total Mg declined from first to third cycle of rubber cultivation. Rubber cultivation

Table Ag.15. **Soil chemical properties under forest and rubber**

Soil parameter	Forest	Rubber			CD
		1st cycle	2nd cycle	3rd cycle	
CEC (cmol/kg)	10.52	6.81	7.44	6.64	1.06
Soil pH	4.85	4.71	4.66	4.65	0.10
OC (%)	3.08	1.40	1.67	1.79	0.34
Total N	0.21	0.15	0.16	0.14	0.03
Total P	0.15	0.27	0.19	0.19	0.04
Total K	1.13	1.85	1.15	0.96	0.51
Total Ca	0.36	0.33	0.25	0.18	0.14
Total Mg	0.72	0.98	0.64	0.55	0.17
Total Fe (%)	9.05	10.14	9.09	8.94	NS
Total Mn (%)	0.06	0.07	0.09	0.06	NS
Total Cu (ppm)	64.13	91.13	102.86	113.13	12.03
Total Zn (ppm)	67.63	78.66	77.38	64.60	NS

resulted in significant increase in total soil copper in comparison with adjacent forest.

In the standardization of analytical protocols, an attempt was made to compare different estimation protocols for organic carbon. It was found that modified Walkley-Black procedure recovered about 30 per cent more carbon. Conservation of heat of dilution by keeping the reaction vessel on an insulating media and sample homogenization by pulverizing the soil to pass through a sieve of 0.15 mm are very crucial steps to be followed and not to be overlooked in standard Walkley-Black

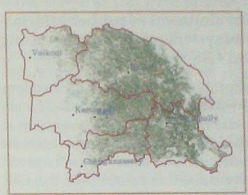


Fig. Ag. 1. **Distribution of rubber area in Kottayam District**

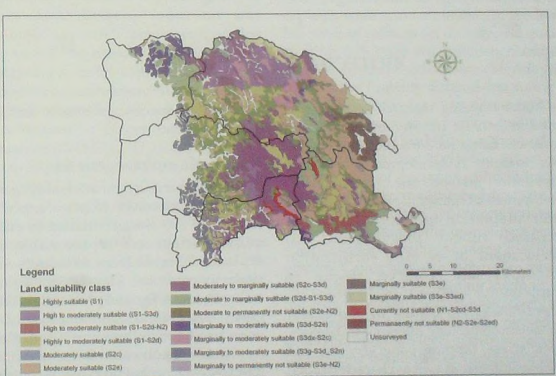


Fig. Ag. 2. **Land suitability map of Kottayam District**

protocol. Conversion factor for OC by standard Walkley-Black procedure was formulated. The regression equation between standard WB-carbon and quantitative external heat carbon (modified Walkley-Black) values is $y = 0.27 + 1.079 x$ (y = modified WB carbon; carbon; x = Carbon by Walkley-Black).

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

With the help of NBSS & LUP Bangalore, toposheet-based soil information of traditional rubber growing area was regrouped into different soil management units based on soil depth, organic carbon and gravel content and districtwise thematic maps were generated. Districtwise soil management unit maps were distributed to the respective regional offices of Rubber Board.

Geo-referencing and extracting themes from toposheets and soil maps were initiated. Themes like land suitability of Kottayam District for rubber cultivation was extracted and brought into GIS along with DEM and overlay analysis was done to assess the rubber area distribution in relation to soil and landscape attributes. In Kottayam District, total area under rubber (age >4 years) was estimated as 66105.67 ha (Fig. Ag.1). Rubber area is mainly distributed in Meenachil taluk (44.7%) followed by Kanjirapally (23.6%), Kottayam (17.4%), Changanassery (10.4%) and Vaikom taluks (3.9%). Majority of rubber area in Kottayam district is distributed on moderately suitable land followed by highly suitable land (Fig. Ag. 2). About 11 per cent of rubber area is distributed on marginally suitable land having limitation with respect to shallow soil depth, high gravel content and steep slope. Rubber distribution over different slope classes showed that 49 per cent of area is distributed on 5 to 15 per cent slope.

BIOTECHNOLOGY DIVISION

Major ongoing research programmes aimed at genetic improvement of *Hevea brasiliensis* using modern biotechnological tools include: i) development of *in vitro* propagation methods for elite clones, ii) development of transgenic *Hevea* plants for better adaptation to abiotic stress and increased rubber biosynthesis, iii) development of *in vitro* fertilization techniques and embryo rescue to complement conventional breeding programmes, iv) study of molecular mechanism controlling tolerance to diseases, abiotic stress, tapping panel dryness and latex biosynthesis as well as characterization of related genes and v) characterization of laticifer cell-specific promoters and gene expression.

1. Somatic embryogenesis

Experiments on somatic embryogenesis and plant regeneration from leaf explants of clone RRII 105 were continued to make it more efficient and reproducible. Leaf explants collected from different sources such as *in vitro* developed somatic embryogenesis-derived plants, glass house-grown bud-grafted plants and mature trees were used for culture initiation. Callus formation was observed in cultures within four weeks of incubation in media containing BA, 2, 4-D and NAA. Rate of callus induction

was found to be reduced with maturity of the source plant. The highest callus induction frequency (90%) was obtained in leaves taken from somatic plants growing in culture tubes, 40 per cent in leaves collected from budded plants and 15 per cent in explants taken from mature trees. Proliferation was obtained only from callus induced in leaves of bud- grafted plants and somatic plants.

To find the combined effect of calcium, sucrose and medium desiccation on embryogenic callus initiation, $Ca(NO_3)_2.4H_20$ (0-1200 mg/L) was supplemented in modified MS medium along with sucrose (50-150 mg/L). Medium desiccation was provided by using phytagel (2-8 g/L). Time and frequency for friable embryogenic calli formation varied with the explant source. Embryogenic callus initiation was observed in callus induced in leaves taken from *in vitro* somatic plants within three months with a frequency of about 60 per cent and in leaves taken from budded plants, it took nearly five months with a frequency of 30 per cent. Embryo induction was simultaneous with embryogenic callus emergence. Embryogenic calli initiation was not obtained in callus obtained from leaves collected from mature trees. Embryos could be induced from proliferated embryogenic calli with a frequency of 60 per cent in modified MS medium ($Ca(NO_3)_2.4H_2$ - 360 mg/L and sucrose - 80 g/L) containing phytohormones BA (0.2 mg/L), kin (0.3 mg/L), GA_3 (0.8 mg/L) and NAA (0.1 mg/L). Maturation and apex induction could be achieved in WPM medium containing organic supplements such as CW (10%), malt extract (200 mg/L), casein hydrolysate (400 mg/L) and phytohormones BA (0.5 mg/L), IBA (0.1 mg/L) and GA_3 (1.0 mg/L). When the cultures were dark-incubated, the embryos enlarged and apex induction occurred within two weeks.

For maturation of embryos induced in callus derived from leaf explants taken from *in vitro* somatic plants, a liquid phase introduced before transferring to the maturation medium was found favourable. Half-strength maturation medium was used for liquid culture. After keeping the embryos in the liquid medium for 3-5 days, they were transferred to the solid maturation medium. Mature cotyledonary embryos were transferred to the germination media standardized earlier for *H. brasiliensis* embryos, derived from other explants. The germinated embryos upon transfer to plant regeneration medium devoid of growth regulators, advanced into well-developed plantlets having both shoot and root growth. Regenerated plants with mature leaves were transferred to earthenware pots and kept in the environmental growth chamber (90% RH) and left uncovered. No leaf wilting or fungal infection was observed and the initial survival rate was 90 per cent.

To support genetic transformation experiments, somatic embryogenesis and plant regeneration using immature anther explants were also continued. To study the influence of calcium on the rate and quality of callus induction different concentrations of calcium nitrate and calcium chloride were tried. Callus friability differed depending on the concentration of calcium nitrate / calcium chloride in the callus induction medium. At very high level, (15 mM) callus obtained was highly friable. To study the effect and concentration of sugars on callus induction, sucrose, glucose, maltose and fructose was added individually at two concentrations (20 and 30 g/L). Among the four sugars tested, sucrose induced callus showing maximum growth. Callus was also induced in glucose as well as maltose containing medium, but the growth of callus was comparatively low. A separate experiment was also carried out to find the optimum level of sucrose required for inducing friable callus. Very friable callus could be induced at higher concentrations of sucrose (60-80 g/L).

With ovule explants, callus induction was noticed from the unfertilized ovules in MS medium containing growth regulator combinations of NAA (1.0 mg/L) and kin (0.5 mg/L) supplemented with high levels of sucrose (9%). The callus was proliferated in the same medium supplemented with 5 per cent sucrose and reduced levels of growth regulators. The calli were sub-cultured to growth regulator-free N6 medium for the emergence of embryogenic callus. Embryo induction was achieved in modified MS medium containing organic supplements and growth regulators. Frequency of embryo induction was 80 per cent when the medium was supplemented with kin (2.0 mg/L) and GA_3 (1.0 mg/L). Mature embryos were subjected to desiccation either rapidly for 4 h or slowly for 48 h. After desiccation, the embryos were subcultured for germination in ½ MS medium supplemented with kin (0.5 mg/L) and BA (0.5 mg/L). The plantlets obtained were transferred to polybags for hardening.

2. *In vitro* fertilization and embryo rescue

Embryo rescue and plant regeneration experiments were continued. Open-pollinated fruits were collected at different maturity (1-5 weeks) and inoculated in different combinations of nutrient media for the recovery of plantlets. Basal media such as MS, WPM, SH and Nistch along with different growth regulators were tried. GA_3 (1.0 mg/L) was included in all the experiments since, in earlier experiments, it was proved to be highly essential for embryo development. Along with GA_3, kin (1.0-3.0 mg/L), 2, 4-D (1.0-3.0 mg /L) and NAA (1.0-3.0 mg /L) were also tried. Embryos could be recovered from two-week-old fruits onwards, with low frequency, in a medium containing NAA (2.0 mg/L) along with GA_3 (1.0 mg/L). The percentage of recovery increased with age with five-week-old fruits giving maximum plant recovery (20%).

To induce polyembryony and production of multiple seedlings from a single ovule, immature fruits (8-10 weeks) were collected from the field and inoculated in Nitsch medium with three growth regulators; GA_3, kin and zeatin individually and in combinations. When kin or GA_3 was given alone, single embryos were obtained. When the medium was supplemented with different levels of zeatin (1.0-5.0 mg/L), keeping concentration of GA_3 and kin (2.0 mg/L), polyembryony was observed in 12 per cent of the ovules cultured. Among the 12 polyembryonic ovules obtained, about 40 embryos could be recovered from one particular ovule, in a growth regulator combination of GA_3 (2.0 mg/L) + kin (2.0 mg /L) + zeatin (3.0 mg /L). Among them, 25 embryos were germinated, out of which 20 were planted and eight plants hardened.

3. Genetic transformation

Agrobacterium-mediated genetic transformation experiments were continued to develop transgenic plants with increased tolerance to tapping panel dryness, abiotic stresses and higher latex yield. The genes coding for superoxide dismutase under the control of CaMV 35S and FMV 34S promoters, sorbitol-6-phosphate dehydrogenase, *hmgr*1, and osmotin were used in these studies.

Several putatively transgenic lines were obtained when embryogenic calli raised from anther were infected with *Agrobacterium* harbouring the gene coding for superoxide dismutase (SOD). Emerging transgenic lines were proliferated and they were routinely sub-cultured for embryogenesis. Histochemical GUS assay confirmed

transformation event. Five GUS positive transgenic lines integrated with SOD gene under the control of FMV 34S promoter were obtained with reduced bacterial overgrowth from two-month- old anther callus when a simple and more economic transformation procedure was used.

Forty-day-old anther calli were cultured on callus proliferation medium supplemented with different concentrations of calcium nitrate (0 - 1200 mg/L) to study the effect of pre-culture of the target tissue on transformation frequency. Callus proliferation was faster in medium containing 600 and 800 mg/L calcium nitrate and friability of the callus was also found improved. When *Agrobacterium* infection was carried out with the callus pre-cultured for two weeks in medium containing 400, 600 and 800 mg/L calcium nitrate, transformation frequencies of 6, 8 and 12 per cent respectively were obtained. With control compact callus, transformation frequency was four per cent only.

Significant difference among treatments was observed by the addition of acetosyringone (20 - 200 mg/L) in the co-cultivation medium. Transformation frequencies of 6, 10 and 14 per cent were obtained with acetosyringone concentrations 20, 40 and 80 mg/L respectively. However, at higher concentration (200 mg/L), no transformed cell lines could be recovered due to excessive over-growth of the bacteria. The transformed cell lines obtained were cultured on proliferation medium fortified with different growth regulators and kanamycin (300 mg/L).

Experiments were also done to enhance the embryogenic potential of the transgenic callus. Effect of osmoticum on callus proliferation and embryo induction was studied by including the non-permeable osmoticum, PEG (4 - 12%), in the embryo induction medium. Varying levels of ABA (0.1 - 8.0 mg/L) was used in the embryo induction medium in combination with the optimum concentrations of PEG. Formation of friable calli was observed when PEG was used along with ABA (Table 1). Non-transformed calli failed to survive probably due to the adverse effect of higher ABA concentrations used in the medium. It was noticed that the highest frequency of friable embryogenic calli (68 %) was obtained in a combination of 1.0 mg/L ABA along with 8 per cent PEG.

Exposure of the transgenic calli to NaCl (200-300 mM) positively influenced the production of friable embryogenic calli. In two transgenic lines, the frequency of friable embryogenic callus formation was improved on transfer of the callus from NaCl containing medium to medium devoid of NaCl

Plants were generated from the root explants of transgenic *Hevea* plants integrated with MnSOD gene, via somatic embryogenesis in order to assess the stability of integrated MnSOD gene in transgenic *H. brasiliensis*. Southern blot analysis was carried out with DNA isolated from the

Table Biotech. 1. **Effect of PEG and ABA on embryogenic callus formation**

PEG (%)	Embryogenic callus (%)					
	ABA (mg/L)					
	0.1	1.0	2.0	4.0	6.0	8.0
6.00	0.2 (7.12)	59.8 (7.76)	28.6 (5.38)	16.0 (4.06)	8.0 (2.91)	0 (0.71)
8.00	62.2 (7.91)	68.0 (8.27)	38.6 (6.24)	23.8 (4.93)	7.2 (2.77)	0 (0.71)

CD (P = 0.05) - 0.19

The values given in parentheses are the transformed values.

leaves of transformed and untransformed plants. PCR amplified 804 bp internal fragment of *npt*II gene of the vector was used as the probe. The αP^{32} labelled *npt*II gene probe generated a band of predicted size (980 bp) with *EcoR*1 digest in the transgenic plant and in positive control (vector plasmid).

For developing transgenic plants with enhanced rubber biosynthesis, fresh genetic transformation experiments were carried out with the gene coding for *hmgr*1 using two *Agrobacterium* strains, EHA 105 and pGV 1303. From the infected embryogenic callus of the ovule, few transgenic lines were obtained and the presence of transgene in these lines was confirmed by PCR. These lines were subcultured for embryo induction in media containing different combinations of ABA (0.1 - 0.5 mg/L), BA (0.2 - 0.8 mg/L) and GA_3 (0.3 - 0.5 mg/L). Transgenic embryos obtained from two embryogenic callus lines were kept for further development. The transgenic lines that emerged from the infected fresh anther callus were proliferated and subcultured in different media combinations to make them friable.

In the genetic transformation experiments with osmotin gene, different target explants were infected with *Agrobacterium* strain GV2260 containing the binary vector with the gene coding for tobacco osmotin protein. Fifteen transgenic lines were obtained from embryogenic calli of anther and 10 lines from two-month-old anther calli. Callus proliferation could be achieved in few of the transgenic lines in a combination of growth regulators, 2, 4-D (0.5 mg/L) + NAA (0.5 mg/L) + kin (0.3 mg/L) along with mannitol (10 g/L). Five lines from the 15 transgenic lines obtained from embryogenic calli, and only one line from the 10 transgenic lines that emerged from the two-month-old calli could be proliferated. Embryos could be induced in a growth regulator combination of 2, 4-D (0.1 mg/L) + kin (1.0 mg/L) + BA (0.5 mg/L) + ABA (0.3 mg/L). Only few embryos germinated in the previously standardized germination medium but they showed abnormal growth. It was observed that culturing the embryos in liquid medium for five days and subsequent transfer to hormone-free medium could improve the growth of embryos and germination. The gene integration was confirmed by PCR using *npt*II- specific primers. The apex-induced embryos were subjected to desiccation treatments under different conditions and for different durations. Preliminary results showed that slow drying of embryos in an unsealed, but closed petri dish for 36 h improved germination. Five transgenic embryos could be germinated and plantlets were kept for hardening.

In the attempts to develop transgenic plants integrated with the gene coding for sorbitol-6-phosphate dehydrogenase for drought tolerance, putatively transgenic calli obtained earlier were proliferated by frequent subculture. These gave rise to small embryoids when subcultured for embryo induction. Root and shoot development was observed in the mature embryos on transfer to plant regeneration medium. Two plantlets were obtained without full leaf development. Histochemical assay of the proliferated calli and embryos showed that they were GUS +ve. PCR analysis revealed the presence of transgene in the transformed calli and embryos.

4. Molecular studies

4.1. Molecular mechanism of disease tolerance

Four different forms of β-1,3-glucanase gene promoter were identified. An attempt has been made to understand whether separate gene sequence also exists for these

different promoter forms. After standardization of PCR conditions, the coding region of the fifth form (corresponding to the 910 bp promoter; β-1,3-glucanase-V) has been successfully amplified. On comparison with the earlier reported promoter and coding region of β-1,3-glucanase gene (U 22147), the new form is showing high similarity in the coding region. However, the difference in the promoter region was more. In the gene portion, variation for "CT" repeat was observed in the intron. In the earlier reported form (β-1,3-glucanase–1), 19 "CT" repeats were observed. The molecular weight of the translated protein of this novel form of β-1,3-glucanase is 41070.72 Da, containing 374 amino acids. The isoelectric point is 8.477 with a charge of 2.560 at pH 7.0. Although, two glycosylation sites, which aids in folding and stability of a protein, are present in the reported sequence, the newly isolated sequence shows only a single glycosylation site. The occurrence of this form is also confirmed in selected clones like, Fx 516, RRIM 600, RRII 414, RRII 429 through PCR. The characterization of coding regions of other forms is in progress.

4.2. Tissue-specific gene expression and characterization of promoters

4.2.1. *Characterization of genomic forms of cis-prenyl transferase gene*

An attempt has been made to characterize the different forms of the genomic sequence coding for *cis*-prenyl transferase enzyme present in clone RRII 105 using specific primers corresponding to HRT1 and HRT2 reported earlier from clone RRIM 600.

The genomic sequence amplified using primers specific for the HRT1 gene contains 1266 bp showing 100 per cent homology with the reported cDNA sequence from our laboratory, whereas it seems highly distinct from HRT2. Sequence is registered at NCBI database under the accession no EF 587242. This is the first report on the characterization of a genomic sequence coding for *cis*-prenyl transferase in *Hevea*. The 100 per cent homology with cDNA sequence indicates the absence of intron. Amplicons obtained from genomic DNA and cDNAs contain an open reading frame of 870 bp excluding the stop codon (TAA). The deduced protein sequence contains 290 amino acids. The molecular weight was predicted as 33169.22 Da and pI value as 7.026. No signal peptide was observed when the amino acid sequence was subjected to signal P prediction as any other non-secretory protein. Two potential glycosylation sites were predicted by Net-N-Glyc software.

The genomic sequence amplified with HRT2 specific primers was 1324 bp and the corresponding cDNA was 1165 nucleotide. On comparison, it was found that the genomic sequence contains a single intron of 167 bp. The intron observed was 39 nucleotides upstream to the translation initiation codon (ATG) *i.e.*, in the 5′ UTR region. The predicted molecular weight for the protein was 34076.31 Da with a pI value 8.11. No signal peptide was predicted to this amino acid sequence. Two potential glycosylation sites were observed, one was at 151th amino acid with a glycosylation potential of 0.5453 and the other at 201th position with a potential of 0.6937. The first glycosylation site, Asn-Asn-Ser (NNS) was identical to that observed in *cis*- prenyl transferase I, whereas a change was observed in second glycosylation site. Asn-Gly-Thr (NGT) was observed in *cis*- prenyl transferase II instead of Asn-Ala-Thr (NAT) in *cis*- prenyl transferase I.

4.2.2. *Characterization of different forms of the hmgr1 gene promoter*

An exploratory work was made to study whether different forms of *hmgr*1 promoter

leaves of transformed and untransformed plants. PCR amplified 804 bp internal fragment of *npt*II gene of the vector was used as the probe. The αP^{32} labelled *npt*II gene probe generated a band of predicted size (980 bp) with *EcoR*1 digest in the transgenic plant and in positive control (vector plasmid).

For developing transgenic plants with enhanced rubber biosynthesis, fresh genetic transformation experiments were carried out with the gene coding for *hmgr*1 using two *Agrobacterium* strains, EHA 105 and pGV 1303. From the infected embryogenic callus of the ovule, few transgenic lines were obtained and the presence of transgene in these lines was confirmed by PCR. These lines were subcultured for embryo induction in media containing different combinations of ABA (0.1 - 0.5 mg/L), BA (0.2 - 0.8 mg/L) and GA_3 (0.3 - 0.5 mg/L). Transgenic embryos obtained from two embryogenic callus lines were kept for further development. The transgenic lines that emerged from the infected fresh anther callus were proliferated and subcultured in different media combinations to make them friable.

In the genetic transformation experiments with osmotin gene, different target explants were infected with *Agrobacterium* strain GV2260 containing the binary vector with the gene coding for tobacco osmotin protein. Fifteen transgenic lines were obtained from embryogenic calli of anther and 10 lines from two-month-old anther calli. Callus proliferation could be achieved in few of the transgenic lines in a combination of growth regulators, 2, 4-D (0.5 mg/L) + NAA (0.5 mg/L) + kin (0.3 mg/L) along with mannitol (10 g/L). Five lines from the 15 transgenic lines obtained from embryogenic calli, and only one line from the 10 transgenic lines that emerged from the two-month-old calli could be proliferated. Embryos could be induced in a growth regulator combination of 2, 4-D (0.1 mg/L) + kin (1.0 mg/L) + BA (0.5 mg/L) + ABA (0.3 mg/L). Only few embryos germinated in the previously standardized germination medium but they showed abnormal growth. It was observed that culturing the embryos in liquid medium for five days and subsequent transfer to hormone-free medium could improve the growth of embryos and germination. The gene integration was confirmed by PCR using *npt*II- specific primers. The apex-induced embryos were subjected to desiccation treatments under different conditions and for different durations. Preliminary results showed that slow drying of embryos in an unsealed, but closed petri dish for 36 h improved germination. Five transgenic embryos could be germinated and plantlets were kept for hardening.

In the attempts to develop transgenic plants integrated with the gene coding for sorbitol-6-phosphate dehydrogenase for drought tolerance, putatively transgenic calli obtained earlier were proliferated by frequent subculture. These gave rise to small embryoids when subcultured for embryo induction. Root and shoot development was observed in the mature embryos on transfer to plant regeneration medium. Two plantlets were obtained without full leaf development. Histochemical assay of the proliferated calli and embryos showed that they were GUS +ve. PCR analysis revealed the presence of transgene in the transformed calli and embryos.

4. Molecular studies

4.1. Molecular mechanism of disease tolerance

Four different forms of β-1,3-glucanase gene promoter were identified. An attempt has been made to understand whether separate gene sequence also exists for these

different promoter forms. After standardization of PCR conditions, the coding region of the fifth form (corresponding to the 910 bp promoter; β-1,3-glucanase-V) has been successfully amplified. On comparison with the earlier reported promoter and coding region of β-1,3-glucanase gene (U 22147), the new form is showing high similarity in the coding region. However, the difference in the promoter region was more. In the gene portion, variation for "CT" repeat was observed in the intron. In the earlier reported form (β-1,3-glucanase–1), 19 "CT" repeats were observed. The molecular weight of the translated protein of this novel form of β-1,3-glucanase is 41070.72 Da, containing 374 amino acids. The isoelectric point is 8.477 with a charge of 2.560 at pH 7.0. Although, two glycosylation sites, which aids in folding and stability of a protein, are present in the reported sequence, the newly isolated sequence shows only a single glycosylation site. The occurrence of this form is also confirmed in selected clones like, Fx 516, RRIM 600, RRII 414, RRII 429 through PCR. The characterization of coding regions of other forms is in progress.

4.2. Tissue-specific gene expression and characterization of promoters

4.2.1. Characterization of genomic forms of cis-prenyl transferase gene

An attempt has been made to characterize the different forms of the genomic sequence coding for *cis*-prenyl transferase enzyme present in clone RRII 105 using specific primers corresponding to HRT1 and HRT2 reported earlier from clone RRIM 600.

The genomic sequence amplified using primers specific for the HRT1 gene contains 1266 bp showing 100 per cent homology with the reported cDNA sequence from our laboratory, whereas it seems highly distinct from HRT2. Sequence is registered at NCBI database under the accession no EF 587242. This is the first report on the characterization of a genomic sequence coding for *cis*-prenyl transferase in *Hevea*. The 100 per cent homology with cDNA sequence indicates the absence of intron. Amplicons obtained from genomic DNA and cDNAs contain an open reading frame of 870 bp excluding the stop codon (TAA). The deduced protein sequence contains 290 amino acids. The molecular weight was predicted as 33169.22 Da and pI value as 7.026. No signal peptide was observed when the amino acid sequence was subjected to signal P prediction as any other non-secretory protein. Two potential glycosylation sites were predicted by Net-N-Glyc software.

The genomic sequence amplified with HRT2 specific primers was 1324 bp and the corresponding cDNA was 1165 nucleotide. On comparison, it was found that the genomic sequence contains a single intron of 167 bp. The intron observed was 39 nucleotides upstream to the translation initiation codon (ATG) *i.e.*, in the 5′ UTR region. The predicted molecular weight for the protein was 34076.31 Da with a pI value 8.11. No signal peptide was predicted to this amino acid sequence. Two potential glycosylation sites were observed, one was at 151th amino acid with a glycosylation potential of 0.5453 and the other at 201th position with a potential of 0.6937. The first glycosylation site, Asn-Asn-Ser (NNS) was identical to that observed in *cis*- prenyl transferase I, whereas a change was observed in second glycosylation site. Asn-Gly-Thr (NGT) was observed in *cis*- prenyl transferase II instead of Asn-Ala-Thr (NAT) in *cis*- prenyl transferase I.

4.2.2. Characterization of different forms of the hmgr1 gene promoter

An exploratory work was made to study whether different forms of *hmgr*1 promoter

are present in the same clone. The *hmgr*1 promoter sequence was amplified from genomic DNA isolated from clone RRII 105, cloned and plasmids from seven bacterial colonies were sequenced. The sequencing information showed that out of the seven clones sequenced, three clones contained 12 TA repeats, one contained 13 and the other three contained nine repeats indicating that three distinct promoter sequences are present in the clone RRII 105 itself.

4.2.3. Identification of transcription factor binding sites in the promoters of hmgr1 and FDP genes

The 505 bp *hmgr*1 and 165 bp FDP gene promoters were analysed to identify the transcription factor binding sites using bioinformatic tools. Several *cis*-acting regulatory elements were observed in the *hmgr*1gene promoter which is listed in Table Biotech. 2. *Cis* elements observed include, light responsive, pathogen-induced and wound-induced elements. *Cis*- acting regulatory elements observed in the FDP gene promoter on PLACE analysis include GATA box, MYBPZM, Root motif, RAVIAAT and CTRMCAMV35S. Major elements observed in this promoter are listed in Table Biotech. 3.

4.2.4. Functional analysis of hevein and hmgr1 promoters in transgenic tobacco

In order to find out the efficiency of the promoters isolated, promoter: GUS fusion binary vectors were developed with *hmgr*1 and the full length of the two forms of hevein promoters. Binary vectors were also developed with 1006 and 272 bps of the two forms of hevein promoters. *Agrobacterium-*

Table Biotech. 2. **Known regulatory elements observed in hmgr1 gene promoters**

Putative cis-element/ consensus	Function/response	No. of elements in *hmgr1* promoter
GT 1 consensus (GRWAAW)	Cell type specific	3
Dof protein binding site (AAAG)	Signal responsive and / or tissue specific gene expression	3
GTG motiff	Expression in pollen	1
GATA box	Light responsive element	2
Root motiff	Expression in roots	6
WRKY element (TGAC)	Stress / pathogen induced expression	2
E box (CANNTG)	Plant pathogen interaction	3
WUSATAg (TTAATGG)	Expression in responding wound	1

Table Biotech. 3. **Known regulatory elements observed in FDP gene promoter**

Putative cis-element/ consensus	Function/response	No. of element in *hmgr1* promoter
Myb PZM (CCWCC)	Dehydration stress responsive Light regulated expression	1
GATA box (GATA)	Expression in roots	1
Root motif (ATATT)	Expression in leaves and root	1
RAVIAAT (CAACA)		1
CTRMCAMV35S		

mediated tobacco transformation was carried out and transgenic plants were developed. Selected transformed plants were analyzed for the presence of GUS activity by histochemical staining of vegetative tissues with X-gluc. Positive GUS activity was observed in all transgenic plants with promoter: GUS fusion constructs. GUS activity was detected on the leaf, petiole, stem and root of the transgenic plants. Also the expression in terms of blue colour was found higher in the wounded tissues and in veins. The results suggest that all the seven promoter fragments analyzed were functional, in terms of their ability to direct expression of *Uid* A gene (GUS gene) in transgenic plants. Irrespective of the size and source of promoter fragment contained, tissues derived from all the transgenic plants turned blue in X-gluc solution. No significant visual difference in GUS gene expression was observed among the transgenic plants integrated with different lengths of promoter: GUS fusions.

4.2.5. Functional analysis of hevein and hmgr1 promoters in Hevea callus through transient assay

An *Agrobacterium*-mediated transient assay of *Hevea* callus was also developed for the functional analysis of the promoter. The *Hevea* calli infiltrated with *Agrobacterium* harbouring the binary vector containing hevein and *hmgr*1 promoter: GUS fusions were analyzed after 48 hours of incubation. Blue colour was developed in the calli infiltrated with *Agrobacterium* harbouring binary vectors containing hevein and *hmgr*1promoter inserted upstream to the gene, by the addition of X-gluc solution. A control experiment in which *Agrobacterium* transformed with the original pCAMBIA vector (control) with promoter less GUS gene was also carried out. No blue colour was observed with the callus infiltrated with *Agrobacterium* containing control plasmid.

4.3. Latex transcriptome analysis of clone RRII 105

To establish ESTs for healthy tree, a cDNA library has been established in lambda vector. Expressed genes (cDNAs) were inserted into lambda vector and this was converted into phagemids following standard protocol. *In vivo* excised phagemid colonies were being used for PCR amplification with T3 and T7 primers to confirm the presence of cDNA inserts. About 800 colonies were screened by PCR and a total of 525 clones with cDNA inserts more than 200 bp were selected for sequencing. Sequences were obtained and editing as well as BlastX analysis were done. Several candidate genes were obtained and based on their predicated functions, genes were classified into 10 groups including, stress/ defense, photosynthesis, cellular metabolism, transcription and signaling, cell division and growth, structural, protein synthesis/degradation, uncharacterized and unknown. 333 Sequences were registered in the NCBI genbank under accession numbers GD272994 to 273326.

BOTANY DIVISION

Development and release of improved clones is the prime mandate of the Division. Hence, research efforts during the year under report were directed towards studying the long-term performance of newer series of indigenous hybrids as well as exotic clones. Evaluation of clones for latex and timber yield, drought and disease tolerance, need-based investigations on propagation aspects and studies on the anatomy of bark and wood were also carried out. Multilocational on-farm and large-scale evaluations of the next series of promising pipeline clones were initiated under the participatory clone evaluation project.

1. Evolving high yielding clones for the traditional area

1.1 Hybridization and clonal selection

Forty three promising hybrid clones, resultant of the 1986 hybridization programme, were evaluated in four SST (1989) laid out at CES, Chethackal. In the 11th year of tapping, 37 hybrid clones maintained higher yield than check clone RRII 105 (Table Bot. 1).

Among the 37 promising hybrid clones planted in 1990 in eight SST, 20 clones continued to maintain the maximum annual mean yield in the tenth year of tapping compared to RRII 105 in the respective trials.

In the two SST planted in 1992, four selections continued to exhibit their superior yield performance in the ninth year of tapping. In Trial A, the hybrid clone 92/380 recorded maximum mean yield (64 g/t/t) followed by clone 92/772 (57.32 g/t/t). The control clone RRII 105 recorded mean yield of 48.31 g/t/t. The above selections also attained maximum girth (80.68 cm in clone 92/380; 86.08 cm in clone 92/772) compared to RRII 105 (59.81 cm). In Trial B, the clone

Table Bot. 1. **Yield of selections in the small-scale trials in the 11th year of tapping**

Trial A		Trial B		Trial C		Trial D	
Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)
86/157	91.78	86/122	151.74	86/428	107.10	86/597	134.96
86/120	75.92	86/79	109.48	86/968	64.55	86/98	92.72
86/60	69.83	86/111	106.75	86/650	61.81	86/99	89.35
86/602	69.62	86/64	78.75	86/424	54.75	86/674	81.30
86/23	68.79	86/44	70.27	86/966	47.16	86/188	72.83
86/607	66.35	86/32	58.80	86/778	39.09	86/400	63.82
86/613	63.18	86/117	58.08	86/908	34.58	86/191	57.24
86/34	62.99	86/174	55.75	RRII 105	35.41	86/68	47.64
86/902	61.52	86/306	52.26			86/70	46.38
86/244	58.51	86/651	41.07			86/599	33.11
86/59	57.17	RRII 105	32.76			RRII 105	42.75
86/304	49.97						
86/957	47.49						
86/660	45.34						
86/110	42.60						
86/5	30.04						
RRII 105	49.83						

92/575 exhibited superior performance recording maximum yield (53.78 g/t/t) and girth (83.72 cm) followed by the clone 92/756 (49.65 g/t/t and 80.06 cm). The control clone RRII 105 recorded comparatively low yield (48.12 g/t/t) and less girth (53.58 cm).

Among 26 hybrid clones and their parents under evaluation in the two SST planted in 1998, four clones were superior in yield compared to the check clone RRII 105, in the fourth year of tapping. Among 40 hybrid clones under evaluation in two SST planted in 1999, three clones were superior in yield to RRII 105.

Thirty four hybrid clones were under evaluation in the SST planted in 1995. Of these, 10 clones recorded better yield than RRII 105 in the sixth year of tapping. The yield and girth of the promising clones are given in Table Bot. 2.

Table Bot. 2. **Mean girth and yield of selected clones**

Clone	Girth (cm)	Yield in the 6th year (g/t/t)
95/7	73.09	72.45
95/27	78.60	74.39
95/64	74.28	50.00
95/79	76.25	46.33
95/95	69.44	46.73
95/102	66.29	41.21
95/124	67.21	52.53
95/308	65.13	50.01
95/309	66.66	66.09
95/349	69.75	52.25
RRII 105	54.59	40.44

Among the 26 W x A hybrid clones under evaluation, 10 clones showed higher yield than the control clone RRII 105 in the sixth year of tapping. The yield and girth of promising clones are given in Table Bot. 3.

Selected hybrids from the hand pollination and half-sib seedlings from progeny selection programmes (2002) were

Table Bot 3. **Mean girth and yield of promising clones**

Clone	Girth (cm)	Yield in the 6th year (g/t/t)
95/10	78.81	79.37
95/21	75.33	44.58
95/25	62.09	50.65
95/29	84.71	44.56
95/34	71.58	53.28
95/170	67.88	49.29
95/171	79.46	45.44
95/241	72.42	51.62
95/271	75.75	54.25
95/340	59.58	45.68
RRII 105	53.71	40.92

planted for evaluation in a clonal nursery trial at CES, Chethackal during 2008. Among the clones, one progeny of the cross PB 330 x RRII 414 registered maximum girth.

After preliminary observations on growth and test tap yield of 231 progenies of full-sib and half-sib origin generated in 2005, 35 progenies were selected. Ranking of selections from full-sibs and half-sibs based on the juvenile yield is given in the Tables Bot. 4 and 5, respectively. The above selections will be further evaluated in replicated clonal nursery trials.

1.2. Ortet selection

In the SST of ortets selected from Plantation Corporation of Kerala Ltd. (Kodumon Estate), OKn 39, a potential latex-timber clone, continued to be the best performer, recording maximum yield (59.92 g/t/t) and girth (83.88 cm) in the sixth year of tapping. The control clone RRII 105 recorded a yield of 58.17 g/t/t and girth of 64.50 cm. OKn 39 was selected for further multiplication and field evaluation. In another trial comprising 25 clones, OKn 49 continued to be the highest yielder in the sixth year of tapping with a yield of 65.00 g/t/t. The control clone RRII 105 recorded 63.16 g/t/t over the same period. In the SST

Table Bot. 4. **Ranking of selected full-sib seedlings based on growth and test tap yield**

Ranking	Parentage	Seedling no.	Girth (cm)	Mean rubber yield (g/t/35 tappings)
1	RRII 429 x PB 330	489	10.4	10.80
2	PB 330 x RRII 105	75	12.2	5.00
3	PB 330 x RRII 414	166	12.0	3.90
4	PB 330 x RRII 414	21	10.8	2.90
5	RRII 429 x PB 330	484	12.8	2.40
6	PB 330 x RRII 414	163	11.6	2.10
7	PB 330 x RRII 414	40	10.3	1.50
8	PB 330 x RRII 414	178	12.2	1.30
9	PB 330 x RRII 414	36	11.8	1.20
10	PB 330 x RRII 414	64	11.0	1.10
11	PB 330 x RRII 414	56	13.4	1.00
12	PB 330 x RRII 414	35	10.3	0.90
13	RRII 429 x PB 330	101	11.2	0.90
14	PB 330 x RRII 105	90	10.4	0.80
15	PB 330 x RRII 105	116	10.8	0.50

Table Bot. 5. **Ranking of selected half-sib seedlings based on growth and test tap yield**

Ranking	Female parent	Progeny no.	Girth (cm)	Mean rubber yield (g/t/35 tappings)
1	RRII 429 OP	405	16.3	19.80
2	RRII 414 OP	601	11.3	6.60
3	RRII 429 OP	350	15.3	3.70
4	RRII 429 OP	309	15.1	3.40
5	RRII 429 OP	265	12.3	3.30
6	RRII 429 OP	337	17.8	3.20
7	RRII 429 OP	436	14.3	2.80
8	RRII 429 OP	355	11.0	2.60
9	RRII 429 OP	432	14.8	2.60
10	RRII 414 OP	139	14.3	2.30
11	RRII 429 OP	288	13.3	2.30
12	RRII 429 OP	296	11.8	2.10
13	RRII 414 OP	11	12.0	1.90
14	RRII 429 OP	314	14.8	1.90
15	RRII 429 OP	435	12.3	1.80
16	RRII 414 OP	126	10.2	1.70
17	RRII 429 OP	276	13.3	1.60
18	RRII 429 OP	295	11.1	1.40
19	RRII 429 OP	376	11.8	0.90
20	RRII 429 OP	241	10.8	0.40

of ortets selected from various smallholdings, yield data collected over four years of tapping showed that the vigorous clones O 73 (61.13 g/t/t) and O 72 (59.10 g/t/t) continued to be the highest yielders whereas, RRII 105 recorded a mean yield of 41.34 g/t/t. In the third year of tapping in the SST of 12 ortets selected from HML Kaliyar Estate, Thodupuzha, OKr 48 recorded the highest yield of 43.10 g/t/t (Table Bot. 6). Over the three years of tapping, OKr 14 with 38.89 g/t/t followed by OKr 48 (36.27 g/t/t) and OKr 75 (35. 30 g/t/t) were found to be the highest yielders, whereas RRII 105 recorded 34.96 g/t/t. Ortets selected from HML Kaliyar Estate, Thodupuzha and Vimala Estate, Kasaragod, comprising 23 clones were opened for regular yield recording by cup coagulation.

2. Evaluation of clones

2.1. Large-scale evaluation

Of the two large-scale trials planted in 1989 at RRII Farm, in Trial 1, RRII 5 and RRII 118 continued to be the top yielders, showing better performance over RRII 105. In Trial 2, the performance of PB clones over 10 years of tapping showed that clone PB 314 recorded the highest yield (78.06 g/t/t) followed by PB 255 (77.43), PB 280 (70.39) and PB 312 (69.99). The results were compiled in a technical report along with the evaluation of these clones in other locations, for upgradation of PB 314, PB 255, PB 280 and PB 312 from Category 3 to Category 2 of the planting recommendation for the traditional region.

Clones in the LST of RRII 400 series planted at CES (1993) were in the eighth year of tapping. In Trial 1, clones RRII 417, RRII 403 and RRII 55 were superior to RRII 105. However, in Trial 2, RRII 430, RRII 422 and PB 330 performed better than RRII 105.

Table Bot. 6. **Yield and girth of the promising ortet clones**

Clone	Girth (cm)	Yield (g/t/t)
OKr 48	61.25	43.10
OKr 75	64.68	34.16
OKr 131	58.25	30.83
OKr 83	69.77	30.31
OKr 87	53.25	30.30
OKr 98	61.10	28.92
OKr 14	51.43	26.70
OKr 70	65.01	25.03
OKr 80	65.85	24.95
OKr 51	56.86	24.10
OKr 97	69.00	23.82
OKr 133	54.41	13.23
RRII 105	68.33	35.76
CD (P = 0.05)	12.11	9.53

In the large-scale evaluation trial of 12 clones of exotic and indigenous origin at CES, Chethackal, HP 44 continued to be the highest yielder with 24.41 per cent yield improvement over RRII 105. Similarly, in the large-scale evaluation trial of the potential hybrid clones planted at RRS Padiyoor, HP 44 performed better than RRII 105 showing 25 per cent yield improvement with a yield of 67.86 g/t/t in the first year of tapping. The above results reveal yield consistency of HP 44. In Trial 2, in the same location, HP 468 recorded 20 per cent yield improvement over RRII 105 during the first year of tapping and also recorded the highest percentage of tappable trees.

2.2. On-farm evaluation

Two indigenous and seven introduced clones are under evaluation in the OFT at Sasthamkotta. Mean girth and yield in the sixth year of tapping are given in Table Bot. 7.

In various large estates, RRII 400 series clones continued to maintain better yield and growth compared to RRII 105. In terms of tappability (7th year) also, the RRII 400 series clones, in general, were superior to RRII 105.

Table Bot. 7. **Mean girth and yield in the sixth year of tapping**

Clone	Mean Girth (cm)	Mean yield (kg/ha/year)
RRII 105	57.60	2584
GT 1	56.65	1707
RRII 203	62.00	1850
PB 217	60.74	1721
PB 255	66.00	2198
PB 260	56.71	1764
PB 311	57.85	2007
PB 314	58.15	2137
PR 255	61.54	2219

In Kaliyar Estate, Thodupuzha, the clone RRII 430 was the highest yielder closely followed by RRII 414, RRII 105, RRII 422 and RRII 417 (Table Bot. 8). The performance of RRII 429 was poor compared to the other clones.

Table Bot. 8. **Tappability and yield in the OFT at Kaliyar**

Clone	Percentage of tappable trees (7th year)	Mean yield over two years (g/t/t)
RRII 414	60.33	35.33
RRII 417	70.94	34.50
RRII 422	57.14	35.03
RRII 429	66.67	26.02
RRII 430	71.87	36.28
RRII 105	55.00*	35.26

* 8th year

Early growth data of RRII 400 series clones from the OFT (2005) at Kanjirapally showed that RRII 414 recorded the highest girth (32.7 cm) followed by RRII 429 (32.2 cm) compared to RRII 105 (26.7 cm). In the OFT (2003) at Punalur, compared to RRII 105 (35.3 cm), RRII 414 attained highest girth (40.0 cm) followed by RRII 429 (37.3 cm). In all other locations, the RRII 400 series clones registered better growth than the check clone RRII 105. Tapping of RRII 400 series clones

was initiated in four smallholdings at the age of 5 ½ years. Preliminary data indicated a better yield in RRII 400 series clones than RRII 105. Incidence of diseases was recorded from eight holdings.

2.3. Investigations on G x E interactions

During the sixth year of tapping, yield data from three locations *viz.* Kanyakumari, Agartala and Nagrakata, were summarised. At HBSS Kanyakumari, RRII 105 was the highest yielder (51.83 g/t/t) followed by PB 217 (49.08), RRIC 100 (47.67) and RRII 430 (45.36). RRII 429 and RRII 203 were the other high yielders (44.00 g/t/t). At RRS Agartala, RRII 429 (66.57 g/t/t) and RRII 422 (63.93) recorded significantly superior yield than RRIM 600 (41.30 g/t/t). At RES Nagrakata, RRII 429, RRII 417 and RRII 422 recorded significantly superior yield than the check clones RRII 105 and RRIM 600. The performance of the above clones in Nagrakata was observed to be consistent over the years. At RRS Padiyoor, during the third year of tapping, RRII 430 continued to be the highest yielder (53.05 g/t/t). The performance of the clones RRII 417, RRII 414 and RRII 422 were also better than that of RRII 105. Yield recording was regularized at IMMT, Bhubanewar and the initial yield trend of RRII 400 series clones is promising.

3. Participatory evaluation of rubber clones

Fourteen trials (2 LST and 12 OFT) were established across 13 locations in the traditional rubber growing area. Twenty pipeline clones and three check clones have been field-planted in two batches for evaluation in various locations under Phase 1 of the project. Multiplication of one batch of clones for Phase 2 was initiated. Nine locations were identified and site selection was completed.

4. Breeding for other specific objectives

4.1. Compact canopy

Evaluation was continued for recombinants of cross between compact canopy and high yielding clones such as RRII 105, RRIM 600 and RRII 118 as female parents. Three progenies of three crosses showed normal growth with compact canopy in the sixth year of growth.

Among the selections from open-pollinated progenies of the compact canopy type there are four different morphotypes under evaluation (Table Bot. 9). Of these, intermediate and normal types registered higher girth than RRII 105 and the mean yield of these morphotypes was 24.8 and 29.4 g/t/t respectively in the fourth year of tapping.

Table Bot. 9. **Girth and mean yield of progenies**

Progenies of natural mutant	Girth (cm) (5th year after opening)	Mean yield (g/t/t) 4th year	Mean yield (g/t/t) Over 4 years
Compact	20.90	–	–
Semi-compact	49.30	18.50	20.00
Intermediate	68.00	24.80	23.50
Normal	79.30	29.40	35.70
RRII 105	65.70	43.40	40.30
CV	7.30	21.18	20.00
CD (P = 0.05)	9.50	12.30	11.50

4.2. Drought tolerance

The yield of the 24 hybrid clones and seven parent clones was monitored in two SST at fortnightly intervals in the fourth year of tapping. Five clones recorded better annual mean yield and 15 clones had better

summer yield than RRII 105. Among the 70 clones under evaluation in the small-scale trials planted in 1999, 15 clones were superior in yield to RRII 105 and four clones showed a combination of high yield and low summer yield drop.

Significant clonal variation for yield and girth in the first year of tapping was observed in the two SST planted in 2001. In Trial A, seven clones were superior in yield to RRII 105 and 18 clones were comparable to the check. Among the superior yielders, clones 95/3, 95/98, 95/489 and 95/253 showed high growth rate in summer. In Trial B, among three clones which were superior to the check, clone 95/397 showed a high growth rate in summer. Nine clones were comparable to check clone of which two showed 100 per cent tappability. Clones 95/280, 95/522, 95/3, 95/28 and 95/279 showed a combination of high yield and tappability.

4.3. Polycross progeny evaluation

Significant variation existed among and within progenies in respect of dry rubber yield and girth in the eighth year of tapping in the two evaluations of polycross progenies comprising 150 clones. Progeny of clone PB 28/83 followed by that of Ch 26 maintained superiority with high recovery of promising clones in terms of dry rubber yield. Twenty five clones with 0.90 to 94.99 per cent improvement in yield were identified.

In the clonal nursery evaluations of polycross progenies at two locations, clone RRII 430 was superior to RRII 105 in terms of juvenile yield. A study on physiological parameters of drought tolerance was undertaken. A fall in Fv/Fm ratio and rise in epicuticular wax content in clones as a consequence of the moisture stress in Padiyoor was observed. Clone P 80 exhibited high test tap yield at Padiyoor, high and stable Fv/Fm values and a high epicuticular wax content indicative of drought tolerance. Clones P 209, P 80 and P 187 were drought tolerant in terms of attributes, *viz.* chlorophyll fluorescence and epicuticular wax content.

5. Anatomical investigations

Histochemical localisation for starch in the bark of healthy and TPD affected trees was carried out. Both ray and axial parenchyma adjacent to the latex vessels showed abundant starch accumulation in the healthy bark. In the affected bark, a considerable depletion in total starch was observed. Starch grains are mostly round in shape and polymorphism is comparatively less. The terminal cell of the vascular ray in the TPD affected area is devoid of starch while it was present in the unaffected area of the same tree. When the trees were applied with ethephon, a drastic depletion of starch grains was noticed both in healthy and TPD affected bark.

A new experiment on variability of wood quality parameters in RRII 400 series clones was initiated. Five clones of RRII 400 series *viz.*, RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430, and their parents RRII 105 and RRIC 100 were selected for the study. Two trees (23 years old) each from three replications (six trees per clone) were subjected to destructive sampling for laboratory studies. Wood discs at three height levels from the trunk were prepared for anatomical investigations. Standard sampling procedures were followed for the study of physical and strength properties.

6. Studies on propagation

The field trial on effect of different types of buds taken from brown budwood on the growth performance of plants showed that scale buds produced more variation in growth than the plants raised from the leaf axillary buds. In another experiment, girth of the plants evolved from different forms of planting materials showed comparable growth between the trees in the 7th year after field planting. The highest variance in girth in the population was recorded by plants raised from the brown budded stumps (Table Bot. 10).

Table Bot. 10. **Mean girth seven years after field planting**

Type of planting material	Girth (cm)	Variance (%)
Brown budded stumps	47.77	23.25
Field budded plants	41.4	11.33
Green budded ploybag plants	51.97	4.92
Brown budded polybag plants	50.7	7.03
Young budded plants (stock 42-day-old)	50.13	4.33
Young budded plants (stock 49- day-old)	47.33	12.81
Young budded plants (stock 56-day-old)	48.87	3.41
CD (P = 0.05)	5.15	

In another study, plants produced using buds collected directly from bud stick of old mature trees recorded significant reduction in growth compared to plants produced using buds from the budwood collected from source bush plants (Table Bot. 11).

7. Morphological characterization of popular clones

Morphological traits such as shape of leaf storey, separation of leaf storey, shape of leaf, pulvinus, petiole and petiolule were studied in 38 pipe line clones in the bud wood nursery established at CES, Chethackal. Preliminary results indicate clonal variation for these traits.

Five clones, *viz.* RRII 33 (low yielder), RRII 105, RRII 414, RRII 429 and RRII 430 (high yielding clones) were selected to study the characteristics of leaf minor veins such as density of areoles, areoles with and without vein endings, branched and unbranched vein endings, shape and number of terminal cell constituting the vein ending *etc.* The preliminary results indicated marked variation between low yielding (RRII 33) and high yielding clones (RRII 105, RRII 414, RRII 417, RRII 429, RRII 430) (Table Bot. 12).

Table Bot. 11. **Girth of plants three years after field planting**

Treatment	Girth (cm)
Budded plants - fresh seed stock	17.22
Budded plants- young budwood stock (Source A)	16.37
Budded plants- aged budwood stock (Source A)	15.13
Budded plants- young budwood stock (Source B)	16.89
Budded plants- young budwood stock (Source C)	16.28
Budded plants- aged budwood stock (Source C)	16.14
Budded plants- budwood collected from old trees	11.04
CD (P = 0.05)	1.25

Table Bot. 12. **Parameters of leaf minor veins**

Clone	No. of veins per areole	Minor veins		Terminal cells per areole	Measurement of terminal cell		
		Branched	Un-branched		Length (μm)	Breadth (μm)	Area (μm²)
RRII 33	1.38 ^{c}	0.41 ^{c}	1.30 ^{a}	1.57 ^{b}	53.61 ^{bc}	14.92 ^{a}	794.38 ^{bc}
RRII 105	2.24 ^{a}	1.34 ^{a}	1.64 ^{a}	4.82 ^{a}	61.34 ^{ab}	16.86 ^{a}	1021.36 ^{ab}
RRII 414	1.86 ^{ab}	0.91 ^{a}	1.49 ^{a}	3.67 ^{a}	55.38 ^{bc}	14.16 ^{a}	743.79 ^{bc}
RRII 417	2.00 ^{ab}	1.29 ^{ab}	1.54 ^{a}	4.37 ^{a}	70.39 ^{a}	15.61 ^{a}	1097.56 ^{a}
RRII 429	2.00 ^{ab}	1.24 ^{ab}	1.39 ^{a}	3.52 ^{a}	47.84 ^{c}	13.40 ^{a}	606.74 ^{b}
RRII 430	1.73 ^{bc}	0.89 ^{b}	1.24 ^{a}	3.81 ^{a}	44.56 ^{c}	13.24 ^{a}	609.77 ^{b}

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

GERMPLASM DIVISION

The major activities of the Division include maintenance of the domesticated gene pool collection, introduction and conservation of remaining *Hevea* species, conservation of the wild germplasm, its agronomic evaluation, screening for diseases, drought and cold stress resistance, timber latex traits and molecular characterization. The three *Hevea* gene pools maintained at RRII, comprising the domesticated gene pool with clones derived from the original Wickham collection of 1876, the wild germplasm belonging to the 1981 IRRDB germplasm collection and the collection of other *Hevea* species, form an important source of variability required for crop improvement.

1. Introduction, conservation and documentation

1.1 Domesticated gene pool (Wickham collection) from secondary centers

Conservation of 183 Wickham clones is being taken up in field gene banks, with one clone museum at RRII Farm, Kottayam and two germplasm gardens established at CES, Chethackal.

Among the five IRCA clones, IRCA 130 and IRCA 111 continued to show superiority

Table Ger. 1. **Girth and dry rubber yield of IRCA clones at the age of 17 years**

Clone	Dry rubber yield** (6th year of tapping) (g/t/t)	Girth in the 17th year of planting** (cm)	Bole volume** (m^{3})	DRC* (%)	PI**
IRCA 130	90.97	72.76	0.21	33.62	1.51
IRCA 109	34.35	63.44	0.14	38.25	3.56
IRCA 111	60.74	72.90	0.15	33.85	2.05
IRCA 18	41.79	66.84	0.12	34.93	4.93
IRCA 230	45.76	64.40	0.11	35.59	3.86
RRII 105	71.38	67.11	0.09	36.66	3.36
CD (P = 0.05)	16.82	5.23	0.03	2.80	0.90

*Significant at P < 0.05; ** Significant at P< 0.01

(Table Ger. 1). Annual girth, monthly yield, plugging index (PI) and timber volume were recorded. Significant clonal differences were recorded for all the characters studied.

Incidence of TPD was less than 5 per cent in this trial. Among the exotic clones, IRCA 130 continued to be the best-clone, followed by IRCA 111.

Among the 20 clones in Germplasm Garden V (Table Ger. 2), RRIM 609, RRII 23 and RRIC 100 continued to be the best clones in terms of yield (74.7 -77.8 g/t/t), while the control clone RRII 105 had a yield of 49.9 g/t/t. These clones also had high girth ranging from 77.6 cm (RRIM 609) to 86.9 cm (RRIC 100), while the control clone RRII 105 recorded 65.9 cm. Plugging index recorded in summer was also relatively low for these clones, ranging from 5.1 (RRIM 609) to 5.5 (RRIC 100), while RRII 105 had a PI of 7.0.

1.2. IRRDB 1981 wild gene pool

Field conservation of a total of 3576 wild accessions was continued in conservation-cum-source bush nurseries (SBN). All the wild accessions were maintained with proper identity.

Re-establishment of the conservation nurseries was continued. Out of 975 wild accessions in Conservation Nursery 2004, top yielding 50 accessions based on first round test tapping were selected for second round test tapping and the potential accessions with good rubber yield were identified. Six accessions (AC 2009, RO 1295, AC 3146, AC 2578, RO 2598, and RO 276) recorded test tap yield higher than RRII 105. Eleven accessions recorded higher or on par yield than RRII 105 and RRIM 600. Five accessions were on par or superior to PB 235. AC 2578 gave exceptionally good test tap yield of 7.75 g/t/t compared to 4.29 g/t/t of RRII 105. Altogether 24 potential accessions were identified out of 975 for Further Evaluation Trial. Accessions with good girth and girth increment were also identified in this SBN. The 701 accessions in SBN 2005 were subjected to test tapping and accessions with high yield were identified for further evaluation. While the control clones yielded 2.7 g/t/t (RRIM 600) to 3.9 g/t/t (PB 235), AC 2421 gave the highest yield of 6.0 g/t/t, followed by MT 3702, RO 2400 and 6/32, whose yield ranged from 4.2 to 4.5 g/t/t. Eight other accessions gave more than 3.0 g/t/t, while 14 more accessions gave more than 2.0 g/t/t. The fourth set of 806 accessions planted in conservation nursery 2006 in an augmented RBD with four controls is maintained properly. The 500 accessions planted in conservation nursery 2007 were morphologically characterized and quantitative growth

Table Ger. 2. **Performance of 20 Wickham clones in the fifth year of tapping**

Clone	Yield (g/t/t)	PI	Girth (cm)	Clone	Yield (g/t/t)	PI	Girth (cm)
RRIM 609	77.80	5.10	77.60	RRII 108	36.40	5.50	68.90
RRII 23	77.60	5.70	81.40	RRII 22	33.70	7.70	55.10
RRIC 100	74.70	5.50	86.90	RRII 15	31.90	7.50	68.30
RRII 178	55.10	5.10	82.40	RRII 27	30.90	6.40	52.70
RRIC 148	50.70	4.40	78.50	RRII 20	29.90	7.10	65.00
RRII 105	49.90	7.00	65.90	PB 255	22.10	7.60	83.30
SCATC 93-114	49.70	6.90	63.10	SCATC 88-13	20.10	6.90	67.20
PR 255	46.60	6.90	54.80	RRIM 618	19.50	7.70	51.80
RRIC 102	42.40	4.40	73.00	RRII 12	15.20	13.10	67.90
RRIC 36	39.20	6.50	64.60				
Haiken 1	38.20	8.70	69.50	CD (P = 0.05)	25.78	2.74	11.03

traits were recorded. These wild accessions are being screened for drought tolerance in collaboration with the Physiology Division. The next set of wild accessions for re-establishment, comprising 201 genotypes, were planted in an augmented RBD with four controls.

1.3. Other *Hevea* species

The arboretum comprising all the available species of *Hevea* planted at CES in 2006, was maintained.

2. Characterization and preliminary evaluation

Growth of the wild accessions in the Preliminary Evaluation Trials (PET) was monitored by recording their girth annually. The PET 94 A, B and CES Chethackal were planted at a close spacing of 2.5 x 2.5 m. In PET 94 A, accession AC 757 (74.19 cm) was superior to RRII 105 for girth, followed by MT 940 (58.94 cm) and RO 895 (55.98 cm). In PET 94 B, AC 643 followed by AC 748 were significantly superior to RRII 105 for girth. In this trial, these two accessions also had the highest girth increment for the last two years, while RRII 105 had the least. None of the wild accessions showed potential for high yield. AC 618, the highest yielder, had only 14 per cent of the yield of RRII 105. In PET 94 C, accession RO 893 (67.52 cm) and AC 465 (65.22 cm) recorded superior girth to RRII 105 (35.91 cm). All the wild accessions in trials 94 A and C had very low yield. None of the nine ortet clones in PET 94 (Ortets) D recorded higher girth or yield than the control clone RRII 105.

The girth of the 46 wild accessions in PET (Ortets) 99 ranged from 18.9 (OR 1145) to 55.2 cm (OM 1182), while that of the controls were 55.6 (PB 260), 55.2 (RRIM 600) and 57.0 cm (RRII 105). OR 1107 (55.1 cm) and OR 1149 (51.7 cm) also had relatively high girth among the wild accessions. The trial was opened for regular tapping.

Girth of the 46 wild accessions in PET 2000 at RRII was recorded and the trial was opened for regular tapping. Two accessions appeared to show potential for high yield.

Annual girth recorded in PET 2000 A at RRS, Padiyoor was the maximum in AC 2537 (47.88 cm) followed by AC 3609 (47.08 cm) and AC 3075 (46.38 cm), while the control clones RRII 105, RRII 208 and RRIM 600 recorded 29.32 cm, 34.98 cm and 30.48 cm, respectively. In PET 2000 B with 166 wild accessions, annual girth and girth at the end of summer period were recorded for identifying accessions with better summer period growth. In PET 2002, AC 567 recorded the highest girth of 31.38 cm followed by AC 1964 (29.67 cm), MT 1717 (27 cm) and AC 824 (26.15 cm), while the control clones RRII 105, RRII 208 and RRIM 600 recorded 26.17 cm, 23.25 cm and 31.54 cm, respectively. In the fifth year, test tapping showed the highest yielder was RO 1241 (4.94) followed by MT 724 (4.83), RO 1313 (4.50), AC 567 (4.31) and AC 824 (3.48 g/t/t) compared to the control clones RRII 105 (5.05), RRIM 600 (4.48) and RRII 208 (1.29 g/t/t).

A set of 55 wild accessions in SBN 2001 at CES, Chethackal was evaluated for their early yield and growth performance in the first six years of growth. Wide variability was observed in girth, girth increment, crotch height, single leaf area, total number of laticifer rows, diameter of latex vessels and test tap yield (Table Ger. 3). The nursery test tap yield (g/t/t) in the fifth year showed that three out of the total 55 accessions had juvenile yield greater than RRII 105, two were on par, while two other accessions showed 50 per cent yield of the control clone.

Table Ger. 3. **Variability for yield, yield components and growth related traits in wild germplasm**

Parameter	Wild accessions			Control clones		CV (%)
	Minimum	Maximum	General mean	RRII 105	RRIM 600	
Yield (g/t/t)	0.04 (RO 5358)	11.19 (RO 5018)	1.17	2.88	5.82	41.97
Bark thickness (mm)	1.00 (AC 5896)	5.30 (MT 4771)	2.76	3.17	3.08	15.14
Total number of laticifer rows	3.00 (MT 5824)	10.67 (RO 2841)	6.39	9.74	8.41	21.98
Diameter (µm) of latex vessels	10.69 (AC 5487)	21.66 (MT 4762)	16.74	17.19	18.42	25.23
Girth (cm) in 6th year after planting	10.75 (AC 5896)	35.60 (RO 5432)	20.62	21.56	20.54	20.91
Girth increment (cm/year) over 3 years	0.50 (AC 5466)	5.47 (RO 5432)	2.35	2.47	2.69	22.45
Crotch height (m)	1.88 (RO 5364)	5.14 (MT 4690)	3.04	2.65	2.46	14.09
Single leaf area (cm^2)	37.51 (RO 5318)	150.40 (RO 5365)	69.34	52.68	56.46	22.93

Note: Figures in parentheses denote the name of accession

3. Further evaluation and selection

The annual girth (at the age of 13 years) and dry rubber yield (5th year after tapping) were recorded and analysed in FET 95 at RRS, Padiyoor. The annual girth was maximum for the accession MT 1032 (74.97 cm) and minimum for MT 188 (38.12 cm). The mean girth of RRII 105 was 59.00 cm. Six accessions (MT 1032, MT 941, MT 1630, MT 1674, MT 999 and MT 1640) showed significantly higher annual girth than the control, RRII 105, while the girth of 61 accessions was statistically on par with the control.

Among the wild accessions, one accession (AC 166) had annual average as well as summer season and peak season dry rubber yield statistically on par with that of RRII 105. The summer season, peak season and annual average dry rubber yield (g/t/t) of this wild accession was 46.03, 66.32 and 56.08 respectively whereas the corresponding yield of RRII 105 was 41.18, 82.43 and 62.27, respectively. Six accessions had timber volume significantly higher than that of RRII 105. Seven accessions were identified as male parents, based on better performance for girth, yield and timber, for hybridization programmes with RRII 105 and RRII 430.

In the FET 2003 at CES, Chethackal, observations were recorded on test tap yield, early growth performance and bark structural traits. The highest test tap yield (g/t/t) was recorded in the accession RO 2629 (7.68) followed by AC 4149 (7.47), and AC 716 (5.02), while the control clones RRII 105, RRII 208 and RRIM 600 recorded test tap yield (g/t/t) of 7.49, 4.01 and 4.36, respectively. Accession RO 2629 recorded the highest girth (39.59 cm) followed by AC 4149 (36.31 cm) and AC 626 (34.07 cm), while the control clones RRIM 600,

RRII 105 and RRII 208 recorded girth (cm) of 29.59, 30.25 and 28.91, respectively. Accession RO 2629 recorded the highest bark thickness of 5.16 mm followed by AC 4149 (4.90) and AC 626 (4.49 mm), whereas the control clones RRII 105, RRII 208 and RRIM 600 recorded 4.19, 3.38 and 3.77 mm, respectively. The highest number of laticifer rows was observed in MT 999 (12.90) followed by AC 4149 (8.83), RO 2629 (8.81) and AC 716 (8.42) while control clones RRII 105, RRII 208 and RRIM 600 recorded 10.10, 7.56 and 8.56, respectively. The highest diameter of latex vessels (µm) was recorded in MT 4529 (26.28) followed by RO 2629 (24.11) and RO 3624 (24.06) as compared to the control clones RRII 105 (27.24), RRII 208 (25.36) and RRIM 600 (20.19).

Test tapping was carried out in FET 2005 comprising 22 wild accessions and three controls. MT 4788 gave the highest yield (g/t/t) of 7.0 compared to 4.7 for PB 235 and RRII 105 and 2.5 for (RRIM 600. AC 2004 had the highest girth, on par with the controls PB 235 and RRII 105.

In another FET, 26 wild accessions selected on the bases of juvenile performances along with two controls (RRII 105 and RRII 208), were planted at RRII Farm during 2008 in RBD with six replications.

4. Screening for stress tolerance

4.1. Biotic stress resistance

Screening of the wild accessions for resistance to Corynespora and Colletotrihcum diseases was continued in collaboration with Pathology Division. Laboratory screening for *Corynespora* tolerance was carried out. Out of 80 accessions screened, 36 were found tolerant. For *Colletotrichum* resistance, out of 630 accessions screened in SBN 2003 and SBN 2006, three accessions were found promising, which have to be reconfirmed.

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

In the field screening trial planted in 2003 at RRS, Dapchari with 130 wild

Table Ger. 4. **Superior accessions with good girth and summer period girth increment**

Accession	Girth (cm)		Accession	Summer
	2008	2009		G.I (%)
RO 2976	20.28	21.83	MT 62	7.94
MT 1681	19.16	21.76	MT 1639	7.84
RO 1769	16.90	20.30	MT 4378	7.81
MT 1660	16.68	19.18	RO 1234	7.04
RO 1348	15.80	17.37	AC 174	6.02
MT 72	15.78	16.12	MT 1710	5.97
RO 2387	15.72	17.84	RO 1204	4.89
RO 2153	15.70	17.82	RO 2835	4.32
RRIM 600	14.10	16.57	RRIM 600	2.17

Table Ger. 5. **Top five accessions/clones for height, girth and girth increment at 2nd year of planting**

Accession/ clone	Height (cm) over 18 months	Accession/ clone	Girth (cm.) Over 14 months	Accession/ clone	GI % over 1 year at 18 months
MT 54	404.58	MT 4788	8.64	93/270	140.16
RO 1761	381.67	MT 54	7.98	MT 1616	119.27
MT 4856	376.67	MT 4856	7.82	RO 1761	110.30
RO 85	372.08	MT 40	7.73	MT 4856	104.90
MT 4788	363.33	93/270	7.63	MT 5100	104.72
RRII 430	342.92	RRII 430	7.97	RRII 430	101.89
RRII 208	297.83	RRII 208	6.57	RRII 208	89.91
RRIM 600	286.25	RRIM 600	6.55	RRII 414	86.86
RRII 414	277.08	RRII 105	6.10	RRIM 600	79.94
Tjir 1	266.67	Tjir 1	5.74	RRII 105	78.55
RRII 105	254.17	RRII 414	5.59	Tjir 1	53.29

accessions, superior accessions with good annual and summer period girth increment were identified (Table Ger. 4).

In the further field evaluation in collaboration with Botany Division of selected rubber clones at RRS, Dapchari for reconfirming their drought tolerance potential, comprising 23 wild accessions, five hybrid clones and six check clones, *viz.* RRII 430, RRII 414, RRII 105, RRIM 600, RRII 208 and Tjir 1, after experiencing two summer periods of 2008 and 2009, there were potential wild accessions (Table Ger. 5) for girth and girth increment over drought tolerant check clones RRIM 600 and RRII 208. Among the five hybrid clones, clone 93/270 was superior. Among the six check clones, modern clone RRII 430 was superior over others and comparing the performance of this clone with RRII 414, clone RRII 430 was found to be more suitable in a drought prone area.

In another pot culture study with nine clones from polyclonal seed garden at HBSS, Nettana and one of assorted origin growth performance and summer survival of the half sib seedlings were assessed during second year (Table Ger. 6). The summer survival potential was assessed by grading based on the yellowing symptoms on leaves.

Clone AVT 73 maintained its superiority throughout the period. The performance of the family of assorted seeds was inferior to all the nine families of clones (except PB 217) both in terms of growth and percentage of yellow leaves. Another experiment initiated was the evaluation of drought tolerance potential of half-sib progenies raised in a seedling nursery, using the seeds from prepotent *Hevea* clones of polyclonal seed garden at HBSS, Nettana (collaborative study with Botany Division). Test tapping was conducted during both peak and summer seasons and the familywise performance of clones assessed. Summer season test tap yield was the highest in the family of the clone PB 28/83.

In the collaborative project on rapid screening of *Hevea* germplasm lines for intrinsic drought tolerance traits and drought-related morphological parameters based on preliminary field level scoring conducted in wild accessions during the summer months, 31 potential accessions were selected for detailed laboratory study. Laboratory screening of selected top, medium and bottom ranking accessions was started based on field screening in SBN 2003, 2004, 2005 and 2006. In the drought evaluation trial 1996 at Rubber Demonstration Centre, Sukma with 36

Table Ger. 6. **Second year growth of seedlings and grading on leaf yellowing**

Clone	Height (cm)	Girth (cm)	Grading on yellowing
AVT 73	201.09	6.90	(12) PYL-2, GL-7, NL-3
PB 242	261.41	5.60	(13) YL-1, NL-3, GL-6, PYL-3
RRII 105	199.66	5.30	(12) GL-6, NL-5, PYL-1
PB 28/83	198.00	4.80	(10) NL-2, GL-6, YL-1, PYL-1
Ch 26	193.25	5.10	(12) PYL-4, GL-7, NL-1
RRII 203	178.64	5.20	(17) GL-8, YL-2, NL-1, PYL-5, FYL-1
PB 5/51	156.80	4.00	(5) YL-2, PYL-1, NL-2
PB 215	148.00	3.90	(4) NL-1, GL-1, PYL-2
Assorted	143.75	3.40	(12) PYL-6, GL-2, NL-4
PB 217	92.00	3.20	(3) NL-3

(Total number of plants in parentheses) GL - green leaves; NL - no leaves;
PYL - partially yellow leaves; YL - yellow leaves; FYL- fully yellow leaves

genotypes, annual girth was recorded. Clone RRII 208 recorded the highest girth (80.46 cm) followed by clones AC 619 (79.33), RO 5554 (78.59), RO 5430 (77.84) and RRII 118 (77.82).

4.2.2. Cold tolerance

A total of 64 wild accessions were screened for cold resistance in two trials at Regional Experiment Station, Nagrakata. Girth of the nine-year-old accessions recorded during pre- and post- winter period showed significant variation. Monthly yield was also recorded. Higher annual girth (cm) was observed in MT 5105 (65.55), RO 2902 (65.19) and AC 3353 (64.23) than SCATC 93-114 (56.35) and RRIM 600 (55.91) in Trial 1. In Trial 2, clone RO 2727 recorded the highest girth of 67.78 cm followed by MT 915 (67.56) and MT 900 (64.75), while the controls Haiken 1 and RRIM 600 recorded 61.22 and 53.47, respectively.

5. Screening for timber characteristics

5.1. Field screening

Annual girth and bole height were recorded and the bole volume was estimated at the age of eight years. The maximum girth (cm) was recorded for MT 999 (43.60) and minimum for AC 651 (27.47). Three accessions (MT 999, MT 941 and MT 915) had the girth on par with PB 235 and RRII 118; six accessions on par with PB 260; 11 accessions on par with RRII 105 and 14 accessions on par with RRIM 600. Two wild accessions had the bole volume on par with RRII 33, RRII 118 and PB 235.

5.2. Screening for timber quality traits through lignin biosynthesis studies

Assessment of Cinnamyl Alcohol Dehydrogenase (CAD) gene expression of selected wild accessions having high lignin percentage along with control clones was made to ascertain whether CAD activity alone was directly correlated with lignification in *Hevea*. Isolated and purified RNA from the bark tissue of 10 wild accessions and two control clones. The processed RNA was used in RTPCR with *Hevea actin* gene primers as control. Significant difference for CAD expression could not be observed among these wild accessions.

6. Utilisation of *Hevea* germplasm

6.1. W x A Open Pollination Garden 2005

The open-pollination garden at RRS, Padiyoor planted in 2005, comprising 24 selected Amazonian and 11 Wickham clones, was maintained.

6.2. Breeding Garden at RRII

A breeding garden established in 2007 at RRII, comprising 46 potential domesticated (Wichkam) clones and six selected wild accessions, was maintained.

6.3. Hand pollination programmes

A hand pollination programme conducted during 2009 at CES, Chethackal involving three wild accessions, two species and six cultivated Wickham clones resulted in 1331 hand pollinations. Another HP programme involving two popular clones and seven wild accessions was also carried out at RRS, Padiyoor, with the objective of combining yield with drought tolerance. Of a total of 506 crosses, 5.1 per cent success was obtained.

6.4. Generation of mapping population

Hybridisation between the popular *H. brasiliensis* clone RRII 105 and the disease tolerant *H. benthamiana* clone F 4542 was carried out with the primary objective of raising a mapping population for identification of QTLs for yield and disease resistance, as well as to identify any useful recombinant. This included 2122 crosses, with 656 reciprocal crosses.

7. Other studies

7.1. Feasibility of ratooning in rubber

Ratoons continued to be superior to their corresponding polybag grown counterparts in the ninth year of growth in terms of girth (Table Ger. 7). 10 more ratoon plants were brought under tapping this year, taking the total number of ratoons under tapping to 300 (86% of the total ratoons). Only 10 polybags plants (5% of the total) could be tapped so far.

Table Ger. 7. **Growth of plants**

	Ratoons	Polybag plants
No. of plants	150.0	201.0
Minimum girth (cm)	33.0	13.0
Mean girth (cm)	61.1	35.3
Maximum girth (cm)	90.0	58.5
Plants with > 50 cm girth	130.0 (86.1%)*	10.0 (5%)

* Figures in parentheses indicate the percentage of tappable plants

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

Bark samples were collected from stimulated trees under regular tapping and newly opened trees of clone RRII 105 of the same age with three stimulations during April, September and November 2008 and studied various bark anatomical and biochemical traits. The results indicated that the bark thickness was increased due to stimulation in both panels. The disorganization of laticifers showed an increasing trend during stimulation in B1-1 panel tapping but the variation was not significant. The total number of laticifer rows was significantly increased in stimulated trees during BO-1 panel tapping. The percentage of lignin and cell wall phenolics was higher in stimulated trees than the unstimulated, but the variation was not significant. The level of biochemical

Table Ger. 8. **Inclination of laticifers and volume of latex in different tapping systems**

Replications	Tree Nos.	Laticifer inclination (Leftward)	Conventional tapping (left top to right bottom slope)		Modified tapping (right top to left bottom slope)	
			Total vol. of latex (ml)	Latex vol./cm panel length (ml)	Total vol. of latex (ml)	Latex vol./ cm panel length (ml)
I	1.00	6.77°	15.50	1.11	22.25	1.46
	2.00	13.39°	23.38	1.43	31.25	2.00
	3.00	0.87°	16.37	0.82	17.25	0.79
	4.00	4.22°	10.00	0.57	28.25	1.61
	5.00	4.87°	20.87	1.18	31.50	1.63
II	1.00	5.41°	11.75	0.69	22.75	1.26
	2.00	0.33°	30.25	1.78	36.38	2.20
	3.00	3.39°	30.00	1.67	29.75	1.69
	4.00	5.38°	6.50	0.43	9.38	0.67
	5.00	7.85°	13.88	1.08	19.25	1.43
III	1.00	11.64°	9.12	0.73	12.75	1.15
	2.00	0.31°	50.00	2.40	51.13	2.59
	3.00	2.57°	42.38	2.57	51.88	3.09
	4.00	3.09°	24.13	1.36	22.88	1.53
	Mean	5.01°	21.72	1.27	27.57	1.66
	SE	1.05	3.42	0.17	3.37	0.18
	p-value		0.23		0.13	

components such as H_2O_2, melondialdehyde (MDA) and cyanide increased due to stimulation whereas the peroxidase activity and the â- CAS (cynade scavenging enzyme) level decreased.

7.3. Studies on the inclination of laticifers and clonal variability in *H. brasiliensis*

Fourteen trees of the clone PB 86 which showed leftward inclination of laticifers (Table Ger. 8) during the previous year's study were subjected to both conventional (left top to right bottom slope) and modified tapping (right top to left bottom slope) at RES, Nettana and the latex yield variation at weekly intervals was observed for a period of one month. All the experimental trees showed higher latex yield during modified tapping than that of conventional tapping.

8. Participatory clone evaluation

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

Field planting of 11 test clones and three controls was carried out, with RRII 105 being used for covariance analysis in each plot, at Shaliacary Estate, Punalur, Mooply Estate, Trissur, TR&T Estate, Mundakayam and Calicut Estate, Calicut.

PLANT PATHOLOGY DIVISION

Monitoring the occurrence of diseases and pests and their management by chemical and biological methods, characterisation of pathogens *etc.* are the main research areas of the Division. Bee keeping, pollution abatement, evaluation of microorganisms for their beneficial activities are the other areas of research.

1. Leaf diseases

1.1. Abnormal leaf fall disease

The field trials at four locations, *viz.* Mundakayam (PB 260), Thodupuzha (PB 260), Pathanamthitta (RRIM 600) and Thrissur (RRIM 600) were continued to evaluate the usefulness of biodegradable oil supplied by M/s. Indian Oil Corporation for abnormal leaf fall (ALF) disease management. Leaf retention assessment proved the efficacy of the biodegradable oil as carrier for oil-based COC micron spraying (Table Path.1).

Table Path. 1. **Per cent leaf retention**

Treatment	Leaf retention (%)	
	Thrissur (RRIM 600)	Mundakayam (PB 260)
Biodegradable oil	65.6	84.5
IOC spray oil	43.1	80.5
Control	6.6	-

Field evaluation of Aspee atomiser, supplied by American Spring and Pressing Works Pvt. Ltd. (ASPEE) was carried out at Laha Estate, Punalur and Pudukad Estate, Thrissur. The above attachment was found useful for ALF disease control. Field screening of HP clones for ALF disease incidence under sprayed condition was continued in the 1993 large-scale trials (Trial I and II) at CES, Chethackal. High leaf retention was recorded in RRII 414, RRII 417, RRII 429, RRII 453, RRII 430, RRII 427 and RRII 105.

ALF disease intensity was recorded in the RRII 400 series clones planted in Clone Trial (1998) at Cheruvally Estate, Erumely

and Clone Trial (1999) at Kaliyar Estate, Thodupuzha. The clone RRII 430 recorded maximum leaf retention at both the locations (95%). In general, leaf retention was good (> 80%) in all the clones in the above locations.

The clones, *viz.* RRII 414, RRII 430, RRII 422, RRII 417 and RRII 429 were evaluated for ALF disease severity in the 1982 trial at RRII farm. The leaf retention assessment indicated that all the new clones were on par with RRII 105 (Table Path. 2).

Table Path. 2. **Leaf retention in RRII 400 series clones under unsprayed condition at RRII**

Clone	Leaf retention (%)			
	2006	2007	2008	Pooled mean
RRII 429	77.19	53.17	74.83	68.27
RRII 422	71.67	54.87	57.67	61.40
RRII 430	81.75	75.41	77.80	78.50
RRII 414	77.84	64.59	68.33	70.25
RRII 417	71.34	63.98	65.00	66.77
RRII 105	78.27	67.29	83.33	76.29
	NS	NS	NS	NS

RAPD analysis of leaves inoculated with virulent strains of *Phytophthora*, by detached leaf technique from the clone RRII 105 and 11 germplasm accessions (MT 935, MT 2212, MT 1070, AC 754, MT 4436, MT 3707, MT 916, MT 2219, MT 1631, MT 1070, RO 1413) showed polymorphic-banding pattern with susceptible parent in five primers (OPA 2, OPA10, OPA 8, OPF 10 and OPO 2) out of 60 random primers.

Characterisation of 82 isolates of *Phytophthora* spp. showed that both mating types co-existed in the plantations of South India and one-third of them belonged to A1 type. Considerable molecular diversity was observed among all the isolates with unique AFLP patterns, indicating that *Phytophthora* spp. undergo sexual reproduction with the occurrence of new genotypes. Population differentiation analysis indicated occurrence of gene flow among populations.

DNA barcoding and barcode fingerprinting of *Phytophthora* spp. were initiated for presenting a genus-wide phylogeny of the four species namely *P. meadii*, *P. botryosa*, *P. colocassiae* and *P. citrophthora* affecting rubber. Two nuclear DNA regions (Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA and the microtubule constituent protein β-tubulin) and one mitochondrial gene, cytochorme oxidase (COX II) were used in the study. Over 800 sequences were generated. The β-tubulin locus provided the highest level of phylogenetic signal across the genus, grouping some of the *P. meadii* isolates within the *P. citrophthora* clade. The highly conserved mitochondrial gene COX II revealed a set of isolates that shows a closer relationship to *P. colocassiae*. Results of the analysis indicate a phylogenetic framework for interpreting the evolutionary history of these four species.

1.2. Powdery mildew disease

To evaluate crop loss due to powdery mildew disease in the clones RRII 105, one plot each was maintained as dusted and undusted at New Ambadi Estate, Kanyakumari. Application of sulphur was undertaken at recommended dose at an interval of 7 - 10 days in the dusted plots. Very low disease incidence was observed in both the plots.

In the crop loss trial at Padiyoor, sulphur dusting was undertaken at recommended dose at an interval of 10 days along with undusted control. The disease intensity was assessed by per cent leaf fall and by visual scoring (Table Path. 3). The monthly block yield was recorded in all the plots.

Table Path. 3. **Powdery mildew disease intensity in different clones**

Clone	Leaf fall (%)	
	Dusted	Undusted
RRII 105	21.27	36.22
RRIM 600	33.57	44.27
PB 235	32.20	43.18
PB 5/51	31.17	61.77

1.3. Corynespora leaf disease

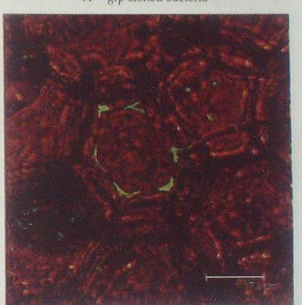
Thirty four bacterial endophytes collected from leaf, flower, stem and root of GT 1 and RRII 105 were tested for detoxification efficacy of phytotoxin from *Corynespora cassiicola*. The culture filtrates of endophytes and pathogen were mixed in different ratios, *viz.* 2:1, 3:1 and 1:0 and tested by leaf puncture bioassay. Culture filtrates of the isolate Rh- 34, at 3:1 concentration detoxified the toxin as evidenced by the reduced lesions produced on rubber leaves.

Ten bacterial isolates showing antagonism to *C. cassiicola* and detoxification of the toxin were tested for their sensitivity against the commonly used fungicides in rubber plantations, *viz.* mancozeb, carbendazim, copper oxychloride, hexaconazole, tridemorph, propiconazole, wettable sulphur and Saaf (mancozeb + carbendazim). All the isolates did not grow at the recommended dose of mancozeb. However, they showed resistant reaction to carbendazim and wettable sulphur in all the doses tested.

The selected antagonistic endophytic bacteria (*Bacillus subtilis*) against *C. cassiicola* were transformed using pGFPuv vector (Clonetech). The transformed bacteria selected using LB ampicillin plate [100µg/ml] showed fluorescence under UV light.

The transformed bacteria were applied to the root [$1x10^8$ cfu/ml] and leaves of the rubber seedlings. After four days, their colonisation in tissues was confirmed (Fig. Path. 1) by scanning through confocal microscope [Leica Confocal System].

A gfp cloned bacteria



B Bacteria in the tissue

Fig. Path. 1. **Localization of gfp cloned endophytic bacteria in the rubber tissue**

Field evaluation of antagonistic endophytic bacteria (mix of 3 *Bacillus* spp.) for Corynespora leaf fall disease control was carried out at two locations in North Kerala

(Adur region) and three in Karnataka, two in Guthigar and one in Jadkal regions). In the two locations at Guthigar, the efficacy of broth bioformulation of the bacterial cultures was comparable with carbendazim and in the other locations the treatments were not effective.

Transcript profiling in relation to biotic stress was initiated to identify and isolate genes that are differentially expressed during Corynespora leaf disease development in *H. brasiliensis*. Gene expression profiles of *Corynespora*-challenged leaf samples along with the uninfected leaves of RRII 105 as control were studied using DD-RT-PCR technique. Over 75 differentially expressed (only up-regulated) major bands were cloned and sequenced to determine the identity of the candidate cDNA. Regulation of several stress responsive genes was identified among the transcripts. Two clones DDCT7 and DDCT12 showed significant homology with Anthocyanidin 3-O-glucosyltransferase (E value: 4^{e-48}) and GRAS transcription factor (E value: 1^{e-15}) respectively, known to be involved in disease resistance in plants. The differential display technique was followed by differential screening using 'Reverse Northern' analysis to screen for cDNA fragments that truly represent differentially expressed mRNAs. The subsequent autoradiograms indicated location of positive differential display clones in the *C. cassiicola* inoculated samples. Northern analyses confirmed quantitative difference existing between the control of RRII 105 and inoculated GT 1 samples.

Germplasm accessions RO 3413, MT 1598, RO 3172, AC 2717, AC 4338 and tolerant clone GT 1 were artificially inoculated with *C. cassiicola* and chitinase assay was carried with the leaf samples collected from 24 to192 h (24 h interval). A marked rise in enzyme activity was obtained from 96 to 144 h in tolerant clones, *viz.* RO 3172, MT 1598, AC 2717 and GT 1, which

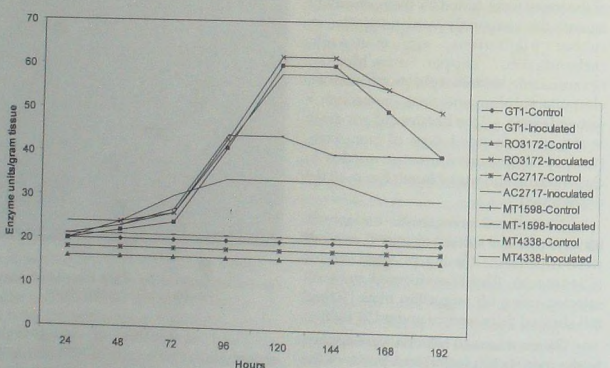


Fig. Path. 2. **Chitinase activity among different germplasm accessions during *C. cassiicola* induction**

declined at 168 h. In RRII 105, AC 4338 and RO 3413, the enzyme activity was constant from 24 to 192 h (Fig. Path. 2).

Based on the sequence data, chitinase-specific primers were designed with restriction sites (*Bam* HI in the forward primer and *Hind* III in the reverse primer).

The amplified products were further cloned in to TA cloning vector [PGMTeasy vector system 11] and sequenced. The sequence data showed the orientation of restriction sites.

Eighty germplasm accessions were screened *in vitro* against *C. cassiicola* for confirming their tolerance. RRII 105, the susceptible clone and GT 1, the tolerant clone were kept as control. The accessions showing tolerance to *Corynespora* were short-listed.

2. Brown root disease

In the experiment at Babu Land Estate, Kanyakumari, to control brown root disease the recovery of fungicide-treated plants and the spread of the disease to neighbouring plants were recorded. The systemic fungicides tridemorph (Calixin 0.5%) and propiconazole (Tilt 0.2%) recorded 60 per cent recovery. Hexaconazole (Contaf 0.04%) and thiram (Thiride 1.5%) were not effective. The spread of the disease was not observed in any of the fungicide treatment. (Table Path. 4). In the evaluation of biocontrol agents against brown root disease at Chimony Estate, Thrissur, the disease incidence varied from 0 to 15 per cent in the treated plots.

Table Path. 4. **Recovery (%) of plants in fungicide treated plants**

Fungicide	Recovery (%)	Spread of disease (%)
Tridemorph (0.5%)	60	0
Thiram (1.5%)	0	0
Hexaconzole (0.04%)	33	0
Propiconazole (0.2%)	60	0

3. Determination of the etiology of TPD of rubber

RT-PCR from total RNA and PCR amplification of cDNA of rubber, yielded products in the range of viroid only in TPD affected trees. To study the transmission of LMW RNA to indicator hosts total RNA was inoculated on tomato and the plants showed epinasty symptoms. Total RNA from inoculated plants showed LMW RNA band in R PAGE indicating that the inoculated LMW RNA can be reisolated. RT-PCR from total RNA isolated from inoculated plants with viroid-specific primers yielded product in the range of viroid. Amplification of a 360 bp product was observed in samples from tomato seedlings inoculated with RNA from TPD trees (Fig. Path.3). Cloning and sequencing of the RT-PCR product showed homology of the sequence to Potato Spindle Tuber Viroid (PSTV) on BLAST analysis (Fig. Path. 4&5).

Fig. Path. 3. **Agarose gel electrophoresis of RT-PCR amplified products obtained from tomato inoculated with rubber total nucleic acid using PSTVd specific primers.**

M - marker (100bp), Lane 1 - TPD tree no. 1 (Primer Abt), Lane 2 - TPD tree no.1 (primer PSTV), Lane 3 - TPD tree no.1 (Primer HAD 3), Lane 4 – Blank, Lane 5 - TPD tree no.2 (Primer Abt), Lane 6 – uninoculated control, Lane 7 – TPD tree no.2 (Primer PSTV)

In comparison with the sequences of PSTV S8, there is an insertion of 'A' residue in both tomato and rubber clones at position 98. Likewise there is deletion of nucleotide 'C' residue in tomato clones at position 267 compared to rubber clones.

A large-scale survey was conducted in six locations covering entire Kerala on TPD incidence in various panels. The TPD incidence increased as the tapping progressed from BO-1 and BI-2 panel at all

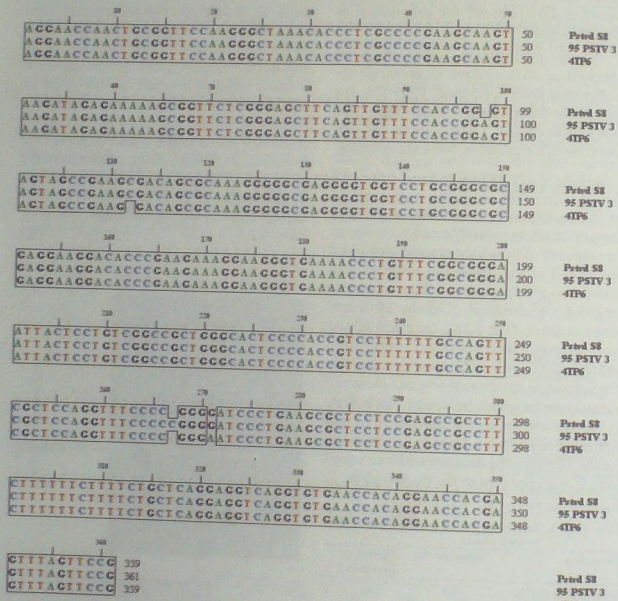


Fig. Path. 4. Alignment of PSTVd S8 (AY492082.1) with rubber (95 PSTV 3) and tomato (4TP6) clones

Table Path. 5. **BLAST analysis of cloned PCR products**

Accession	Description	Query coverage (%)	Maximal identity (%)
AY492082.1	PSTV isolate S8, complete genome	99	100
AF454395.1	PSTV, complete genome	99	100
AY937194.1	PSTV isolate PSTVd_Int5f, complete genome	99	99
AY937188.1	PSTV isolate PSTVd_Int3a, complete genome	99	99

Table Path. 6. **TPD incidence in various panels at different locations in Kerala**

Panel	TPD incidence (%)					
	Pala	Taliparamba	Kanjirappally	Nedumangad	Adoor	Mannarkad
BO-1	6.8	5.2	9.0	8.1	11.4	9.5
BO-2	14.4	14.2	13.9	15.7	17.1	15.4
BI-1	25.4	21.7	19.4	24.3	22.7	20.1
BI-2	27.0	28.2	26.4	29.0	30.1	26.5

locations (Table Path. 6). The survey was also conducted in smallholdings in Dapchari and North East to study the variation in TPD intensity as a result of change in temperature.

4. Pests of rubber

The experiment laid out in a highly white grub-infested soil at Nagapuzha indicated that entomopathogenic nematodes (EPN) and entomopathogenic fungi (*Beauveria brongniartii*) reduced the grub population as well as the damage to the young rubber plants. Imidacloprid (0.0025%) + Carbaryl (0.20%) also performed better than phorate 10% G in controlling the damage (Table Path. 7).

Laboratory studies to evaluate the effect of EPN in controlling bark feeding caterpillar, *Aetherastis circulata*, per cent infesting rubber showed 84 per cent mortality of 2nd instar larvae @ 360 ijls/5 larvae within 24 hours of exposure. Field efficacy of EPN on bark feeding caterpillar was studied at PCK, Kodumon. The results showed that EPN have less effect (only 19.31 and 26.25 per cent control after one and three weeks) against *A. circulata* under field conditions.

A field trial was laid out at RRII farm to evaluate the effect of different doses of EPN on the larvae of Mooply beetle, *Luprops curticollis*. Three doses of EPN such as 2, 4 and 6 lakhs per m^2 were given along with neem gold, chlorpyriphos and untreated control for comparison. Larval mortality recorded 7, 10 and 15 days after imposing

Table Path. 7. **Comparison of treatments on control of white grubs**

Treatment	Reduction of grubs (%)	Retention of plants (%)
EPN (6 lakhs/m^2)	90.00	90.00
Beauveria brongniartii	68.25	90.00
Beaumet	53.08	90.00
Imidacloprid 0.0025%	51.75	77.40
Imidacloprid 0.0025% + Carbaryl 0.20%	52.60	90.00
Phorate 10 G 15 kg/ha.	40.43	67.75
Control	17.17	69.15
CD (P =0.05)	22.91	8.27

treatments (Table Path. 8), indicated the effectiveness of EPN in reducing larval population.

Table Path. 8. **Per cent mortality of larvae after application of EPN**

Treatment	7 days after treatment	10 days after treatment	15 days after treatment
EPN 2 lakhs/m²	12.79	20.50	23.00
EPN 4 lakhs/m²	26.86	28.40	51.62
EPN 6 lakhs/m²	31.02	37.89	52.20
Neem gold 0.03%	10.74	11.78	15.22
Chlorpyriphos 0.05%	75.79	77.51	78.10
Control	3.30	3.63	4.10

However, chlorpyriophos was the most effective treatment giving 75 per cent mortality within a week.

Other pests of rubber recorded during the year

1. Leech, *Hirudo* sp. (Family - Hirudinidae; Order - Hirudinida; Class - Clitellata; Phylum - Annelida). Leaches cause much menace to farm workers and supervisors as they bite on the leg and suck the blood. Reported from many estates during July to December.
2. Porcupine, *Hystrix indica*. (Order – Rodentia; Class - Mammalia; Phylum - Chordata). Reported from Thodamarg, Maharashtra in a non-traditional new planting area during March – July 2009.
3. Shield bug, *Pentatoma* sp. (Family – Pentatomidae; Order – Hemiptera; Class – Insecta; Phylum – Arthopoda). Caused puncture injuries to one-year-old rubber plants during August 2009 in Manimala area.
4. Locust, *Aularches miliaris* (Family - Pygomorphidae; Order - Orthopatera. Class - Insecta; Phylum – Arthropoda). Sporadic occurrence and damage were reported from Pudukkad Estate, Thrissur in August 2008.

5. Vector control

For studying the vector dynamics in rubber plantation and formulating strategies on control of the vectors, a collaborated research project was initiated with Vector Control Research Centre (ICAR), Pondicherry and RRII. *Aedes albopictus*, the vector of Chikungunya and Dengu fever was found to be the predominant species (48%) followed by *Armigerus subalbatus* (43%) in rubber plantation. The biodiversity of mosquito species in rubber plantations was less compared to coastal region. *Mansonia* sp. was the predominant species (25%) in coastal region. The population density of *A. albopictus* is influenced by rainfall and predominant during the post- north east monsoon season.

6. Microorganisms for improving growth of rubber and cover crops

Based on intrinsic antibiotic resistance (IAR) pattern, five isolates of root nodulating bacteria collected from North East India along with the isolate from Kerala were inoculated to germinated seeds of *Mucuna bracteata* to study their competitive ability and nodule occupancy in the presence of native isolates. The occupancy of the NE isolates in *M. bracteata* nodules ranged from 82 – 96 per cent, while Kerala isolate showed 86 per cent nodule occupancy.

In another polybag experiment with different doses of *Azospirillum*, growth parameters of the plants were measured after 12 months by uprooting. Application of culture(5 ml) for six times or 10 ml at the time of planting showed more growth of plants in terms of height, girth, shoot and root dry weight *etc*. Rubber seedlings inoculated with phosphofungi at 50 per cent

P application showed more girth, height, shoot dry weight, root weight, root volume of plants than other treatments.

A total of 84 bacterial isolates (30 rhizosphere isolates, 24 non-rhizosphere isolates and 30 root endophytes) including 30 antagonists of rubber pathogens were isolated from two clones of rubber RRII 414 and RRII 105. Effect of eight commonly used fungicides, *viz*. copper oxychloride, carbendazim, mancozeb, mancozeb+ carbendazim (SAAF), sulphur, tridemorph, propiconazole and hexaconazole at different concentrations on the isolates was studied. Among the fungicides, wettable sulphur and carbendazim were safer. At recommended level, they inhibited only two and five antagonists and were not harmful to 58 and 60 bacterial isolates, respectively. Mancozeb inhibited 11 antagonists was found more harmful. Except tridemorph and hexaconazole, all fungicides were safe to the isolates studied at 25 per cent recommended level. Among the 30 antagonists, three isolates identified as *Bacillus subtilis/ amyloliquefaciens*, *Burkholderia cepacia* and *Bacillus subtilis* were showing antagonism to the four pathogens, *viz. C. cassiicola*, *Cortiium salmonicolor*,*Phellinus noxius* and *Phytophthora meadii*. They produced antifungal metabolites like volatile organic compounds, HCN and siderophore, solubilised phosphates, showed nitrogenase activity and produced IAA and ammonia and improved the growth of rubber seedlings upon inoculation. The eight fungicides tested reduced the growth of these isolates at the recommended level. Mancozeb and hexaconazole at recommended levels prevented their growth. Even at 25 per cent of the recommended level hexaconazole was inhibitory to the two *Bacillus* isolates. These antagonists were compatible with the fungicides copper oxychloride, carbendazim and sulphur at 50 per cent level and at the recommended level the harmful effect was less.

Out of 80 bacterial isolates including rhizobacteria and endophytes selected in the previous years twelve isolates showed more growth improvement of rubber seedlings in polybags and were selected for further evaluation.

An experiment on biological farming of rubber was initiated to compare the efficiency of beneficial microbes using microorganism identified at RRII along with organic manures in promoting growth and protecting the plants from diseases with that of recommended chemicals. Compatibility of the efficient isolates was studied by cross streaking and selected for co-inoculation. A seedling nursery was raised as per treatments and 1500 plants were green budded with RRII 430. The girth and height of the plants at the time of budding were comparable in the different treatments.

7. Waste management in rubber processing

The digester of the anaerobic immobilized growth digester has been modified by fixing "bio media" for the attachment of microbes and also rearranging the inlet and outlet. The bottom of the digester has been designed to maintain bacterial flocks as sludge blanket for the efficient degradation of the organic waste. The bacterial seeding also has been done. The reed bed system has also been designed and is being installed.

Based on the methane recovery from the RSS processing effluent in the Group Processing Centres and by avoiding fossil fuel and electricity usage in TSR factories, the Project Design Documents (PDD) for the bundled methane recovery from sheet

processing effluent and bundled project for the replacement of fuel oil and electricity with biomass gasifier for heat generation were developed and submitted for the host country approval.

The projects involves 25 group processing units for the methane project and 25 TSR factories for biomass gasifier project. The annual average estimated credit in terms of CER was 758 for methane and 8647 for biomass gasifier projects respectively.

8. Post harvest storage of sheet rubber

The evaluation of bacterial antagonists against post harvest mould growth in sheet was carried out on baled sheets of RSS 4 during monsoon season at Meenachil Rubbers, Palai and Rubber marketing Society, Konny. The bimonthly observation showed that the bioformulation treated bales were free from mould growth up to five months.

PLANT PHYSIOLOGY DIVISION

Plant Physiology Division conducts research in the areas of environmental physiology, physiology of growth and yield, stock-scion interaction, tapping panel dryness, nutrition physiology, gene expression studies, secondary metabolites and ecological impact of 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

One-year-old poly bag plants of *Hevea brasiliensis* (clones RRII 105, RRIM 600, RRII 430 and PB 260) were subjected to drought stress by withholding irrigation for eighteen days during summer 2009. The rate of photosynthetic oxygen evolution and effective quantum yield of PSII (Ö PSII) were inhibited significantly in drought affected plants. On the contrary, dark respiration rate of leaf increased during early drought. The clones RRII 430 and RRIM 600 recorded less inhibition in PSII and photosynthetic rate as compared to other clones under drought conditions, which was attributed to their tolerant clonal characters. RRII 105 and PB 260 were shown to be drought susceptible clones (Table Phy.1).

A consistently over-expressing 23 kDa chloroplast small heat shock protein (sHSP) was detected in drought and high light-stressed plants. Under drought condition, the level of expression of the sHSP was higher in RRII 430 and RRIM 600 than other clones. The protein was not over-expressed in clone PB 260 and PR 255. The over-expression of the sHSP in the chloroplast of these clones suggests a possible role in abiotic stress tolerance in young plants of rubber.

1.2. Identification of molecular basis for drought tolerance

(a). Polybag plants of RRII 105 and RRIM 600 were subjected to drought stress for two weeks. Total RNA was isolated from leaf and cDNA was synthesized. Real-Time PCR analyses were carried using seven gene transcripts which showed deregulation in microarray analyses in response to drought stress. The following were the gene transcripts analysed.

Table Phy.1. **Effect of drought stress on photosynthesis, fluorescence and respiratory activities in four clones**

Clones	Dark Respiration (μMol/m²/s)		Photosynthetic Oxygen Evolution (μMol/m²/s)		Dark Fv/Fm		Φ PSII actinic light (μMol)		ETR of PS II actinic light 230 (μMol)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
RRII 105	1.3[a]	2.1[b]	6.7[a]	2.1[b]	0.81	0.7	0.46[a]	0.32[b]	44	30
RRIM 600	1.3[a]	2.0[b]	6.6[a]	3.0[c]	0.8	0.75	0.49[a]	0.34[b]	44	33
RRII 430	1.9[a]	1.6[a]	5.8[a]	3.7[c]	0.79	0.73	0.47[a]	0.4[c]	49	40
PB 260	1.8[a]	2.1[b]	6.2[a]	2.5[b]	0.8	0.73	0.48[a]	0.38[b]	43	34

n= 5 Values followedby same alphabets are not critically different

1. HbDRT 5b (NAC 1 protein)
2. TPD 24 (NAC 1 protein)
3. HbNRG 21 (un known)
4. TPD 27 (unknown)
5. HbNRG 18 (unknown)
6. HbDRT 50 (unknown)
7. HbDRT 82b (hypothetical protein)

The relative level of amplified mRNA was normalized to mRNA expression of the house keeping gene, 18 S.

The transcript level of DRT 5b was over-expressed in RRII 105 about a relative quantification value of 95.63 times than the control. While in the case of RRIM 600, it was only 21.04 times than the control. Same trend was observed for the gene transcript, TPD 24. Up regulation of DRT 5b and TPD 24 (coding for NAC 1 protein) indicate that they might be expressed as a response to drought stress in *Hevea*. The transcripts such as DRT 50, DRT 82 b and TPD 27 showed up-regulation in clone RRIM 600 while these transcripts showed down-regulation in RRII 105. Up-regulation of these transcripts in clone RRIM 600 may be associated with drought tolerance.

(b) Polybag plants of RRII 105 and RRIM 600 were subjected to drought stress for two weeks. mRNA was isolated from leaf samples and cDNA was synthesized. Subtractive hybridization analyses were performed and the subtracted library was further cloned and sequenced. Sequence data of about 40 drought responsive transcripts were finally obtained.

1.3. Xylem sapflow measurements in mature rubber plants

The seasonal variation in xylem sapflow rate was analyzed. The wintering or the defoliation period of the year exhibited gradual reduction in the rate of sapflow concomitant with defoliation rate and this was followed by gain in the rate of flow during the refoliation period. There was summer (March-April) decline in sapflow rate in mature rubber trees and the rate was marginal during intensive rainy days (June-July) and it is attributed to the low transpiration pull due to stomatal closure. The sapflow rate was directly influenced by the sunlight intensity and sunshine hours during summer season but no significant relationship was noticed with other environmental parameters like rainfall and potential evaporation. The maximum temperature influenced the sapflow rate only during pre-monsoon period. The latex yield did not have any relation with the xylem flow. The average water use of a mature rubber tree is around 20-25 litres per day.

1.3.1. Irrigation requirement of mature rubber in North Konkan region

The study was to examine whether irrigation could be reduced from the maximum level of (1 ETc) to 0.25 or even lesser in mature yielding plantation with no adverse effect on its physiology. Irrigation

was provided during summer 2008 and 2009. The treatments were, (A) rainfed trees, (B) partially irrigated (deep soil area, 0.2 ETc in 2007-08) and (C) 1.0 ETc irrigation (shallow soil).

The dry rubber yield was recorded for the summer months and compared with previous year yield (Table Phy. 2).

Table Phy. 2. **Dry rubber yield of clone RRIM 600 under different levels of irrigation in Dapchari**

Irrigation	Projected yield (kg/ha) (Jan–May)		Yield (g/t/t)	
	2008	2009	2008	2009
Unirrigated- Plot A	368[a]	385[a]	21.0[a]	24[a]
Partially irrigated- 0.20 ETc - Plot B (deep soil)	985[c]	1050[c]	54.7[c]	65[d]
1.0 ETc- Plot C (shallow soil)	721[b]	780[b]	40.0[b]	49[c]

* Values followed by same alphabets are not critically different.

The dry rubber yield in rainfed-control was significantly lesser than the irrigated plants. In shallow soil, 1.0 ETc irrigation (C) during summer season did not give any yield improvement over the partially irrigated (deep soil area) plot B. The deep soil area recorded significantly higher yield than shallow soil area. It was reconfirmed that partial irrigation (0.2 level) is enough for deep soil area in a mature plantation.

1.4. Measurement of flux in CO_2 and water vapour in rubber canopy

The eddy covariance system was installed at Central Experimental Station, Chethackal, inside a four-year-old rubber plantation during March 2009 to monitor the ecosystem level carbon dioxide and water flux and canopy level photosynthesis in rubber. The canopy level net CO_2 assimilation rate of young immature plantation area was worked out to be in the range of -1.2 – 11.4 g /m²/day for the month of March 2009 (Table Phy. 3).

Table Phy. 3. **Net carbon dioxide (g/m²/day) and water vapour (mm/day) flux in a four-year-old immature rubber plantation**

Day	Carbon dioxide flux (Net carbon assimilation) Fc (g/m²/day)*	Water vapour flux evaporation (mm/day)*
16 March 2009	3.9	3.3
17	3.1	2.7
18	5.1	2.3
19	5.0	2.2
20	5.1	2.4
21	8.6	2.6
22	3.0	3.7
23	8.0	3.9
24	1.6	5.2
25	-1.2	2.7
26	7.3	3.8
27	11.4	3.0
28	5.4	3.3
29	3.8	3.3
30	3.4	5.3
31	6.6	4.2

* The values were corrected for density effects (WPL corrections)

The total water evaporation (soil and plant canopy level) rate ranging from 2.2-5.2 mm/day was also recorded during the month. The data on flux rates are being downloaded and processed on a daily basis.

1.5. Cultivation of elite rubber clones in high altitude

The 10 clones under evaluation at Haileyburia Tea Estate Ltd., Elappara were RRIM 600, RRII 105, RRII 208, RRII 422, RRII 414, PB 311, PB 260, PB 235, PR 261 and GT 1. Clone PB 260 showed maximum girth followed by GT 1, RRIM 600 and PB 235 in the trial initiated in 2006. Clone PR 261 recorded the minimum girth. In 2007, RRIM 600 reached maximum height followed by PB 311 in the next year. RRII 105 was the lowest in terms of plant height. No major disease incidence was noticed. Serious stress-

induced injuries were also not observed either due to drought or low temperature. Cover crop establishment was below average compared to the traditional belt.

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

1.6.1. Field scoring of germplasm accessions for drought tolerance

Field screening of 3570 wild genotypes for drought tolerance was carried out during the months of February- March in germplasm source bush nurseries (SBN) raised during 2003, 2004, 2005, 2006 and 2007 at CES, Chethackal. In summer, the percentage of leaf yellowing and leaf senescence was recorded by visual scoring and the accessions were sorted and ranked numerically. Genotypes that were exhibiting low senescence and yellowing in field under drought conditions were ranked top as tolerant ones and *vice versa*. Based on this, the genotypes were ranked as top, middle and bottom (Table Phy. 4). A total of 172 germplasm accessions were identified as top ranking ones based on field tolerance to drought stress.

1.6.2. Nursery evaluation of selected germplasm accessions

Four wild accessions found tolerant to drought stress in the laboratory screening were evaluated at nursery stage for various traits. Polybag plants of MT 5100, MT 5078, MT 4788, MT 4856 and MT 4694, RO 4615 and control clones RRIM 600 and RRII 105 were evaluated for water deficit stress by withdrawing irrigation for three weeks. PS II photochemical efficiency, leaf water potential, total soluble sugars and leaf chlorophyll content were estimated in irrigated (C) and unirrigated (S) plants.

Table Phy. 4. **Wild germplasm accessions selected from source bush nurseries by visual scoring of field tolerance for drought**

Field No.	Total accessions	Top ranking	Middle ranking	Bottom ranking
SBN 2003	588	79	29	12
SBN 2004	976	51	50	31
SBN 2005	700	19	51	35
SBN 2006	806	11	13	32
SBN 2007	500	12	47	13
Total	3570	172	190	123

The accessions MT 5078, MT 5100 and clone RRIM 600 maintained higher photochemical efficiency in drought. The mid-day leaf water potential showed drastic decline in drought stressed plants compared to irrigated plants. The leaves of clone RRIM 600 and accession MT 5100 maintained higher leaf water potential under drought. Among drought-stressed plants, clone RRIM 600 exhibited higher sugar content in the leaves followed by MT 5078. Accessions MT 5078 and RO 4615 showed higher chlorophyll content in drought stressed plants. A comparative evaluation of the drought tolerance capacity was estimated on the basis of their percent inhibition in drought compared fo irrigated plants. Accessions MT 4856, MT 4694, RO 4615 and clone RRII 105 exhibited higher percentage of drought inhibition compared to clone RRIM 600, followed by accessions MT 5078, MT 5100 and MT 4788. Clone RRIM 600, accessions MT 5078 and MT 5100 emerged as top ranking ones.

1.7. Physiological, biochemical and molecular basis for the adaptation of photosynthetic apparatus under drought stress

Four clones of *Hevea brasiliensis* (RRII 105, RRIM 600, RRII 208 and PR 261) were used for various studies under drought stress. The drought stress was imposed on one-year-old polybag plants of the above clones by withholding watering. Diurnal response curve of photosynthesis in these plants was measured and compared with

well-irrigated plants. This revealed the differences in the rate of photosynthesis in different clones. The effects of drought stress on seedlings grown in soils with three different pH levels were also studied. The results indicated that the seedlings grown in soils with acidic pH were more susceptible to drought stress.

To understand the events of light reactions and electron transport rate (ETR) across the photosystems, the ETR across PSII and PSI was measured using a DUAL PAM. It was found that in all the clones of *Hevea* and also in various species measured, the PSI ETR was always higher than that of PSII ETR. This difference is due to the cyclic electron flow across the PSI. The present study revealed the occurrence of cyclic electron flow across PSI even in non-stressful conditions.

When the PSI and PSII electron transport rates were measured in *Hevea* clones exposed to drought stress, it was found that the PSII ETR is highly decreased while the PSI ETR was found to be more or less unaffected. This study revealed that the PSI is more resistant to abiotic stresses like drought than PSII.

2. Physiology of growth and yield

2.1. Yield and yield components

Twelve clones planted at CES, Chethackal in 1982 were subjected to long-term yield evaluation under normal tapping. Trees were tapped for 16 years (S/2 d2 6d/7) from 1991-2007. Annual mean (mean yield for 12 months) and grand mean (mean yield for 16 years tapping for a clone) were calculated. The duration of yield having either the mean yield or above were considered as the peak yielding period of a clone. From this, mean peak yield was determined for each clone. To estimate the yield decline in a clone the per cent decrease from mean yield and peak yield was calculated.

Majority of the clones exhibited a good yield trend for 10 years and peak yielding period ranged between 4th - 13th year of tapping (Table Phy. 5). Among the clones, the period of peak yield varied from 2nd to 15th year of tapping. Clones PB 235, RRII 118, RRIM 600, GT 1 and Gl 1 maintained a long period of higher yield compared to other clones. Clones RRIM 501, RRII 300 and Tjir 1 maintained a short period of higher yield. At 16th year of tapping, considerable reduction in yield was observed in clones RRIM 703, RRIM 300, RRIM 600, RRIM 612, RRIM 501, RRII 105 and GT 1. The maximum decrease from mean yield was noticed in RRIM 703 and RRII 105 recorded 20 per cent decline. It was almost negligible in clones RRII 118 and GT 1. When peak yield was compared with yield at 16th year of tapping, the RRIM 703 showed the maximum reduction. About 30 per cent reduction from peak yield was observed in clones RRII 105, RRIM 600, GT 1 and RRIM 612. Yield declining phase in *Hevea* clones varied from 11th – 16th year of tapping. In clones RRII 300

Table Phy. 5. **Yielding pattern of *Hevea* clones based on peak yield and yield declining phase with respect to year of tapping**

Clone	Tapping period (years)	No. of peak yielding years	Peak yielding phase (year of tapping)	Yield declining phase (year of tapping)
RRII 300	16	8	3rd -10th	11th
PB 235	16	11	5th -15th	16th
RRII 105	16	9	3rd -11th	12th
RRIM 600	16	11	4th -14th	15th
GT 1	16	10	5th -14th	15th
PR 107	16	9	5th -13th	14th
Gl 1	16	10	5th -14th	15th
RRIM 501	16	8	5th -12th	13th
RRII 118	16	11	4th -14th	15th
RRIM 703	16	10	2nd -11th	12th
Tjir 1	16	7	4th -10th	11th
RRIM 612	16	10	4th -13th	14th
Mean	16	9.5 (~10)	4 - 13	14

and Tjir 1 the yield decline was noticed from 11th year, whereas, in high yielding clone RRII 105 the decline started from 12th year of tapping. In clone PB 235 the good yield out put was up to 15 years and decline started from 16th year.

Based on long-term yield, the clones were categorised as long duration peak yielding clones and short duration peak yielding clones (LDP/SDP). Seven clones fall under SDP category and five clones under LDP (Table Phy. 6). From this classification, the minimum year of tapping for maximum exploitation from a clone under normal tapping and the recommended year for controlled upward tapping (CUT) in a clone can be derived. To overcome the declining trend of yield after sixteen years of tapping, CUT was initiated (S/3 d/2 6d/7 ET 5%) in 12 clones. A significant increase in yield was observed in all clones subjected to CUT. Two-three fold increase in yield was observed in the first year of tapping irrespective of clones. More than three fold increases was found in clones PB 235, RRII 118 and GT 1 and in clones RRII 105, Tjir 1, RRIM 703 and Gl 1 it was by two fold. Similar trend was not observed in the second year of tapping under CUT; the yield was significantly declined in most of the clones.

Table Phy.6 **Classification of clones based on duration of peak yield and recommended tapping year of a clone for CUT (RTCUT)**

Clone	Classes	RTCUT (year)
RRII 300	SDP	11th
PB 235	LDP	16th
RRII 105	SDP	12th
RRIM 600	LDP	15th
GT 1	LDP	15th
PR 107	SDP	14th
Gl 1	LDP	15th
RRIM 501	SDP	13th
RRII 118	LDP	15th
RRIM 703	SDP	12th
Tjir 1	SDP	11th
RRIM 612	SDP	14th

SDP – Short duration peak yielder
LDP – Long duration peak yielder

2.2. Tapping induced loss of biomass

To understand the mechanism of missing biomass in tapped trees, a study was started in July 1999 with five clones in a 1987 plantation at HBSS, Nettana, Karnataka. The trees were opened for tapping during July 1997 and untapped trees were used as control. The clone RRII 105 consistently recorded lowest annual biomass increment upon tapping and it lost around 32 and 37 per cent of shoot biomass compared to the respective untapped tress during 2007 and 2008, respectively (Table Phy. 7). The popular clone RRII 105 proved to be a tapping sensitive clone. The annual biomass increment under d3 system of tapping was higher than d2 tapping. The clone PB 235 followed by PB 260 continuously recorded high biomass

Table Phy. 7. **Difference in annual biomass increment (kg/tree) between tapped and untapped trees (2008)**

Clone	Annual shoot biomass increment (kg/tree)			Standing shoot biomass (kg/tree) (21-year-old plant)			Biomass loss with respect to untapped trees (%)	
	Untapped	d2	d3	Untapped	d2	d3	d2	d3
RRII 105	67±18	12±5	26±7	655	410	451	37	31
RRII 300	47±12	19±7	51±11	516	398	505	23	2
PB 235	88±15	28±4	41±11	1130	769	826	31	26
PB 260	55±5	27±4	40±4	915	624	619	31	31
PB 311	36±3	24±7	36±6	736	541	540	26	26.5

increment both in untapped and tapped trees.

The average yield potential of five clones in S/2 d2 6d/7and S/2 d3 6d/7 system at HBSS, Nettana was analyzed. The results (Table Phy. 8) shows that the clone PB 235 is the top yielder both in S/2 d2 and S/2 d3 systems of tapping followed by PB 260 and PB 311. The popular clone RRII 105 was shown to be a medium yielder, particularly under S/2 d3 6d/7 tapping system though there was no significant difference in yield from other clones for S/2 d2 6d/7 system. RRII 300 has consistently been ranked as a poor yielder for Nettana conditions. The clones PB 235 and PB 260 consistently recorded greater biomass increment as well as high yield, required for considering them as latex-timber clones for the South Karnataka region.

Table Phy. 8. **The yield potential of five clones in d2 and d3 system of tapping**

Clone	Ten years average dry rubber yield (kg/ha)	
	d2	d3
RRII 105	1983[a]	1544[b]
RRII 300	997[c]	1183[c]
PB 235	2160[a]	2068[a]
PB 260	1912[a]	1921[a]
PB 311	1892[a]	1967[a]

* Values with same alphabets are not critically different from each other.

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

With the aim of selecting latex-timber clones, *viz.* RRII 105, PB 260, PB 235 and PB 217 from Malankara estate, were selected during the year 2006-07. The clones were under tapping on S/2 d3 system. The annual girth increment and annual block yield during 2006- 2007 and 2007-2008 were recorded. Among the four clones, RRII 105 was the highest yielding clone followed by PB 260 and PB 217. The shoot biomass accumulation was greater in PB 235 and PB 260 but this was reflected in yield of PB 235.

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

Screening of 84 HP clones, was done by measuring the ATP content in latex. Among the 84 clones, 22 showed high latex ATP content than the control clone, RRII 105.

2.5. Clonal variation on latex regeneration mechanism

Functioning of *in vitro* protein biosynthesis in the latex was studied by measuring the incorporation of ^{14}C labelled amino acids (Leucine, Arginine, Lysine and Phenylalanine) to the cytosol proteins. Seven clones with different yield potentials (high, medium and low) were used for this study. Incorporation of ^{14}C labelled amino acids into protein in the C-serum showed differences between clones. The highest incorporation was noticed in clones RRIM 600, GT 1, PB 217 and PB 260 compared to RRII 105. Low yielding clones RRII 33 and RRII 38 showed relatively low protein biosynthetic capacity.

2.6. Studies on rubber biosynthesis

Prenyl transferase (RuT) enzyme activity was studied in the latex using ^{14}C labelled IPP as the substrate in healthy trees of varying yield potential. This enzyme activity was significantly high in high yielding clones (RRII 414, RRII 429 and RRII 105) and low in low yielding clones (RRII 33 and RRII 38). The high yielding clones had 3-6-fold increase in the enzyme activity than low yielding clones. Among the high yielding clones, RRII 429 had the highest enzyme activity. Work is in progress to assay the enzyme activity in more number of trees and more number of clones with different yield potentials. The investigations are also

in progress to study the HMGR enzyme protein content in the bark tissues of different clones in relation to latex yield.

3. Stock-scion- interaction

3.1. Stock influenced differential expression of genes in the scion of *Hevea*

Gene expression pattern of field-grown bud-grafted, growth chamber acclimatized bud-grafted field plants and tissue-cultured plants was studied. Transcript level changes were more in bud-grafted plants compared to tissue cultured plants. The more variability in the gene expression was found in the normal bud-grafted plants compared to tissue- cultured plants. This may be due to the influence of rootstock on the scion. These results strongly suggest the possibilities of rootstock influence on the scion of *H. brasiliensis*.

3.2. Scion to scion communication

The differentially expressed genes in two scions (RRII 105 x RRIM 600) grown on a single root stock was studied through differential display analysis. The gene expression pattern was found to be similar in single bud-grafted RRII 105, double bud-grafted RRII 105 and double bud-grafted RRIM 600, whereas it was different in single bud-grafted RRIM 600. The results indicate the possible communication between two different scions when they are budded on to a common root stock. The present results agree with the earlier wintering behaviour data of this budding combination. During wintering, RRIM 600 showed early wintering behaviour as RRII 105, whereas RRIM 600 is a late wintering clone.

4. Tapping panel dryness

4.1. Effect of stimulation in the laticiferous tissues

Physiological, biochemical and cytological changes in the laticiferous tissue of mature trees after ethylene stimulation (periodic intervals) were compared with that of unstimulated trees. Bark samples were collected from the tapping panels (B0-1 and B0-2) of trees under regular tapping with and with out ethephon stimulation (2.5% bark application, three times per year). Biochemical components like cyanide (CN), β-cyanolalanine synthase (β-CAS), peroxidase (PX), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), *etc.* were analysed in the soft bark tissue during the post-stimulation periods. Histological analyses were carried out simultaneously in both stimulated and unstimulated groups and significant variations were observed in the levels of biochemical components in the bark tissues of trees tapped both BO-1 and BO-2 panels as stimulation effect. Biochemical analysis showed the development of stress-related changes in the bark tissues due to stimulant application, irrespective of the tapping panels.

4.2. Involvement of ethylene in rubber yield and TPD incidence.

Levels of CN, β-CAS, H_2O_2 and PX in the bark tissues of healthy and TPD trees were analysed to reconfirm the earlier results. Production of β-CAS was much lower in TPD-affected bark and hence CN accumulation was higher in the bark tissues. Similarly, the levels of H_2O_2 were much higher than its scavenging by enzymes and thus CN accumulated in the TPD-affected tissues. In normal healthy trees, CN accumulation was significantly low due to higher β-CAS activity.

The coding region for β-CAS was PCR-amplified and cloned into the pGEM-T Easy vector. Later, the coding region was restriction-digested with specific enzymes and was inserted into the corresponding sites of the pET expression vector. Cloning

was confirmed by restriction digestion analysis of the plasmid DNA. Attempts were made to get the protein extracted. Protein extraction was explored using standard protocols. However, the protein could not be extracted. The pET vector containing the coding region of CAS was also sequenced and the sequencing data was analysed.

4.3. TPD management through prophylactic application of compounds

AGROCARE (a homeopathic preparation) was tested for remission of TPD at Malankara Estate, Thodupuzha by applying it on 100 per cent TPD affected trees. Yield and TPD incidence was monitored to test its efficiency.

After three months of application, 55 per cent of trees with TPD yielded latex and the rest remained as in full TPD. Among the latex yielded trees, 25 per cent were yielding normal. After nine months, there was further reduction in TPD incidence with more trees (60%) yielding latex.

4.4. Molecular basis of TPD

4.4.1. Investigations on the molecular physiology of tapping panel dryness syndrome(TPD): cloning and characterization of TPD responsive genes by subtractive hybridization

The third round of subtraction was done between mRNA isolated from bark tissues of healthy and TPD-affected trees and DNA sequencing of the subtracted clones was carried out. The sequencing data indicated the presence of few important genes specific to both the conditions (Table Phy.9).

Candidate genes were identified from all the three rounds of subtraction and primers were designed and synthesized for 60 transcripts for performing quantitative PCR (qPCR). The specificity and efficiency of the primer combinations were determined. The primer combinations with a slope between -0.32 and -0.34 under various dilutions were selected. Standardization of primer combination was done for 20 transcripts (ASR protein, CRT/DRE binding factor 1, senescence associated protein,

Table Phy. 9. **Important transcripts from third round of subtractive hybridization**

TPD	Healthy
Chaperonin, CPN60-2, mitochondrial precursor	Proline Rich Protein1, PRP1
RAP2-like protein	NAD-depdt sorbitol dehydrogenase
GDA protein	Peroxidase
P66	ATP Synthase CF1 alpha subunit
ADP ribosylation factor	Lecithin retinol acetyltransferase
Avr9/Cf-9 rapidly elicited protein75	Inositol phosphate phosphatase
Aquaporin	Sodium/calcium exchanger protein
Ubiquitin conjugating enzyme2	Calpain like protease
Class II LMW HSP	Peptidase M28 family protein
WD-40 repeat family protein	Shaggy-related protein kinase delta
Type IIB calcium ATPase (MCA5)	Acireductone dioxygenase
Lipoxygenase	LEA5
1 beta glucosidase	Phosphoinositide 3-kinase
hsp17.5	Endoglucanase (cel5)
CCR4-NOT transcrip. Complex	Rac2
Transcription factor MBF1 (tcf gene)	Drought inducible 22 kDa protein
Guanine nucleotide regulatory protein	Lght harvesting chlorophyll a/b binding protein

tonoplast intrinsic protein (TCTP), drought induced 22kD a protein, hsp70, SOD, MAP kinase, VAD1, HbNRG18, HbNRG21, HbNRG5b, HbTPD24, HbTPD27, HbDRT50, HbDRT82b, â actin, 18S rRNA, and ubiquitin). The primer combination for five transcripts such as CRT/DRE binding factor 1, senescence associated protein, hsp70, MAP kinase and VAD1 were selected based on the results obtained.

5. Nutrition physiology

5.1 Management of soil characteristics to reduce abiotic stress and incidence of TPD in rubber trees

Three blocks of newly tapped experimental plantations (400 trees each) were maintained under different soil pH at Malankara estate, Thodupuzha to study the influence of soil pH in the development of abiotic stress in rubber trees. Lime application, analysis of soil for pH and leaf for nutrient contents, yield recording, growth measurements, *etc.* were carried out at periodic intervals and compared the data between experiment and control groups. Out of the three experimental blocks, lime application could reduce the soil acidity in two blocks. The control block (without liming) has soil pH of 4.3. Observations on growth and yield did not show significant variations between treatments.

Effect of soil pH in the growth and stress responses in the rubber seedlings was studied by growing the seedling plants in polybags with different soil pH conditions. The results showed that plants growing in soil pH 6.8 are performing well with optimum growth, while plants growing in high acidic soil (3.8 pH) showed severe oxidative stress and retarded growth with low soil nutrient uptake capacity.

6. Gene expression studies

6.1. Construction of an expression vector for *Bacillus subtilis* and over-expression of chitinase in endophytes of *H. brasiliensis*

The Bacillus expression vector pHT43, which has the capacity to transport the expressed protein across the cell wall into the medium, had been obtained commercially. The coding region for chitinase gene was restriction-digested as *Bam*H I and *Xba* I fragment from the clone pHCMC and introduced into the corresponding sites of pHT43. Transformed cells were selected on a selection medium and the clones were confirmed by colony PCR. However, the protein expression could not be confirmed. The clones had not been stable after few subcultures. Further restriction digestions were not successful.

7. Secondary metabolites

7.1. Quantification and identification of inositols

The experiment was continued to find out the clonal and seasonal differences in quebrachitol content. The efficiency of the methods of extraction for quebrachitol from RRII 105 and RRIM 600 was evaluated. The protocol developed was found suitable for the isolation of quebrachitol from all types of latex sera. A difference was observed in the extraction rate for different clones. The C-serum of RRII 400 series clones showed higher extraction percentage. Analysis of isolates obtained from RRII 105 and RRIM 600 showed no variation in the extractable amount of quebrachitol irrespective of the source of the serum.

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

Study on latex ionic composition and osmotic concentration was continued to

Table Phy. 10. **Clonal and seasonal differences in K^+ content, contribution of K to osmotic potential, osmotic concentration, and osmoregulation**

Clone	October - November			February - March			
	K (mg/100g)	Contribution of K to osmotic potential (bar)	Osmotic concentration (mOsmol/ kg)	K (mg/ 100g)	Contribution of K to osmotic potential (bar)	Osmotic concentration (mOsmol/kg)	Osmotic regulation (bars)
RRII 43	212.43	-2.489	456.10	161.98	-2.477	475.11	-0.48
RRII 118	208.11	-2.429	448.32	211.56	-2.429	524.32	-0.10
PB 311	239.77	-2.765	397.09	259.21	-3.025	525.36	-3.2
RRII 105	240.56	-2.948	405.36	231.67	-2.714	531.18	-3.15
GT 1	257.68	-3.008	344.58	257.13	-3.001	511.8	-4.19
RRII 308	268.05	-3.136	384.85	254.94	-2.903	466.71	-2.05
RRIM 600	270.05	-3.099	400.86	197.38	-2.304	480.23	-1.98
GI 1	254.69	-2.973	390.10	251.47	-2.972	458.72	-1.72

CD (P = 0.05)

K	- Clone - 26.57;	Season - 13.28;	Clone x Season – 37.57
Contribution of K to Osmotic potential	- Clone - 0.26;	Season - NS;	Clone x Season – 0.37
Osmotic concentration	- Clone - NS;	Season - 23.84;	Clone x Season – 67.43

confirm the role of cations to water relations of latex. Osmotic concentration of C-serum was calculated from the osmotic potential values. The activity of myo-inositol-1-phosphate synthase enzyme from serum samples of different clones was determined. Osmotic concentration was higher during the stress season in all the clones than the peak yielding season. It was found that K^+ concentration significantly influences the osmotic potential of latex. Clonal difference was observed in osmoregulation (Table Phy. 10).

7.3. Microbial hydrolysis of rubber wood sawdust for ethanol production

Cellulosic and starch materials present in the rubber wood sawdust were converted into simple sugars using suitable microorganisms isolated from rubber wood waste. This study was a preliminary step to understand the possibility of utilizing suitable microorganisms in cellulose and starch degradation of rubber wood and further fermenting to ethanol using the reducing sugars generated through enzyme hydrolysis. The study indicated the possibility of increasing the efficiency of the selected microorganisms by optimizing the culture conditions and/or by genetic transformations for cellulosic/starch degradation to gear up the entire process.

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

During this period, data on the CO_2 emission and GDP of all countries were collected from different sources. With this data the long-term trend of total CO_2 emission, carbon intensity per capita emission *etc.* of all developing and developed countries were studied. During this period, the Eddy covariance system (EC) was installed in the agro meteorology observatory of RRII for trial running. The data from EC system were downloaded and analyzed for studying various parameters as a preliminary study. Selected a proper site for EC system at CES, Chethackal for actual data through permanent installation in rubber plantation. Continuing the analysis of weather data from different rubber growing regions of India.

RUBBER TECHNOLOGY DIVISION

The activities of the Division were focused mainly on developing improved techniques in processing, refinement in test procedures, chemical modification of NR, development of blends of NR with other rubbers and rubber nanocomposites.

1. Primary processing

A 500 L capacity freezer was installed at CES, Chethackal for carrying out large-scale trials on low temperature preservation of latex. Latex (100 L/day) treated with mild dosage of preservative was stored in the freezer for five days at 4-5°C and then processed into TSR at PCRF. ISNR 3L could be produced by the above method.

Field latex preserved with the new preservation system, in which TMTD of conventional LATZ system is replaced, was centrifuged and its properties were compared with that of LATZ. Storage behaviour of the latices is being assessed.

Rectified the heat loss from the drying chamber of the existing solar drier and the drier was made fully operational. Four driers were fabricated and supplied to different research stations for drying sheet rubber / cup lumps. A modified sheet processing machine was developed which requires 50 per cent less space for installation and cheaper than the conventional dual rollers.

Trials were initiated to explore the possibility of removing white spots from block rubber using radio frequency drying.

2. Latex technology

The method of quick determination of dry rubber content for ammonia preserved latex was tested at different laboratories and it was confirmed that the error could be reduced to less than 0.1 per cent. The data were analysed statistically (Table Chem. 1) compared and the salient features of the methods are in Table Chem. 2.

Table Chem. 1. **Dry Rubber Content (DRC) estimation of ammonia-preserved latex using conventional and new method**

DRC(%) using standard laboratory method	33.22
DRC(%) using new method	33.24
Difference in DRC(%) between two methods	0.012
Standard deviation of the difference	0.025
Maximum variation between the two methods	0.018

n = 50

Table Chem. 2. **Advantages of the new method over conventional method**

Parameter	New method	Conventional method
Accuracy of the method	± 0.1 %	± 0.1 %
Drying time	2 - 3 h	16 - 20 h

Study on fatty-acid sensitized coagulation of NR latex was continued. The effect of carbon black in blends of NR obtained through soap-sensitized coagulation with BR was studied. These blends had a higher level of vulcanization along with better mechanical properties (Table Chem. 3) and ageing properties as compared to blends prepared from conventional rubber.

Table Chem. 3. **Mechanical properties of carbon black-filled blend vulcanizates**

Parameter	NR/BR 80/20		NR/BR 60/40	
	Sample	Control	Sample	Control
100% Modulus (MPa)	2.16	1.78	2.71	2.63
200% Modulus (MPa)	4.20	3.34	5.38	5.15
300% Modulus (MPa)	6.78	5.50	8.55	8.28
Tensile strength (MPa)	20.00	21.1	11.70	11.10
Elongation at break (%)	690	700	400	390
Tear strength (N/m)	66	51	39	30
Hardness Shore A	66	65	68	67
Heat buildup (DT°C)	19	20	20	20
Din abrasion loss (mm^3)	79	80	74	75

Storage behaviour of the *in situ*-formed NR-silica composite was studied and it was observed that the technological properties of the composite remained more or less unaffected on storage. DPNR was prepared using the standardized method and its raw rubber (Table Chem. 4) and technological properties (Table Chem. 5) were compared with those of a commercial sample. The properties of DPNR prepared in the laboratory were comparable to those of the commercial sample.

Table Chem. 4. **Raw rubber properties of DPNR**

Parameter	DPNR	
	Commercial	Laboratory sample
Dirt content (%)	0.002	0.002
Volatile matter (%)	0.29	0.13
Ash content (%)	0.43	0.12
Nitrogen content (%)	0.16	0.14
P_o	36	30
PRI	19	17
ASHT	2	4
Mooney viscosity ML(1+4) (100°C)	68	68
Acetone extract (%)	4.78	2.98

Table Chem. 5. **Technological properties of DPNR**

Parameter	DPNR	
	Commercial	Laboratory sample
Tensile strength (MPa)	25	26.1
300% Modulus (MPa)	8.2	7.4
Elongation at break (%)	556	582
Tear strength (N/mm)	56.3	67.8
Hardness (Shore A)	54	54
DIN Abrasion loss (mm^3)	159	158
Rebound resilience (%)	65	63
Heat build up (ΔT°C)	13	13
Compression set (%)	11.4	12.6
Water absorption (increase in mass (mg/cm^2)	0.621	0.334

3. Blends

Optimized the blending conditions of field latex and MG30 (NR-g-PMMA) latex in the pilot plant for preparing PVC dispersible grade of natural rubber (PDNR). This rubber was melt-blended with PVC at 70:30 ratio (PDNR/PVC). A blend of NR, MG30 and PVC in the same ratio (NR/NR-g-PMMA/PVC) was also prepared in the dry stage. Control blends of NR and PVC (70:30) without NR-g-PMMA and NBR/PVC (70:30) were taken for comparison and vulcanizate properties were determined (Table Chem. 6). Both the NR-g-PMMA containing samples showed better mechanical properties than that of control NR/PVC, while the PDNR/PVC showed comparable properties to NBR/PVC.

Morphology and thermal stability of the vulcanizate containing polymeric filler were studied using TEM and TGA respectively. To study the percolation threshold of the composite, vulcanizates containing 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 parts polymeric filler were prepared and its modulus at 50 per cent elongation was determined and the percolation threshold was calculated.

Collaborated with a leading tyre tread manufacturing unit and evaluated the properties of tread rubber prepared using the polymeric filler. Two different proportions of polymeric filler were added and designated as NR/PF10 and NR/PF12. Both the compounds showed almost similar properties as that of a standard tread compound. The technological properties are given in Table Chem. 7.

Table Chem. 6. **Vulcanizate properties of NR/PVC and NBR/PVC blends**

Parameter	NR/ PVC	NR/NR-g-PMMA/PVC	PDNR/PVC	NBR/PVC
Tensile strength (MPa)	13	22.6	17.7	15.8
300% Modulus (MPa)	2.8	9.1	5.7	5.1
Tear strength (N/mm)	23	47.6	38	37.2
DIN abrasion loss (mm^3)	289	175	189	-
Compression set (%)	58.8	58.1	58.3	-

Table Chem.7. **Technological properties of the tread compounds containing different proportions of polymeric filler**

Parameter	NR/PF 10			NR/PF 12		
Cure time (min.) at 159 °C	7	10	15	7	10	15
300% Modulus (MPa)	12.55	12.24	11.33	13.33	13.82	13.47
Tensile strength (MPa)	34.29	32.71	29.58	31.98	30.98	30.38
Elongation at Break (%)	572	577	564	562	551	560

4. Nanocomposites

Dynamic mechanical properties and thermal stability of the NR/PF1 and NR/PF2 nanocomposites were studied using DMA and TGA. Compared with the control samples, both the nanocomposites showed better dynamic and thermal properties. Effect of molecular weight and chain branching of NR molecules on production of NR nanocomposites was studied using the two rubber fractions obtained through centrifugation of NR latex. Nano-silver with secondary capping agents such as polyvinyl alcohol (PVA), polyethylene oxide condensate and carboxy methyl cellulose were prepared and the chemical stability against acids, bases and salts were determined. Particle size and its distribution were determined using dynamic light scattering technique (Figs. Chem. 1 and 2).

It was found that nano-silver with PVA as capping agent had better chemical stability (Fig.Chem. 3). Absorption of UV light in the range 390-400 nm is attributed to nano- silver particles below 30 nm. This absorption of UV radiation was maintained for PVA capped sample after addition of NaOH up to one per cent (Fig. Chem. 3).

The latex stage incorporation of nano-silver suspension to RVNRL was done followed by the preparation of nanocomposite film. Nano-silver- dry rubber composites were prepared and its dynamic and mechanical properties were evaluated.

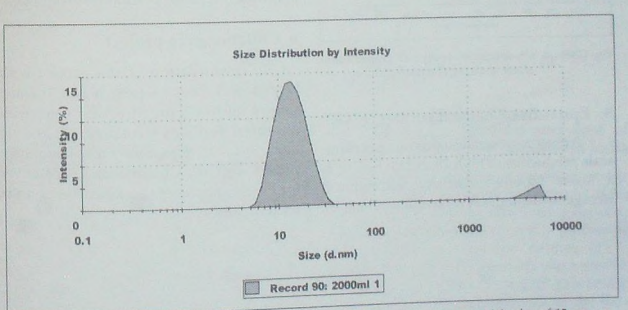


Fig. Chem. 1. Dynamic light scattering of nano-silver indicating the average particle size of 12 nm

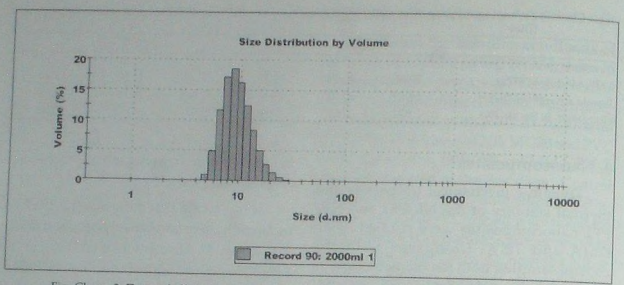


Fig. Chem. 2. Dynamic light scattering of nano-silver showing the particle size distribution

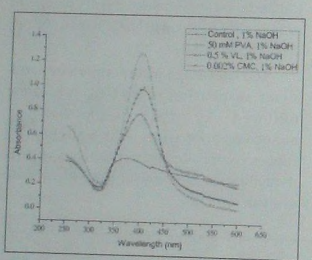


Fig. Chem. 3. UV spectrum of polymer- capped nano-silver showing chemical stability

5. Epoxidised natural rubber (ENR)

A protocol was developed for pilot plant-scale production of ENR 25 and ENR 50 with a lower Mooney viscosity and reduced storage hardening behaviour (Table Chem. 8).

Table Chem. 8. Change in Mooney viscosity of ENR during storage

	Mooney viscosity M_L (1+4) at 100° C			
	Initial	After 3 months	After 7 months	After 12 months
ENR 25	80.0	86.0	92.0	92.2
ENR 50	76.0	83.0	85.7	92.3
NR	87.6	92.0	93.0	94.0

The evaluation of ENR of varying epoxy content for tyre application was continued.

6. Collaborative project

In continuation of the project with Schieffelin Leprosy Research and Training Centre (SLRTC), microcellular soles with improved flexibility were prepared and are being evaluated. Initiated a collaborative project with M/s Chittaranjan Locomotives for the development of rubber components used in railways.

TECHNICAL CONSULTANCY DIVISION

The activities of the Division were focused mainly on establishment of rubber-based industries, product development, quality control, advisory services, training programmes and schemes on diversified uses of natural rubber (NR).

1. Product development

The following products were developed as per request from the entrepreneurs:

- NR/EPDM compound for valves
- Floor mat using waste crumb
- Nitrile rubber compound
- Latex carpet backing
- Latex adhesives for different applications
- Rubber grommet
- Vulcanized latex sheet for floor covering in firework factories
- Rubber rollers
- Stop log rubber seal

2. Quality control

Support was provided to various industries by way of testing the raw materials, rubber compounds, rubber products *etc.* The Division received 1300 samples for testing and 6300 parameters were tested. Evaluation of 24 rubber chemicals, *viz.* accelerators, stabilizers, *etc.* was also taken up.

3. Advisory services

Advisory service through factory visit was offered to one firm. Technical advise was given for queries received from 600 firms. The Division has also been associated with establishment of rubber park at Tripura.

4. Scheme on diversified uses of natural rubber

Rubberisation of roads: Final report was received from NATPAC (National Transportation Planning and Research Centre, Trivandrum) regarding this study. The study endorsed the significant improvement in performance related characteristics of Natural Rubber Modified Bitumen (NRMB) used for road construction under KST Project, Government of Kerala.

The Division collected a total amount of Rs. 4,56,520/- towards testing, development and consultancy.

ECONOMICS DIVISION

The Division is concentrating on studies pertaining to economic aspects of natural rubber (NR) cultivation, processing, marketing, end-uses, ancillary sources of income and by-products. Inter-divisional collaborative studies are also undertaken by the Division. During the period under review three projects were completed and reported by the Division. The summaries of the results pertaining to the completed projects are given in the following sections.

1. Capacity utilisation and cost of processing of TSR industry in India

A detailed analysis at the disaggregate level was taken up in the background of the observed insignificant relationship between capacity utilisation and the cost of processing in technically specified rubber (TSR) processing industry. The study was based on a census conducted among 48 processing units under different sectors and the data pertained to the five-year period from 2001-

02 to 2005-06. The capacity utilisation of the industry was only 54 per cent with an installed capacity of 171450t during 2005-06. The five-year average cost of the industry was Rs.5.52 per kg. Private sector was the only sector which could achieve an average unit cost lesser than the industry average whereas the costs of all other sectors were higher than the industry average. Lower unit costs were achieved through the managerial strategies by reducing the direct cost of processing. It is evident from higher shares of operational costs and lower shares of overhead and other costs, use of labour on contract, piece rate and production-linked incentive schemes and use of Biomass gasifier in the private sector. Cooperatives and public sector units incurred losses due to the higher shares of overhead and other costs. Scale of processing and capacity utilisation in the industry were circumscribed by contract processing and frequent fluctuations in the prices of both raw material and output. The position of processors as price takers in both the input and output markets, increasing cost of processing, lack of margin, *etc.* were found to have adversely influenced the production and capacity utilisation of the processing industry. Therefore, given the constraints, a consortium approach to confront the rigidities in the input and output markets is essential from a long-term policy perspective.

2. Impact of NR price volatility on small-scale non-tyre rubber products in Kerala

The first phase of the survey which was focused on the raw material intensive latex-based products indicated the vulnerability of the manufacturing units to frequent fluctuations, especially the unprecedented rise in NR prices since 2003. The units manufacturing latex-based products such as rubber band and gloves had either temporarily closed down the operations or cut down the volume of production. Many of the closed units attempted to retain the labour by paying half the wages expecting a fall in NR prices. During the second phase when the NR prices consolidated at a higher level, the survival strategies adopted by the units included dilution of the quality of finished products by hiking the proportion of fillers to the extent of 85, 80, 97 and 70 per cent respectively for mats and moulded rubber, tyre flaps, gum and rubber band.

The units which were successful in implementing the survival strategy also could raise the prices of the products and consolidated their position in the market. The unique case of tread rubber manufacturers revealed the exit of smaller units by leasing out to the larger ones including all the infrastructural facilities and labour. The observed trends are synonymous with emerging pattern in the unorganised non-tyre segment at the national level. This segment is confronted with the uncertain raw material prices and steadily increasing imports over the past one decade. This situation calls for policy interventions relevant to the production conditions at the regional level with a consortium approach to confront the uncertainties in the raw material and product markets.

3. Commercial yield performance of rubber clones in the estate sector in India

This report is the fifth in the series based on a continuous yield evaluation scheme initiated by the Rubber Research Institute of India in 1974. The database consists of yield and yield related aspects of 25 clones obtained from 706 fields covering an area of 9669 ha under the estate sector. One of the highlights of the analysis is the growing share of area under the yield declining phase

which has important policy implications. It was observed that the share of area under this phase increased from 12 per cent during the 1970s to 36 per cent in the recent years. Since there is an inverse relationship between yield and age of the tree beyond its peak yielding phase, the observed trend will adversely affect the average yield and production in the years ahead.

The combined life cycle annual average yield of the 25 selected clones was 1340 kg per ha. The yield profile showed that the mean yield had stabilised from the fourth year of tapping as mean yield during this year surpassed the 25-year life cycle mean yield (1340 kg/ha). The period from the 4th to 16th year of tapping can be considered as the stabilized phase since the mean yields during this period were above the 25-year life cycle mean yield. The highest yield was reported in the 7th year (1593 kg/ha). During the 1-5-year phase, the mean yield was the highest for PB 260 (1658 kg/ha) and RRII 105 occupied the second position (1629 kg/ha). The yield and related components of 25 clones for the 1-10 year phase is given in Table Eco. 1.

In the 1-10-year phase also PB 260 recorded the highest yield (1741 kg/ha) followed by RRII 105 (1736 kg/ha). During the 1-15-year phase, RRII 105 occupied the first position in yield with 1732 kg/ha followed by PB 260 (1725 kg/ha). For PB 260, data were available only for the first 15 years of tapping and for RRII 105 data were available for 22 years. During the 1-20 and 1-25-year phases, RRII 105 reported the

Table Eco. 1. **Mean yield and related components during 1-10-year phase**

Clone	Yield (kg/ha)	Yield per tree (kg)	Share of FC (%)	Tappable stand per ha (No)	Tapping intensity (%)
GI 1	1219.40	3.58	30.21	340	90.97
GT 1	1303.56	3.79	27.86	351	79.24
LCB 1320	1042.56	3.57	31.61	294	92.07
PB 217	1437.07	4.53	22.29	324	75.04
PB 235	1366.15	4.12	30.79	335	71.91
PB 252	1288.87	4.80	35.54	281	76.38
PB 260	1741.44	4.68	27.87	376	60.67
PB 28/59	1478.60	4.51	29.43	327	71.36
PB 310	1389.27	4.26	30.79	326	70.45
PB 311	1367.45	4.34	31.80	316	69.68
PB 5/139	1367.63	3.74	25.35	374	99.63
PB 5/51	1220.30	3.79	27.43	324	75.97
PB 5/60	954.06	2.89	34.81	330	91.00
PB 5/63	1072.52	4.11	37.82	261	74.06
PB 6/9	1138.08	3.13	23.48	366	67.67
PB 86	1220.42	3.60	24.61	348	88.13
RRII 105	1735.54	5.10	23.35	348	66.68
RRII 116	1214.06	3.55	36.39	347	96.70
RRII 118	1253.33	3.49	31.59	358	93.61
RRII 208	1299.33	3.59	27.87	366	80.32
RRIM 600	1324.79	4.33	28.52	315	84.52
RRIM 605	1217.92	3.62	29.81	339	86.40
RRIM 623	1146.01	3.57	30.47	329	86.62
RRIM 628	1031.78	3.66	37.91	281	76.46
RRIM 701	1085.30	3.60	34.51	304	81.13
Mean	1340.46	4.08	28.24	335	78.92

highest yield (1713 kg/ha and 1712 kg/ha). The average yield during 1-30-year phase was 1312 kg per ha and the yield during this phase was the highest for PB 217 (1506 kg/ha).

The monthly and seasonal yield composition and seasonal stability during 1-10-year phase showed that the peak season, which approximates the winter season (October - January), accounted for 47 per cent of the annual yield and the highest yielding month was December (13%). The share of the lean season, approximating the summer season (February - May) was 18 per cent and the lowest yielding month was March (< 3%). It was also observed that there was a steady decline in the grand mean yield of all the clones together from Tamil Nadu in the south (1451 kg/ha) to Karnataka (991 kg/ha) in the north.

The estimated Mean Yield Index (MYI) and Commercial Yield Performance Index (CYPI) showed higher values for PB 260 followed by RRII 105 among the top 12 clones during the 1-10-year phase. Hence, during the 1-10-year phase, PB 260 was found to be the best clone suiting Indian conditions, from the commercial angle. However, during the 1-15 year phase the yield performance of RRII 105 was found to be better than PB 260. In accordance with the recommendation of popularising multiclonal planting policy, it is necessary to evolve region-specific recommendations based on comparative performance and the respective secondary characteristics.

EXPLOITATION TECHNOLOGY DIVISION

The Division continued applied research on crop harvesting aspects of rubber. A collaborative programme for popularising Low Frequency Tapping (d3) among smallholdings was initiated with the active participation of regional offices of Rubber Board. The participating team includes five small growers under each Regional Office, scientists of the Division and extension officers of the concerned Regional Office. The Division has entered into an agreement with the Indian representatives of M/s Hevea-Tech SDN BHD, Malaysia for evaluation of G-Flex technology (ethylene gas stimulation) for Controlled Upward Tapping (CUT).

The increasing share of senile plantations adversely affects rubber production, for which CUT is a viable option to boost productivity. For the large-scale implementation of CUT in smallholdings, training of trainers (Extension officers and Rubber Tapping Demonstrators) was organised in traditional and non-traditional regions. Tappers at RRS, Guwahati and RRS, Agartala were also trained in use of modified gouge knife. A demonstration trial on CUT was also initiated at Taranagar farm. Other activities included testing and evaluation of various products like rainguard materials and yield stimulants etc. used in crop harvesting.

1. Low frequency tapping systems

Experiment on the yield performance of low frequency tapping with different levels of stimulation in clone RRII 105 (from opening onwards) at EFU, RIT was continued. Similar yield trend was observed during the reporting year also. Under d4 and d6 frequencies, tapping was still on virgin bark compared to renewed panel tapping under d2 and d3 frequencies.

In the S/2 d6 6/d7 demonstration plot at CES the highest yield of 10.9 kg/tree and mean annual yield of 83.8 kg/tap was obtained in

Table Exp. 1. **Monthly yield response of Low Frequency Tapping (S/2 d6 6d/7) in clone RRII 105 at CES, Chethackal**

Month	Yield 4500 trees) kg/tap	kg/month	No. of taps per month
April	43.3	173.2	4
May	97.6	488.0	5
June	144.3	577.2	4
July	120.0	600.0	5
August	134.4	537.6	4
September	110.9	443.6	4
October	60.1	300.5	5
November	77.9	311.6	4
December	70.9	283.6	4
Jannuary	73.3	366.5	5
February	41.0	164.0	4
March	32.1	128.4	4
Total	83.8 (mean)	4373	52
Yield (kg)/tree		10.9	

clone RRII 105 (Table Exp. 1). The high yield was due to change of tapping panel to BO-2.

In an exploratory trial on once in 10 days (d10) tapping frequency at CES, Chethackal, promising yield of 2453 kg/400 trees was obtained from 36 tappings. The trees were tapped under d6 frequency for initial two years in BO-2 panel. From third year, frequency was modified to d10. As on 2008, TPD incidence was found to be very low (1.4%).

In the trials at Kulasekharam region, annual rainfall was considerably low (716 mm in 54 days) compared to previous years (1016 mm) and hence, all the scheduled stimulations were not carried out. The yield was 1743 kg/400 trees at Kanthimathy Estate (weekly tapping) and 1576 kg/400 trees at Hariharaputra Estate (only 10 months tapping during 2008-09).

In the on-farm trial on weekly tapping initiated during 2002-03, collection of yield data and other informations from two locations, *viz.* Manickel Estate, Mundakkayam and Tropical Farms, Adivaram, Calicut were continued to monitor long-term performance. Tropical Farms are maintaining four d4 frequency

Table.Exp. 2. **Dry rubber yield (kg/400 trees) under Low Frequency Tapping**

Month	Tropical Farms d4	d7	Manickel Estate d7
April	94	112	119
May	176	124	175
June	210	173	166
July	249	244	208
August	209	255	158
September	192	217	194
October	149	181	179
November	207	208	237
December	296	331	325
January	344	311	241
February	135	111	108
March	134	104	79
Total	2396	2371	2187

Table Exp. 3. **Yield response of LFCUT with rain guard in clone RRII 105 at CES, Chethackal**

Treatment	Yield* (kg/400 trees)	Number of taps
S/4U d3 6d/7 ET5.0% La 12/y (m)	3880^{b}	105
S/2 d3 6d/7 ET2.5% Pa 3/y	5649^{ab}	105
S/4U d4 6d/7 ET5.0% La (stim.after every 5th tapping)	5791^{ab}	78
S/4U d4 6d/7 ET5.0% La (stim.after every 4th tapping)	5166^{ab}	78
S/2 d4 6d/7 ET2.5% Pa 6/y	7218^{a}	78
S/3U d6 6d/7 ET5.0% Ga (stim.after every 4th tapping)	4604^{b}	52
S/2 d6 6d/7 ET2.5% Pa 12/y (m)	5358^{ab}	52
S/3U d6 6d/7 ET5.0% Ga (stim. after every 2nd tapping)	4038^{b}	52

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

blocks along with the seven blocks under weekly tapping.

2. Low Frequency Controlled Upward Tapping (LFCUT)

In an experiment at CES on LFCUT with rainguards and annual panel change in clone RRII 105, three treatments were tapped half-spiral on basal panel from April 2008 to March 2009. During fifth year of tapping, yield under CUT in all frequencies with various levels of stimulation was comparable (Table Exp. 3).

In another LFCUT trial in clone RRII 118 (basal panel is unsuitable for tapping), during fourth year of tapping (panel HO-2), good yield was obtained under d4 and d6 frequencies of tapping (Table Exp. 4). Yield under S/4U d3 was significantly lower than S/4U d4, S/3U d4 and S/3U d6 frequencies of tapping.

3. Other experiments

The experiment on long-term evaluation of rainguard to study the problem of production losses due to rain and recovery through stimulation was continued at RIT. The 2008-09 data showed that, crop loss in non-rainguarded trees can be fully compensated by stimulation under d2 or d3 frequency of tapping. (Table Exp. 5).

In many plantations, panel change on basal panel (first two years on BO-1, next year on BO-2 and thereafter alternate the panels) is practised due to the general belief that it can give higher yield with low incidence of TPD. However, present study

Table Exp. 4. **Yield response of LFCUT with rain guard in clone RRII 118 at CES, Chethackal**

Treatments	Yield* (kg/400 trees)	kg/tree	Number of taps
S/4U d3 6d7 ET5% La (6w)	2285^{b}	5.7	105
S/4U d4 6d7 ET5% La (3w)	3506^{a}	8.7	78
S/3U d4 6d7 ET5% La (6w)	3394^{a}	8.4	78
S/3U d6 6d7 ET5% Ga (3w)	3626^{a}	9.0	52
S/3U d6 6d7 ET5% Ga 12/y (m)	2810^{ab}	7.0	52

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

Table Exp. 5. **Effect of stimulation under d2 and d3 frequencies of tapping in clone RRII 105 without rainguarding on recovery of yield loss at EFU, RIT, Pampady**

Treatments	Yield (400 trees)			DRC	Number	Panel/
	kg/year	kg/tap	kg/tree	(%)	of taps	year
S/2 d2 6d7 with RG	3161^{abc}	23.1^{d}	7.9^{abc}	39.3	137	BI-2(1)
S/2 d2 6d7 without RG	3507^{a}	31.5^{cd}	8.8^{a}	40.3	111	BI-1(1)
S/2 d2 6d7 ET2.5% Pa. 3/y (without RG)	3395^{ab}	30.7^{cd}	8.5^{ab}	40.4	111	BI-1(1)
S/2 d2 6d7 ET2.5% Pa. 5/y (without RG)	2547^{bc}	22.9^{d}	6.4^{bc}	39.4	111	BI-1(1)
S/2 d3 6d7 ET2.5% Pa. 3/y (with RG)	2269^{c}	22.8^{d}	5.7^{c}	38.3	100	BI-1(2)
S/2 d3 6d7 ET2.5% Pa. 3/y (without RG)	2964^{abc}	41.9^{ab}	7.4^{abc}	40.0	70	BO-2(5)
S/2 d3 6d7 ET2.5% Pa. 5/y (without RG)	2609^{abc}	34.3^{bc}	6.5^{abc}	40.6	76	BO-2(5)
S/2 d3 6d7 ET2.5% Pa. 7/y (without RG)	3305^{ab}	44.8^{a}	8.3^{ab}	39.3	74	BO-2(5)

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

Table Exp. 6. **Dry rubber yield (kg/ha) in clone RRII 105 under different frequencies of tapping with and without panel change (1997-2008)**

Treatment	1997-98	1998-99	1999-00	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08
S/2 d2 6d7 (without PC)	1722^{c}	2677^{a}	2671^{a}	2392	3976^{a}	3652^{a}	2923^{bcde}	2669^{bcd}	3839^{a}	2698^{ab}	1917^{ab}
S/2 d2 6d7 (with PC)	2291^{a}	3372^{ab}	2066^{bcd}	2416	2193^{bc}	3832^{a}	3177^{ab}	4000^{a}	3695^{a}	2235^{bc}	1697^{b}
S/2 d3 6d7 ET 2.5%. Pa.4/y (without PC)	1704^{c}	2874^{abc}	1873^{d}	2529	2834^{b}	3723^{a}	2958^{abcd}	1990^{d}	2255^{b}	1669^{c}	2321^{a}
S/2 d3 6d7. ET 2.5%. Pa.4/y (with PC)	1734^{c}	2890^{abc}	1888^{d}	2581	2472^{bc}	2692^{b}	2536^{cde}	3335^{ab}	3015^{ab}	2448^{ab}	1893^{ab}
S/2 d4 6d7. ET 2.5%. Pa.7/y (without PC)	1690^{c}	2671^{c}	2042^{cd}	2232	2083^{c}	2323^{b}	3125^{abc}	2074^{d}	2240^{b}	2389^{abc}	1817^{ab}
S/2 d4 6d7. ET 2.5%. Pa.7/y (with PC)	2036^{b}	3468^{a}	2388^{abc}	2162	2173^{bc}	2808^{b}	2487de	2338^{cd}	2842^{ab}	2895^{ab}	1846^{ab}
S/2 d4 6d7. ET 2.5%. Pa.9/y (without PC)	1762^{c}	2948^{abc}	2150^{bcd}	2600	2494^{bc}	2595^{b}	3570^{a}	2829^{bc}	3026^{ab}	3062^{a}	2195^{ab}
S/2 d4 6d7 ET 2.5%. Pa.9/y (with PC)	1508^{d}	2780^{bc}	2453^{ab}	2613	2812^{b}	2811^{b}	2317^{e}	2308^{cd}	2594^{b}	2743^{ab}	1999^{ab}

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

over 11 years, indicates no beneficial impact of panel change on yield increase. Benefit of panel change was observed only in the initial years. Within a short period, panel management became a serious issue and the problem was severe under d2 and d3 frequency. In order to manage the panel under d2 and d3 frequency of tapping , CUT was introduced during 2004-06. Higher yield could be obtained with upper panel change. Comparable yield could be obtained under various frequencies of tapping (Table Exp. 6). Information from the trial and growers field also indicate high incidence of TPD.

GENOME ANALYSIS LABORATORY

Research activities of the Genome Analysis Laboratory are organized around three major areas, *viz.* (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, optimisation and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping

Various marker technologies have been adopted and successfully used in characterisation of rubber genome.

1.1. Microsatellite markers for characterization of *Hevea* clones and germplasm

Development of microsatellite markers in *Hevea* was continued with the isolation and characterisation of *Hevea* genomic clones containing microsatellite/ simple sequence repeats (SSR). Evaluation of polymorphic microsatellites was carried out using RRII 400 series clones along with their parental clones: RRII 105 and RRIC 100. Microsatellite alleles were identified with ease through allelic segregation among RRII 400 series clones. Microsatellite markers were also used for characterization of 40 popular clones and 60 wild *Hevea* germplasm accessions.

1.2. Single Nucleotide Polymorphisms (SNPs)

1.2.1. Characterisation of SNPs in latex biosynthesis genes in RRII 400 series rubber clones

Allele-specific PCR amplification was optimized for validation of SNPs identified in four major latex biosynthesis genes: *geranyl geranyl diphosphate synthase, farnesyl diphosphate synthase, mevalonate kinase* and *HMG-CoA synthase* in *Hevea*. Touchdown PCR amplification procedure was adopted. Allele-specific amplification of SNP was found consistent either with 'C' or 'G' alleles.

1.2.2. SNPs in wild Hevea accessions

SNP detection and genotyping were also carried out in wild *Hevea* accessions. Four representatives of wild *Hevea* accessions from each of the three different provinces: Acre, Mato Grosso and Rondonia were used in SNP detection. Twelve genes were amplified at their 3' untranslated region (3'UTR) and sequenced. Eighteen SNPs and one indel in *glutathione peroxidase*, seven SNPs in *mevalonate kinase*, three in *HMG synthase* and two SNPs in *latex abundant protein* genes at their 3'UTR and its associated coding sequences were identified. Earlier studies with cultivated clones could not detect any SNP in *glutathione peroxidase* and latex abundant protein genes. SNPs detected in the Mato Grosso genotypes showed similarity with popular clones. While analyzing SNPs in *glutathione peroxidase* gene, clear discrimination of the accessions provincewise indicating geographic distinctness of the wild accessions was observed. This supports earlier findings on genetic relationship using RAPDs and microsatellites. Effective haplotypes were predicted which could be used as geographical marker for wild accessions.

1.3. Retrotransposons and retro-based markers in rubber

The abundance of retrotransposons in *Hevea* genome was reported for the first time. Genomic clones bearing retro-sequences from a genomic library were identified through plaque hybridization using 'reverse transcriptase' (RT) gene as the probe. Work is in progress in developing retro-based marker system in rubber.

1.4. Genetic linkage map in rubber

Total 52 RAPDs (20 RAPDs - RRII 105 specific, 19 RAPDs - RRII 118 specific and 13 RAPDs - present in both the parents) were analyzed for segregation in progeny population. Genomic microsatellite markers (di- and tri-nucleotide repeats) were developed and tested together with EST derived (gene-derived) microsatellite markers, generated through mining of the GenBank sequences, for polymorphisms using two parental clones (RRII 105 and RRII 118) of a progeny population being used for linkage mapping. Thirty seven polymorphic SSR markers (13 genomic and 24 EST-derived SSRs) were used to genotype the progeny population and segregation data was recorded for linkage map construction.

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

2.1.1. Resistance gene analogue (RGA) in rubber

Isolation and characterization of genomic RGAs in rubber from RRII 105 were reported earlier. For comparative analysis, RGAs were also amplified from RRIM 600 (RGA-M) and *H. benthamiana* (RGA-B). Sequences were analyzed to generate phylogram. Most of the RGAs isolated from *Hevea benthamiana* were clustered together indicating their species specificity. Functional RGAs (RT-RGAs) from *Corynespora* challenged RRII 105 were also analyzed. Substantial nucleotide sequence variability was detected among these RT-RGAs. Sequences of 28 clones were further analyzed as they coded for open reading frame (ORF). Conceptual translation of the 28 clones revealed characteristic motifs of "R" genes. Alignment of amino acid sequences of RT-RGAs also revealed variation at translation level and ultimately clustered into nine major groups. Reverse northern analysis of the representative RT-RGA clone of each group was carried out both with control and treated (*Corynespora* infected) cDNA probes (radio-labelled). Both constitutive (expressed both in control as well as in infected plants) and induced expression of RT-RGAs (only in infected plants) was identified which had significant levels of sequence variability.

2.1.2. Genes involved in host tolerance to Corynespora leaf disease

An effort was made to isolate genes induced during the interaction of *Corynespora cassiicola* with RRII 105 during disease development, using differential display of mRNA (DDRT-PCR) by challenge inoculation of rubber plants with spore suspension of *C. cassiicola* in glass house condition. Fifty seven differentially expressed (over-expressed) transcripts/bands from *C. cassiicola* challenged leaf samples were cloned and sequenced. Full-length cloning was initiated for two genes *anthocyanidin 3-O-glucosyltransferase* and transcriptional regulators of the *GRAS* family. All the cDNA clones were subjected to reverse northern analysis using infected and non-infected total RNA from RRII 105 and RRIM 600 as probe to identify the cDNA clones showing consistent expression profiles.

2.2. Gene regulation under abiotic stresses

To understand stress adaptation processes, transcript profiling of both tolerant and sensitive responses under different abiotic stresses have been continued. This will provide a comprehensive understanding of stress adaptation and clues for identification of genes, which are useful for improvement of abiotic stress tolerance.

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 for functional genomic studies. Out of 180 differentially expressed major bands/cDNA (down or up-regulated), 144 bands could successfully be cloned and sequenced. Finally 131 cDNA sequences (59 down-regulated and 72 up-regulated cDNAs) were analyzed, which revealed 110 unique sequences comprised of 13 clusters/contigs and 97 singletons. Most of them had no match with the GenBank sequences. However, several differentially expressed genes encoding catalase, phosphatidylinositol/ phosphatidylcholine transfer protein, NADH dehydrogenase, Myb transcription factor, downward leaf

curling protein, epimerase/ dehydratase, Na^+/H^+ antiporter, chloroplast Ycf2 and chloroplast FtsH protease involved in cold adaptation process in rubber were also identified along with the unique transcripts. Cold-induced accumulation of FtsH-like protease has already been detected in *Medicago sativa*, and induction of FtsH transcripts is specifically regulated by low temperature and light independently and is not part of a general response to stressful conditions.

2.2.2. Rubber EST Project

Ninetysix stress responsive cDNA clones (31 contigs + 65 singletons) were subjected to reverse northern dot-blot analysis to screen for truly differentially expressed cDNA fragments. Duplicate blots of the 96 stress responsive cDNA clones (subtracted) were hybridized with labelled cDNA probes from cold treated and control RNA samples to screen for truly differentially expressed cDNA fragments. Out of 96 clones, 56 gave quantitatively more signals with the cDNA probe from cold treated PR 261 plants indicating over-expression of the respective genes under cold stress. Among these genes encoding carbonic anhydrase, glutathione peroxidase, metallothionein, chloroplastic Cu/Zn SOD, serine/threonine protein kinase, transcription factor, DNA binding protein *etc.* showed significant increase in expression levels. Northern analysis will be carried out with some of the selected over-expressing clones. To generate more number of cold responsive ESTs, a second plasmid library containing more than 600 subtracted cDNA clones was constructed.

2.3. Methylation dynamics of *H. brasiliensis* genome

Preliminary study was conducted on *Hevea* genome to establish the presence of methylation in its genome. Experiments were designed to analyze the changes in the pattern of methylation in the promoter regions of certain genes of interest in three *H. brasiliensis* clones (RRII 105, RRIM 600 and PB 260) in response to cold stress. Existence of methylation in *Hevea* genome was proved through restriction digestion analysis using methylation sensitive and non-sensitive isoschizomers and bisulfite sequencing. Methylation analysis was done using CyMATE software. Bisulfite sequencing data was collected for *HMGR1* and *coronatine-insensitive* (*COI1*) gene promoter regions for two consecutive years (2007-08) and for other gene promoters it was done for a single year only (2008). Significant difference in the methylation pattern was observed in the promoter regions of selected genes like *HMGR1* gene, *COI1* gene, *farnesyl diphosphate synthase* (FDP), *HMGS1* and *REF* when samples from two different locations were analyzed. In HMGR promoter, 5.67 per cent of the probable sites were methylated and RRII 105 plants were found more prone to methylation changes in the region studied. Significant changes in methylation pattern were observed at some of the important motifs related to stress response, defense and promoter enhancement. In *HMGS*, on average 6.34 per cent sites were found methylated. *HMGS* gene promoter region was also showing the same trend as that of *HMGR1* in terms of methylation at CAAT-box. Characteristic methylation pattern was observed in the ACE motif and CAAT-box in COI1 gene promoter of RRII 105 plants planted in Elappara. In *FDP* gene promoter 3.57 per cent sites were methylated, which comprised of CGN and CHH types of methylation. Whereas, all three types of methylation CGN, CHG and CHH in varying degree were noticed in *REF* gene promoter. Frequency of methylated sites was found to be 5.59 per cent in *REF* gene promoter. The results from this study indicate stress is

playing a direct role in the expression of genes in rubber and methylation is acting as a control mechanism by which plants adapt and respond to the environmental conditions.

3. Cloning and characterization of agronomically important genes

3.1. Cloning and characterization of lignin biosynthesis gene(s)

Wood quality relies on secondary xylem formation and more particularly on lignin deposition in secondary cell walls. Therefore, a substantial effort has been put on the functional characterization of genes involved in lignin production.

3.1.1. Cinnamyl alcohol dehydrogenase

Successful cloning and characterisation of cDNA encoding cinnamyl alcohol dehydrogenase (CAD) was reported earlier. Corresponding full-length genomic clone was also generated from RRII 105. Nucleotide sequence information showed that the protein-coding region of the gene is ~ 2091 bp in size and the coding sequence was interrupted by the presence of four introns.

To identify promoter sequences in the upstream to the 5'end of the *CAD* gene, two clones were generated through genome walking. Between the two, only the 'CAD_Stu' fragment (713 bp) showed homology in the coding region of the *CAD* gene at the down stream (from 547 bp) of the sequence. Therefore, the upstream sequence of 546 bp of this clone is a putative promoter sequence characterized by the presence of a TATA box ("TATATATAA") at position 88 bp from ATG start codon of the gene. Further characterization of the promoter sequence and also the expression study with the *CAD* gene are in progress.

A bacterial expression cassette for the *CAD* gene controlling lignin biosynthesis in rubber was constructed. The *CAD* cDNA fragment was sub-cloned into an expression vector (pRSET-C) to generate recombinant protein in *E. coli* under the control of a T7 promoter. Recombinat pRSET plasmid was transformed into *E. coli* BL21(DE3) pLysS cells to express the recombinant protein from the expression vector containing the *CAD* gene. About 49 kD protein (fusion protein) band was noticed on SDS-PAGE within an hour of induction of the transformed *E. coli* cells with 1 mM IPTG.

3.1.2. Cinnamoyl-CoA reductase

Partial cDNA fragment was successfully amplified using degenerate primers synthesized based on conserved regions of *cinnamoyl-CoA reductase* gene sequences derived from different plant species, cloned and sequenced. RACE (rapid amplification of cDNA ends) was performed to amplify both 5' and 3' cDNA ends of *CCR* gene. Sequence information of the full-length cDNA, was deduced through the sequencing of both 5' and 3' RACE products of the gene. Full-length cDNA sequence of the *CCR* gene was found to be 1397 bp in length including 1017 bp of coding and 380 bp of non-coding sequences (114 bp of 5'-UTR and 266 bp of 3'-UTR), which was cloned successfully.

3.2. Cloning of *metallothionein* gene (s) in rubber

Earlier studies indicated the presence of *metallothionein* gene (*MT*) in subtracted cDNA library derived from cold-stressed leaf samples of rubber. *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. An effort was made to identify the functional isoforms of the gene in stressed leaf samples. Reverse transcriptase reaction was carried out with gene-specific primers to amplify the respective cDNA. The amplified product was cloned successfully, sequenced and confirmed as *metallothionein* gene.

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 works under A and B Divisions 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.

During the reporting period, the total crop realized was 140222 kg. A total of 302 tapping days was possible in the year and 63 tappers were engaged for tapping. The total mandays engaged were 50942. 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 5426.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

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

1. Crop improvement

1.1. Evaluation of clones

Data on pre and post-winter girth and yield (g/t/t) were recorded in normal and continuously tapped trees in 1985 and 1986 clone trials. The highest girth was observed in RRII 118 (86.8 cm) closely followed by RRII 203 (85.2 cm) and RRIM 600 (82.6 cm) in 1985 clone trial. In 1986 trial, the highest girth was recorded in RRIC 102 (87.2 cm) closely followed by RRII 118 (86.1 cm). Out of 18 *Hevea* clones from two clone trials (Tables Ghy. 1 & 2), PR 255 ranked first (55.4) in terms of annual mean yield (g/t/t) in the 14th year closely followed by RRII 203 (47.3), RRII 118 (47.3), RRIC 102 (46.1), GT 1 (44.9), RRII 208 (44.5) and the lowest was for PB 260 (21.6) under normal tapping system. The annual mean yield in most of the clones under continuous tapping system was comparatively lower than in normal tapping system. The project is concluded.

1.2. Evaluation of polyclonal population

Data on pre and post-winter girth and yield were recorded for evaluation of growth and yield performance in polyclonal seedling population and results are shown in Table Ghy. 3. Amongst the 10 promising polyclonal seedling trees, the highest girth (cm) over 21 years of planting was observed in selection S2 (128.7) followed by S10 (125.7), S7 (117.9) and the lowest in S8 (90). The highest annual mean yield (g/t/t) in the 14th year of tapping was observed in selection S2 (312.7) followed by S7 (115.5) and S10

Table Ghy. 1. **Growth and yield performance clones in 1985 clone trial**

Clone	Growth (cm)		Annual mean yield (g/t/t)		Projected yield (kg/ha/year)*	
	Girth	Girth increment	NT	CT	NT	CT
RRII 105	68.3	0.6	35.9	29.2	1565.0	1752.0
RRII 118	86.8	2.1	47.3	30.8	2062.0	1848.0
RRII 203	85.2	1.2	47.3	46.8	2062.0	2808.0
RRIM 600	82.6	0.8	39.5	33.6	1722.0	2016.0
RRIM 605	74.2	0.5	31.5	30.2	1373.0	1812.0
PB 86	77.4	0.7	35.4	34.1	1543.0	2046.0
PB 235	78.0	1.0	40.9	28.0	1783.0	1680.0
PB 5/51	57.7	1.5	26.9	29.7	1172.0	1782.0
GT 1	78.4	1.0	44.9	39.2	1957.0	2352.0
Gl 1	63.2	0.5	24.2	20.7	1055.0	1242.0

NT: Normal tapping; CT: Continuous tapping; * Yield based on 400 trees in 150 tapping days/ha/year (CT) and 109 tapping days/ha/year (NT).

Table Ghy. 2. **Growth and yield performance of 10 clones in 1986 clone trial**

Clone	Growth (cm)		Annual mean yield (g/t/t)		Projected yield (kg/ha/year)*	
	Girth	Girth increment	NT	CT	NT	CT
RRIC 102	87.2	0.7	46.1	25.8	2009.0	1548.0
RRIC 105	74.7	1.0	27.8	23.7	1212.0	1422.0
RRII 5	71.6	1.4	36.5	30.9	1591.0	1854.0
RRII 105	69.7	1.3	33.5	25.9	1460.0	1554.0
RRII 118	86.1	1.6	29.1	32.5	1268.0	1950.0
RRII 208	75.0	1.0	44.5	37.3	1940.0	2238.0
PB 260	71.0	0.5	21.6	28.7	941.0	1722.0
PB 310	81.2	1.4	41.6	40.2	1813.0	2412.0
PB 311	74.6	0.9	29.1	40.7	1268.0	2442.0
PR 255	79.2	1.0	55.4	32.8	2415.0	1968.0

NT: Normal tapping; CT: Continuous tapping; * Yield based on 400 trees in 150 tapping days/ha/year (CT) and 109 tapping days/ha/year (NT).

(107.1). Out of 10 promising polyclonal selections, selection S2 ranked first (126.7) in terms of annual mean yield (g/t/t) over the first 14 years of tapping followed by S1 (93.3), S9 (72.5) and S10 (68.7) under normal tapping system. The experiment is concluded.

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 at RRTC, Hahara, RRS, Guwahati. The initial data on girth did not show significant difference among treatments. An increase in the population of fungi, *Pseudomonas*, *Azotobacter* and phosphobacteria after three months of treatment was observed in the rhizosphere soil of rubber.

A decrease in the populations of bacteria and actinomycetes populations was observed when compared with pre treated soils. Only a few fungal species were isolated with high percentage of relative abundance. Among the species *T. viride*, *Pencillium* spp., *Cladosporium* sp., *Mucor hiemalis* and yeasts populations was found to be dominant.

Table Ghy. 3. **Performance of seedling selections**

Selections	Girth (cm)	Annual girth increment (cm)	Annual mean yield (g/t/t)	Mean yield over 14 years (g/t/t)
S1	93.5	1.5	84.9	93.3
S2	128.7	1.8	312.7	126.7
S3	113.0	3.8	74.1	60.1
S4	96.0	1.3	90.6	63.6
S5	108.6	3.3	58.2	50.9
S6	95.9	3.0	88.6	62.7
S7	117.9	3.5	115.5	66.5
S8	90.0	1.0	78.7	58.9
S9	101.5	0.7	98.8	72.5
S10	125.7	3.9	107.1	68.7
Mean	107.8	2.4	110.9	72.4

3. Crop protection

3.1. Survey on pests and diseases

Survey on pests and diseases of rubber was carried out in 25 pockets covering 13 different locations in Assam and Meghalaya. Severity of powdery mildew disease was high (above 60 %) in smallholdings (Mitani, Umling, Umsiang, Sarutari, Killing and Byrnihat) and caused die-back in branches. The clone PB 235 and RRII 105 showed extensive die-back due to powdery mildew disease. Minor incidence of brown root disease (below 2%) was observed on mature plants of smallholdings at Umsiang, Mitani and also at RRTC, Hahara. High incidence of Periconia leaf blight disease (above 75%) was noticed on tender leaves in seedling nursery at Umling and Umsiang in Meghalaya. Incidence of thread blight disease (30%) was observed in mature plantation at Umling. Minor infestation of scale insect (below 10%) and termites was noticed in most of the locations surveyed. Appropriate control measures were suggested for all diseases.

3.2. Isolation and identification of fungal pathogens of rubber

Isolation of fungal pathogens from diseased samples of Periconia leaf blight, brown root, thread blight and leaf anthracnose of rubber collected during survey was carried out and identified as *Periconia heveae, Phellinus noxius, Pellicularia filamentosa* and *Colletotrichum gloeosporioides* respectively. A total of five isolates of *P. heveae* collected from nursery in different locations were subcultured in oatmeal agar medium for studies on strain differentiation.

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

A total of 457 wild accessions of *Hevea* germplasm is available in germplasm conservation garden at Sarutari Research Farm under RRS, Guwahati. Severity of powdery mildew disease was assessed on short-listed wild accessions conserved at Sarutari Research Farm. Nine out of seventeen short-listed accessions of wild germplasm showed consistent tolerance to powdery mildew disease.

3.4. Investigation on tapping panel dryness

A detailed investigation on the occurrence of TPD has been carried in Assam region under different years of exploitation. It was observed that the incidence of TPD is increasing with the age of the plants and years of exploitation. The young plants were generally showing lower incidence of TPD syndrome. The results showed that 30 per cent trees showed TPD incidence in BI-1 panel under fourth year of tapping where 76 – 100 per cent of the total panel length was affected.

3.5. Improvement of P uptake of rubber through Arbuscular mycorrhizal fungi (AMF) inoculation

Monthly recording of different growth parameters of AMF as well as other beneficial microorganisms treated rubber plants are being continued. The treated plants are performing better in terms of girth, height,

leaf number and whorl number. The combined inoculations of two beneficial organisms (*G. fasciculatum* and *G. mosseae*), are always showing better growth performances as compared to single incoulam. Plants inoculated singly with *G. fasciculatam* showed the highest growth rate followed by *G.mosseae* and *G. margarita* respectively.

Similarly, rubber seedlings treated with dual inocula of *G. fasciculatam* and phosphate solubilising microorganism (PSM) are found to be superior in enhancing the growth rate followed by combined inocula of *G. fasciculatam* and *Azotobacter* treated seedlings. In single inoculation, seedlings treated with *G. fasciculatam* showed better performance followed by PSM and *Azotobacter* inoculum.

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The Station continued its research activities on cropping system models, nutritional requirement of rubber, clone evaluation, physiological studies, latex harvesting and latex technology. The Station was also engaged in socio-economic survey of rubber growers and provided advisory services to growers.

1. Crop improvement

1.1 Evaluation of clones

In the evaluation trial (1995) involving 10 clones, PB 260 (52 g/t/t), RRIM 600 (46.2 g/t/t), PB 235 (45.5 g/t/t) and PB 311 (43.1 g/t/t) were the high yielding clones. Yield data from G x E trial revealed that RRII 429 (67 g/t/t) was the highest yielder followed by RRII 422 (64.8 g/t/t) and PB 235 (48.6 g/t/t). Check clone RRIM 600 recorded mean yield of 41.3 (g/t/t). In another on-farm trial (OFT) involving six clones planted in 1998, check clone RRIM 600 exhibited the highest yield (27.8 g/t/t) followed by PB 235 (25.5 g/t/t) and RRII 208 (21.5 g/t/t). In ortet evaluation trial, selection 114 gave the highest yield (34.5 g/t/t) followed by 144 (21.91 g/t/t) and 98 (21.75 g/t/t), where as the check clone RRIM 600 gave 18.92 g/t/t.

In a block trial planted in 2000 with eight clones, Haiken 1 showed the highest girth (54.8 cm) followed by RRIM 600 (52.7 cm), RRII 118 (49.39 cm) and PB 260 (48.15 cm). In another OFT involving seven clones, RRII 429 showed highest girth (17.2 cm) followed by RRII 430 (16.7 cm) and RRII 417 (15.7 cm) in the third year after planting.

In recombination breeding, 12 seedling selections that gave high yield were cloned and planted in field for clonal nursery evaluation. Four new clonal nursery evaluations have been initiated involving approved cultivars, pipeline clones and ortets from traditional area and HP clones.

2. Crop management

Data on soil organic carbon and pH recorded from 390 and 572 samples, collected at 0-60 cm depth from rubber plantations of different locations of Tripura state during 1986-1988 and 2006-2008 respectively were statistically analysed. An increase in soil organic carbon percentage (0.91%) was observed during 2006-2008 period when compared to 1986-1988 period (0.74%). The majority of samples analysed in 2006-07 were in the medium range where as in 1986-87, most of the samples recorded low level of organic carbon (Fig. Agr. 1). The increase was also evident in both, surface

(0.18%) and sub-surface soil (0.16%). There was no significant increase in soil pH (4.40 and 4.46) during that period. The increase in soil organic matter may be due to the addition of biomass by growing cover crop in immature phase, weeds in rubber plantation and also of leaf litter.

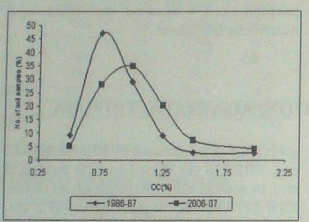


Fig. Agr. 1. **Variation in organic carbon content of rubber soils (0-60 cm) over a period of 20 years**

In tea intercropping trial, average rubber yield was 1414 kg per ha in the fourth year of tapping. Tea green leaf yield was 718 kg per ha during the year. Tea yield showed declining trend due to shading of rubber canopy.

An OFT on application of FYM was initiated in clone RRIM 600.

In fertilizer trial, clone RRII 429 showed maximum immature girth (37.0 cm) in the fifth year in response to high dose of fertilizer compared to other clones, *viz.* RRII 417, RRII 430 and RRIM 600.

An experiment was also initiated with two cropping system models in rubber with different geometry by maintaining 440 rubber trees per ha. In this system, intercrops like banana, pineapple and arecanut were planted in space available in between rubber rows. Upland rice which is very popular among the locals was planted in the space available in between the rubber during first year and it yielded 240 and 321 kg paddy per ha in Model I and Model II respectively with zero tillage operation. The paddy occupied 20 and 30 per cent of area in Model I and II respectively. Vegetable crops like amaranthus, cowpea and okra were grown in one line in between the rice and rubber in both the models. The cowpea yield was higher compared to other intercrops. Two tree species, *i.e.* Acacia and Gamhari were planted in the borders in both models, which can be used as fire wood and timber in the later stage.

3. Crop physiology

In physiological studies, the photosynthetic parameters were recorded in two shaded levels (70% & 25% height) and open seedlings of *Hevea brasiliensis* during summer. Plants grown under open condition maintained the highest photosynthetic rate of 12.6 iMol $CO_2/m^2/S$. However, photosynthetic rate was low in 25 per cent light environment (4.28 iMol $CO_2/m^2/S$.)

Leaf lamina and petiole dry biomass in seedlings under various levels of shade showed that 11.6 per cent of total leaf biomass was portioned into petiole under full irradiance condition when compared to 12.0 per cent in 35 per cent shade and 14.5 per cent in 75 per cent shade.

Activity of antioxidant in leaf during winter revealed that cold tolerant clone Haiken 1 had higher Glutathione reductase and Ascorbate peroxidase activities compared to RRIM 600, RRII 105 and PB 235. Antioxidant enzyme activities were low in the susceptible clone RRII 105. Growth parameters revealed a very low growth rate for RRII 105 in winter. Photosynthesis rate (P_N) in winter showed a significant difference among clones with Haiken 1 having the highest and RRII 105 having the lowest rate.

4. Latex harvest technology

In latex harvest study, RRII 105 continued to give high yield in S/2 d2 frequency of tapping system without stimulation. Yield under S/4 and S/3 d2 systems of tapping with application of five and three stimulations respectively was comparable with S/2 d2 system without stimulation. 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 system of tapping. However, the highest per tap yield was observed in S/2 d6 system compared to d/4 or d/3 systems of tapping. In another experiment, S/3 double cut alternate panel tapping system (DCA) with six stimulations (3+3) showed higher yield compared to S/2d2 system of tapping in RRIM 600, PB 235 and GT 1. However, changing panel after two years of S/2 d2 system of tapping has shown higher yield compared to without change of panel.

5. Crop protection

Survey of diseases and pests of rubber was carried out in 16 locations covering 36 sites of West and South Tripura. Incidence of powdery mildew, *Periconia* leaf spot, *Colletotrichum* and *Corynespora* leaf disease and brown root disease was observed. Powdery mildew was observed in moderate to severe form in mature rubber plantations, while it was severe to very severe in nurseries. Incidence of *Periconia* leaf spot, *Colletotrichum* and *Corynespora* leaf disease was moderate in young plants. Few disease-prone pockets were identified and powdery mildew and other leaf diseases will be monitored to understand the trend in spread and severity over space and time.

Screening of six accessions of wild *Hevea* germplasm, *viz.* RO 3794, RO 5055, RO 5407, RO 5365, RO 24/105 and MT 4859, showing moderate tolerance to powdery mildew disease were selected for further evaluation by staggered cut technique. Low incidence (2-4.3%) of brown root disease was observed in sporadic locations. Minor incidence of scale insects and associated sooty mold fungus, slugs and termite was observed in some areas.

Seasonal variation of tapping panel dryness (TPD) was assessed in 2146 trees in rubber plantations of Tripura covering 18 small rubber holdings. Average TPD was observed to be 14.5 per cent while it was 6.5, 14.9, 17.8 and 18.8 per cent in BO-1, BO-2, BI-1 and BI-2 panel, respectively. Seasonal average TPD was observed to be 12.1, 13.3, 15.5 and 17.2 per cent during summer (Apr.-May), rainy (June-Sept.), pre-winter (Oct.-Nov.) and winter period (Dec.-Jan.), respectively. The highest TPD was observed during winter period with more fresh incidence. Partially affected panels were found to show good recovery during summer period, while the recovery was very low in 50 per cent or more affected panels. Few clusters of TPD affected trees were observed in certain locations during winter period.

6. Rubber technology

In connection with study on the effect of storage on properties of sheets, it was observed that the sheets were affected with mould growth after five months of storage. However, the sheets stored under controlled conditions of humidity and temperature did not show any mould growth even after 12 months of storage. The physical properties were not affected in sheets stored under controlled conditions. However, the technological properties were affected (Table Agr. 1). The Mooney viscosity had crossed 100 units and the strength of rubber did stabilize after 18 months of storage. It was

Table Agr. 1. **Properties of dry rubber after storing for 12 months**

Property	Control (Zero time)	After storage for 12 months			
		Controlled humidity and temperature	Location 1 (Agartala)	Location 2 (Rudijala RPS)	Location 3 (Taranagar)
Mooney viscosity	88.00	96.00	97.00	97.00	98.00
Po	51.00	59.00	58.00	59.00	62.00
PRI	80.00	74.00	73.00	70.00	69.00
Gel content (%)	6.00	39.00	46.00	39.00	50.00
Volatile matter (%)	0.29	0.23	0.33	0.38	0.25
Acetone extract (%)	1.80	1.70	1.80	1.30	1.20
Strength of raw sheets (kg/cm)	31.00	35.00	35.00	30.00	33.00

Table Agr. 2. **Clonal variation in rubber particle size**

Clone	Summer (nm)	Pre-winter (nm)	Mean (nm)
RRII 414	337	352	345
RRII 417	363	368	366
RRII 422	488	430	459
RRII 429	414	361	388
RRII 430	351	410	381
RRII 105	397	407	402
RRII 600	312	337	325
Mean	380	380	380

also found that the effect of storage on sheets in all locations is the same.

In connection with the study on the seasonal variations in properties of rubber, the particle size study was conducted in seven high yielding clones, *viz.* RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, RRII 105 and RRIM 600, for two seasons. The study revealed that RRII 422 (459 nm) had the biggest particle size and RRIM 600 (325 nm) had the smallest particle size (Table Agr. 2).

The study on the evaluation of technology transferred to group processing centres of Tripura showed all the group processing centres are practising next day sheeting system and trained people are available in all centres. However, there were some deviations from the recommended practices like dilution and sieving of the latex, dosage and mixing of the acid, thickness of the sheets, temperature of the smoke house, *etc.* in group processing centres. It was found that 32 per cent of the group processing centres were storing the sheets directly on floor which is causing deterioration of quality.

7. Economics

The impact of the block planting scheme on the socio-economic status of the beneficiaries was studied by surveying 480 rubber growers of nine Block Planting Units (BPU) and two Group Planting Units (GPU) in different regions of Tripura. Survey results showed that NR holding size of households under BPU (1.31 ha.) and GPU (1.72 ha.) are higher compared to the all India average holding size (0.5 ha). Rubber is the major source of income for growers of BPU or GPU. It is found that the major social parameters like literacy, education, percentage of income earners, *etc.* in block planting units are comparable with the growers under the Group Planting Units. But, major economic parameters including indebtedness of the family and performance of households under the Group Planting Units are better. In this context, to raise the overall well being of the socially marginalised societies covered under the Block Planting Scheme, policy initiatives catalytic to the overall development of the rural areas through

public investments conducive for private investments are necessary.

8. Advisory work

Discriminatory fertilizer recommendations based on soil and leaf analysis were offered to 288 rubber growers. A total of 546 soil samples were analysed for this purpose. A total of 161 latex samples were analysed for dry rubber content and other latex parameters. Bud wood of high yielding clones was supplied to Rubber Production Department for distribution to growers. The Station also provided advice regarding disease remedial measures on request from the growers.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

The Regional Research Station, Tura continued its research activities on evaluation of clones and polyclonal population, evolving suitable crop harvesting systems and crop management.

1. Crop improvement

Yield data from the 1985 and 1986 field experiments were analysed for clonal and monthly variations. The highest yield (g/t/t) was recorded in clones RRIM 600 (46.7 g/t/t), PB 311 (43.7 g/t/t), RRII 105 (42.3 g/t/t), RRII 203 and PB 235 (39.2 g/t/t). Monthly yield was higher in November followed by December, October and September.

On-farm evaluation of selected clones, *viz.* RRII 417, RRII 422, RRII 429, RRIM 600, PB 235 and RRII 203, in farmers' fields in West and East Garo Hills is in progress.

To evaluate the performance of half-sib progenies, seeds were collected from the 1985 and 1986 clone trial area from RRIM 600, RRII 203, PB 5/51, RRIC 105 and PB 86 and seedlings raised in the field at RRS Tura, Ganolgre Farm.

To find out the availability of assorted seeds of rubber in NE region and its viability, a sample survey was conducted and the results revealed that in NE region seeds are available between August and October, which showed 40 to 60 per cent viability.

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 (drc). Maximum volume of latex (ml/t/t) was recorded between October and December which was significantly higher than other months while minimum volume of latex was recorded between January and February. Maximum drc (%) was recorded in June (33.46) and July (34.13) while minimum in January (23) and February (22). Maximum yield (g/t/t) was recorded in November (60.02). Due to low temperature and prolonged dry spell (October, 2008 to March, 2009) early defoliation occurred in the month of January. Refoliation and flowering were also observed in the month of February.

To find out the suitable tapping system for Garo hills under the different temperature regimes (no tapping rest, rest at < 10 °C, rest at < 15 °C and rest at < 20 °C), yield data were recorded under S/2 d2 and S/2 d3 tapping systems in clone RRIM 600. In both the tapping systems, maximum yield was recorded in control treatment followed by treatments comprised of tapping rest at < 10 °C, < 15 °C and < 20 °C temperature

regimes. Control treatment (no tapping rest) recorded a maximum yield (g/t/t) of 57.18 under S/2 d2 tapping system and 64.53 g/t/t under S/2 d3 system. In terms of yield, S/2 d2 system for < 10 °C temperature regime and tapping rest from February to March is suitable for agroclimatic conditions of Garo Hills.

3. Crop management

3.1. Nutritional studies

The results from nutritional trial in on-farm at Borgang, Assam indicated that the highest girth (81.43 cm), girth increment (2.80 cm), yield (65.71 g/t/t), drc (35.55%) and latex volume (192.60 ml/t/t) were recorded in treatment $N_{60}P_{30}K_{45}$ kg/ha and the lowest in treatment $N_0P_0K_0$. In general, improved soil and leaf nutrient contents in NPK applied plots was observed.

3.2. Soil moisture retention characteristics under the rubber growing area of Meghalaya

Soil samples, up to the depth of 120 cm were collected from six rubber growing areas of Meghalaya and analyzed for physical parameters and available nutrients. Results showed a soil bulk density of 1.29 - 1.58 g/cm^3, particle density of 2.36 – 2.60 g/cm^3, porosity of 39.23 – 46.55 per cent and the textural class was clay loam in all the locations. Organic carbon status was low to medium (0.59 – 1.31%), available P was low (0.04-0.56 mg/100g), available K was medium (4.1 – 11.1 mg/100g) and soil pH was 4.61 - 5.10. Soil moisture increased with depth and annual mean was between field capacity and permanent wilting point (17.82 - 19.53%).

3.3. Advisory work

Collected soil samples from the rubber growing areas of Meghalaya and analysed for physical properties and available nutrients. Fertilizer recommendations were offered to the farmers based on the results. Existing soil analytical data were pooled and analysed and it was observed that more than 75 per cent of the samples were having more than one per cent organic carbon. Majority of the samples were low in available P. In case of soil pH, majority of the soil samples were having the value of 4.75 or more.

In the case of shifting cultivation study, a declining trend in soil pH, organic carbon, CEC, exchangeable cations and available N, P & K was observed with increase in duration from one to four years.

REGIONAL EXPERIMENT STATION, NAGRAKATA, WEST BENGAL

The main objectives of research in the Station are focussed mainly on evaluating clones and developing agromanagement practices suited to the locality.

1. Crop improvement

1.1. Evaluation of clones

Experiments to identify clones with better yield and secondary characteristics were continued. In Trial I, the girth was significantly higher in clone Haiken 1 followed by SCATC 93-114 and RRIM 703. Clone RRIM 612 registered higher girth followed by PB 86 in Trial II. In Trial III, PB 235 followed by Haiken 1 and in Trial IV, PB 280 followed by SCATC 93-114 and Haiken 1 (Table Nag.1) were the best performers in terms of growth.

With regard to yield (Table Nag. 2), RRIM 703 followed by PB 235 and SCATC

Table Nag.1. **Growth of plants in different clone trials**

Trial I	Girth (cm)	Trial II	Girth (cm)	Trial III	Girth (cm)	Trial IV	Girth (cm)
GT 1	66.40	Gl 1	62.92	Haiken 1	68.06	RRIC 104	56.76
Haiken 1	74.69	PB 86	71.15	PB 235	72.20	RRII 308	57.67
PB 235	68.54	PR 107	63.63	PB 260	65.68	RRII 105	60.28
PB 311	64.76	RRII 105	69.34	PB 310	63.00	RRII 300	60.83
PB 5/51	57.19	RRII 208	69.90	PB 86	64.73	PR 261	61.40
RRII 118	71.11	RRIM 605	69.55	PR 107	61.52	PB 235	62.34
RRII 203	70.18	RRIM 612	72.80	RRIC 102	67.01	RRII 208	62.43
RRII 300	67.35			RRII 208	60.88	RRIM 600	62.97
RRIM 703	72.54			RRIM 600	65.32	Haiken 1	63.35
SCATC 88-13	67.61			RRIM 612	64.41	SCATC 93-114	64.76
SCATC 93-114	72.74			SCATC 93-114	67.45	PB280	66.68
CD (P = 0.05)	2.72	CD (P = 0.05)	2.72	CD (P = 0.05)	2.15	CD (P = 0.05)	2.05

Table Nag. 2. **Yield in different clones**

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	32.77	PB 86	31.92	PR 107	26.56	SCATC 93-114	31.73
Haiken 1	35.52	RRII 105	43.81	RRIM 612	29.29	RRIC 104	35.29
RRII 300	38.39	RRIM 605	44.02	PB 86	31.63	PR 261	46.33
PB 311	40.62	RRIM 612	47.09	SCATC 93-114	32.39	RRII 300	47.93
GT 1	40.94	RRII 208	47.21	RRIC 102	35.78	Haiken 1	52.97
RRII 118	42.52	Gl 1	55.38	PB 260	38.34	RRII 308	55.29
RRII 203	42.65	PR 107	64.36	Haiken 1	41.84	PB 235	55.41
SCATC 88-13	44.47			PB 310	46.12	PB 280	56.36
SCATC 93-114	47.12			RRIM 600	46.63	RRIM 600	56.47
PB 235	47.56			RRII 208	50.07	RRII 208	62.38
RRIM 703	48.38			PB 235	55.40	RRII 105	63.37
CD (P = 0.05)	2.71	CD (P = 0.05)	6.51	CD (P = 0.05)	4.48	CD (P = 0.05)	4.84

Table Nag. 3. **Growth and yield in different accessions**

Accession	Girth (cm)	Yield (g/t/t)	Accessions	Girth (cm)	Yield (g/t/t)
AC 1950	57.00	28.14	RO 2635	62.50	1.40
AC 607	32.70	3.38	RO 2890	65.20	6.34
AC 619	54.30	2.17	RO 3172	66.60	1.46
AC 623	43.60	8.49	RO 5329	52.90	16.53
AC 68	59.90	5.70	RO 5348	63.30	3.16
AC 763	50.10	23.25	RO 5363	58.60	58.25
Gl 1	53.30	40.96	RO 5408	48.50	4.51
GT 1	62.80	46.86	RO 5430	61.90	12.93
MT 196	55.80	23.54	RO 5557	65.00	4.90
MT 2229	55.80	6.67	RO 6139	51.00	4.61
MT 2594	47.60	6.00	RRII 105	54.10	42.36
MT 44	60.90	8.94			
PB 260	59.30	27.19			
RO 2629	63.90	5.22			
CD (P = 0.05)	3.11	5.16			

Table Nag. 4. Growth characteristics of Hevea seedlings raised from seeds of different origin

Origin of seeds	Per cent germination	Survival rate (%)	Plant height (cm)	Growth after 8 months		
				Girth (cm)	Number of whorls	Number of leaves/ whorl
Nagrakata	50	85	69	7.5	2	5
Kanyakumari	70	94	100	10.3	2	6
Sarutari	40	97	44	4.6	1	4
Ganolgre	50	100	58	5.7	1	4

93-114 in Trial I were the best clones. In Trial II, PR 107 showed high yield followed by Gl 1. The clone PB 235 recorded high yield in Trial III followed by RRII 208 and that in Trial IV, RRII 105, followed by RRII 208, RRIM 600 and PB 280 performed better.

1.2. Evaluation of germplasm

The experiment, initiated in 1998 to conserve and to evaluate the germplasm accessions' adaptability in the region was continued. Within the wild accessions, maximum girth (Table Nag. 3) was noticed in RO 3172 followed by RO 2890. Among Wickham clones, GT 1 showed highest girth.

Among all germplasm accessions, RO 5363 recorded the highest yield (Table Nag. 2) followed by AC 1950. The clone GT 1 was the highest yielder among the four Wickham collections.

1.3. Performance of polyclonal seedlings

The experiment in which performance of polyclonal seedlings are being evaluated, the mean girth recorded for the trees was 62.6 cm with average block yield of 58.5 g/t/t. Among the population, 51 per cent plants showed above-average yield.

1.4. Performance of polycross progeny raised from locally available seeds

The performance of the polycross rubber seeds collected locally and from North-East was compared with that from traditional region during 2008-09. Seeds were procured from a) Nagrakata, West Bengal; b) Sarutari, Assam, c) Ganolgre, Meghalaya and d) HBSS, Kanyakumari, Tamil Nadu. Germination was more in the seeds procured from Kanyakumari followed by Nagrakata and therefore, the growth of the seedlings raised from seeds of Kanyakumari was better than others (Table Nag. 3).

1.5. Performance of rubber in abandoned tea growing areas of Dooars belt of North Bengal

Attempt was made to introduce rubber in the soil of high pH (8.3). Four clones, *viz.* RRIM 600, RRII 208, RRII 105 and RRII 429, were planted in blocks in soils having different pH (5.6 & 8.3). The survival rate of these clones after one month in high pH ranged from 85 to 100 per cent with clone RRII 208 having cent per cent survival. After six months the survival rate got reduced to 35 per cent.

2. Crop management

2.1. Nutritional trials

The 3:3:3 NPK factorial trial was continued. No regular pattern on yield or girth was noticed among different treatments.

2.2. Intercropping trials

The experiment on intercropping tea in Rubber was continued in Dooars area of West Bengal with four different planting combinations of rubber and tea along with monoculture as controls. In general, yield of rubber was better in intercropping situation compared to that of monoculture. Yield of tea was significantly higher in monoculture compared to different intercropping treatments. In intercropped situations, more pest attack was noticed on tea.

REGIONAL RESEARCH STATION
DAPCHARI, MAHARASHTRA

The mandate of the Station is to identify suitable clones and develop location-specific agrotechnology for drought-prone regions. The ongoing experiments cover crop improvement and environmental physiology.

1. Environmental physiology

For the identification of suitable clones having physiological tolerance to water stress and high temperature, three experiments based on irrigation scheduling and method of irrigation are being conducted in this station.

The irrigation experiment was started during 1987 with ET_C based irrigation scheduling in two methods of irrigation (basin and drip) with the objective to standardise and evaluate the advantages of drip irrigation over basin irrigation in terms of water saving. The treatment comprised of 1.00, 0.75 and 0.50 ET_C in basin and 0.75, 0.50 and 0.25 in drip method of irrigation. From February 2000, the 0.75 ET_C in the basin and 0.50 ET_C in the drip method were reduced to 0.25 ET_C to find out whether irrigation requirement could further be reduced. Among the irrigation schedules of reduced level of 1.00 ET_C recorded higher growth and yield was on par with reduced level of 0.25 ET_C level of irrigation. It was found that the trees under different levels of basin irrigation showed higher girth as compared to drip system. The same trend was noticed in yield also (Table Dap.1).

Table Dap. 1. **Effect of irrigation methods and irrigation scheduling on girth and yield**

Treatment		Girth (cm)	Yield (g/t/t)
Control (Rainfed)		62.5	50.6
Basin	1.00 ETc	72.9	66.6
	0.25 ETc*	69.5	63.1
	0.50 ETc	68.6	63.7
Drip	0.75 ETc	67.9	53.9
	0.25 ETc**	67.6	50.1
	0.25 ETc	64.4	53.8
SE		1.06	2.92
CD (P = 0.05)		2.3	6.03

* changed from 0.75 ET_C to 0.25 ET_C

** changed from 0.50 ET_C to 0.25 ET_C

In the trial to study the effect of different levels of irrigation (1.00 ET_C' 0.75 changed to 0.25 ET_C from February 2000 and 0.50 ET_C) on yield and yield components of two clones *viz.* RRII 105 and RRII 118, fortnightly yield, monthly girth and seasonal DRC percentage were monitored. Results indicated that clone RRII 105 performed better in terms of yield while clone RRII 118 recorded better growth in response to different levels of irrigation (Table Dap. 2).

Table Dap. 2. **Effect of irrigation on growth and yield**

Treatments	Girth (cm)			Yield (g/t/t)		
	RRII 118	RRII 105	Mean	RRII 118	RRII 105	Mean
Control	63.69	77.13	70.40	43.27	36.69	40.00
1.00 ETc	70.29	88.65	79.47	53.66	47.34	50.50
0.25 ETc	69.25	89.11	79.18	65.13	53.40	59.30
0.50 ETc	67.31	83.67	75.48	42.57	41.42	42.00
Mean	67.63	84.63		51.16	44.71	
	Clone	Irrigation	CxI	Clone	Irrigation	CxI
SE	1.15	2.27	3.01	2.61	5.01	7.90
CD (P = 0.05)	4.95	4.95	NS	5.61	10.35	14.64

The cost evaluation trial started during 1987 to find out the expenses incurred towards various inputs, farm practices and irrigation was continued. The treatments included irrigated and unirrigated trees of RRIM 600 and the irrigated trees were divided into two parts based on soil depth. Among them, one being maintained under reduced irrigation of 1/5th ET_C (deep soil) and another under 1.0 ET_C (shallow soil) level of irrigation. The reduced level of irrigation was further reduced from 1/4th to a minimum level of 1/5th to find out the optimum irrigation requirement for mature trees in deep soil area. The results (Table Dap. 3) indicated that the trees under the reduced level of irrigation in deep soil area had better growth and yield performance than the rainfed and higher level of irrigation (1.0 ET_C).

Table Dap. 3. **Effect of different depths of soil and irrigation schedules on yield**

Months	Yield (g/t/t)		
	Group A	Group B	Group C
April	8.82	40.82	28.27
May	4.45	28.13	20.02
June	9.48	31.45	24.85
July	11.03	42.18	31.94
August	20.78	39.00	31.51
September	24.69	56.43	36.71
October	35.94	96.68	63.85
November	27.69	102.43	65.48
December	29.43	81.57	47.06
January	46.34	122.26	92.35
February	21.99	84.52	68.52
March	15.00	63.67	47.33
t-test	0.001	0.094	0.002

A - rainfed B - 1/5ET_C C – 1.0 ET_C irrigation

2. Crop improvement

2.1. Clone evaluation

The clone evaluation trial started in 1985 to evaluate growth and yield of 15 modern clones and to select suitable high yielding and drought tolerant clones for North Konkan region was continued. All the clones under this trial were maintained under life-saving irrigation since 1985. Monthly girth, DRC percentage and fortnightly yield were recorded. Results indicated that RRIC 52 recorded higher girth of 67.5 cm than RRII 105. RRII 5 recorded higher yield of 38.56 g/t/t, which is on par with the clones RRII 6, RRII 105, RRII 208, RRIC 100 and RRIC 102 (Table Dap. 4).

Table Dap. 4. **Growth and yield of various clones**

Clone	Girth (cm) 2009	Yield (g/t/t) 2009
RRII 5	62.2	38.56
RRII 6	65.4	38.32
RRII 105	58.8	36.35
PB 260	63.8	27.76
PB 310	63.8	28.94
PB 311	60.2	30.51
RRIC 52	67.5	16.78
RRIC 100	61.1	33.51
RRIC 102	63.0	23.18
RRIC 105	59.2	22.52
PR 255	61.4	33.28
PR 261	59.6	23.30
RRII 208	67.4	37.86
RRII 308	60.1	17.84
RRIM 600	61.1	25.46
SE	1.98	3.66
CD (P = 0.05)	4.07	7.34

2.2. Ortet selection

The large-scale evaluation of 14 ortets of Dapchari was started during 2008 to evaluate the growth and yield performance of ortets selected from polycross seedlings planted at this station with four control clones (RRII 105, RRII 208, RRII 430 and RRIM 600). The results showed that OS 317 recorded significantly higher girth than clone RRII 208, but it was on par with other control clone (Table Dap.5).

2.3. Germplasm screening

Screening for wild accessions against drought under Dapchari conditions commenced in 2003 using 130 accessions

Table Dap. 5. **Growth performance of different ortets under Dapchari condition**

Ortet	Height (cm)	Girth (cm)	No. of whorls
OS 1	4.01	152.8	2.9
OS 8	3.71	150.2	2.4
OS 34	4.10	143.5	2.8
OS 35	3.80	142.8	2.3
OS 36	3.33	117.6	2.4
OS 37	3.46	139.2	2.7
OS 42	3.66	133.9	2.6
OS 111	4.08	174.6	2.7
OS 135	3.14	115.4	2.4
OS 136	3.13	123.4	2.4
OS 173	3.73	128.0	2.7
OS 216	3.93	156.2	3.0
OS 236	3.43	119.5	2.6
OS 317	4.57	169.1	2.5
RRII 105	4.03	135.5	2.5
RRII 208	3.76	125.4	2.4
RRII 430	4.39	141.7	2.8
RRIM 600	4.23	177.0	3.4
SE	0.39	15.79	0.42
CD (P = 0.05)	0.79	32.10	NS

along with three selected clones, *viz.* RRII 105, RRIM 600 and Tjir 1 as checks in Augmented Block Design was continued. The observation on pre-and post-drought growth and RWC was recorded. The accessions showed wide variability for all characters studied. In general, Mato Grosso accessions were superior for the growth characters than those from Rondonia and Acre provenances. Twenty five potential drought tolerant accessions were identified based on 3-4 years field performance for further detailed studies.

Field evaluation of 25 clones selected from earlier drought tolerance studies was initiated. Five HP clones, *viz.* RRII 105, RRIM 600, Tjir 1, RRII 430 and RRII 208 were also included in the experiment laid out in Rectangular Lattice Design. The observation on growth was recorded and the clones showed variability for the characters studied.

REGIONAL RESEARCH STATION, DHENKANAL, ORISSA

The Regional Research Station located in Dhenkanal district of Orissa State represents dry sub-humid climate. The station continued its research activities, with the particular objective of identifying clones suited to this region.

1. Crop improvement

Crop improvement includes seven ongoing trials mainly on evaluation of clones and polyclonal population including one on-farm trial.

1.1. Clone evaluation

In the clone trial (1987), both GT 1 (72.9 cm) and RRIM 600 (70.8) recorded significantly higher girth over RRII 105 (67.5). The clone RRII 105 recorded the highest mean yield (g/t/t) of 33.1, followed by clone RRIM 600 (31.7). The clone RRIM 600 in general is found superior in terms of growth and RRII 105 is superior yield.

Table Ori. 1. **Girth and yield of elite clones**

Clone	Girth (cm)	Yield (g/t/t)
RRII 105	67.5	33.1
RRIM 600	70.8	31.7
GT 1	72.9	27.6
Mean	70.4	30.8

In the clonal trial (1990), no significant difference in girth among the clones was recorded. The best growth performance in terms of girth was recorded in SCATC 93-114 (79.4 cm) and RRII 208 (77.0 cm) whereas for rubber yield (g/t/t), SCATC 88-13 had

recorded the highest mean yield (41.1), followed by RRII 208 (40.8), while SCATC 93-114 recorded the lowest yield (17.7). Among the clones, RRII 208 showed overall superior growth, yield and adaptability.

In the clone trial (1991), the performance of clones with polyclonal seedlings was compared. Polyclonal population recorded the highest girth (93.7 cm) and adaptability, followed by GT 1 (84.3) and RRII 208 (80.2). Among the clones, the highest mean yield (g/t/t) was recorded by RRII 208 (42.5) followed by RRII 105 (35.4). In this trial also, RRII 208 registered the highest growth and yield.

In the GxE on-farm trial (1996), at Bhubaneswar, RRII 430, RRII 414 and RRIC 100 showed better growth. Yield recording of the clones has been commenced.

In the clone trial (1999) for evaluation of modern clones, the highest mean girth was recorded in RRII 208 (46.6 cm) and IRCA 111 (45.8) closely followed by RRII 105. The lowest girth was recorded in RRII 51 (34.6 cm).

1.2. Polyclonal seedling evaluation

In the trial laid out in 1989 to evaluate the growth, yield performance and adaptability of polyclonal seedlings in Orissa conditions, the highest mean girth was recorded in tree no.471 (119.8 cm) followed by tree no. 482 (116.7 cm). The highest annual mean yield was recorded in tree no. 154 (83.1 g/t/t). Ten elite polyclonal seedlings were identified for further selection and evaluation.

1.3. Ortets evaluation

Ten ortets along with a few modern clones were field planted for further evaluation during 2008. OR 1, OR 6 among ortets and RRIM 600 and SCATC 93-14 showed comparatively better preliminary growth.

REGIONAL RESEARCH STATION, PADIYOOR

Long-term research programmes initiated with the objective of identifying clones suited to the region and evaluation of clonal tolerance to drought /disease are in progress. The field trials laid out include evaluation of germplasm, screening of clones for timber /latex traits, investigations on Genotype x Environment interaction, large-scale testing of potential hybrid clones, water requirement studies and disease evaluation of clones.

1. Crop management

1.1. Physico-chemical characterisation of soil

Electrical conductivity and exchangeable potassium were significantly (R^2 0.72 and 0.86 respectively) influenced by slope class. Regression analysis indicated almost complete dependence of first layer actual soil volume on first layer thickness and first layer coarse fragments, with an R^2 value of 0.99.

Work on registration of boundary map using GPS and preparation of DEMs was completed. Visualisation techniques for understanding the terrain characteristics in 3D using DEM at 90 m resolution acquired during the shuttle radar topography mission is also being attempted (Table Pad. 1).

1.2. Water requirement studies

An experiment was initiated in an immature rubber plantation for assessing irrigation levels at IW/CPE ratios of 0.3, 0.6,

Table Pad. 1. **Slope, altitude and physical properties expressed on First Layer Actual Soil Volume (FLASV)**

Slope class	Slope (%)	Alt (m)	FLT (m)	FLCF (m^3)	FLASV (m^3)	OC (Kg/ m^3)	Ex K (g/m^3)	CEC Eq/m^3	EC (dS/m)
1	9.8	95	0.13	0.64	2.48	20	161	113	0.041
1	7.5	74	0.13	0.71	2.41	30	160	149	0.073
1	8.1	87	0.10	0.63	1.77	49	209	101	0.042
1	5.6	85	0.12	0.65	2.23	37	94	90	0.041
2	11.8	86	0.14	0.76	2.6	32	209	148	0.049
2	11.7	105	0.13	0.74	2.38	35	279	177	0.050
3	16.0	88	0.14	0.96	2.41	38	263	225	0.051
3	15.8	140	0.12	0.85	2.03	67	234	150	0.050
3	17.7	99	0.13	1.24	1.88	48	275	146	0.057
3	19.5	95	0.13	0.74	2.38	41	214	201	0.048
4	25.4	103	0.17	1.44	2.64	51	333	157	0.094

FLT – First Layer Thickness; FLCF – First Layer Coarse Fragments

0.9, 1.2 and an unirrigated control. Application of irrigation water was discontinued due to frequent rains during the season. Total biomass was found to be significantly higher for the irrigated treatments. A significant increase in the number of tappable trees was also observed (Table Pad. 2).

Table Pad. 2. **Effect of different levels of irrigation on girth**

Treatment	Girth 9th year (cm)	Girth increment (cm)	
		Dry months	Wet months
IW/CPE 1.2	55.7	1.76	3.31
IW/CPE 0.9	54.5	2.20	2.91
IW/CPE 0.6	57.9	2.76	3.60
IW/CPE 0.3	53.6	2.44	3.68
Control	50.8	2.46	4.05
CD (P = 0.05)	NS	NS	NS

1.3. Response to applied fertilizers in high yielding clones

The experiment laid out in June 2002 was continued. The treatments comprised of three clones (RRII 105, RRII 414 and RRII 429) with four fertilizer N, P_2O_5 and K_2O levels in the ratio of 30:30:20, 60:30:20, 90:60:40 and 120:60:40 kg/ha. Higher doses of fertilizers did not improve the girth of the plants significantly (Table Pad. 3).

Table Pad. 3. **Effect of fertilizer levels on growth**

Fertilizer levels	Girth (cm) in different clones		
	RRII 105	RRII 429	RRII414
30:30:20	35.04	31.27	34.58
60:30:20	33.02	33.55	34.58
90:60:40	31.16	28.83	35.27
120:60:40	34.56	32.85	34.84

CD(P = 0.05): NS

2. Crop improvement

2.1. Large-scale evaluation of clones

Among the 11 clones tested, PB 330 and IRCA 18 showed significantly higher girth

Table Pad. 4. **Growth and yield in different clones**

Clone	Mean girth (cm)	Mean yield (g/t/t)	Summer yield (g/t/t)
RRII 105	56.84	48.39	28.56
PB 314	59.33	44.61	25.48
IRCA 130	61.19	65.58	43.28
PB 28/59	56.24	47.73	25.24
IRCA 109	56.05	32.81	19.18
PB 330	62.06	41.34	15.71
IRCA 18	61.43	39.73	13.02
RRIM 703	50.65	37.78	16.29
IRCA 111	56.36	35.23	20.12
PB 255	56.38	51.93	31.99
IRCA 230	57.88	39.38	15.69
CD (P = 0.05)	4.47	14.72	13.98

than that of RRII 105. The girth of IRCA 130 was on par with that of RRII 105. The average annual yield of IRCA 130 (65.58 g/t/t) was significantly superior to that of RRII 105 (48.39 g/t/t). The summer yield also showed a similar trend (Table Pad. 4).

2.2. Evaluation of rubber clones /selections at high altitude situations

In the trial at Ambalavayal, a high altitude location, RRII 105 recorded the lowest girth (42.4 cm) over other popular clones/selections tested. The clones RRIC 100, PB 86 and RRII 203 and selections P 270, P 213, P 1, P 296 and Iritty showed significantly higher girth over that of RRII 105 (Table Pad. 5). Tapping has been initiated in the trial.

Table Pad. 5. **Growth of plants at high altitude**

Clone	Girth (cm)
RRII 105	42.4
RRII 203	60.5
RRIC 100	64.3
RRIC 102	53.3
PB 86	63.1
P 296	58.4
P 90	54.9
P 270	69.9
P 280	54.9
P 2	54.7
P 121	54.4
P 213	66.8
P 1	63.3
P 155	45.3
Iritty	57.9
CD (P = 0.05)	12.7

HEVEA BREEDING SUB-STATION, NETTANA, KARNATAKA

Evaluation of clones under different biotic and abiotic stress conditions and identification of clones suitable for commercial cultivation are the major areas of research in this station. Experiments on latex harvest technology and crop protection are also given importance.

1. Crop improvement

1.1. Small-scale clone trials

Among the six small-scale clone trials, three trials (1988A, 1988B and 1988C) planted in 1988 are small-scale evaluation of selected ortets. Three trials (1991A, 1991B and 1991C) planted in 1991 are small-scale evaluation of indigenous and imported clones.

In the 1988A trial, 15 ortet clones and three control clones, *viz.* RRII 105, RRIM 600 and GT 1 are under evaluation. Mean yield over seven years of tapping indicated T 2 to be the highest yielder with 73.0 g/t/t, followed by O 17 and GT 1 (Table Kar. 1). The other two control clones, RRIM 600 and RRII 105 recorded mean yield of 55.1 and 50.3 g/t/t respectively. In the 1988B trial, among the 16 ortet clones and three control clones (RRII 105, RRIM 600 and GT 1) under evaluation, T 1 and GT 1were high yielding with 69.4 and 69.3, respectively after seven years of tapping. In the 1988C trial with 14 ortet clones and three control clones, GT 1 recorded maximum yield (84.4 g/t/t) followed by ortet C 140 (77.5) and clone RRII 105 (71.6) after seven years of tapping.

Performance of top yielding clones in two SSTs planted in 1991 is presented in Table Kar. 2. In the 1991A trial, 36 indigenous and exotic clones are under evaluation. Mean yield over five years of tapping indicated that the clones PB 235 (85.3 g/t/t) and PB 314 (84.3 g/t/t) were the highest yielders,

Table Kar. 1. **Performance of top yielding ortets from the 1988A, 1988B and 1988C trials**

Trial	Clone	Girth* (cm)	Mean yield during 2008 (g/t/t)	Mean yield over seven years of tapping (g/t/t)
1988A	T 2	106.4	96.9	73.0
	O 17	100.0	91.3	67.5
	GT 1	79.3	68.2	65.7
	O 15	86.9	79.2	65.6
	C 1/2	86.0	65.9	60.4
1988B	T 1	107.6	75.0	69.4
	GT 1	89.4	78.5	69.3
	O 40	84.4	37.2	53.7
	O 53	86.5	30.2	52.4
1988C	GT 1	90.8	113.4	84.4
	C 140	95.0	85.3	77.5
	RRII 105	74.4	65.8	71.6
	O 26	92.3	82.9	69.1
	O 49	85.3	100.1	66.6
Control clones#	RRII 105	73.0	44.3	57.5
	GT 1	86.5	86.7	73.1
	RRIM 600	71.8	33.1	42.9

*Girth recorded in 21-year-old trees during December 2008
#Mean of data from three trials

Table Kar. 2. **Performance of top yielding clones in the small-scale trials 1991A, 1991B and 1991C**

Trial	Clone	Girth (cm) *	Mean yield during 2008 (g/t/t)	Mean yield over five years of tapping (g/t/t)
1991A	PB 235	84.6	83.3	85.3
	PB 314	70.2	64.8	84.3
	PB 280	70.7	70.5	77.7
	PB 312	67.5	78.7	75.3
	PB 311	70.9	60.7	71.6
1991B	RRII 5	75.8	65.8	69.1
	RRII 3	69.4	78.7	60.6
	Nab 17	77.8	50.8	49.4
	RRII 105	60.0	32.4	44.5
1991C	HP 83/224	82.4	57.6	61.8
	PB 28/59	83.5	70.0	54.0
	HP 83/225	72.4	51.1	45.8
	PR 261	71.6	56.1	45.7
Control clones#	RRII 105	60.1	41.5	48.2
	GT 1	72.2	46.5	41.6
	RRIM 600	52.8	25.3	31.7

* Girth recorded in 18-year-old trees during December 2008;
#Mean of data from three trials

followed by PB 280 (77.7), PB 312 (75.3), PB 311 (71.6) and RRII 105 (65.2). In the 1991B trial, after five years of tapping, mean yield was the highest for the clones RRII 5 (69.1 g/t/t), RRII 3 (60.6), Nab 17 (49.4) and RRII 105 (44.5). In the 1991C trial, HP 83/224 was the highest yielding clone with 61.8 g/t/t followed by PB 28/59 (54.0) and HP 83/225 (45.8).

1.2. Large-scale clone trials

Growth and yield performance of clones in the large-scale clone trial (1989) is summarised in Table Kar. 3. Among the 14 clones under evaluation, maximum yield was noted in clone RRII 203 (67.90 g/t/t) followed by KRS 25 (59.60) and PB 255 (52.40). Control clones RRII 105 and RRIM 600 recorded mean yield of 48.50 and 32.00 g/t/t, respectively.

Table Kar. 3. **Growth and yield of clones in the large scale clone trial (1989)**

Clone	Girth* (cm)	Mean yield (2008) (g/t/t)	Mean yield over seven years of tapping (g/t/t)
RRII 203	92.9	67.3	67.9
KRS 25	82.6	62.8	59.6
PB 255	73.2	49.9	52.4
KRS 163	74.4	49.2	52.1
KRS 128	79.0	45.8	51.4
RRII 105	72.3	41.3	48.5
RRII 308	80.5	56.4	46.2
SCATC 88-13	63.5	36.0	41.6
PR 255	60.2	38.7	38.2
Haiken 1	55.8	52.7	35.4
PR 261	68.1	26.8	32.8
RRIM 600	69.0	35.6	32.0
RRII 300	73.6	33.1	30.1
SCATC 93-114	64.8	17.2	12.3
CV (%)	5.6	32.6	21.4
SE (±)	2.3	8.2	5.3
CD (P = 0.05)	6.8	23.9	15.4

* Girth recorded in 20-year-old trees during Dec 2008

Growth and yield performance of clones in the large-scale clone trial 1990A is summarised in Table Kar. 4. Among the clones, PB 260 recorded a maximum yield of 61.10 g/t/t followed by PB 235 (59.70) and PB 217 (55.40). Tjir 1 was the lowest yielder (19.80 g/t/t).

Table Kar. 4. **Growth and yield performance of clones in the large-scale clone trial 1990A**

Clone	Girth* (cm)	Mean yield (2008) (g/t/t)	Mean yield over six years (g/t/t)
PB 260	82.4	72.5	61.1
PB 235	86.4	85.3	59.7
PB 217	76.7	97.0	55.4
PB 311	73.9	75.2	55.0
HP 372	88.2	83.4	53.2
RRII 105	71.3	60.4	51.8
HP 223	81.2	73.8	48.9
GT 1	76.9	64.5	48.3
Gl 1	65.8	28.0	30.3
HP 185	72.4	44.6	28.7
Mil 3/2	76.6	48.1	28.6
HP 187	70.2	31.4	28.3
Hil 28	70.6	28.6	25.9
HP 204	65.0	31.9	22.2
Tjir 1	60.8	21.6	19.8
CV (%)	4.9	24.1	9.9
SE (±)	2.1	7.8	2.4
CD (P = 0.05)	6.0	22.7	6.8

* Girth recorded in 19-year-old trees during December 2008

Table Kar. 5. **Growth and yield performance of clones in the large scale clone trial (2000)**

Clone	Girth* (cm)	Overall mean yield** (g/t/t)
RRII 414	60.7	73.1
RRII 430	59.5	72.4
RRII 422	41.1	65.3
RRII 429	50.6	55.6
RRII 403	41.7	41.1
RRII 105	46.3	40.5
RRIC 100	53.0	34.1
RRII 407	47.3	32.6
CV (%)	7.4	21.6
SE(±)	2.1	6.5
CD (P = 0.05)	6.5	19.6

*Girth recorded in nine-year-old trees during December 2008

**Mean over five months of tapping

1.3. Large scale trial (2000)

The trial is comprised of eight clones with six RRII 400 series clones and their parent clones, *viz.* RRII 105 and RRIC 100. The growth performance after nine years (Table Kar. 5) revealed maximum girth in clones RRII 414 (60.70 cm) and RRII 430 (59.5 cm). Five month yield data indicated clones RRII 414 and RRII 430 as the top performers with a yield of 73.1 and 72.4 g/t/t respectively. Parent clones, *viz.* RRII 105 and RRIC 100 recorded minimum yield of 40.50 and 34.10 g/t/t, respectively.

2. Trial on estimation of genetic parameters

The trial planted in 1990 consists of 12 parent clones and their half-sib progenies. Based on mean yield over six years, three parent clones, *viz.* PB 235, RRII 203 and RRII 105, were the top yielders with 77.0, 54.8 and 52.5 g/t/t respectively. Parent clone PB 235 (90.7 cm) followed by RRII 203 (85.4 cm) recorded the highest girth, while IAN 45/873 recorded the lowest girth (Table Kar. 6).

Among the progenies, half-sibs of RRII 203, GT 1 and PB 235 recorded a yield of 43.9, 42.2 and 41.3 g/t/t, respectively. In general, some of the half-sibs of high yielding clones recorded more yield. Similarly, a few half-sibs of low yielding clones also recorded minimum yield.

Table Kar. 6. **Growth and yield performance of clones in the Trial 1990B**

Clone	Girth* (cm)	Mean yield during 2008 (g/t/t)	Mean yield over six years (g/t/t)
Parent clone			
PB 235	90.70	97.00	77.00
RRII 203	85.40	70.80	54.80
RRII 105	71.70	49.80	52.50
GT 1	78.00	52.50	42.00
PB 5/51	71.80	44.10	41.00
PB 242	73.70	39.00	39.40
PB 213	71.00	39.60	38.60
PB 252	78.70	39.40	36.40
RRIM 600	60.80	31.20	34.50
IAN 45/873	56.40	33.00	30.00
PB 86	60.30	23.10	28.00
Tjir 1	67.30	25.80	27.00
CV (%)	4.80	15.30	8.90
SE(±)	2.00	4.00	2.10
CD (P = 0.05)	5.80	11.80	6.30
Family			
RRII 203	102.60	33.70	43.90
GT 1	103.30	36.70	42.20
PB 235	110.40	32.50	41.30
PB 5/51	97.90	31.20	37.30
PB 242	103.10	25.50	35.90
IAN 45/873	95.20	31.70	34.50
RRII 105	97.50	34.00	33.80
PB 252	93.90	29.00	33.80
RRIM 600	88.60	23.40	32.80
PB 86	86.10	35.20	32.50
PB 213	109.90	44.30	28.40
Tjir 1	90.40	34.60	24.50
CV (%)	7.20	34.90	16.10
SE(±)	4.10	6.60	3.30
CD (P = 0.05)	11.90	19.30	9.60

*Girth recorded in 19-year-old trees during December 2008

Table Kar. 7. **Yield (kg/400 trees) of promising clones of *Hevea* under different tapping systems (1987 trial)**

Clone	Without stimulation		With stimulation		
	S/2 d2	S/2 d3	S/2 d3	S/2 d4 level I	S/2 d4 level II
RRII 105	2311	1502	1761 (4)	1245 (6)	1489 (9)
RRII 300	841	1167	1133 (4)	1045 (6)	1692 (9)
PB 260	1812	1774	1882 (4)	1262 (6)	1134 (9)
PB 311	1723	1610	1699 (1)	1053 (2)	1438 (4)
PB 235	2183	2556	2860 (2)	1375 (3)	1838 (5)
No. of tapping days	142	98	98	73	74

4. Latex harvest technology

In the 1987 trial, variation in performance of different clones under different tapping and stimulation systems was observed. In this trial, five clones, *viz.* RRII 105, RRII 300, PB 260, PB 311 and PB 235, are under evaluation. Among the clones, PB 235 recorded maximum yield in all treatments. All the clones responded well to low frequency tapping (LFT) with stimulation. Yield of trees under LFT system was comparable to the yield of trees under d2 tapping system with lesser number of tapping days (Table Kar. 7).

HEVEA BREEDING SUB-STATION, PARALIAR, TAMIL NADU

The agroclimate of Kanyakumari District is ideal for good seed set and the region is also well known for the rare occurrence of leaf diseases. So, thrust was given for hybridization and generation of polycross planting materials. Being a highly potential region, clone evaluation was also actively pursued during the period under report.

1. Clone evaluation

In the large-scale clone trial (1994) tapping on BO-2 was initiated during the month of June 2008 and the pooled data up to the seventh (6+1) year of tapping is presented in Table Par. 1. Clone PB 255 continued to be the best yielder (80.24 g/t/t), followed by PB 314 (76.51). Among the exotic clones introduced recently, IRCA 109 (74.64 g/t/t) and IRCA 111 (73.19) exhibited better yield performance than RRII 105 (57.69). However, the other two clones IRCA 18 (58.93 g/t/t) and IRCA 230 (55.66) exhibited poor yield. In the block evaluation experiment (1994) consisting of 13 modern popular clones, RRII 105 (60.19 g/t/t) outperformed all the other clones during the seventh year of tapping (Table Par-2). Comparable yield trend was exhibited by PB 311 (59.57 g/t/t) and PB 28/59 (57.93), but the

Table Par. 1. **Mean yield of clones in the large scale clone trial at Keeriparai (1994)**

Clone	Yield (g/t/t)	
	2008-09	Mean over 7 years
PB 255	110.68	80.24
PB 314	113.11	76.51
IRCA 109	74.63	74.64
IRCA 111	87.53	73.19
IRCA 130	91.74	67.25
RRIM 703	90.27	64.83
IRCA 18	69.28	58.93
RRII 105	89.46	57.69
PB 28/59	78.53	55.92
IRCA 230	66.30	55.66
PB 330	64.52	54.15
Mean	85.09	65.36
CD	16.07	14.79

Table Par. 2. **Mean yield on the block trial (Keeriparai, 1994)**

Clone	Yield (g/t/t)	
	2008-09	Mean over 7 years
RRII 105	109.12	60.19
PB 311	75.61	59.57
PB 28/59	87.54	57.93
PB 235	79.63	56.32
PB 260	72.99	50.38
RRIC 102	76.32	50.14
PR 255	50.44	50.08
PB 217	46.38	48.76
PR 261	47.59	47.11
RRII 5	59.87	44.36
RRII 50	51.37	44.24
RRII 176	57.56	42.28
RRII 51	63.81	37.60
Mean	67.55	49.92
SE	4.27	2.64

yield performance of RRII 51 (37.6) and RRII 176 (42.28) was found to be very poor.

Tapping was completed on BO-1 in the trial G x E interaction of selected *Hevea* clones' (1996) and the data are presented in Table Par. 3. In this trial, RRII 105 exhibited an increasing yield trend from the third year onwards and RRII 203 (55.78 g/t/t) was the only clone which continued to exhibit better yield than the control clone RRII 105 (54.54). The hybrid clones belonging to the 400 series showed a yield trend on par with RRII 105, but the initial yield trend of RRII 429 (40.35 g/t/t) was poor. In the observational trial at Vaikundam Estate (2000) all the hybrid clones exhibited an initial yield trend better than RRII 105 (37.96 g/t/t) and RRII 422 (50.49) was the best performer (Table Par. 4). The initial yield performance of the hybrid clones belonging to the 400 series exhibited wide variations from their performance reported from Kerala. So, five large-scale on-farm trials were initiated representing five different microclimates in this region. Observations on initial establishment success, juvenile growth, disease incidence, *etc.* are being recorded.

Table Par. 3. **Mean yield on the trial G xE interaction (1996)**

Clone	Yield (g/t/t)		TPD incidence (%)
	2008-09	Pooled data on BO-1 panel	
RRIM 600	43.70	42.53	2
RRII 429	46.35	40.35	–
RRII 203	44.47	55.78	–
PB 217	41.03	35.51	1
RRII 51	39.79	36.83	1
RRII 414	40.53	46.27	1
RRII 430	44.40	52.85	–
RRIC 100	47.34	42.71	–
RRII 422	45.44	50.53	3
RRII 105	49.48	54.54	4
RRII 417	40.12	47.16	1
RRII 176	42.59	38.99	–
Mean	43.77	45.33	
CD (P = 0.05)	6.44	5.06	

Table Par. 4. **Mean yield on the observational trial (Vaikundam 2000)**

Clone	Mean yield over 3 years (g/t/t)
RRII 422	50.49
RRII 429	49.94
RRII 430	47.37
RRII 414	46.13
RRII 417	45.40
RRII 427	39.81
RRII 105	37.96
Mean	45.30

2. Breeding orchard

The breeding orchards (1987 and 1988) were maintained and hand pollination was attempted using various parental combinations during 2008-09. The potential hybrids and ortets selected in the previous years were test tapped and latex samples were subjected to ATP fingerprinting as part of evaluation and final selection. The hybrids obtained in hand pollination carried out during 2007-08 were raised in a nursery for preliminary evaluation and selection.

3. New generation polyclonal seed garden

The polyclonal seed garden (2000) established with nine modern clones as parents was maintained and disease incidence was controlled by spraying/ dusting at appropriate time. Polycross seeds were collected and raised at HBSS, Paraliar and also at a private estate at Punalur for evaluation and selection of potential high yielders (ortets). The polycross seedling trees raised during 2007 was test-tapped and seven promising ortets were selected for further evaluation and selection.

4. Root trainer planting technique

In the field trial initiated at Churulacode (2002), root trainer plants continued to exhibit better yield (42.61 g/t/t) than polybag

plants (40.97 g/t/t). The experiment initiated to identify an alternative potting medium for root trainers was repeated during 2009.

The experiment on young budding in root trainers was also repeated during 2008-09 at Vadakel Nursery, Perumbavoor and Cheerakuzhy Nursery, Mannarkkad. Observations on budding success, sprouting on cut back, growth parameters *etc.* were recorded at periodic intervals. The initial results obtained from the experiment indicated that young budding in root trainers has several advantages over the conventional methods of root trainer /polybag plants.

5. Effect of solar radiations on incidence of TPD

Under this experiment, one large-scale clone trial and one block trial were initiated during 2008. Tapping panel was protected with coconut fronds and arecanut sheet throughout the year and observations were recorded on initial establishment, sunscorch incidence, initial growth *etc.* Sufficient plants were raised at HBSS, Paraliar for destructive sampling in the third year.

6. Participatory clone evaluation

Eleven pipeline clones and three controls were planted at Tharuvaiyar Estate during 2008 and all the vacancies were filled during 2009. All the plants were paint marked and plot boards installed. Soil samples were analysed and fertilizer was applied as recommended. Disease severity was also assessed. Action has already been initiated to lay out two more experiments under the project during 2010 at Bethany Estate, Mukampalai.

LIBRARY AND DOCUMENTATION CENTRE

During the year, 80 books were added to the stock of the library. The library subscribed 63 foreign journals and 81 Indian journals. About 32 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 two issues of *Current Content Bulletins* were compiled and distributed. The database was updated by adding 1844 records. Distribution of 670 Press Clippings and 153 SDI bulletins was also taken up.

The Institute's journal, *Natural Rubber Research*, Volume 20 (1&2) 2007 was published and 250 copies of the journal including the back volumes were distributed. Organised the distribution of 700 copies of Annual Report (2005-06). The sale of 48 no. of RRII publications was also arranged. Photocopies of about 70067 information materials were taken during the period.

AGROMETEOROLOGY

1. Climate resource characteristics of rubber growing tracts

1.1 Extreme weather events in traditional region

A study of the extreme weather events in Kottayam was undertaken. The trends of extreme temperature events showed a high significant increasing trend, while the rainfall trend was not significant (Fig. Agromet. 1a & 1b). Analysis was carried out for the number of warmer days/month of maximum and minimum temperature events (Table Agromet. 1a & 1b).

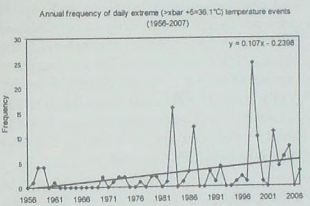


Fig. Agromet. 1a. Extreme temperature events > 36.1°C for Kottayam

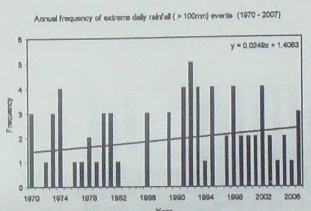


Fig. Agromet. 1b. Extreme rainfall events > 100 mm for Kottayam

Table Agromet. 1a. **Number of warmer days with maximum temperature events**

	Maximum temperature			
	Mean	Slope	R^2	z value
January	32.4	0.46	0.49	4.608
February	33.2	0.29	0.26	3.009
March	33.9	0.43	0.42	4.406
April	33.6	0.36	0.36	3.619
May	31.9	0.39	0.43	3.933
June	29.6	0.25	0.41	3.851
July	28.7	0.28	0.38	3.513
August	29.1	0.40	0.56	4.641
September	29.6	0.33	0.23	3.727
October	30.3	0.41	0.48	3.951
November	30.6	0.31	0.38	3.818
December	31.7	0.45	0.45	3.944

Table Agromet. 1b. **Number of warmer days with minimum temperature events**

	Minimum temperature			
	Mean	Slope	R^2	z value
January	21.4	0.28	0.24	2.071
February	22.4	0.17	0.14	1.485
March	23.5	0.33	0.28	1.920
April	23.7	0.24	0.20	1.776
May	23.9	0.29	0.25	2.865
June	22.8	0.23	0.16	1.689
July	22.4	0.32	0.26	2.625
August	22.6	0.31	0.25	-0.054
September	22.6	0.32	0.23	2.297
October	22.5	0.32	0.21	2.477
November	22.4	0.35	0.28	2.647
December	21.6	0.15	0.07	0.914

All the days with maximum temperature events per month were significant while some months were not significant for the minimum temperature events. The positive slope values were high for the maximum temperature events during the December and January months signifying that there is a high increase in warmer days during winter. The highest correlation was obtained in the case of August. These results signify indications of a climatic shift which is slowly delaying the usual

rainfall and temperature seasonal events encountered earlier.

1.2. Extreme climatic events in NE

Along with the various techniques for trend analysis, time series of cold spells have been used to detect any possible trend/shift over a span of 26 years from 1984-2009 during winter in West Tripura. Low temperature time series of winter showed a warming trend with time. The presence of data gaps in the time series of February revealed that warmer nights prevailed over cooler nights during the initial years indicating a climate shift and vice versa (Fig. Agromet. 2).

Though February in general exhibited a warming trend, the reduced rate of increase of temperature (.0002 °C) during the last decade (2000 – 2009) confirms the shifting of the low temperature regime to the February. Cusum charts indicate the period 1997-1999 could be identified as the major change point in the minimum temperature time series of winter.

1.2.1. Does winter precipitation has any bearing on the occurrence and intensity of low temperature spells and to the severity of the event?

Preliminary analysis indicated that significant decrease of rainfall during the peak winter months of December and January from 2000-01. It was observed that half of the warm winters had less or practically no rainfall occurred during winter. No direct link to the severity of the event could be found out, though the latter half of the period (1984-2009) is characterized by warm winters with deficient rainfall.

1.2.2. Impact of cyclones on immature rubber plantations in West Tripura

Results indicated that 50 per cent of the surveyed area suffers a stand loss of <5% and five per cent of the area suffers a stand loss of >25 per cent.

2. Forewarning of pests and diseases

2.1. Corynespora leaf disease (CLF)

Jadkal in Kundapura district of South Karnataka (13° 48'N, 74° 47'E, 35 amsl) has a history of repeated attacks of CLF. The morning and afternoon relative humidity and vapour pressure deficit at this area were analysed based on a time series approach with the diseased leaf count number. The leaf count was compared with the Percent Disease Intensity (PDI). Humidity Thermal Index (HTI) which is known to influence plant diseases has also been worked out and included in the time series analysis (Fig. Agromet. 3).

From the earlier analysis conducted in HBSS, Nettana from 1996 to 2006, the morning HTI showed >6.0 during the years with high incidence of the disease. During the previous disease season (February-March, 2008) the period which coincided with the phenological leaf emergence after defoliation, the favourable period observed

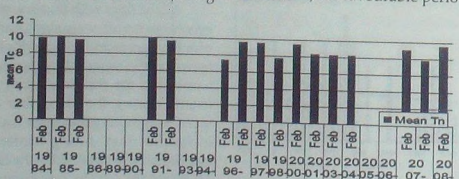


Fig. Agromet. 2. Occurrence of cold spells (Tn = 10 °C) during February

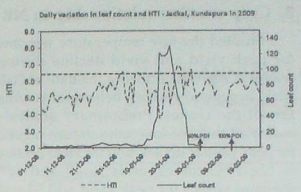


Figure Agromet. 3. **Daily data on leaf fall and morning HTI in Jadkal, Kundapura**

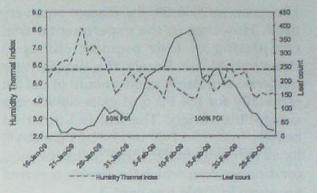


Fig. Agromet.4. **Daily data on leaf fall and morning HTI in Sullia, Dakshin Kannada**

was from 28.02.08 to 12.03.08 (14 days). During this period there were 11 days with intermittently >5.5 HTI values and therefore the disease was mild. This was in sharp contrast to the period from 22.12.08 to 10.01.09 (20 days) having only 3 intermittent days containing values < 5.5 HTI (Fig.Agromet. 3). A very high level of mean leaf fall per plot was observed from 12-01-09 to 25-01-09. A correlation co-efficient of 0.43 was obtained at a lag period of 7 days after the HTI value remained continuously above the 5.5 mark. It was also observed that there was a sudden decrease from the peak leaf fall after the HTI went below the 5.5 mark for seven days. The rest of the parameters did not show significant influence on the incidence of the disease.

In the experiment carried out in the hot spot area of Sullia Taluk, Dakshin Kannada, Karnataka (12° 38'N 75° 32'E 162 msl), revealed similar patterns in infestation of the disease. The calculated HTI showed a value of >5.5 from 18-01-09 to 02-02-09 and the leaf fall showed a significant increase from 01-02-09 to 21-02-09.

Here the leaf fall count increased after one week when the HTI crossed the 5.5 value for a continuous seven days. A sudden decrease from the peak leaf fall occurred after the HTI went below 5.5. This place is situated in lower latitude than Jadkal, Kundapura. Here, due to the delay in leaf development, the disease incidence along with the leaf emergence was slightly delayed compared to Jadkal. The mean PDI worked out at the end of February was 45.2% for the plot.

The leaf count and HTI values during the leaf emergence period for Adur, Kasaragode district, Kerala (10° 40'N 76° 08'E, 30 msl) are given in figure Agromet. 5. The duration of days with HTI values above 5.5 was very low and crossed the 5.5 mark only on a single day and there was no leaf fall due to *Corynespora*.

2.2 Powdery mildew disease

Powdery mildew disease assessment by leaf collection procedure was initiated in the popular clones of RRIM 600 and RRII 105 in Vaikundam Estate, Kanyakumari district where powdery mildew is quite common.

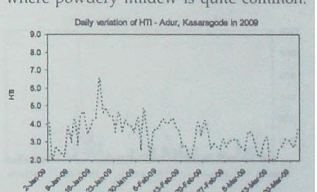


Fig. Agromet.5. **Humidity Thermal Index and leaf count in the absence of leaf fall**

However, in 2008-09, the disease was very mild. However in the experimental trial comprising of four popular clones at Padiyoor, North Kerala the disease was moderate with fairly good amount of leaf fall especially for the PB 5/51 clone which is a susceptible variety. The leaf fall occurred heavily from 25-01-09 to 19-02-09 (26 days) for all the clones under study. Meteorological conditions under which the disease occurred are given in Table Agromet. 2.

Table Agromet.2. Meteorological parameters for Powdery Mildew occurrence at Padiyoor

Parameter	Range
Maximum temperature	33.0-38.0°C
Minimum temperature	22.6-17.2°C
Relative humidity (Morning)	21-55%
Relative humidity (Afternoon)	81-92%
Sunshine hours	1.4-9.6

In Padiyoor, North Kerala, an increase in afternoon relative humidity from 41 to 62 per cent within a day coupled with a decrease of sunshine hours from 7.6 to 4.6 resulted in the spread of the disease bringing about leaf fall after about 10 days with a variation of two days (Fig. Agromet. 6). However, it was observed that leaf fall continued at certain times even when the sunshine hours were >6.0. Similar conditions in the afternoon relative humidity were also recorded in the region with intense leaf fall after nine days.

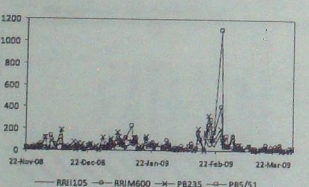


Fig. Agromet. 6. **Daily diseased leaf fall data caused by *Oidium hevea* at Padiyoor**

3. Crop-weather relationship in NE

Studied the low temperature window for peak yield and yield decline during winter at Agartala utilizing the data of GxE trial. In general yielding behaviour is same for all the clones. Preliminary analysis indicated that the low temperature regime for peak yield is 15± 2 °C and for yield decline is 12± 1 °C corresponds to 47 and 48th standard week's for peak yield and 50 and 51st weeks for yield decline.

Another study on low temperature spell versus yield showed that yield depression of 15-32 per cent occurs when mean minimum temperature of the spell remains 12.5 ±1°C for more than 12 days. Above normal yield depression during January corresponds to a continuous spell of 12 days or more with mean minimum in the range of 7-9 °C. Increment in December yield was recorded when the minimum temperature remains = 15 °C continuously for more than 12 days.

4. Agromet database management

Archival of all previous data had been completed for all stations. Thermohygrograph and Self-Recording Rain Gauge have been installed in HBSS, Nettana for closely monitoring the rubber diseases affecting the station. Field-based meteorological stations were started for diseases as well as for the study on the project of the "Development and demonstration of Integrated Vector Management for prevention of Chikungunya/ Dengue in Rubber plantation area" in collaboration with the Vector Control Research Centre (VCRC). Stevenson Screen with thermometers and Manual Rain Gauges were established in the Aimkombu RPS and Malankara Estate. The data will be utilised to study the effect of weather parameters on the occurrence and spread of vector-borne diseases, *viz.* Chikungunya/Dengue in rubber plantations.

ANNUAL EXPENDITURE

Expenditure at a glance (2008-09)

Head of Account	Expenditure (Rs. In lakhs)
Non-Plan	
General charges	642.60
Projects (CES)	307.71
Total	**950.31**
Plan	
General charges	1805.06
NERDS Research Component	313.98
Total	**2119.04**
Grand Total	**3069.35**

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Shammi Raj, M.Sc., Ph.D.	Agrometeorologist (Scientist C)
Shaji Philip, M.Sc, Ph.D.	Scientist C
E. Edwin Prem, M.Sc. (Ag.)	Scientist S2
C. Bindu Roy, M.Sc., Ph.D.	Assistant Mycologist
Sadanand K. Mushrif, M.Sc. (Ag.)	Scientist S2 (study leave up to 16.01.09)
T.V. Thomas	Assistant Farm Superintendent

Plant Physiology Division

R. Krishnakumar, M.Sc., Ph.D.	Senior Scientist
P. Sobhana, M.Sc., Ph.D.	Scientist C (up to 01.01.09)
Molly Thomas, M.Sc., Ph.D.	Scientist C
K. Annamalainathan, M.Sc., M.Phil, Ph.D.	Scientist C
D. Bhuvanendran Nair, M.Sc., Ph.D.	Scientist C
S. Sreelatha, M.Sc., Ph.D.	Scientist C
M.B. Mohammed Sathik, M.Sc., M.Phil, Ph.D.	Scientist C
Jayasree Gopalakrishnan, M.Sc., M.Phil.	Scientist S3
N. Geetha, M.Sc., Ph.D.	Scientist S2
S. Visalakshy Ammal, B.Sc.	Technical Officer
T.V. Somaraj	Asst. Farm Superintendent
A.K. Peeusmon	Asst. Farm Superintendent
Rajen Mathew	Asst. Farm Superintendent (w.e.f.06.06.08)

Exploitation Technology Division

K.U. Thomas, M.Sc., Ph.D.	Joint Director
R. Rajagopal, M.Sc., M.Phil., Ph.D., Dip. Stat.	Scientist C
K. Karunaichamy, M.Sc., Ph.D.	Scientist C
M. Jojomon	Asst. Farm Superintendent

Rubber Technology Division

K.T. Thomas, M.Sc., LPRI, M.Tech., Ph.D.	Joint Director
N.M. Claramma, M.Sc., Ph.D.	Senior Scientist
Rosamma Alex, M.Sc., LPRI, M.Tech, Ph.D.	Senior Scientist
K. Mariamma George, M.Sc.	Scientist C
N. Radhakrishnan Nair, M.Sc., M.Tech., MBA, Ph.D.	Scientist C
Jacob K. Varkey, M.Sc., M.Tech., Ph.D	Scientist C
Siby Varghese, M.Sc., Ph.D.	Scientist C
K.N. Madhusoodanan, M.Sc.	Scientist C
Leelamma Varghese, M.Sc., PGDHRM	Scientist C
Benny George, M.Sc., Ph.D.	Scientist C

Manoj Kurian Jacob, M.Sc.	Scientist A
Valsa George, M.Sc.	Assistant Technical Officer
Treasa Cherian, M.Sc.	Senior Scientific Assistant
M. Susamma Joseph, M.Sc.	Senior Scientific Assistant
M.L. Geethakumariamma, M.Sc., Ph.D.	Senior Scientific Assistant
C. Madheswaran, B.A.	Technical Assistant (Glass Blowing)(HG)

Technical Consultancy Division

P. Viswanathan Pillai, M.Sc	Deputy Director (up to 31.07.08)
G. Rajammal, M.Sc., M. Tech., LPRI, PGDBA	Scientist D
K.C. Mary, B. Sc.	Assistant Technical Officer (up to 19.09.08)
P. Nishiath Rahman, M.Tech.	Assistant Rubber Technologist
K.S. Mohandas, M.Sc., M.Tech.	.Assistant Rubber Technologist
Sheela Joseph, M.Sc., B.Ed., M. Tech.	Senior Scientific Assistant
K.I. Elizabeth, M.Sc., M.Tech., Ph.D.	Senior Scientific Assistant

PCRF

P.S. Sadeesh Babu, B. Tech., PGDBA	Scientist C
I. John Britto	Radiation Safety Officer

Economics Division

K. Tharian George, M.A., Ph.D.	Joint Director
Toms Joseph, M.A.	Scientist C (working arrangement at H.O. w.e.f. 17.08.07)
Binni Chandy, M.A.	Scientist S3
S. Mohanakumar, M.A., M.Phil.	Economist (up to 02.01.09)
S. Veeraputhran, M.A., M. Phil.	Scientist S3

Project Monitoring

M.A. Nazeer, M.Sc., Ph.D.	Joint Director

Library and Documentation Centre

Mercy Jose, B.Sc., M.L.I.Sc.	Documentation Officer
Accamma C. Korah, B.Sc., M.L.I.Sc.	Senior Librarian
A.S. Ajitha, M.A., M.L.I.Sc.	Librarian (Documentation)
V.R. Sujatha, B.Sc., M.L.I.Sc.	Technical Assistant (HG)
N. Latha, M.Sc., M.L.I.Sc.	Technical Assistant (HG)

Statistics and Computer

Ramesh B. Nair, M.Sc. (Ag. Stat.)	Assistant Director (up to 01.01.09)
B. Biju, M.Sc., M.C.A.	Assistant Director (Systems)
Suma George, P.G.D.C.A., M.C.A., B.Ed.	Computer Assistant
P. Aneesh, M.Sc., P.G.D.C.A.	Statistical Inspector

Instrumentation

S. Najamul Hussain, M.Tech., AMIETE	Instrumentation Engineer (up to 31.05.08)
Thomas Baby, M.Sc., M.Phil., Ph.D.	Instrumentation Officer
R. Rejikumar, M.Sc., M.Tech.	Assistant Instrumentation Officer
M.R. Anilkumar, Dip. in Inst. Tech.	Assistant Instrumentation Officer

Administration

V.Ramakrishnan	Dy. Secretary (Admn.) (up to 31.07.08)
P.Sari	Dy. Secretary (Admn.) (up to 31.01.09)
M.S.Nirmala	Dy. Secretary (Admn.) (w.e.f. 09.02.09)
K.K. Saramma	Assistant Secretary (up to 31.07.08)
P.V.Raveendran Nair	Assistant Secretary
R. Babu	Assistant Secretary (up to 15.01.09)
G.Asokan	Assistant Secretary (w.e.f. 25.02.09)
Sheela Peter	Section Officer

Accounts	
P.V. George, M.Com., M.B.A.	Joint Director (Finance) (up to 31.10.08)
Mohanan.A.R.Menon	Deputy Director (Finance)
K. Vijayamma, B.Com., P.G. Dip(FM)	Assistant Director (Finance) (upto 30.09.08)
Benoy Varghese, B.Sc., P.G.D.C.A., A.I.C.W.A.	Accounts Officer
V.V. Suresh Kumar	Assistant Accounts Officer
P. Seetha Lakshmi	Section Officer
Maintenance	
K.P. Sajeev, B.E.	Estate Officer
T. Manoj, B.E.	Assistant Engineer (Civil)
Experiment Station at RRII	
P.M. Narayanan	Assistant Estate Superintendent (up to 31.12.08)
Mary Mathew	Sr. Pharmacist
Security Wing	
M.T. Varghese, M.A.	Assistant Security Officer
Central Experiment Station, Chethackal, Kerala	
Sabu P. Idicula, M.Sc.(Ag.)	Deputy Director
Jacob Abraham, B.Sc., M.B.B.S.	Medical Officer
Zachariah Kurian, M.Com., A.C.A.	Assistant Director (Finance)
Mary Varghese	Junior Scientist
V.S. Govindankutty	Farm Superintendent
T.R. Divakaran	Assistant Estate Superintendent
P.J. George	Assistant Estate Superintendent
P.V.Omana	Section Officer
Annamma Andrews, H.S.C.	Nurse (HG)(up to 31.12.08)
K.G. Vijayan	Assistant Farm Superintendent
C.C. Joseph	Assistant Farm Superintendent
P.M.Sebastian	Assistant Farm Superintendent
P. Babuji	Assistant Farm Superintendent
Regional Research Station, Padiyoor, Kerala	
Radha Lakshmanan, M.Sc. (Ag.), Ph.D.	Senior Scientist
K.J. Joseph	Assistant Farm Superintendent
Regional Research Station, Guwahati, Assam	
D. Chaudhuri, M.Sc.(Ag.), Ph.D.	Deputy Director
G.C. Mondal, M.Sc., Ph.D.	Plant Pathologist
H.K. Deka, M.Sc., Ph.D.	Scientist C
M. Choudhury, M.Sc. (Ag.)	Scientist B (on study leave w.e.f. 01.10.08)
Ashith Raj, M.Sc. (Ag.)	Scientist A
P. Ajithkumar	Asst. Farm Superintendent
Regional Research Station, Agartala, Tripura	
Sushil Kumar Dey, M.Sc., Ph.D.	Deputy Director
P. M. Priyadarshan, M.Sc., Ph.D.	Plant Breeder
T. Sailajadevi, M.Sc.	Agrometeorologist (Scientist C)
Krishna Das, M.Sc., Ph.D.	Scientist S3
Debasis Mandal, M.Sc.	Scientist S3
Joy Joseph, M.A.	Scientist S3
Debabrata Ray, M.Sc. (Ag.)	Scientist S2
Joby Joseph, M.A.	Scientist A
N.K. Gogoi, M.Sc. (Ag.)	Scientist A
P. Deepthi Antony, M.Sc. (Ag.)	Scientist A
Bhaskar Datta, M.Sc. (Ag.)	Scientist A
Jiban Chakraborty, B.Com.	Assistant Director (Finance)
Amal Chandra Sarma, B.Sc., ALC, LLB, PGDBM	Senior Scientific Assistant

Tapan Kumar Pal, M.Sc.	Senior Scientific Assistant
S. Dinesh, B.Com.	Section Officer
Haradhan Bhowmik, B.A.	Assistant Estate Superintendent
Regional Research Station, Tura, Meghalaya	
A.P. Thapliyal, M.Sc., Ph.D.	Deputy Director
R.P. Singh, M.Sc. (Ag.), Ph.D.	Scientist S3
M.J. Reju, M.Sc.	Scientist S3
Pramesh Khoyumthem, M.Sc., Ph.D.	Scientist A
Regional Experiment Station, Nagrakata, West Bengal	
Gitali Das, M.Sc., Ph.D.	Senior Scientist
K. Satheesan, B.Sc.	Assistant Farm Superintendent
Regional Research Station, Dapchari, Maharashtra	
Meena Singh, M.Sc. (Ag. Bot.), Ph.D.	Plant Physiologist
S. Ravichandran	Scientist A
K. Gopi, B.Sc.	Section Officer
P.G. Sasikumar, B.A., MET	Assistant Farm Superintendent
Regional Research Station, Dhenkanal, Orissa	
Balkrishan, M.Sc., Ph.D.	Scientist C
S.C. Mallik	Section Officer
C. Krishnan	Assistant Farm Superintendent
Hevea Breeding Sub-station, Nettana, Karnataka	
T.R. Chandrashekar, M.Sc., M.Tech. Ph.D.	Senior Scientist
K.K. Vinod, M.Sc. (Ag.)	Plant Breeder
M.J. Manju, M.Sc. (Ag.)	Scientist S3
K.V. Sumesh, M.Sc., Ph.D.	Scientist A
V.J. George	Assistant Estate Superintendent
Hevea Breeding Sub-station, Paraliar, Tamil Nadu	
T.A. Soman, M.Sc., M.Phil., Ph.D.	Scientist C
M. Suryakumar, M.Sc.	Scientist S2
Regional Soil Testing Laboratory, Adoor, Kerala	
Thomas Eappen, M.Sc., B.Ed.	Scientist S3
K.C. Jayasree, B.Sc.	Assistant Technical Officer
Regional Soil Testing Laboratory, Muvattupuzha, Kerala	
C.P. Mary, M.Sc.	Technical Officer
Regional Soil Testing Laboratory, Kozhikode, Kerala	
Joyce Cyriac, M.Sc.	Scientist B
P.K. Madhusooddhanan, B.Sc.	Assistant Technical Officer
K. Jayasree, M.Sc.	Senior Scientific Assistant
Regional Soil Testing Laboratory, Nedumangad, Kerala	
S. Sheela, M.Sc.	Assistant Technical Officer
Regional Soil Testing Laboratory, Palai, Kerala	
Valsamma Mathew, M.Sc.	Senior Scientific Assistant
Regional Soil Testing Laboratory, Thrissur, Kerala	
C. Viswambharan, B.Sc.	Senior Scientific Assistant
Regional Soil Testing Laboratory, Taliparamba, Kerala	
Suresh Babu Ayyappanchalil, M.Sc.	Senior Scientific Assistant

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Research divisions and functions

The major research divisions are Agronomy/ Soils, Biotechnology, Botany, Germplasm, Plant Pathology, Plant Physiology, Exploitation 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 include 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 establishements 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 (IRRDB), 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

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