



RUBBER RESEARCH INSTITUTE OF INDIA

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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 the RRII in its scientific worth and research contributions has won it the recognition as an International Centre of Excellence on NR research.

Location

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

continued on inside back cover

ANNUAL REPORT 2006-2007



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, 1982 and in 1994. 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 six departments under the Board *viz.*, Administration, Rubber Production, Processing & Product Development, Finance & Accounts, Training & Technical Consultancy and Licensing & Excise Duty.

Chairman

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Rubber Research Institute of India

Dr. N.M. Mathew (up to 31.10.2006)

Dr. James Jacob (from 29.12.2006)

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Rubber Production Department

Dr. A.K. Krishnakumar

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Administration Department

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DIRECTOR'S REVIEW

The Golden Jubilee celebrations of RRII inaugurated by His Excellency, President Dr. A.P. J. Abdul Kalam on 29 July 2005 came to a formal close with a Rubber Growers' Conference on 24 October 2006 and an Interactive Meeting with manufacturers of non-tyre rubber products on 27 October 2006. After successfully organizing a series of events marking the Golden Jubilee year of the Institute and effectively stewarding the activities of RRII as its ninth Director for nine years, Dr. N.M. Mathew retired from service on 31 October 2006. The Institute is indebted to him for the services that he has rendered to it and we wish him all the best.

Inaugurating the Golden Jubilee celebrations, the President also released two new clones, namely RRII 414 and RRII 430 for large-scale commercial cultivation which are still being accepted by the rubber growing community with great enthusiasm. An orientation programme on identification of RRII 400 series clones was organized for the extension officers of Rubber Board during August 2006. The planting season of 2006 witnessed very high demand for the new clones. Rubber Board promotes a multiclone planting policy. In the coming couple of years, we expect to release possibly another one or two more new clones from the RRII 400 series. All the growers who participated in the Rubber Growers' Conference held at RRII on 24 October 2006 were of the opinion that both RRII 414 and RRII 430 were performing very well in the field. With more than 150 experimental clones which include hybrids, ortets and poly cross progenies, the Institute aims to release one or two new

clones every 5-10 years. This is not an easy task, but with the large number of promising clones in our possession we are confident of achieving this ambitious target which will eventually solve the

present problem of large-scale monoclonal cultivation. No other Rubber Research Institute in the world can claim possession of such a large number of promising clones in the pipeline; thanks to our breeders who have done a marvelous job.

The breeders will have greater responsibilities in the years ahead. Over the years, we have been focusing mostly on breeding and selecting high yielding clones, but we had been lucky that the high yielding RRII 400 series clones also have faster growth rates, and therefore, higher timber production than RRII 105. As far as disease tolerance is concerned, the newly released clones from the RRII 400 series are either on par with RRII 105 or better. With climate stress increasingly becoming a serious factor limiting the growth and productivity of rubber, the Institute will be looking up to the breeders for evolving clones which will have high latex and timber production and tolerance to drought. Developing location-specific clones from the various Regional Research Stations of the Institute scattered in the eight different states in India, and



especially from the North Eastern states also should be a priority for the breeding team of RRII. The selections from the different Regional Research Stations will have to be simultaneously field tested in the different agroclimatic locations. To speed up our breeding and selection cycle, certain fundamental changes are being envisaged which include setting up of poly cross gardens, evaluation of large number of half-sib progenies, ATP-finger printing of seedlings for possible early prediction of high yielding plants, participatory clone evaluation with growers from different agroclimatic regions etc.

RRII has a large repository of wild *Hevea* germplasm accessions which are being screened for specific agronomic traits such as tolerance to drought, cold and various diseases, timber production etc. Some promising wild accessions are being evaluated in field trials for their agronomic performance and some are also being used as parents in hybridization programmes.

Classical breeding and selection of progenies gave RRII rich dividends in the form of RRII 105, RRII 414 and RRII 430 and several dozens of promising lines. But this approach suffers from the inevitable problem of "gene drag" (of unwanted traits from the parents to offspring) which is more problematic when wild accessions with promising secondary traits such as disease or environmental stress tolerance, but lacking in rubber yield, are used as parents. Integrating molecular tools with classical breeding programme, therefore assumes great significance in the years ahead.

RRII has been one of the first institutes of its kind that embarked upon an ambitious molecular biology and biotechnology

programme. The transgenic rubber plants integrated with MnSOD gene developed by RRII showed better tolerance to moisture deficit stress under laboratory conditions. These plants need to be tested under field conditions for which mandatory bio-safety clearance needs to be obtained from Government of India. The Institute has a separate Genome Analysis Laboratory in addition to a full fledged Biotechnology Division. Molecular Physiology and Molecular Pathology Laboratories function attached to the Pathology and Physiology Divisions of RRII. It is proposed to bring together all the molecular biology and biotechnology related research activities taking place in the different laboratories of RRII into one single functional group called Advanced Centre for Molecular Biology and Biotechnology. In addition to improving the efficiency of use of manpower and other resources, this will help in better co-ordination and impart a better sense of purpose to those working in this important area of modern biology. Historically, classical breeding has contributed to evolving new clones. In future, the role of molecular biology in crop improvement cannot be undermined. Physiological mechanisms of adaptation to diverse agroclimatic conditions and the molecular basis behind them require to be understood in greater detail in order to help the breeders evolve location-specific clones.

Using the techniques of differential display RTPCR and subtractive hybridization several genes associated with drought, cold, TPD, fungal disease etc. have been identified. Different types of DNA markers such as microsatellites, AFLPs and SNPs were developed and validated in the Wickham clones as well as in wild accessions.

Various long-term field trials in Crop Management such as fertilizer trials were continued. A collaborative work with the National Bureau of Soil Survey & Land Use Planning, Bangalore for regrouping soil resources data of traditional rubber growing regions into district-wise thematic maps was initiated. Fertilizer recommendations were given to more than 6000 growers based on leaf/soil analysis and tens of thousands of DRC samples were analyzed through the Regional Laboratories. Location-specific agronomic practices, especially for the non-traditional regions need to be developed on a priority basis.

As part of ecosystem level studies by the Crop Physiology team three new programmes were initiated during the reporting year. They include measuring the interception of solar energy by rubber canopy, measuring the water consumption by rubber trees and measuring the net flux of carbon dioxide in rubber holdings. A protocol developed by Crop Physiology Division for isolation and purification of *L-quebrachitol* from *Hevea* latex has been submitted for patenting.

Anaerobic microorganisms have been successfully used for treating effluents from sheet processing for making biogas. A high rate reactor has been designed and commissioned to treat sheet processing effluent and generate biogas which can be used for partially replacing firewood for drying sheets. The Pathology Division has identified and isolated microorganisms capable of protecting rubber sheets from mould growth during storage. They have also isolated large number of species of endophytic bacteria that are antagonistic to various fungal pathogens of *Hevea*. Developing environment-friendly crop

protection practices will remain a major priority for the Pathology team in the years ahead. An international training under the CFC funded project on *Corynespora* leaf disease – “Strategies for Management of *Corynespora* Leaf Disease of *Hevea brasiliensis*” was organized at RRII during April 2006.

An important study by the Economic Research team showed that there has been a steady increase in the share of older rubber holdings (which were in the yield declining phase) in the recent years. This can have significant adverse impact on the productivity of rubber in the years ahead. A comprehensive data base on international trade in all forms of rubber and rubber products has been developed.

Natural Rubber Research, the research journal of RRII, continued its publication during the reporting year. There were 47 research articles, 5 books and 3 working papers published by RRII during the reporting year. In addition to these, 40 papers were presented by our scientists in national and international conferences. RRII collaborated with IRRDB in publishing a “Coffee Table Book entitled “Portrait of Global Rubber Industry”. Dr. Vinoth Thomas won the “Grand Prize” in the international photography competition conducted by IRRDB in connection with the publication of the Coffee Table Book.

A quick method for the determination of DRC in field latex and production of surgical gloves with low antigenic protein content in collaboration with the industry was standardized by our Rubber Technology team. Nanocomposites with better mechanical properties were prepared and rubber/plastic blends were evaluated in relevant rubber products/formulations. Deproteinised skim rubber with enhanced

mechanical and ageing properties were prepared. A collaborative research programme was initiated with the Schefflin Research and Training Centre, Tamil Nadu for development of special footwear for physically handicapped. Industry advisory services were continued.

RRII has made tremendous impact on the rubber cultivation sector of the country during the past 50 plus years of its existence. It is now felt that there has to be greater emphasis in the years ahead in technology research leading to industrial applications. In the liberalized economic scenario, the manufacturing industry is under pressure to

come up with innovative ideas in order to improve its competitiveness. This should give adequate opportunities for RRII to collaborate with the industry so that it can take its findings from the laboratory to the floor of the factory. Commissioning an Advanced Centre for Rubber Technology at RRII has been a step in the right direction although it will take some more time for it to become fully operational.

After the retirement of Dr. N.M. Mathew, Director of Research on 31 October 2006, Dr. James Jacob took over charge as the new Director on 29 December 2006.

AGRONOMY / SOILS DIVISION

Development and periodic refinement of the agromanagement techniques for better growth and yield of rubber are the major research themes of the Division. Research programmes are also undertaken to assess and sustain the quality of rubber growing soils and to reduce the cost of cultivation. Experiments on different aspects of nutrient management, intercropping and cropping systems, soil and water conservation and planting techniques are in progress to achieve the major goals. Experiment to develop agronomic package to reduce the long unproductive gestation period of rubber is being continued. For a better site-specific management, the soil series were regrouped into management units based on important soil parameters with the help of NBSS & LUP Bangalore. Development of the rubber information system for the traditional region of rubber cultivation using remote sensing and GIS technique is also in progress.

1. Nutrient management

The field experiment initiated in 1989 at Kodumon Estate, Adoor to study the nutrient requirement of clone RR11 105 was concluded. Seventeen years after

commencing the experiment with graded levels of fertilizers, available P status in soil was higher in P applied treatments. Soil organic carbon and other nutrients were not significantly influenced by the treatments. There was no significant difference between the control and fertilizer-applied treatments in the leaf nutrient status. Response in yield to fertilizer application was inconsistent.

The experiment on sequential skipping of fertilizer application in mature rubber (RR11 105) at Kodumon Estate was continued. Yield (2006-07) and girth increment (2002-07) were not significantly influenced by skipping of fertilizers (Table Ag.1). Significant difference was also not noticed in soil and leaf nutrient status.

Experiment to explore the possibility of substitution of potassium fertilizer with sodium chloride in mature plantation of clone PB 217 was continued at Malankara Estate, Thodupuzha. Yield with 100 per cent recommended level of potassium (30 kg/ha) and 25 per cent substitution with sodium (7.5 kg/ha) was comparable and was significantly higher compared to the control (Table Ag.2).

Table Ag. 1. Effect of skipping of fertilizers on growth and yield of RR11 105

Treatment	Girth increment (cm) 2002-2007	Yield (g/t) 2006-07
Application of full dose in two splits (30:30:30 N, P ₂ O ₅ & K ₂ O kg/ha)	8.30	88.18
Application of full dose every year (Pre-monsoon)	9.24	106.30
Skipping pre-monsoon fertilizer application	8.90	100.63
Skipping post-monsoon fertilizer application	8.67	94.38
Skipping for one complete year	8.87	102.37
Skipping for two complete years	9.18	120.17
Continuous no manuring	8.85	104.15
SE	0.84	10.57
CD (P=0.05)	NS	NS

The field experiment to study the effect of long-term use of inorganic and organic manures on the growth and yield of rubber and on the physico-chemical properties of the soil was continued. No significant difference was observed between treatments on girth and girth increment.

The study on coir pith manure (C-POM) as pit manure for rubber (2002 planting, RRII 105) indicated significant improvement in girth with the application of farmyard manure (12 kg/pit) and C-POM (2.5 kg/pit) over no pit manure control at CES,

Chethackal. However, no significant difference in girth was observed between farmyard manure and C-POM application. Soil analysis indicated no significant difference in organic carbon and available nutrient status between treatments.

The field experiment to study the phosphorus mobilization efficiency of the two commonly cultivated legumes in rubber plantations, viz. *Pueraria phaseoloides* and *Mucuna bracteata* was concluded. Available P status was significantly higher in the rhizosphere of legumes compared to that of natural cover. Acidification and enhanced acid phosphatase activity were observed in the rhizosphere soil of legumes indicating the solubilization of P from less available soil P fractions (Table Ag. 3).

Studies on biomass accumulation, nutrient uptake and nutrient removal in 30-year-old rubber trees indicated clonal difference in these parameters. Accumulation of Ca was higher in clone RRII 105 while that of K was higher in clone RRII 118 among the clones studied.

In a pot culture experiment, application of lime along with fertilizer increased the growth, dry matter production and nutrient uptake of plants. Liming increased the

Table Ag. 2 Effect of sodium application on yield

Levels of potassium and sodium (kg/ha)		Yield (g/t)
K ₂ O	Na ₂ O	
30.0	0	68.04
30.0	7.5	62.84
30.0	15.0	52.18
22.5	0	57.28
22.5	7.5	68.53
22.5	15.0	63.10
15.0	0	56.79
15.0	7.5	67.75
15.0	15.0	57.91
0	0	51.30
SE		4.82
CD (P=0.05)		14.48

Table Ag. 3 pH and available P status of rhizosphere soil

P levels (kg/ha)	pH				Available P (ppm)			
	<i>Pueraria</i>	<i>Mucuna</i>	Natural cover	Mean	<i>Pueraria</i>	<i>Mucuna</i>	Natural cover	Mean
0	4.38	4.57	4.72	4.56	35.5	28.3	21.1	28.3
15	4.53	4.58	4.62	4.58	33.0	31.7	25.2	29.9
30	4.61	4.64	4.72	4.66	32.2	28.4	25.5	28.7
45	4.64	4.53	4.70	4.62	33.4	29.5	26.3	29.4
Mean	4.54	4.58	4.69		33.5	29.5	24.5	
CD (Crops)	0.092						1.33	
CD (P level)	NS						NS	
CD (Crop x P level)	NS						NS	

calcium concentration in different plant parts indicating higher uptake of calcium by rubber plants under conditions of higher availability. Among the liming materials, dolomite and quick lime were found to be equally effective on the growth of rubber seedlings.

The comparative study on composted household waste and chemical fertilizers indicated no significant difference between treatments on growth of rubber plants.

A nursery experiment to study the effect of sulphur addition along with rock phosphate on growth of rubber seedlings indicated that application of sulphur enhanced the number of buddable plants in 10 months period. Soil P availability increased with increasing level of sulphur application.

2. Rubber growing soils

Studies on assessment of quality and health of different soil ecosystems such as mature rubber, rubber with *Mucuna*, rubber with pineapple, cassava, teak and forest were continued. Analysis of different physical size fractions of soil indicated that carbon and nitrogen tend to accumulate in the finer (clay and silt) fractions of the soil in all the systems and the finer fractions were higher in soils under rubber.

Assessment of microbial population in different soil systems indicated significantly higher bacterial population in forest soil compared to all other soils (Table Ag. 4). The difference in bacterial population was higher during February compared to November period.

The study on characterization of acidity and its management in rubber growing soils was concluded. Profile samples were collected from eight major soil series to characterize the nature of soil acidity. High aluminium saturation and soluble

Table Ag. 4. Soil bacterial population (per g soil) in different systems

System	Nov '06 (x10 ⁶)	Feb '07 (x10 ⁶)
Forest	9.32	84.20
Teak	7.74	27.00
Rubber	5.08	37.00
Pineapple	6.04	52.40
Cassava	7.10	58.20
<i>Mucuna</i>	6.84	20.60
CD (P = 0.05)	2.43	10.32

aluminium was observed in the subsurface layers of most of the series. Inverse relation was observed between exchangeable Al and exchangeable Ca in the soil, indicating the deleterious effect of soil acidity on the availability of Ca.

Variation in soil nutrient availability in soils under rubber and adjacent forest was compared. Clay, silt, cation exchange capacity, organic C, pH and available K content registered a decrease in soils under rubber compared with the forest soil (Table Ag.5). However, the available P content showed an increase in soils under rubber.

Table Ag. 5. Physical and chemical properties of soils under rubber and forest

Soil properties	Rubber	Forest
Gravel (%)	46.35 (1.10)	41.61 (0.73)
Sand (%)	47.85 (0.33)	32.46 (0.28)
Silt (%)	10.5 (0.22)	12.10 (0.30)
Clay (%)	30.43 (0.29)	41.48 (0.21)
CEC (cmol/kg)	7.08 (0.43)	13.72 (0.42)
pH	4.84 (0.06)	5.10 (0.07)
Organic C (%)	1.91 (0.12)	2.48 (0.12)
Av. P (mg/100 g soil)	1.88 (0.86)	0.23 (0.03)
Av. K (mg/100 g soil)	10.32 (1.09)	19.14 (1.50)

Values in parenthesis indicate SE

Studies were initiated to identify soil factors influencing the yield in low and high yielding rubber plantations. The study indicated low soil exchangeable aluminium in high yielding fields compared to that in low yielding areas.

In a study on sulphur availability in rubber growing soils, sulphur availability varied from 12 to 23 mg per kg in four soil series. Available S content was high in Kanjirapally and Thiruvanchoor series and was significantly correlated with soil pH, clay and sand.

A study on soil fertility changes in three rubber plantations under different cycles in the estate sector was initiated. Preliminary results indicated a decreasing trend in organic C and available K content towards the end of first cycle of rubber cultivation. However, accumulation of soil P was noticed. There was no change in soil pH.

In vitro studies indicated that organic sources applied to the lateritic soil decreased the exchangeable Al and raised the pH of the soil thereby improving soil conditions for plant growth.

In the experiment on effective soil volume and fertility assessment of rubber growing soils, a reduction in yield with increasing gravel content was noted.

3. Root studies

The allocation of biomass to roots was studied in 7, 18 and 29-year-old rubber trees. As the age of the tree increased, the allocation of biomass to roots decreased (Table Ag. 6).

The experiment to study fine root production of rubber trees (clone RRII 105)

Table Ag. 6. Biomass allocation to roots in trees of different age groups

Age (year)	Biomass (kg)			Allocation to roots (%)
	Above ground	Below ground	Total (kg)	
7	217.5	40.79	258.29	15.79
18	1219.3	149.07	1368.37	10.89
29	2287.0	186.66	2473.70	7.55

in relation to precipitation was continued at two locations viz. CES, Chethackal and RRII Kottayam with varying soil nutrient status. Soil nutrient status was higher at CES compared to RRII. Fine root production was higher at RRII compared to CES. A time lag of 35-45 days was observed between the onset of rains and highest fine root production at both the locations.

4. Planting techniques

Study on the effect of density of planting on growth and yield of rubber (clone RRII 105) at Chethackal was continued. Plants in the lowest density of 420 trees per ha recorded significantly higher yield, girth and girth increment (Table Ag.7). Annual yield was significantly higher in the highest density (749/ha) and was at par with densities 549 per ha and 638 per ha. The methods of fertilizer application (M1 on area basis and M2 on per plant basis) did not influence yield of trees.

Table Ag. 7. Mean yield for the period April '06 - March '07

No. of trees/ha	Yield (g/t/l)			Yield (kg/ha/year)		
	M1	M2	Mean	M1	M2	Mean
420	81.38	79.46	80.42	2124.50	2077.75	2101.13
479	58.53	59.14	58.99	2126.25	2206.25	2166.25
549	64.45	61.14	62.80	2529.75	2655.00	2592.38
638	58.55	55.25	56.90	2805.00	2544.50	2674.75
749	55.40	55.14	55.27	2809.75	2596.25	2703.00
Mean	63.72	62.02		2479.05	2415.95	

Main plot treatment SE-4.21, CD-9.18
Sub plot treatment SE-2.91, CD-NS

Main plot treatment SE-129.93, CD-283.12
Sub plot treatment SE-104.91, CD-NS

The experiment to study the effect of planting pits of various dimensions on the growth and yield of rubber was continued. Girth of rubber plants was not significantly influenced by size of planting pits.

5. Reduction in immaturity period

The field experiment initiated at Malankara estate, Thodupuzha to develop an agronomic package to reduce the immaturity period of *Hevea* (Clone RR11 105) was continued. Preliminary results indicated better growth under integrated management.

6. Intercropping and cropping systems

Experiment on intercropping coffee and cocoa, established during third year of rubber cultivation (clone RR11 105) was continued. Growth and yield of rubber trees were not significantly influenced by growing coffee and cocoa as intercrops. Soil moisture status during January was higher when intercrops were grown along with rubber. Yield of rubber during summer season (December-February) was also higher in the presence of intercrops. Coffee yield continued to be very low but yield of cocoa was comparatively higher. The experiment on intercropping coffee and cocoa in mature rubber at Ponkunnam was also continued. In this experiment also, the growth and yield of rubber were not significantly affected by growing intercrops. Coffee yield continued to be low. The monthly average yield of cocoa was 6.1 pods.

Experiments to explore the feasibility of growing perennial intercrops with rubber, under normal system of planting and under wide-row system at CES, Chethackal were continued. In both the experiments, significant differences were not observed between treatments on girth of plants. Yield of *Garcinia* and *Vanilla* was higher in the experiment where rubber was planted at the

normal spacing indicating the beneficial effect of shade under conditions of severe drought. Coffee yield was comparable in both the experiments.

In the cropping system experiment at CES, Chethackal, growth and yield of rubber continued to be higher in the inter-cropped area with altered spatial arrangement of planting compared to monoculture under normal spacing.

The on-farm experiments on interaction between rubber (RR11 105) and wild jack progressed well. At Ponthenpuzha, wild jack standing close to rubber (< 2.0 m) significantly reduced the growth of rubber compared to rubber without wild jack. However, at Mallikasseri, Pala, girth of rubber at all the distances from wild jack was low compared to rubber without wild jack. At both locations, wild jack density showed significant effect on growth of rubber (Fig. Ag. 1). Girth of rubber showed declining trend with increasing wild jack density.

At Ponthenpuzha, wild jack density did not show any significant effect on latex yield. However, at Mallikasseri (Pala) wild jack density showed significant negative effect on total latex yield during dry period (November-February).

Experiment on inter-planting of rubber with teak, wild jack and mahogany at CES, Chethackal was in progress. Girth and girth increment of rubber were not significantly influenced by row spacing, type of timber and their interaction. Among the timber species, growth of wild jack was better followed by teak and mahogany.

Experiment on effect of density of teak and mahogany inter-planting on growth of rubber was continued. Girth of rubber did not vary significantly due to density of timber trees. Among the timber species, only teak girth varied significantly with density. Girth of teak was significantly better at the highest density.

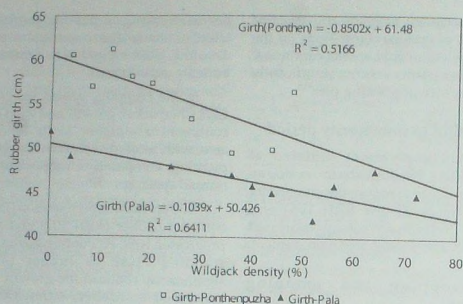


Fig 1: Effect of wild jack density on rubber

7. Soil and water conservation

The experiment to study the influence of silt pits on the growth and yield of mature rubber (clone RR11 105) and to explore the possibility of applying fertilizer for rubber in silt pits was continued at TR&T Estate, Mundakayam. Yield was significantly higher in treatments with 250 pits per ha compared to that in no-pit control (Table Ag.8). The girth increment was not significantly influenced by number of pits. The mean soil moisture content was the

highest in plots with 250 pits per ha. Yield and girth increment were not significantly influenced by different methods of fertilizer application.

8. Rubber information system

Development of rubber information system in traditional region using remote sensing and GIS technique was in progress. With the help of National Bureau of Soil Survey & Land Use Planning, Bangalore, the soil series were regrouped in to seven management units based on soil parameters, depth, gravel content and organic carbon which have the highest variability in rubber growing soils.

9. Advisory service

The activities of the DRIS Unit were to offer advisory services to rubber smallholdings through discriminatory fertilizer recommendation based on soil and leaf analysis and latex testing for DRC estimation. During 2006-07, 10,500 soil and 450 leaf samples were collected from

Table Ag. 8. Effect of conservation pits on growth and yield of rubber

No. of pits / ha	Girth increment 2001-2007 (cm)	Yield (g/t)
150 (S)*	8.59	54.36
150 (P)**	7.72	53.33
250 (S)	8.48	60.39
250 (P)	8.86	58.59
No pit & standard practice	7.86	52.26
No pit & no fertilizer	7.77	50.46
SE	0.94	2.51
CD (P = 0.05)	2.83	7.57

S* - Surface application ; P** - Application in pits

smallholdings located in the traditional rubber growing areas of Kerala, Karnataka and Tamil Nadu and analyzed in the central, regional and mobile soil testing laboratories. Based on the test results, around 4,850 discriminatory fertilizer recommendations were offered to rubber growers.

Nine hundred and fifty two individual Discriminatory Fertilizer Recommendations (DFR) were given to 38 large estates based on analysis of 1325 soil and 835 leaf samples. Facilities for latex testing for dry rubber content (DRC) were also provided to small growers. During the reporting period, 57,690 latex samples were tested for DRC and 40 samples for volatile fatty acid.

BIOTECHNOLOGY DIVISION

The major ongoing research programmes in the Biotechnology Division are i) development of large-scale *in vitro* propagation methods for elite *Hevea* clones, ii) development of superior transgenic *Hevea* plants for better latex yield, disease tolerance, adaptation to environmental stress and recombinant protein production in the latex, iii) production of haploids and triploids and development of *in vitro* fertilization techniques to complement conventional breeding programmes, iv) study of molecular mechanism and characterization of genes controlling tolerance to diseases, abiotic stress, tapping panel dryness and latex biosynthesis and v) study of laticifer cell-specific gene expression and characterization of laticifer cell-specific promoters.

1. Somatic embryogenesis

The plant regeneration system using immature anther explants of clone RRII 105 developed earlier was extended to the RRII 400 series clones. Immature anther of clones RRII 414 and RRII 417 were inoculated into callus induction medium keeping RRII 105 as control. Callus initiation, proliferation and embryo induction were obtained from both the clones.

To confirm the genetic uniformity of the somatic plants developed earlier, RAPD analysis was repeated with 25 random primers using DNA isolated from 12 somatic plants, the mother plant and 12 seedlings raised from monoclonal seeds. No variation in banding pattern was detected among the somatic plants and the mother plant. Clear variation in banding pattern was detected in the seedlings with different primers. The protein and isozyme profiles of peroxidase and esterase of the different developmental stages during somatic embryogenesis were also studied. More proteins in terms of number and quantity were observed in the embryogenic callus and embryos. At the torpedo and cotyledonary stage, the protein accumulation was low. Number of proteins as well as their accumulation was very low in the non-embryogenic callus. Peroxidase and esterase isozyme profile differed in different developmental stages. In embryogenic calli and embryos, the peroxidase activity was more. Esterase activity was high in embryos and plantlets and displayed a single zone of activity. Both peroxidase and esterase showed no activity in non-embryogenic calli. To correlate the histo-morphological differences between embryogenic and non-embryogenic callus, histological study was also attempted.

Histochemical detection of starch, protein and lipid was also done. The embryogenic callus was characterized by small cells with thick cell wall, prominent nuclei and dense cytoplasm. Embryogenic callus was densely accumulated with starch grains, lipids and proteins. Non-embryogenic callus seems to have large cells with thin cell wall and less cytoplasm. In non-embryogenic callus, content of starch, lipids and proteins was very low.

Refinement experiments were continued at various stages from callus induction to subsequent embryogenesis and plant regeneration using immature inflorescence explants. Addition of calcium nitrate and a mixture of amino acids such as asparagine (50 mg/L), arginine (50 mg/L), glutamic acid (150 mg/L) and proline (100 mg/L) significantly enhanced the embryo induction frequency to about 80 per cent.

Mature embryos at the cotyledonary stage were transferred to plant regeneration medium containing the growth regulators GA_3 (1.45 μ M) and BA (8.8 μ M) along with adenine hemisulfate (50 mg/L). Plant regeneration could be improved by the addition of organic supplements casein hydrolysate (250 mg/L), banana powder (100 mg/L) and malt extract (40 mg/L) along with 10 per cent coconut water.

In some of the earlier experiments on somatic embryogenesis, proembryo cultures in certain media combinations were observed to be capable of inducing repetitive embryogenesis and these cultures could be multiplied very fast. A set of experiments were conducted with the objective of optimizing growth regulators as well as the developmental stage of embryos. A protocol could be standardized for secondary embryogenesis

Utilization of root segments of somatic plants as the initial explant for further embryo induction and subsequent plant regeneration was experimented and proved to be an effective system. In this pathway, actively growing root tips of somatic plants were used as the initial explant for callus induction, somatic embryo induction and subsequent plant regeneration. Many somatic plants could be regenerated by employing the above mentioned systems.

Experiments were also continued to enhance the efficiency of embryogenesis and plant regeneration with the system developed recently with leaf explants. Presence of NAA in the callus induction medium helped in getting good quality callus. Calli could be induced in 40 days avoiding liquid preculture and these were subcultured for embryogenic calli formation. Callus proliferation was obtained in the same medium with reduced auxin and increased sucrose. Proliferation and embryo induction occurred in the dark. Yellow friable embryogenic calli that emerged from the cultured calli were subcultured for embryo induction. Several modifications were made in embryo induction medium by changing major salts, amino acids and reducing the level of phytohormones for effective and normal embryogenesis and to increase the rate and its quality. Embryogenic calli were subcultured every two months into fresh media for proliferation and embryo induction. Embryogenesis could be achieved in modified MS medium (KH_2PO_4 , 270 mg/L and $Ca(NO_3)_2$, 360 mg/L), which contained B_5 vitamins, organic supplements and phytohormones. A good embryo induction frequency of above 60 per cent could be obtained with normal embryos in a reduced hormone combination of BA (0.3 mg/L), kin (0.2 mg/L), GA_3 (0.8 mg/L) and NAA (0.1 mg/L) and containing the amino acids, glutamine

(300 mg/L), alanine (50 mg/L), serine (20 mg/L) and arginine (37 mg/L). Subculturing single embryos and maintenance in darkness were found to be more effective for their enlargement and conversion to cotyledonary stage. Maturation and apex induction could be achieved in WPM medium containing vitamins, organic supplements such as CW (10%), malt extract (100 mg/L), casein hydrolysate (400 mg/L) and phytohormones BA (0.5 mg/l), Kin (0.3 mg/L), IBA (0.1 mg/L) and GA₃ (1.0 mg/L). Good plant development occurred when single germinated embryos were transferred to MS medium containing sucrose (40 g/L). Fully developed plantlets were obtained within 3-4 weeks of culture. In the optimized protocol, the embryo induction and plant regeneration frequency could be increased considerably.

Plant regeneration experiments were carried out with the somatic embryos obtained from ovule culture in basal media (MS, WPM, N6), organic supplements (malt extract, banana powder, yeast extract, casein hydrolysate, coconut water), amino acids (asparagine, arginine, glutamine, proline, glycine) and various growth regulators alone and in combination. MS basal medium was found to be optimum for germination. Organic supplements like malt extract (200 mg/L), banana powder (500 mg/L) and coconut water (20%) had a beneficial effect, when supplied along with proline (200 mg/L) and glutamine (500 mg/L). A combination of BA (1.0 mg/L) and GA₃ (0.8 mg/L) favoured germination. However, leaf expansion and retention were still found to be difficult.

For microspore culture, experiments were conducted to induce fresh callus from intact anthers at the late uninucleate to the early binucleate stage. The anthers were pretreated in MS medium containing maltose

(10%) at 4° C and directly inoculated for callus induction in N6 medium with zeatin (3.0 mg/l) and kinetin (3.0 mg/L) containing high levels of sucrose (150 g/l). After one month in culture, the anthers turned brown and they were subcultured to medium supplemented with low levels of sucrose (50 g/L). Emergence of calli was observed from the pollen grains.

2. *In vitro* fertilization and plant recovery

Experiments were performed for immature embryo rescue to develop a system for plant regeneration from immature embryos as well as to utilize the media compositions for the growth of *in vitro* fertilized embryos. Fruits at different stages of maturity were collected from the field and sterilized using 0.1 per cent HgCl₂. Ovules were isolated and cultured in MS, WPM and Nitsch medium. Growth regulator combinations of GA₃, kinetin and zeatin along with different levels of sucrose ranging from 50-180 g/L were tried. A protocol has been developed for the rescue of embryos at the age of 2-3 months. It was observed that increased sucrose level (50 g/L) is ideal for normal development of the embryo. Nitsch basal medium was found to be ideal for embryo growth. Embryos could be germinated in MS basal medium supplemented with BA (0.3 mg/L) and GA₃ (0.3 mg/L). Plants were hardened and transferred to polybags.

3. Genetic transformation

Genetic transformation experiments were continued to develop transgenic plants with increased tolerance to tapping panel dryness, drought and environmental stress, enhanced rubber yield and for the production of recombinant proteins in the latex.

The genes coding for superoxide dismutase, sorbitol-6-phosphate dehydrogenase, cis-prenyl transferase, HMGR1, farnesyl diphosphate synthase, rubber elongation factor, osmotin, Myb1 transcription factor and TB antigen were used in these studies.

The transgenic plants integrated with superoxide dismutase gene under the control of CaMV 35 S promoter were further multiplied by budgrafting along with RR11 105 control plants. Physiological and biochemical studies such as PSII activity, cell membrane stability and antioxidant enzyme levels were done in the Physiology Division. An elevated SOD enzyme level was observed in the transgenic plants. In order to get more independent transgenic lines, fresh genetic transformation experiments were carried out with MnSOD gene under the control of CaMV 35 S promoter as well as FMV 34 S promoter. Embryogenic callus derived from immature anther was used for the genetic transformation of SOD gene with CaMV 35 S promoter whereas fresh callus was used for the genetic transformation of SOD gene with FMV 34 S promoter. New transgenic lines were developed with both the constructs. Histochemical GUS assay as well as PCR analysis confirmed the presence of the inserts. Refinement experiments were also carried out to enhance the embryo induction frequency with SOD gene under the control of FMV 34 S promoter and it was observed that addition of 6 per cent PEG in the medium enhanced the embryo induction frequency.

Fresh genetic transformation experiments were carried out with the gene coding for sorbitol-6 phosphate dehydrogenase and the gene coding for osmotin protein, using embryogenic calli derived from anther as the explant. Putatively transformed lines obtained were further cultured for proliferation. For

incorporating genes involved in the rubber biosynthetic pathway to enhance rubber production, experiments were carried out with the genes *viz.* HMGR1, FDP and cis-prenyl transferase. Putatively transgenic lines could be obtained from tissues infected with HMGR1 and FDP. Embryos and few plantlets could also be regenerated from these tissues. Experiments were also done to incorporate TB antigen gene for recombinant protein production. Embryos could be induced from the putatively transgenic lines obtained from leaf embryogenic calli.

4. Molecular studies

4.1. Molecular mechanism of disease tolerance

Extensive optimization experiments were carried out to amplify the promoter elements upstream to the earlier reported sequence of β -1,3-glucanase gene involved in abnormal leaf fall disease tolerance, through random amplification of the genomic DNA ends (RAGE). However, no right products were amplified. Attempts were also made to amplify the promoter elements through inverse PCR. The genomic DNA of clone RR11 105 was digested with six nucleotide blunt end cutting restriction enzyme *Ssp* I. The digested product was allowed to circularize at very low molecular concentrations. The circularized product was later linearised by another enzyme *Bgl* II. The product was later used for PCR amplification of the unknown promoter sequence. Three amplicons were obtained. They were sequenced and found to be the upstream elements of β -1,3-glucanase. One fragment was about 195 bp corresponding to the earlier reported sequence and the other two fragments are 550 and 579 bps in size.

4.2. Tissue-specific gene expression and characterization of promoters

To examine the expression pattern of *HbFDP* gene in *Hevea brasiliensis*, semi-quantitative RT-PCR analysis using total RNAs extracted from mature and immature leaves, latex and seedlings were performed. The amplified PCR products for the *HbFDP* gene were noticed in all *Hevea* tissues examined. *HbFDP* gene exhibits a differential expression pattern among tissues and the level of transcripts was most abundant in latex. This pattern of expression is likely to reflect an active role of the *HbFDP* enzyme in polyisoprene production within the laticiferous tissues during the natural rubber biosynthesis.

Attempts were made to isolate the promoter elements of HMGR 1 and FDP genes through RAGE. Genomic DNA (clone RR11 105) was digested with various six nucleotide-specific blunt end cutting restriction enzymes (*Nru* I, *Pvu* I, *Nae* I, and *Hpa* I). The digested DNA fragments were purified and ligated with adapter. After extensive PCR trials with adapter-ligated fragments of various enzyme digests, a 506 bps promoter sequence upstream to the ATG codon of HMGR 1 and 165 bps promoter sequence upstream to the ATG codon of FDP got amplified. The HMGR 1 gene promoter contains TATA box at -199 position and FDP gene promoter contains TATA box at -86 position relative to the ATG codon. For the isolation of more promoter regions of the above genes, reverse primers were designed based on the 5' upstream end of the isolated promoters.

An interesting feature was observed with HMGR 1 gene promoter with 'TA' dinucleotide repeats. An attempt was made to find out whether this nucleotide repeats have any correlation with yield. With this objective HMGR 1 promoter fragment of

various *Hevea* clones like RR11 430, RR11 414, RR11 33 and RR11 38 with varying yield potentials were amplified, cloned and sequenced. On sequence comparison, variations in number of repeats were observed but no correlation could be made with respect to yield potential. Sequences were deposited in the NCBI site under accession numbers EU 159430 and DQ 785798.

In addition to the earlier reported hevein gene promoter, another promoter sequence upstream to the intronless hevein isoform was also amplified through PCR using a specially designed reverse primer. Since a 32 nucleotide deletion of the hevein promoter was observed in certain clones and later genomic intronless isoforms were also observed, attempts were made to see whether isoforms of hevein gene promoter are also present in the same clones. In the earlier attempts the 32 nucleotide deletion was not present in the clone RR11 105. Initially attempts were made to amplify the promoter isoforms from the genomic DNA of clone RR11 105. The primers were designed in such a way that it offers specific amplification of intronless isoform of the gene. Remarkable variation in the number and sequence of nucleotides were observed between the two promoter forms. Major variation observed was a deletion of 32 nucleotides between -200 and -232 position relative to the TIC. This deletion was observed immediately upstream (six nucleotide) to the putative CAAT box motif. There are reports that the insertion or excision of nucleotides in close proximity to the CAAT box or TATA box have regulatory role in gene expression. This new version of hevein gene promoter was amplified from different clones (RR11 430, RR11 38 and PB 86) and compared.

4.3. Development of Chimeric promoter: GUS fusion constructs

In order to study the integrity and efficiency of the isolated gene promoters of hevein intronless isoform and HMGR 1, binary vector constructs were made. Different levels of hevein gene promoter (1831 bps, 1006 and 272 bps) and a 506 bp HMGR 1 promoter fragment were directionally inserted in to pCambia vector between *Eco* RI and *Avr* II sites in the multiple cloning site. Constructs were made in such a way that a GUS reporter gene was placed under the control of the isolated gene promoter fragment. Chimeric *Hevea* gene promoter:GUS gene fusion constructs were successfully transferred into *Agrobacterium tumefaciens*.

5. Isolation and characterization of TPD-induced/regulated cDNAs from *Hevea brasiliensis* by subtractive hybridization analysis and mRNA differential display

Two SSH libraries (forward and reverse) were developed earlier and a total of 352 clones which were differentially expressed were identified. Differentially expressed genes were classified into 10 functional categories according to the putative function of their homologous genes in the databases generated by BLAST analysis. Based on BLAST analysis results, putative functions were assigned to 134 clones (non-redundant cDNAs) in which (i) 57 genes were with known functions, (ii) 48 EST had matches with genes in GenBank database of unknown functions, and (iii) 29 had no matches (uncharacterized/novel). The sequences generated in this study have been deposited in the GenBank database and can be viewed on the NCBI website (<http://www.ncbi.nlm.nih.gov>). There were differences in the distribution of expressed

genes among functional classes between the healthy and TPD trees.

Seven genes (from forward library) involved in signaling (1), rubber biosynthesis (1), unknown (1), stress/defense (3) and a MYB transcription factor and 6 genes (from reverse library) representing stress/defense (3), protein metabolism (1), signaling (1) and unknown (1) were selected to validate their differential expression. Initially semi-quantitative RT-PCR was performed with them to verify whether the corresponding genes were differentially regulated. It was found that seven genes [(Myb transcription factor gene (MYB), translationally controlled tumor protein gene (TCTP), calcium-binding protein (CBP), rubber elongation factor protein gene (REF), thioredoxin H-type gene (TRX-H), F box family protein (F-box) and REF-like stress related protein 1 (RLP1)] were significantly up-regulated in healthy trees. Further, mRNA transcript accumulation for six genes [cysteine protease like mRNA (CP), PR-osmotin precursor gene (PRO), ethylene biosynthesis-related gene (EB), annexin-like protein R14 (ALP), phosphatidic acid phosphatase-related gene (PAP), ASR like protein 2 (ASR-2)] was found to be significantly higher in TPD affected trees. Subsequently, two unigenes MYB and TCTP expressed in healthy trees were selected for Northern analysis. The size of Myb and TCTP genes was 970 bp and 670 bp, respectively. As predicted from the SSH results and RT-PCR, the levels of the Myb and TCTP mRNA transcripts were higher in healthy trees. The semi-quantitative RT-PCR analysis showed that the mRNA levels of Myb and TCTP genes increased by nearly 4 and 3 folds, respectively in healthy trees. However analysis of intensity of radioactive signals in Northern blots indicated that Myb and TCTP were induced by nearly 15 and 12 folds respectively in healthy trees.

BOTANY DIVISION

Botany Division continued its research on crop improvement with greater thrust for evolving promising clones. Evaluation of potential clones for latex and timber yield and tolerance to drought and disease was continued. Investigations on propagation techniques and studies on the anatomy of bark and wood were also carried out. Hist-chemical studies in relation to tapping panel dryness were undertaken. A system of clone identification using morphological descriptors for the RR11 400 series clones was standardized. A mega project on participatory plant breeding for multi- locational on-farm evaluation of the next series of promising clones in the pipeline was initiated.

1. Evolving high yielding clones for the traditional area

1.1. Hybridization and clonal selection

The 1985 small-scale trial (SST) of RR11 400 series clones was concluded. The five

recommended clones *viz.* RR11 414, RR11 417, RR11 422, RR11 429 and RR11 430 maintained their superior yield performance over RR11 105 in the long term. Studies on disease incidence of selections under unsprayed conditions were undertaken.

Eleven clones from the progenies of 1983 hybridization programme *viz.* 83/24, 83/35, 83/29, 83/102, 83/173, 83/181, 83/372, 83/191, 83/17, 83/224 and 83/234 which exhibited higher yield compared to RR11 105 over five years of tapping in the SSTs conducted at the Kerala Agricultural University (KAU) campus in Trichur were identified for further multiplication and evaluation. Eighty promising hybrid clones from the progenies of 1986 HP evaluated in 12 SSTs at CES are being maintained in source bush nurseries.

Among the 30 hybrid clones selected from the seven SSTs planted in 1990, 15 clones continued to record superior mean yield (Table Bot.1). Twenty six clones

Table Bot. 1. Yield of the promising hybrid clones in the eighth year of tapping

Trial B		Trial C		Trial D		Trial E	
Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)
389	96.93	158	99.15	715	68.05	312	72.80
390	120.02	740	42.43	889	53.10	392	84.07
702	63.97	631	71.81	887	105.02	643	69.34
652	55.07	954	95.92	911	61.24	928	75.44
959	75.29	RR11 105	54.98	RR11 105	83.52	RR11 105	55.76
RR11 105	69.15						

Trial F		Trial G		Trial H	
Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)
698	51.93	6	86.68	140	56.13
730	83.50	65	62.58	763	63.84
776	104.73	91	45.85	799	78.80
780	87.93	114	28.91	800	82.77
788	47.29	RR11 105	69.79	RR11 105	69.30
RR11 105	90.57				

recorded better summer yield compared to RRII 105 in the respective trials in the eighth year of tapping.

In the seventh year of tapping in the 1992 SSTs, (Trials A and B) four clones viz. 772, 380, 575 and 756 continued to record higher yield than RRII 105. In Trial A, the clone 948 recorded the highest girth of 89.96 cm. The clones 772, 380 and 424 also registered above average girth. In Trial B, the promising clone 575 recorded the highest girth of 81.36 cm. Five top yielding clones were multiplied for further evaluation and maintained in the polybag nursery.

Among the 76 hybrid clones including those from W x A crosses under evaluation in three SSTs planted in 1995, 11 clones recorded better yield than RRII 105 in the fourth year of tapping. The yield and girth of some of the promising clones are given in Table Bot. 2.

Table Bot. 2. Yield and girth of some selections (4th year of tapping)

Clones	Girth (cm)	Yield (g/t/t)
Trial A		
7	69.08	74.27
27	73.98	65.03
64	70.03	67.71
79	62.35	71.02
95	63.00	66.30
102	63.31	61.55
308	62.00	65.86
309	62.13	65.44
356	68.33	62.05
RRII 105	51.75	41.24
Trial B (W x A crosses)		
10	72.46	89.09
29	77.83	56.58
34	64.82	65.73
170	64.08	50.14
193	62.58	32.36
271	71.50	51.56
RRII 105	49.64	61.01

The small-scale trials initiated in 1998 and 1999 at CES Chethackal were in the second and first year of tapping respectively. Eight clones were on par with RRII 105 in the 1998 SSTs in terms of yield while in the 1999 SSTs, nine clones recorded better yield than RRII 105.

Hybrids and half-sib seedlings resultant of hand pollination programme 2002 were test-tapped. Rubber yield (g/t/10 taps) ranged from 13.4 to 41.4. Twenty two promising yielders and their respective parents were multiplied and planting materials were raised for evaluation in clonal nursery trial. The clonal nursery of 36 hybrid clones from another set of W x A crosses laid out at CES were test-tapped. Six clones showed promising yield ranging from 15.62 to 35.72 g/t/10 taps compared to RRII 105 (15.06 g/t/10 taps).

A total of 231 hybrid seedlings from the 2005 hand pollination programme were established and maintained in the nursery for further studies and subsequent selection.

1.2. Ortet selection

Nineteen potential ortets selected from three large estates after small-scale evaluation were established in large scale trials. Among the ortets selected from PCK Estate, Kodumon in the sixth year of tapping in the small-scale trial, clone O 39 recorded the highest yield of 70.70 g/t/t with a mean girth of 78.8 cm whereas RRII 105 recorded 63.38 g/t/t and 60.9 cm (Table Bot. 3). Another set of 25 ortets selected from PCK Estate, Kodumon are in the fourth year of tapping in the SST in which mean yield of clone O 36 (50.45 g/t/t) was superior to RRII 105 (45.27 g/t/t).

Monthly yield recording of the clones of ortets selected from various smallholdings and CES Chethackal was continued in the

Table Bot. 3. Yield and girth in the sixth year of tapping of the ortet clones

Clone	Yield (g/t/t)	Girth (cm)
O 28	62.35	80.4
O 11	52.52	69.7
O 59	23.77	64.5
O 54	35.65	79.0
O 63	50.10	61.7
O 75	52.48	74.1
O 22	41.17	66.5
O 25	31.70	58.9
O 01	19.25	57.1
O 12	35.10	66.2
O 39	70.70	78.8
O 74	50.77	63.8
O 27	43.40	65.7
O 19	29.20	49.4
RRIM 600	62.17	59.7
RRII 105	63.38	60.9

third year of tapping. Data collected over three years showed that clone O 73, a selection from a smallholding at Kanjirappally with 50.98 g/t/t and O 72, a selection from the selfed progeny of RRII 105 with 53.98 g/t/t recorded yield superior to RRII 105 (41.63 g/t/t).

Table Bot. 4. Performance of clones in large-scale trial

Clone	Mean yield (7 th to 9 th year)
PB 235	75.49
PB 311	92.19
PB 280	94.96
PB 314	84.96
PB 312	85.52
PB 217	79.63
PB 260	86.70
PB 255	63.92
KRII 105	74.34
G. Mean	81.97
V.R.	6.14*
C.D. (0.05)	10.82

2. Evaluation of clones

2.1. Large-scale evaluation

The mean yield in BO 2 panel between the seventh and ninth year of tapping in the 16 introduced clones under evaluation in the large-scale trials (LSTs) planted in 1989 is shown in Table Bot. 4.

Statistical analysis of the data revealed significant clonal variation in yield over the

Table Bot. 5. Yield of the RRII 400 series clones in large-scale trial

Trial 1			Trial 2		
Clone	6 th year yield (g/t/t)		Clone	6 th year yield (g/t/t)	
	Annual mean	Summer		Annual mean	Summer
RRII 446	36.26	17.68	RRII 454	41.03	27.28
RRII 55	69.63	49.44	RRII 430	79.90	40.81
RRII 54	27.48	22.26	RRII 434	29.27	19.03
RRII 417	90.23	48.48	RRII 427	71.57	41.25
RRII 407	68.07	40.16	RRII 53	30.67	20.80
RRII 403	72.10	46.82	RRII 422	73.75	52.18
RRII 449	34.31	24.83	PB 330	73.47	43.20
RRII 429	76.69	47.65	RRII 410	60.43	43.34
RRII 402	69.00	48.33	RRII 52	57.37	47.30
RRII 453	37.26	23.07	RRII 105	70.09	42.46
RRII 414	83.12	51.02			
RRII 105	80.02	52.36			
G.M.	61.93	39.34	G.M.	58.76	37.76
V.R.	48.13**	14.61**	V.R.	14.87**	5.25*
C.D. (0.05)	14.43	9.61	C.D. (0.05)	14.43	14.65

three years in panel BO-1. Seven clones exhibited higher yield compared to RRII 105, with four clones viz., PB 311, PB 280, PB 312 and PB 260 proving to be significantly superior.

Evaluation of RRII 400 series clones in the LST indicated superiority of RRII 417, RRII 422, RRII 430, RRII 427, RRII 414 and PB 330 in terms of mean annual yield and RRII 52, RRII 410, RRII 422 and PB 330 in terms of summer yield over RRII 105 (Table Bot.5).

In the large-scale evaluation of exotic and indigenous clones, mean yield over four years showed that HP 44 (PB 242 x RRII 105) recorded 39 per cent improvement over RRII 105. Yield of the introduced Malaysian clone RRIM 722 (62.23 g/t/t) was also superior to RRII 105 (Table Bot. 6).

Table Bot. 6. Yield and girth of exotic and indigenous clones in LST

Clone	Mean yield (g/t/t)	Girth (cm) 4 th year of tapping
RRII 50	39.20	64.90
RRIM 712	43.70	58.68
RRIM 728	27.36	59.54
RRIM 722	62.23	65.41
RRII 51	35.87	64.04
HP 44	71.34	66.62
65	25.55	73.28
RRIM 600	29.60	63.14
70	24.05	63.69
180	32.50	65.90
120	51.60	73.09
RRII 105	53.00	63.09

2.2. On-farm evaluation

At Sasthamkotta, the on-farm evaluation trial was in the fourth year of tapping. Clones RRII 105, PR 255 and PB 255 performed better (Table Bot. 7).

Table Bot. 7. Mean yield of clones in the fourth year of tapping at Sasthamkotta

Clone	Mean yield (kg/ha/year)
RRII 105	2347
GT 1	1400
RRII 203	1524
PB 217	1515
PB 255	2304
PB 260	1927
PB 311	2196
PB 314	2234
PR 255	2338

On-farm trials of the RRII 400 series clones are under various stages of growth and exploitation. Clones in trials in four locations viz. Aiyemcombu, Cheruvally, Kaliyar and Koney are under tapping. The RRII 400 series clones maintained superior yield and growth compared to RRII 105 in all the locations. Girth at opening and mean yield of the clones as per estate records from two locations are given in Table Bot.8.

Other on-farm trials of the RRII 400 series clones in the immature phase are at Kanjirappally, Punalur and Kulathupuzha estates and in 20 small growers' plots. The RRII 400 series clones in all these locations recorded superior growth compared to RRII 105.

2.3. Investigations on genotype x environment interactions

Yield recording was continued in three locations viz. Kanyakumari, Agartala and Nagrakata. Clone RRII 203 continued to be the top yielder at Kanyakumari in the fourth year of tapping, followed by clones RRII 105, RRII 417 and RRII 422. The yield of clone RRII 429 was poor. At RRS Agartala, clone RRII 422 showed the highest yield (53.33 g/t/t) followed by clones RRII 417 and RRII 429. At Nagrakata, clone RRII 429 was consistently the top yielder, with significantly higher yield. Clones RRII 422,

Table Bot. 8. Girth at opening and mean yield of five RR11 400 series clones from two large estates

Clone	Cheruvally Estate		Kaliyar Estate	
	Girth at opening (cm)	Dry rubber yield (kg/ha) (mean over 4 years)	Girth at opening (cm)	Dry rubber yield (kg/ha) (mean of one year)
RR11 414	45.40	1717	52.86	1413
RR11 417	45.31	1561	53.18	1146
RR11 422	45.25	1563	48.38	1034
RR11 429	41.12	1659	54.14	1141
RR11 430	44.35	1626	50.75	1443
RR11 105	43.63	1519	40.52	1371

RR11 417 and RR11 105 were the other high yielders. The performance of clone RR11 414 was poor in both Agartala and Nagrakata. At RRS Padiyoor, trees were opened for tapping. Initial yield trends of RR11 400 series clones, except clone RR11 429 was better than RR11 105. Physiological and biochemical parameters from three locations were compiled.

3. Participatory evaluation of rubber clones

A new project on farmer-participatory research for the large-scale and on-farm evaluation of clones in the pipeline was formulated and approved. Rubber planters at various locations in the states of Kerala, Karnataka and Tamil Nadu were contacted. Thirty four planters expressed interest in the newly evolved pipeline clones. Twenty planters were selected to participate in phase 1 of on-farm evaluation programme of 20 pipeline clones. A suitable site for laying out two central research trials was selected at CES, Chethackal. The locations for initiating the satellite on-farm trials in phase 1 were also selected. Source bush nurseries of the pipeline clones were planted and maintained properly at CES, Chethackal to ensure the availability of sufficient bud wood for multiplication and supply to various locations.

4. Breeding for other specific objectives

4.1. Compact canopy

In the evaluation trial of recombinants of the cross between genetic variant and high yielding and vigorous clones such as RR11 105, RR11 600 and RR11 118 as female parents, the data revealed high variability with respect to height and canopy diameter of the trees. Three clones selected from progenies of the crosses Genetic Variant x RR11 118 and RR11 600 x Genetic Variant showed normal growth with compact canopy in the fifth year of growth. Cross combination Genetic Variant x RR11 118 recorded normal girth (39.0 cm) and height (8.5 m) and a compact canopy (canopy diameter 4.1 m) whereas the cross RR11 600 x Genetic Variant recorded 37.8 cm girth, 10.9 m height and 3.9 m canopy diameter. The control in comparison recorded the respective values of 32.5 cm, 9.6 m and 4.3 m.

4.2. Drought tolerance

Fifteen drought tolerant ortets selected from the drought-prone North Konkan region of Maharashtra are under evaluation for their yield potential in Kerala. In the second year of tapping, five of the ortet clones gave higher yield than RR11 105, of which two clones, D 111 with 98.54 g/t/t and D 95 with 61.02 g/t/t were significantly

superior to RRII 105 which gave a mean yield of 39.94 g/t/t. Among the 24 hybrid clones developed by crossing parents with high yield and various drought tolerance attributes, three clones in the second year of tapping recorded significantly superior yield compared to RRII 105. Five clones short-listed based on yield potential and physiological and anatomical parameters of drought tolerance were planted in a statistically laid out small-scale evaluation trial for *in situ* screening for adaptability to drought in the hot spot area of RRS Dapchari.

4.3. Polycross progeny evaluation

The 150 clones comprising polycross progenies of 10 parent clones evaluated in two trials for girth, dry rubber yield, volume of latex and dry rubber content on a seasonal basis are in the sixth year of tapping. In Trial 113 clones were better than clone RRII 105 which gave 59.34 g/t/t. Progeny of PB 215 recorded the highest mean yield. A high recovery of vigorous clones was obtained from within progenies of PB 217, PB 252, PB 215, PB 28/83 and RRII 105. In Trial 2, 13 selections were identified to be better performers compared to RRII 105. The highest mean yield and recovery of high yielding clones was recorded by the progeny of PB 252, followed by that of Ch 26 and PB 28/83.

Forty-eight clones selected from half-sib progenies of clones viz. PB 260, GT 1, RRII 203, PB 255, PB 217, RRII 105, PB 28/83 and RRIM 600 based on juvenile yield were planted in clonal nurseries in two locations viz. CES, Chethackal and RRS, Padiyoor. Growth of plants and juvenile yield by test incision were monitored. In general, the growth and yield of clones were better at CES, Chethackal, compared to that at the drought-prone location of RRS, Padiyoor. In terms of juvenile yield, clone RRII 105 ranked

23rd at Padiyoor (0.09 g/ plant/ test incision) and 18th at Chethackal (0.23 g/ plant/ test incision). Clones RRII 430, RRII 422 and RRII 417 were superior to RRII 105 in both the locations. Clone P 138, the progeny of PB 28/83 (0.22g/ plant/ test incision) was the best at RRS, Padiyoor, while clone P 263, the progeny of PB 255, with 0.50g/ plant/ test incision was superior at CES, Chethackal. Two clones, P209 (progeny of PB 28/83) and P 266 (progeny of PB 255) performed well in terms of juvenile yield and were among the top three rankers at both locations. These clones were superior to RRII 105 and the RRII 400 series. Twenty clones were short-listed for the study of drought tolerance parameters in the two locations.

5. Anatomical investigations

P-protein and definitive callose deposition in the sieve elements in TPD trees were studied. The P-protein appeared as a narrow elongated structure in the sieve plate of healthy tree and unaffected area of TPD trees, where as the TPD-affected area showed a thick P-protein plug in most of the sieve elements. Stimulation with ethephon triggered its intensity. The P-protein gradually disappeared, followed by the deposition of callose on the sieve plate to make the element permanently inactive. The occurrence of definitive callose was found to be more in the sieve plate of TPD-affected area than that in the unaffected area. Ethephon stimulation triggered the deactivation of sieve element through intense deposition of definitive callose. The non-functional sieve elements at a later stage showed the deposition of lignin.

A preliminary step in the localization of endophytic bacteria in *Hevea* trees was carried out by using the vital stain 2,3,5-Triphenyl Tetrazolium Chloride. In TPD affected trees the bacterial population was

found to be much less in the affected area especially in the area adjacent to the cambium compared to the unaffected area of the same tree.

5.1 Wood anatomy

In connection with documenting the clonal variations of rubber wood quality from non-traditional region, an experiment was initiated to evaluate the wood quality parameters of popular *Hevea* clones from RRS, Agartala. Seven clones viz., RRII 105, RRII 600, GT 1, PB 235, RRII 208, RRII 203 and RRII 118 planted during 1979 were selected for the study. Wood samples from a total of 56 trees were collected for testing the physical, mechanical and structural properties, in collaboration with P&PD Department.

6. Studies on propagation

Comparative growth performance of different forms of planting materials comprising brown budded plants, field budded plants, green and brown budded polybag plants, young budded plants and polyclonal seedlings showed that green budded polybag plants recorded the highest mean girth of 44.96 cm followed by brown budded polybag plants (43.87 cm) after six years of growth in the field. Field budded plants recorded significant reduction in growth. Polyclonal seedlings recorded a high girth of 56.3 cm at a height of 50 cm from the ground.

Plants raised by budgrafting using budwood collected from young stock on fresh seed stock recorded better growth,

Table Bot.9. Survival and growth of green budded plants in polybags

Treatment	Establishment success (%)	Height of plants(cm)
IBA 250 ppm	66.0	21.36
IBA 500 ppm	75.5	21.00
IBA 1000 ppm	64.5	20.32
Water	76.5	21.90
Retaining healthy laterals	64.0	19.11
Retaining no laterals	58.5	19.06

whereas plants raised from budwood collected directly from the old trees recorded poor growth in the field after one year of planting. Brown budded plants raised from the prominent semigreen buds recorded better growth and tender green buds recorded relatively poor growth after one year. In the experiment on controlling die back of green budded plants in polybags, treatment of tap root with IBA (500 ppm) and dipping tap root in water for 2-3 hours before planting in polybags were found to be effective methods for better establishment and growth of green budded plants in polybags (Table Bot.9).

7. Morphological characterization of popular clones

A study was undertaken to elucidate the discriminating morphological features of RRII 400 series clones for their early identification. A book detailing the identifying features of these clones was published and an orientation programme on identification of the RRII 400 series clones was organized for the extension officers of Rubber Board for extending information among the planting community.

GERMPLASM DIVISION

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. The major activities of the Division include 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 tolerance, timber-latex traits and molecular characterization.

1. Introduction, conservation and documentation

1.1 Domesticated gene pool (Wickham collection) from secondary centers

183 Wickham clones are being conserved in three field gene banks (one clone museum at RRII Farm, Kottayam, and two germplasm gardens at CES, Chethackal).

Annual girth and monthly yield were recorded from Gardens IV and V. Among the five IRCA clones, IRCA 130 and IRCA 111 continued to show superiority over RRII 105 (Table Ger.1) and were put in the breeding garden at RRII.

Table Ger.1. Girth and dry rubber yield of IRCA clones at the age of 14 years

Clone	Annual girth (cm)	Dry rubber yield (g/t/t)
IRCA 130	68.39	76.04
IRCA 111	68.29	58.53
IRCA 109	61.24	41.99
IRCA 230	59.09	39.85
IRCA 18	59.93	38.92
RRII 105	59.31	56.49
CD (P=0.05)	6.058	16.546

In Germplasm Garden V, clone RRIC 100 maintained its superiority among the 20 clones in terms of yield (66.6 g/t/t), followed by RRII 609 (64.3 g/t/t). Girth (84 cm) also was the highest for RRIC 100, while the control clone RRII 105 had a girth of 63.4 cm.

1.2. IRRDB (1981) wild gene pool

Wild accessions numbering 3576 are being conserved and maintained with proper identity in conservation-cum- source bush nurseries (SBN).

Re-establishment of the conservation nurseries is in progress. The fourth set consisting of 806 accessions was planted in the conservation nursery in 2006 at CES, Chethackal in a statically laid out design. These wild accessions are being screened for drought tolerance, in collaboration with Plant Physiology Division. Multiplication of another set of 500 wild accessions was carried out for planting in the next phase in 2007.

1.3. Other *Hevea* species

An arboretum comprising all the available species of *Hevea* was planted at CES for characterization, evaluation, and assessment of the extent of genetic diversity at morphological and molecular levels. This includes the four imported accessions (*H. pauciflora* and *H. nitida* from Sri Lanka, and *H. pauciflora* and *H. camargoana* from Indonesia) along with *H. benthamiana*, *H. spruceana*, an inter-specific hybrid Fx 516, five floral variants discovered earlier in the 1981 wild germplasm, and two *H. brasiliensis* clones.

2. Characterization and preliminary evaluation

Three Preliminary Evaluation Trials (PET 94A, 94B & 94C) were continued to

monitor the growth of wild accessions. Girth of the plants was recorded on an annual basis. In PET 94A, accession AC 757 (66.53 cm), was superior to RRII 105 in girth, followed by RO 895 (52.64 cm) and MT 940 (51.09 cm). All the 24 wild accessions in PET 94B recorded very low yield, compared to that of the control clone. However, accession AC 643 recorded a girth of 56.7 cm and was superior compared to other clones in the trial. Accession AC 643 also recorded the highest bole volume (0.22 m³), significantly higher than that of clone RRII 105 of the same age. Accession AC 643 was significantly superior to all others for girth increment before and after the commencement of tapping. In PET 94C, accession RO 893 (60.06 cm) and AC 465 (58.73 cm) recorded higher girth than RRII 105.

In the ortet trial PET 94D, clone RRII 105 recorded the highest girth, while OR 1130, OR 1181, OM 1124, OR 1175 and OR 1187 were on par with it. All the accessions except OR 1176 were on par for bole volume. Accession OR 1130 recorded the highest girth increment per year after tapping commenced and was superior to clone RRII 105. Five other accessions were on par with OR 1130 for this trait. In PET (Ortets) - 99, girth of the wild accessions ranged from 18.9 cm (OR 1145) to 55.2 cm (OM 1182), while that of the controls were 55.6 cm (PB 260), 55.2 cm (RRIM 600) and 57.0 cm (RRII 105). Accessions OR 1107 and OR 1149 had relatively higher girth among the wild accessions (55.1 cm and 51.7 cm respectively) and 30 per cent of the plants have attained tappable girth (>45 cm).

Annual girth recorded in PET 2000A was the maximum in accession AC 3406 (38.94 cm), followed by RO 4363 (36.50 cm) and AC 3609 (32.14 cm). In PET 2000 B with 166 wild accessions, annual girth during summer period were recorded. Accession

AC 3638 recorded the highest girth (44.45 cm) followed by RO 3669 (40.32), RO 2883 (39.85) and RO 3647 (39.08 cm). Accession AC 3318 recorded highest annual girth increment (53%) followed by X/3817 (45%) and RO 883 (43%). Girth increment during summer period was highest in accession AC 513 (27%), followed by RO 4368 (21%), AC 4261 (21%) and RO 3169 (17%). Accessions AC 542, RO 3010 and AC 341 exhibited prominent variability in terms of canopy growth and branching pattern.

In PET 2002, accession AC 567 (22.15 cm) recorded the highest girth followed by AC 1963 (19.66 cm) and RO 2165 (19 cm), while the control clones RRII 105, RRII 208 and RRIM 600 recorded a girth of 16.20, 17 and 18.10 cm respectively.

The second round of test tapping in SBN 03 confirmed that 16 accessions out of the total of 589 in the nursery, had juvenile yield greater than RRII 105, while another 16 accessions showed yield levels between 50 and 100 per cent of the controls, which can be used in hybridization programmes.

3. Further evaluation and selection

The annual girth, dry rubber yield (3rd year after tapping) and volumetric timber (bole) yield at the age of 11 years were recorded and analyzed in the PET 95. The annual girth was maximum for the accession MT 54 (62.99 cm) and minimum for MT 188 (34.32 cm). The mean girth of clone RRII 105 was 49.18 cm. Six accessions (MT 54, MT 1032, MT 1674, RO 2385, RO 1517 and MT 191) recorded significantly higher annual girth than the control, RRII 105. Seventy three accessions were statistically on par with the control clone for girth.

Among the wild accessions, the dry rubber yield average of eight tappings was maximum for MT 179 (45.75 g/t/t) and

minimum for AC 637 (1.00 g/t/t), whereas the mean yield of RRII 105 was 48.25 g/t/t. Seventeen accessions had the yield statistically on par with the control, RRII 105, of which six accessions were from Acre, three from Rondonia and eight from Mato Grosso. Thirteen accessions (AC-5, RO-3 and MT-5) showed 80-95 per cent of the yield of RRII 105 and 34 accessions (AC-7; RO-11 and MT-16) had 50-79 per cent of the yield of the control clone.

Four accessions with better performance for yield and girth and another set of four accessions with better yield and timber potential were included in the breeding garden established at RRII during 2007. The timber (bole) volume was maximum for MT 941 (0.072 m³) and minimum for RO 1739 (0.020 m³). Fifty accessions (AC-10; RO-7 and MT-33) had bole volume higher than that of clone RRII 105 (0.042 m³).

The trial was screened for the incidence of TPD. Of the 934 trees under regular tapping, 120 trees (13%) showed symptoms of TPD. Among these 77 trees showed above 50 per cent and 43 trees below 50 per cent panel dryness.

In the Further Evaluation Trial (FET) 2003, highest girth was recorded for accession RO 2629 (22.44 cm) followed by MT 2233 (17.63 cm) and MT 1012 (16.11 cm),

while the control clones RRII 600, RRII 105 and RRII 208 recorded 17.84, 16.72 and 15.94 cm respectively. In another evaluation trial 2005, juvenile growth data were recorded.

4. Screening for stress tolerance

4.1. Biotic stress resistance

Screening of the wild accessions for resistance to *Phytophthora*, *Corynespora* and *Colletotrichum* was continued in collaboration with Pathology Division. The third round of *in vitro* screening was completed and 151 wild accessions were reported to be resistant to *Phytophthora* sp. Laboratory screening for *Corynespora* resistance so far has shown tolerance in 82 out of 215 accessions.

First round of screening in SBN 2006 for *Colletotrichum* resistance indicated field tolerance in some of the accessions which, however, need further screening for confirmation.

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

In the field screening trial planted in 2003 at RRS, Dapchari with 130 wild accessions, monthly girth was recorded and the annual and summer period girth increment was worked out to assess the drought tolerance potential of these accessions (Table Ger. 2).

Table Ger.2. Potential accessions with good annual and summer period girth increment

Accession	Girth(cm)at 3 rd year	Accession	Annual girth increment (%)	Accession	Summer girth increment (%)
RO 2976	20.41	MT 1602	43.86	AC 3353	23.38
MT 1681	20.35	RO 96	37.95	MT 1655	10.0
RO 1769	18.84	MT 48	35.94	RO 3204	7.53
AC 3057	17.58	AC 4861	34.35	MT 82	5.65
RO 85	17.22	AC 3353	31.94	RO 1520	5.56
MT 1660	17.05	MT 915	31.92	AC 4861	4.76
MT 1710	17.02	RO 3660	30.52	RO 1269	4.65
RO 2152	16.74	MT 1689	30.33	RO 1540	4.32
RRII 600	14.07	RRII 600	16.40	RRII 600	4.40

These accessions were also test-tapped to assess the juvenile yield potential under drought stress. Accessions RO 1769 (33.34 g; test tap yield of 10 tappings) showed superiority with respect to juvenile yield under drought stress. Lowest yield was recorded for MT 1660 (9.47 g).

A clonal nursery evaluation of half-sib progenies (25 clones) for drought tolerance is in progress in collaboration with Botany Division, with the objective of evaluating their yield and related attributes in the drought-prone area of RRS, Padiyoor, and traditional area at CES, Chethackal. Another objective is to screen the clones for intrinsic anatomical and physiological attributes of drought tolerance. Hand pollination programmes incorporating wild drought tolerant accessions and potential domesticated clones were also initiated with the objective of developing high yielding drought tolerant *Hevea* clones. The cross combination of RR11 429 x RO 1769 resulted in successful progenies which are being evaluated.

In a collaborative project with Plant Physiology Division on rapid screening of *Hevea* germplasm lines for intrinsic drought tolerance traits, a preliminary field-level scoring was conducted in 806 wild accessions during the summer months, based on drought-related morphological parameters and 31 potential accessions were selected for further laboratory studies.

A FET of potential genotypes from wild and hand pollination progenies, identified for drought tolerance was also initiated at RRS, Dapchari in collaboration with Botany Division towards developing drought tolerant *Hevea* clones with good yield. Another experiment initiated was the evaluation of drought tolerance potential of half-sib progenies raised using the seeds from prepotent *Hevea* clones of polyclonal

seed garden at HBSS, Nettana (collaborative study with Botany Division).

In the drought evaluation trial 1996 at Rubber Demonstration Centre, Sukma with 36 genotypes, annual girth was recorded. Clone RR11 208 recorded the highest girth followed by clones RO 5430 and RR11 118.

4.2.2 Cold tolerance

A total of 64 wild accessions were screened for cold resistance in two trials at Regional Research Station, Nagrakata. Girth of the seven-year-old accessions recorded during pre- and post-winter period, showed significant variation. Higher annual girth was observed in MT 5105 (53.03 cm), RO 3204 (52.22 cm), AC 3353 (52.06 cm) and RO 2976 (50.09 cm) compared to clones SCATC 93-114 (46.49 cm) and RR1M 600 (48.44 cm) in trial 1. In trial 2, clone RO 2727 recorded the highest girth of 53.19 cm followed by MT 915 (53.03 cm) and MT 900 (51.15 cm), while the controls RR1M 600 and Haiken 1 recorded 46.83 cm and 50.02 cm respectively.

Response in growth to cold stress was assessed using the girth increment values over the stress period in the two trials. In trial 1, accession AC 3300 had the highest girth increment of 1.69 cm. The five accessions which showed higher girth values had only medium rate of growth during the stress period as indicated by their girth increments. In trial 2, the highest girth increment during the stress period was in accession RO 3043 (1.93 cm). Four accessions with higher annual girth recorded in the pre-winter phase had higher girth increment during the stress period.

5. Screening for timber characteristics

5.1. Field screening

Annual girth, bole height, bole volume, percentage of lignin and cell wall phenolics

were recorded and analyzed at the age of six years. The mean girth of wild accessions ranged from 23.42 – 36.67 cm. The maximum girth was recorded by MT 999 and minimum for AC 651. Among the six Wickham clones, RRII 33 (42.88 cm) showed the highest girth followed by PB 260 (41.37 cm), RRII 118 (40.85 cm), PB 235 (40.18 cm), RRII 600 (35.01 cm) and lowest in RRII 105 (34.26 cm). One accession (MT 999) had the girth on par with RRII 33, PB 260, RRII 118 and PB 235 and two accessions *viz.* MT 915 and MT 941 showed the girth statistically on par with RRII 118 and PB 235. Fifteen accessions had girth on par with RRII 600 and RRII 105.

Wide variability was noticed in the bole height in the wild accessions and Wickham clones. Among the wild accessions, the bole height was maximum for AC 650 (4.75 m) and minimum for MT 935 (2.31m). In

Wickham clones, RRII 105 (3.47 m) had the highest bole height followed by PB 235 (3.11 m), RRII 118 (2.89 m), RRII 33 (2.76 m), RRII 600 (2.63 m) and lowest in PB 260 (2.44 m). The wild accession, AC 650 showed significantly higher tree height than that of six Wickham clones and another accession, AC 637 had the tree height significantly higher than RRII 118 and RRII 33. Two accessions (AC 637 and MT 941) were superior to RRII 600 and PB 260 for tree height. The bole volume in the wild accessions and Wickham clones ranged from 0.01m³ – 0.03m³ and the variation was not statistically significant.

5.2. Screening for timber quality traits through lignin biosynthesis studies

The protocol for the estimation of Cell Wall Phenolics (CWP) was standardized and

Table Ger. 3 Cell wall phenolics content in wild accessions compared to the Wickham clones

Wild Accessions	CWP (μ OD/mg/dry wt. of EXR)	Cell Wall Phenolics over control clones (%)					
		PB 235 (0.310)	RRII 33 (0.295)	RRII 105 (0.304)	RRII 118 (0.307)	RRII 600 (0.319)	PB 260 (0.359)
MT 1021	0.372	+20.8	+25.9	+22.3	+21.3	+16.6	+3.6
MT 1020	0.368	+18.4	+24.4	+20.9	+19.0	+15.2	+2.4
MT 935	0.362	+16.5	+22.5	+19.0	17.9	+13.4	+1.0
MT 922	0.321	+3.4	+8.7	+5.6	4.6	+0.5	-11.0
MT 919	0.349	+12.6	+18.3	+14.8	13.8	+9.4	-2.7
MT 999	0.294	-5.3	-0.5	-3.3	4.1	-8.0	-18.0
MT 1032	0.279	-10.0	-5.4	-8.0	8.9	-12.4	-22.1
MT 915	0.318	+2.6	+7.8	+4.8	3.8	-0.2	-11.3
MT 941	0.276	-11.3	-6.7	-9.4	10.2	-13.7	-23.3
AC 635	0.297	-4.3	+0.6	-2.3	3.1	-6.9	-17.2
RO 322	0.252	-19.2	-15.0	-17.5	18.2	-21.4	-30.4
RO 255	0.338	+8.8	+14.4	+11.2	10.2	+5.9	-5.8
AC 685	0.339	+9.2	+14.8	+11.6	10.6	+6.3	-5.6
RO 879	0.460	+48.1	+55.7	+51.3	49.9	+44.1	+28.1
AC 637	0.289	-6.7	-1.9	-4.7	-5.6	-9.2	-19.3
AC 650	0.374	+20.4	+26.6	+23.0	+21.9	+17.2	+4.2
AC 655	0.329	+5.9	+11.4	+8.2	+7.2	+3.1	-8.4
AC 651	0.341	+9.8	+15.5	+12.2	+11.2	+6.9	-5.0
AC 707	0.347	+11.7	+17.5	+14.1	+13.1	+8.7	-3.4
CD (P<0.05)	0.09						

* OD – Optical density EXR – Extractive free Xylem Residue

the percentage of CWP (μ OD/mg dry weight of EXR) was estimated in the timber screening trial at RRS, Padiyoor (Table Ger. 3). One accession, RO 879 had significantly higher CWP than that of six Wickham clones. In comparison with the controls (Wickham clones) the percentage of CWP was higher in 12 wild accessions and lower in three.

The estimation and quantification of lignin and CWP were carried out in five clones of RR11 400 series. Among the five clones, lignin percentage was numerically higher in RR11 429 (21.06%) than the rest but less than that of RR11 105 (22.77%). All the five RR11 400 series clones had the CWP less than that of RR11 105 (0.307 μ OD/mg/ dry weight of EXR). Among the clones it was highest in RR11 417 and lowest in RR11 430.

5.3. Studies on variability in structure and properties of wood of *Hevea* clones

The major mechanical properties of wood such as shear strength and tensile strength were tested in 10 clones viz. PB 310, RRIM 600, RR11 45, RR11 44, PB 311, RR11 105, PB 235, PB 260, PR 255 and PR 261.

5.3.1. Radial shear strength

The maximum load bearing capacity at

radial plane of the timber of clone PB 310 was significantly higher than that of all the other nine clones studied. RRIM 600 ranked second for this character which is statistically superior to PR 261 and PR 255. All other clones are statistically on par for this trait. The maximum shear stress was also significantly superior for clone PB 310 over all the other clones except RRIM 600 which was on par with the former. RRIM 600 was also significantly superior to PB 260 for this trait.

5.3.2. Tangential shear strength

The maximum load bearing capacity at tangential plane of clone PB 310 was significantly superior to six clones viz. PR 255, PR 261, PB 260, PB 235, PB 311 and RR11 44. Similarly clone RRIM 600 was superior to PR 255, PR 261 and PB 235.

The maximum shear stress was also significantly superior for PB 310 over five clones viz. PR 255, PR 261, PB 260, PB 235 and PB 311. RRIM 600 was superior to PR 255, PR 261 and PB 235 (Table Ge. 4).

5.3.3. Tensile Strength

Among the clones studied, tensile strength at maximum load was the highest for clone PB 260 and the lowest for RR11 44.

Table Ger. 4. Shear strength properties of wood of *Hevea* clones

Clone	Radial		Tangential	
	Max. load (kg)	Max. shear stress (kg/cm ²)	Max. load (kg)	Max. shear stress (kg/cm ²)
PB 310	3120.70	125.30	2837.70	113.50
RRIM 600	2941.40	117.70	2748.30	109.77
RR11 45	2855.13	114.20	2662.80	106.53
RR11 44	2839.03	112.07	2630.20	105.23
PB 311	2827.57	112.33	2590.50	103.67
RR11 105	2819.33	113.33	2719.67	106.97
PB 235	2817.97	112.70	2473.17	99.10
PB 260	2747.03	109.90	2597.90	103.93
PR 261	2713.93	108.10	2448.60	96.00
PR 255	2710.87	108.43	2512.40	100.50
CD (P=0.05)	204.66	7.97	179.52	7.09

were recorded and analyzed at the age of six years. The mean girth of wild accessions ranged from 23.42 – 36.67 cm. The maximum girth was recorded by MT 999 and minimum for AC 651. Among the six Wickham clones, RRII 33 (42.88 cm) showed the highest girth followed by PB 260 (41.37 cm), RRII 118 (40.85 cm), PB 235 (40.18 cm), RRIM 600 (35.01 cm) and lowest in RRII 105 (34.26 cm). One accession (MT 999) had the girth on par with RRII 33, PB 260, RRII 118 and PB 235 and two accessions *viz.* MT 915 and MT 941 showed the girth statistically on par with RRII 118 and PB 235. Fifteen accessions had girth on par with RRIM 600 and RRII 105.

Wide variability was noticed in the bole height in the wild accessions and Wickham clones. Among the wild accessions, the bole height was maximum for AC 650 (4.75 m) and minimum for MT 935 (2.31m). In

Wickham clones, RRII 105 (3.47 m) had the highest bole height followed by PB 235 (3.11 m), RRII 118 (2.89 m), RRII 33 (2.76 m), RRIM 600 (2.63 m) and lowest in PB 260 (2.44 m). The wild accession, AC 650 showed significantly higher tree height than that of six Wickham clones and another accession, AC 637 had the tree height significantly higher than RRII 118 and RRII 33. Two accessions (AC 637 and MT 941) were superior to RRIM 600 and PB 260 for tree height. The bole volume in the wild accessions and Wickham clones ranged from 0.01m³ – 0.03m³ and the variation was not statistically significant.

5.2. Screening for timber quality traits through lignin biosynthesis studies

The protocol for the estimation of Cell Wall Phenolics (CWP) was standardized and

Table Ger. 3. Cell wall phenolics content in wild accessions compared to the Wickham clones

Wild Accessions	CWP (μ OD/mg/dry wt. of EXR)	Cell Wall Phenolics over control clones (%)					
		PB 235 (0.310)	RRII 33 (0.295)	RRII 105 (0.304)	RRII 118 (0.307)	RRIM 600 (0.319)	PB 260 (0.359)
MT 1021	0.372	+20.8	+25.9	+22.3	+21.3	+16.6	+3.6
MT 1020	0.368	+18.4	+24.4	+20.9	+19.0	+15.2	+2.4
MT 935	0.362	+16.5	+22.5	+19.0	17.9	+13.4	+1.0
MT 922	0.321	+ 3.4	+ 8.7	+ 5.6	4.6	+ 0.5	-11.0
MT 919	0.349	+12.6	+18.3	+14.8	13.8	+ 9.4	- 2.7
MT 999	0.294	- 5.3	- 0.5	- 3.3	4.1	- 8.0	-18.0
MT 1032	0.279	- 10.0	- 5.4	- 8.0	8.9	- 12.4	-22.1
MT 915	0.318	+ 2.6	+ 7.8	+ 4.8	3.8	- 0.2	-11.3
MT 941	0.276	-11.3	- 6.7	- 9.4	10.2	- 13.7	-23.3
AC 635	0.297	- 4.3	+ 0.6	- 2.3	3.1	- 6.9	-17.2
RO 322	0.252	-19.2	-15.0	- 17.5	18.2	- 21.4	-30.4
RO 255	0.338	+ 8.8	+14.4	+11.2	10.2	+ 5.9	- 5.8
AC 685	0.339	+ 9.2	+14.8	+11.6	10.6	+ 6.3	- 5.6
RO 879	0.460	+48.1	+55.7	+51.3	49.9	+ 44.1	+28.1
AC 637	0.289	- 6.7	- 1.9	- 4.7	- 5.6	- 9.2	-19.3
AC 650	0.374	+20.4	+26.6	+23.0	+21.9	+17.2	+ 4.2
AC 655	0.329	+ 5.9	+11.4	+ 8.2	+ 7.2	+ 3.1	- 8.4
AC 651	0.341	+ 9.8	+15.5	+12.2	+11.2	+ 6.9	- 5.0
AC 707	0.347	+11.7	+17.5	+14.1	+13.1	+ 8.7	- 3.4
CD (P<0.05)	0.09						

* OD – Optical density EXR – Extractive free Xylem Residue

the percentage of CWP (μ OD/mg dry weight of EXR) was estimated in the timber screening trial at RRS, Padiyoor (Table Ger. 3). One accession, RO 879 had significantly higher CWP than that of six Wickham clones. In comparison with the controls (Wickham clones) the percentage of CWP was higher in 12 wild accessions and lower in three.

The estimation and quantification of lignin and CWP were carried out in five clones of RR11 400 series. Among the five clones, lignin percentage was numerically higher in RR11 429 (21.06%) than the rest but less than that of RR11 105 (22.77%). All the five RR11 400 series clones had the CWP less than that of RR11 105 (0.307 μ OD/mg/ dry weight of EXR). Among the clones it was highest in RR11 417 and lowest in RR11 430.

5.3. Studies on variability in structure and properties of wood of *Hevea* clones

The major mechanical properties of wood such as shear strength and tensile strength were tested in 10 clones viz. PB 310, RRIM 600, RR11 45, RR11 44, PB 311, RR11 105, PB 235, PB 260, PR 255 and PR 261.

5.3.1. Radial shear strength

The maximum load bearing capacity at

radial plane of the timber of clone PB 310 was significantly higher than that of all the other nine clones studied. RRIM 600 ranked second for this character which is statistically superior to PR 261 and PR 255. All other clones are statistically on par for this trait. The maximum shear stress was also significantly superior for clone PB 310 over all the other clones except RRIM 600 which was on par with the former. RRIM 600 was also significantly superior to PB 260 for this trait.

5.3.2. Tangential shear strength

The maximum load bearing capacity at tangential plane of clone PB 310 was significantly superior to six clones viz. PR 255, PR 261, PB 260, PB 235, PB 311 and RR11 44. Similarly clone RRIM 600 was superior to PR 255, PR 261 and PB 235.

The maximum shear stress was also significantly superior for PB 310 over five clones viz. PR 255, PR 261, PB 260, PB 235 and PB 311. RRIM 600 was superior to PR 255, PR 261 and PB 235 (Table Ge. 4).

5.3.3. Tensile Strength

Among the clones studied, tensile strength at maximum load was the highest for clone PB 260 and the lowest for RR11 44.

Table Ger. 4. Shear strength properties of wood of *Hevea* clones

Clone	Radial		Tangential	
	Max. load (kg)	Max. shear stress (kg/cm ²)	Max. load (kg)	Max. shear stress (kg/cm ²)
PB 310	3120.70	125.30	2837.70	113.50
RRIM 600	2941.40	117.70	2748.30	109.77
RR11 45	2855.13	114.20	2662.80	106.53
RR11 44	2839.03	112.07	2630.20	105.23
PB 311	2827.57	112.33	2590.50	103.67
RR11 105	2819.33	113.33	2719.67	106.97
PB 235	2817.97	112.70	2473.17	99.10
PB 260	2747.03	109.90	2597.90	103.93
PR 261	2713.93	108.10	2448.60	96.00
PR 255	2710.87	108.43	2512.40	100.50
CD (P=0.05)	204.66	7.97	179.52	7.09

The clone PB 260 was significantly superior to nine clones for this trait and RRII 105 was statistically on par with eight clones (Table Ger.5). In general, the timber of PB 310 was superior for shearing strength and PB 260 was superior for tensile strength properties.

Table Ger. 5. Tensile strength of wood of *Hevea* clones

Clone	Max. load (kg)	TS at LP ¹ (kg/cm ²)	TS at ML ² (kg/cm ²)
PB 235	380.81	279.24	840.89
PB 260	478.16	355.89	1069.32
PB 310	373.37	296.79	833.98
PB 311	401.24	283.54	843.48
PB 255	342.69	358.93	735.26
PR 261	449.25	345.29	991.87
RRIM 600	404.99	323.42	907.19
RRII 45	362.23	242.49	830.82
RRII 44	307.17	239.57	665.12
RRII 105	359.69	315.75	862.63
CD (P=0.05)	70.97**	NS	158.84**

TS at LP: Tensile strength at limit of proportionality.

TS at ML: Tensile strength at maximum load

6. Utilization 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.

7. Feasibility of ratooning in *Hevea*

Girth was recorded in both sets of plants in the seventh year of growth. The ratoons continued to be superior to their corresponding polybag-grown plants in terms of girth. Ratoons recorded mean girth of 52.9 cm while polybag plants recorded

26.5 cm. Variability for yield has been observed, and there are indications that there could be clonal differences for response to ratooning, as in other crops.

8. Effect of stimulation in the laticiferous tissues of *Hevea*

Bark samples were collected from 10 untapped and tapped trees of RRII 105 at the age of 24 years for comparison of parameters such as bark thickness and number of laticifer rows (both functional and disorganized). The tree girth was higher in untapped trees than the tapped ones whereas the bark thickness was less in the untapped trees. The total number of latex vessel rows was also higher in tapped trees in comparison with untapped trees. The proportion of functional and disorganized latex vessels was 52 and 48 per cent respectively in the untapped trees whereas in tapping trees it was 76 and 24 per cent indicating that more number of latex vessels is getting disorganized in untapped trees than that of tapped trees.

9. Studies on the inclination and orientation of laticifers and phloic elements in *Hevea brasiliensis*

Studies on the inclination of laticifers is being continued with the objective of categorization of *Hevea* clones based on laticifer inclination and accordingly to develop suitable tapping system. Bark samples were collected from 15 mature trees from another trial to reconfirm the earlier finding of left-ward inclination of laticifers in clone PB 86.

PLANT PATHOLOGY DIVISION

Keeping vigil on the occurrence and spread of diseases and pests and research on their management are the prime activities of the Division. Etiology of TPD, utilization of microorganisms in plant growth and protection, bee keeping, waste management in rubber processing and mould-free storage of sheets are the other areas of research.

1. Leaf diseases

1.1. Abnormal leaf fall disease

The field trial carried out at Pudukkad Estate (RRIM 600) and Chemoni Estate (RRII 105), Trichur, to evaluate the effectiveness of neem seed oil (NSO) in combination with the spray oil (SO) for low volume spraying against abnormal leaf fall disease showed that the addition of NSO with SO was comparable to rubber seed oil (RSO) (Table Path.1).

Table Path.1. Effect of combination of carrier oils on leaf retention

Treatment	Leaf retention (%)	
	RRIM 600	RRII 105
NSO:SO (1:2)+ COC*	27.5	61.4
RSO:SO (1:2) + COC*	24.5	56.1
NSO:RSO: SO (1:1:2) + COC*	25.2	38.7
RSO:SO (1:2) (Control)	9.5	6.0

*6 kg COC in 40 L oil/ha

The study on the spray oil emulsion from M/s. Indian Oil Corporation (IOC), Cochin, in combination with water-based COC was carried out at Kulathupuzha Estate, Punalur. Water-based COC at the rate of 8 kg per ha was tested with different doses of IOC emulsion *i.e.* 3, 4 and 6 per cent. Leaf retention assessment indicated that the addition of 6 per cent IOC emulsion (67.5% leaf retention) was comparable with oil-based spraying (75% leaf retention).

Bordeaux mixture spraying in combination with a non-ionic adjuvant using micron sprayer at Pudukad Estate, Trichur in clone RRIM 600 did not show any positive effect on leaf retention. The field evaluation of oil-based COC in water using adjuvant @ 1 ml/L showed promising results.

In vitro bio-assay and field evaluation using the clone RRIM 600 of bio-solution from M/S Sovereign Innovations, Malaysia, against *Phytophthora*, showed that it was not effective.

Field screening on HP clones in the 1993 large-scale trials for ALF disease incidence at CES Chethackal, under sprayed conditions by visual scoring of the trees recorded highest leaf retention for RRII 55 in Trial I followed by RRII 429 and RRII 105. In Trial II, RRII 430 and RRII 422 recorded higher leaf retention.

Among the RRII 400 series clones, RRII 430, RRII 417 and RRII 422 recorded high (90%) leaf retention in Cheruvally Estate (Clone Trial 1998). In Kaliyar Estate (Clone Trial 1999) the clones RRII 430 (95%), 417 and 422 (90%) recorded high leaf retention while RRII 414 recorded 85 and 80 per cent leaf retention, respectively. In RRII Farm, Kottayam, the RRII 400 series clones under unsprayed conditions recorded more than 70 per cent leaf retention.

Mature leaves of F₁ plants of RRII 105 were inoculated with virulent strains of *Phytophthora* by detached leaf technique. Eight F₂ progenies showed tolerance to *Phytophthora* similar to that of RRII 105. RAPD analysis of isolated DNA from tolerant clones and F₂ population of RRII 105 was carried out with 90 random primers. Six primers (OPA 2, OPA 10, OPA 18, OPF 10, OPF 13 and OPO 2) showed polymorphic banding pattern with susceptible parent Tjir 1.

A total of 154 morphologically different endophytic bacteria were isolated from three rubber clones, viz., GT 1, RRII 105 and RRII 600 of which 36 showed *in vitro* antagonism against *Phytophthora meadii*.

Consortium of six efficient endophytic bacteria significantly reduced the disease intensity compared to untreated plants upon challenge inoculation of *P. meadii* on RRII 105 and RRII 600. Lesions of 0.8 to 2 cm were observed in control leaves while only very minute lesions were seen on the treated leaves of susceptible clone (RRII 600). Leaves of untreated tolerant plants (RRII 105) also showed minute lesions.

1.2. Powdery mildew disease

A trial was initiated at New Ambadi Estate, Kanyakumari to evaluate crop loss due to powdery mildew disease in clone RRII 105. Pre-treatment application of sulphur was undertaken at the recommended dose at an interval of 7 to 10 days using Micron power duster. Intensity of powdery mildew was low during the season. Possibility of using water-based fungicides for the control of powdery mildew disease was attempted in another trial using a 5.5 HP mist blower.

1.3. Colletotrichum leaf disease

Higher doses of fungicides at various spraying frequencies were evaluated on the first year plants of clone RRII 105 at TR& T Estate, Mundakayam. Mancozeb (Indofil M-45 and Carbendazim (Bavistin) at normal dose (7 day interval) and double dose (14 and 21 day interval) were sprayed. The results indicated that by increasing the dose, the interval cannot be extended beyond 14 and 7 days, which is reflected in the disease intensity on mature and light green leaves respectively (Table Path. 2).

The addition of non-ionic adjuvant (APSA 80) with recommended fungicides did not improve the disease control significantly.

Field screening of 806 accessions (4030 plants) in the Source Bush Nursery (2006) at CES Chethackal for Colletotrichum leaf disease incidence was carried out by visual scoring on a 0-5 scale which indicated 161 accessions in grade 0 (no disease), 237 accessions in grade 1 (very few spots on very few leaves), 84 accessions in grade 2 (few spots on many leaves), 55 accessions in grade 3 (many spots on many leaves), 131 accessions in grade 4 (heavy spotting, crinkling of lamina and leaf tip drying on many leaves) and 138 in grade 5 (heavy

Table Path. 2. Effect of fungicide dose and interval on Colletotrichum leaf disease

Treatment	Disease intensity (%)							
	Mature leaves				Light green leaves			
	7 days	14 days	21 days	Mean	7 days	14 days	21 days	Mean
Carbendazim	0.6	1.1	2.6	1.4	0.5	1.5	3.0	1.7
Mancozeb	1.4	0.9	1.3	1.2	0.3	2.0	2.5	1.6
+ Carbendazim	1.5	0.4	1.9	1.3				
Propiconazole	1.6	2.8	1.8	2.1	0.8	2.9	1.6	1.8
Mean	1.3	1.3	1.9		1.5	3.5	1.1	2.0
					0.78	2.5	2.05	

CD ($P=0.05$) for treatments = 0.60

CD ($P=0.05$) for intervals = 0.52

Interaction = 1.05

Treatments not significant

CD ($P=0.05$) for intervals = 0.49

Interaction = 0.98

spotting leaf fall, more than half of the leaf area drying).

1.4 *Corynespora* leaf disease

Endophytic bacteria from GT 1, a tolerant clone to *Corynespora cassiicola*, were tested for bio- efficacy to detoxify the phytotoxin from *C. cassiicola*. The culture filtrates of the pathogen and bacteria were mixed in different ratios and inoculated to the leaves of RR11 105, a susceptible clone. One isolate was effective in detoxifying the toxin (at 1: 3 concentration).

Out of the 185 Hevea germplasm accessions screened in the laboratory, 81 were showing tolerance to *Corynespora* and were short-listed.

Both tolerant (GT 1) and susceptible clones (RR11 105) artificially inoculated with *C. cassiicola* showed symptom expression at 96 hours. Chitinase assay was carried out with the leaf samples collected from 24 hours to 192 hours (at 24 hour-interval) after inoculation. A marked rise in enzyme activity was observed at 96 hours onwards up to 144 hours. The decline of enzyme activity was observed at 168 hours in tolerant clone GT 1 and no increase was observed in susceptible clone (RR11 105). The cloned chitinase gene from GT 1 on blast analysis showed homology to chitinase gene sequence reported earlier from rubber.

Among the endophytes isolated from *H. brasiliensis*, 42 isolates showed *in vitro* antagonism against *C. cassiicola*. Seedlings treated with consortium of selected antagonistic isolates showed resistance to *C. cassiicola* infection. The leaves of treated seedling on *in vitro* inoculation showed lesion size of 0.1 to 0.2 cm whereas that of control were 0.8 to 1.6 cm.

A consortium of efficient endophytic bacteria was evaluated at field level for the

control of *C. cassiicola* at Sheradi, Karnataka using four-year-old plants of RR11 105. The disease incidence was generally low in the experimental area. The plants treated with bacteria refoliated faster than control plants. Comparatively more infection was observed in control treatments.

Endophytic bacteria were localized in the intercellular spaces of various tissues using 2, 3, 5-triphenyl tetrazolium chloride staining (TPTZ). The bacterial cells stained dark red to purple. Green fluorescent protein (Gfp) was cloned in endophytic bacteria. Confocal scanning revealed the colonization of Gfp cloned bacteria in the tissues.

A rapid and reliable method of identification (at genus level) of *Alternaria alternata* causing Alternaria leaf spot and *C. cassiicola* was developed using sequence characterized amplified region (SCAR) markers as it is difficult to distinguish between them due to similarity in the symptoms they produce and in the season of occurrence. Six pairs of SCAR primers, derived from unique RAPDs were successfully used in diagnostic PCR to discriminate between the pathogens. This technique could detect the pathogens when the fungal DNA concentration was as low as 1 ng in standard PCR reaction volume of 25 µl. DNA samples from diseased lesions (0.2 to 0.5 cm diameter) were successfully used in the identification of these pathogens using SCAR markers. This molecular tool is useful for epidemiological studies on Alternaria leaf blight and *Corynespora* leaf fall diseases in rubber.

The toxin from *Alternaria* was tested using leaf wilt bioassay and leaf puncture bioassay. The clones GT 1 and PB 260 showed tolerance and the clones RR11 105 and RRIM 600 showed susceptibility to the toxin. Irrespective of the clone, light green stage of the leaf was most susceptible to the toxin.

Thirteen fungicides (systemic, contact and their combinations) were evaluated *in vitro* for the control of *Alternaria* leaf blight pathogen. As the pathogens *Corynespora* and *Alternaria* occur together in the field and produce similar symptoms, the sensitivity of both these pathogens to different fungicides were tested. The systemic fungicides propiconazole (Tilt) and tridemorph (Calixin) were effective in controlling the growth of *Alternaria*. Carbendazim (Bavistin) was efficient in controlling the growth of *Corynespora*, but had very little inhibition on the growth of *Alternaria*. The systemic fungicides hexaconazole (Contaf) and difenconazole (Score) were efficient in limiting the growth of both *Alternaria* and *Corynespora*.

AFLP analysis of 60 *Corynespora* isolates collected from different regions of South India during 2000-2007 and their pathogenicity studies showed a great deal of variations among the isolates.

Two isolates of *Corynespora* were studied for their response to the systemic fungicides, carbendazim (Bavistin) and hexaconazole (Contaf) by supplementing (10 and 50 ppm) in their growth media. Growth measurements were recorded for up to 10 generations. DNA was extracted after each generation and used for genetic characterization. There was no genetic variation in the isolates subjected to the fungicides even after passing through 10

generations indicating that the chances for build up of resistance to these fungicides on repeated spraying in the field are low.

1.5. *Cylindrocladium* leaf disease

Cylindrocladium disease incidence in different clones planted at Kaliyar Estate was recorded. The clone RRII 410 showed higher disease incidence followed by RRII 414 and it was mild for all other clones. The isolate was identified as *Cylindrocladium reteauii* by International Mycological Institute (IMI).

Five fungicides were screened against *Cylindrocladium* using poisoned food technique. Among the fungicides mancozeb + carbendazim (SAAF) was found to be the most effective with 100 per cent inhibition at 500, 750 and 1000 ppm (Table Path. 3).

Infected leaves collected after disease season and during wintering period were stored in the laboratory (30-32°C) and tested for viability of the fungus at monthly intervals. The fungus could be isolated from the diseased leaf up to seven months after collection.

ERIC PCR based genomic finger printing of *Cylindrocladium* isolates from rubber (collected during 2005 and 2006), clove and *Eucalyptus* (obtained from KFRI, Peechi) generated a total of 12 bands ranging from 200 to 2000 bp. Comparison of banding pattern revealed that all the five isolates were similar.

Table Path. 3. Efficacy of fungicides against *Cylindrocladium* (% inhibition)

Fungicides	Concentrations tested (ppm)								
	25	50	100	200	250	400	500	750	1000
Difenoconazole	—	42.30	43.33	—	51.33	—	52.30	53.71	—
Propiconazole	—	64.52	69.23	—	70.51	—	83.33	85.26	—
Mancozeb + carbendazim	58.05	—	62.21	—	62.85	64.6	100	100	100
Copper oxychloride	—	25.35	25.35	28.34	—	29.22	—	40.55	42.48
Phosphorous acid	—	—	13.31	—	18.32	—	22.09	26.12	38.12

2. Stem diseases

2.1. Pink disease

The experiment in TR&T Estate, Mundukayam in RR11 105 indicated that the systemic fungicide difenconazole (Score) (2 ml/L) was comparable to recommended fungicides. The Bio-formulation (supplied by M/s Plantrich, Kottayam) was not effective.

2.2. Bark rot disease

A new panel protectant compound from M/s. Anna Industries, Cochin was tested and recommended for use.

2.3. Patch canker disease

Field trial on patch canker disease control was continued at Lahai Estate, Pathanamthitta in PB 28/59. Higher recovery of plants was noticed with the fungicide Cymoxanil M8 (Curzate M8) compared to Chlorothalonil (Kavach), azoxystrobin (Amistar), Mancozeb (Indofil M-45) and metalaxyl MZ (Ridomil MZ).

3. Brown root disease

In the brown root disease control experiment at Babu Land Estate, Kanyakumari, the fungicide drenching was undertaken for the second year with tridemorph (Calixin 0.5%), propiconazole (Tilt 0.2%) hexaconazole (Contaf 0.04%) and thiram (Thiride 1.5%). The spread of the disease was not observed in any of the fungicide treatment. In the evaluation of bio-control agents against brown root disease at Eachipara division of Chimony Estate, Trichur, second year application of the bio-agents *Trichoderma* and rhizobacteria was carried out. The disease incidence was not observed in any of the treatments.

4. Maintenance of pathogens

4.1. Long-term preservation of fungal cultures

Long-term storage of plant pathogens is important to maintain cultures for research

and identification purposes. Five different techniques were employed with *Corynespora* and *Phytophthora* to identify the best method of long-term preservation. Viability and virulence of the fungus were assessed at periodic intervals after subjecting to the treatment up to three years. The techniques adopted were (1) continuous growth method, (2) immersion in sterile distilled water, (3) desiccation, (4) cryopreservation and (5) lyophilization. For storage of *Corynespora*, cryopreservation and immersion under water were the most efficient up to two years but for *Phytophthora* storage under sterile water was efficient up to six months only.

5. Tapping panel dryness (TPD)

Screening for the presence of LMW-RNA showed that irrespective of the symptom and clone the LMW-RNA was present in the TPD affected plants (Table Path. 4).

Table Path. 4. Presence of LMW RNA in plants with different symptoms

Symptom	No. tested	LMW- RNA	
		Positive	Negative
Cracking	20	15	5
Cracking + bulging	15	15	0
Scaling	20	12	8
Sloughing	20	15	5
Necrosis	20	16	4

For studying the transmission of LMW-RNA, 250 seeds each from TPD affected and apparently healthy trees were collected and planted in polybags after monitoring TPD status of the source tree for two years. Transmission through budgrafts was studied and the results (evaluated by R-PAGE) showed that all the plants tested from the group in which both stock and scion were viroid +ve, showed viroid bands in R-PAGE. Preliminary studies on the

infectivity test showed epinasty of indicator plants on which total RNA isolated from TPD affected trees were inoculated.

Attempts were made to characterize the LMW-RNA through cloning and sequencing. RT-PCR of total RNA extracted from diseased and healthy samples and gel band purified RNA were done using random hexamers and viroid-specific primers. PCR amplification of the cDNA was done with random hexamers and viroid-specific complementary primers. When primers of Posp. viroid group specific primers and PSTVd specific primers were used, products in the range of viroid were observed only in TPD trees which were cloned and sequenced. The results of blast (NCBI) gave two types of results: one viroid-specific and another non-specific hits. Based on the viroid-specific sequences obtained, a set of abutting primers to cover the upper right and lower half of the viroid RNA were designed and used in RT-PCR reaction. Amplification of a product of 300+bp was consistently obtained in R-PAGE positive TPD affected samples and was always absent in R-PAGE negative healthy samples.

A large-scale study on the incidence of TPD in clone RR1105 in smallholdings at Pala region was conducted using 21000 trees under various stages of tapping. The study

showed an increasing trend in the incidence of TPD from BO 1 panel to B1 2 panel. An increase in DRC (%) with increase in intensity of TPD was noted. The number of TPD trees in clusters was found to be higher compared to single trees as the year of tapping progressed (Fig. Path. 1).

Observations on the incidence of TPD in various regions like Kanjirappally, Adoor, Nedumangad, Thaliparampa and Mannarkkad were completed. In order to study the seasonal effect on the incidence of TPD a detailed study has been initiated in Tripura and Assam.

A study was conducted in various estates in Nagercoil region of Tamil Nadu to estimate the incidence of TPD in different clones (RR1105, RR11600, PB 28/59 and GT 1) in estate sector. Irrespective of the clone, there was an increase in the incidence of TPD from BO 1 to B1 2 panel. However, when individual panels are taken into account, the increase in the incidence within a period of 16 months was not very significant with respect to RR11600 and PB 28/59.

5.1. Characterization of TPD in CUT panel

This study was conducted at Malankara Estate, Thodupuzha. A total of 10 blocks were selected which comprised of three clones (RR11118, GT 1 and RR11600) belonging to different age groups and under different years of exploitation in the CUT system. Twenty five percentage of the trees with TPD in the basal panel continued to be TPD affected in the CUT panel also. Nearly 40 per cent of the trees were healthy in the basal panel and continued to be healthy in CUT also. There was a considerable variation in the remaining 35 per cent of the trees. Some of the trees which were healthy in the basal panel, became affected by TPD in the CUT

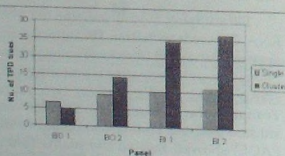


Fig. Path. 1 Distribution (in cluster and at random) of TPD trees at different stages of tapping

panel while some of the TPD affected plants in the basal panel did not show any TPD symptoms in the CUT panel.

6. Pests of rubber

In a field trial, the arrowroot powder (*Curcuma zedaria*) at 10 per cent gave 100 per cent control of termites followed by *C.aeroroginosa* which gave 82.75 per cent control, for six months

Lamdacyhalothrin 0.005 per cent spray gave 95.71 per cent control of bark feeding caterpillars followed by deltamethrin 0.0056 per cent giving 77.14 per cent control. Among the eco-friendly materials, IOC agro spray 10 per cent gave only 29.30 per cent control of caterpillars. The entomopathogen, *Beauveria bassiana* infected 20 per cent of the pupal population of the caterpillar.

Two applications of carbaryl 0.50% + lamdacyhalothrin 0.05% at an interval of one week was the most effective treatment for the management of borer beetles affecting standing rubber trees (Table Path. 5).

Table Path. 5. Effect of insecticide combination and IOC oil for control of borer beetles on rubber trees

Treatment	Control of borer (%)
Carbaryl 0.50+Cyper methrin 0.25%	68.50
Carbaryl 0.50+Deltamethrin 0.028%	75.50
Carbaryl 0.50+ Lamdacyhalothrin 0.05%	97.00
Carbaryl 0.50 + Quinalphos 0.25%	87.00
IOC Agrospray oil 10%	5.75
CD	5.98

A field trial at RR11 for the control of mooply beetle, *Luprops curticolis* showed 88 per cent mortality with deltamethrin (Decis) 0.0056% followed by quinalphos 0.1%. Eco-friendly applications such as neem gold (0.3%), spray oil (6%) and entomopathogenic

fungus *B.bassiana* were on par (9-14% mortality) and were not as effective as chemical treatments.

Soil samples from different rubber growing areas of Kanyakumari, Pathanamthitta, Ernakulam, Palakkad and Kozhikode districts were tested for the presence of entomopathogenic nematodes (EPN). The larvae of greater wax moth, *Galleria mellonella* was used as bait for the isolation of EPN. In nine soil samples out of 60, the baited larvae picked up the infection of nematodes. The larvae were dead within 2-3 days of baiting. The nematodes were confirmed to be entomopathogenic as they invaded and multiplied within rebaited wax moth larvae. Pathogenicity of the indigenous isolate was also tested by using wax moth larvae.

Intensity of infestation of plant parasitic nematodes in healthy and TPD-infected plants showed 12 per cent increase in their incidence in TPD-infested plants than those in healthy plants. Clonal variations in nematode incidence was also noticed by collecting root samples from different clones such as RR11 105 and GT 1. Incidence of nematode infestation recorded in CT 1 was 28 per cent less compared to RR11 105.

7. Bee keeping in rubber plantation

The gross cost, gross income and annual net income per hive for *Apis cerana indica* were Rs.785, Rs.1156 and Rs.371 respectively. The annual net income per hive to the bee keepers who had utilized the family labour was Rs.736. The average honey yield per hive was 12 kg with a range of 7-17 kg per hive. For *Apis mellifera* the gross cost, gross income and annual net income per hive were Rs.1590, Rs.2598, and Rs.1008 respectively. The annual net income per hive to bee keepers who have utilized family labour was Rs.1593. The average honey yield per hive

was 30 kg with a range of 28-35 kg per hive. The total honey production in rubber sector was estimated to be 3750 tonnes.

Evaluation of eight-frame standard bee hive and six-frame Janata bee hive for *A. cerana indica* colonies showed eight frame standard beehive to be less beneficial as honey yield was less (7.5 kg/hive) compared to six frame Janata bee hive with a higher honey yield (10.80 kg/hive). The demand for the eight-frame colony also was found to be less compared to the others.

The availability of different bee forage plants helps the bees for their growth and development and the bee keepers can reduce the artificial feeding in the off season/dearth period. Nineteen different plants were identified in and around the rubber planting areas as good bee forage plants during off season.

A field study was conducted to find out the effect of artificial feeding on the brood rearing activity of Indian honey bee, *A. cerana indica* using twelve colonies of similar strength. The feedings were given at an interval of five days. Comb and brood area was measured before and after 20 and 40

days of feeding. The brood rearing activity was found to be more (82%) in honey bees fed with sugar syrup containing vitamin C and turmeric powder than that of control. The percentage increase in brood development in honey bees fed with sugar syrup containing only vitamin C was 73 per cent. The brood development in control and bees fed with sugar syrup+ turmeric powder were on par (Table Path. 6).

9. Microorganisms for improving growth of rubber and cover crops

Three selected isolates of root nodulating bacteria of *Mucuna bracteata* from north eastern states were studied for their single and combined inoculation effect. *M. bracteata* plants inoculated with the Agartala isolate showed higher shoot weight, root weight and root volume while that with Tura isolate had more shoot length and number of nodules. Combined inoculation of the three north eastern isolates did not show any superior effect than their single inoculation.

The effect of *Azospirillum* cultures on root development of young rubber seedlings was studied. Root branching, volume and

Table Path. 6. Effect of artificial feeding on the brood rearing activity of *Apis cerana indica*

Treatment	Total average area before feeding (sq.cm.)		Total average area after four feedings(sq.cm.)		Average development percentage (sq. cm.)		Total average area after eight feedings		Average development percentage (sq.cm.)	
	Comb area	Brood area	Comb area	Brood area	Comb area	Brood area	Comb area	Brood area	Comb area	Brood area
Sugar syrup alone (control)	454.0	363.6	543.3	401.6	19.67	10.7	649.67	476.7	43.0	31.1
Sugar syrup + Vitamin C	465.0	325.3	585.0	422.0	25.6	19.5	680.3	500.0	46.2	53.4
Sugar syrup + Vitamin C + Turmeric powder	405.0	246.0	54.0	306.0	33.6	24.4	626.0	385.0	54.6	56.5
Sugar syrup + Turmeric powder	442.0	286.0	533.0	352.0	20.5	23.1	632.0	380.0	43.0	32.9

weight were found to be high for inoculated plants compared to control. The rubber seedlings inoculated with different phosphofungi showed better growth than uninoculated plants. More shoot growth was recorded for plants inoculated with phosphofungus isolate 6 while more root growth was for phosphofungus isolate 9. Phosphofungal population in the soil was found to increase upon inoculation. The phosphofungi reisolated from the different treatments were morphologically similar to the inoculated ones. Available phosphorus content was more in soil inoculated with isolate 9, while P content in plants was more when inoculated with isolate 6.

Rhizosphere colonizing and endophytic bacteria collected from root, stem and leaf of different clones of rubber from different locations were tested for their growth promoting activity on young rubber seedlings. A total of 178 isolates were inoculated and after 45 days various growth parameters *viz.*, length, weight, volume and branching of roots, height and girth of shoots and number of leaves were recorded. Isolates which showed more response in these parameters were selected for further screening.

A study on the effect of different stock scion combination on rhizosphere and endophytic microbial population was carried out using five clones *viz.*, RR11 105, GT 1, GI 1, RR11 203 and RR11 118 planted in 1984 at CES, Chethackal. Microbial population was found to vary in the rhizosphere of different clones and also in plants raised on own stocks as well as assorted stock. Among the plants raised on own stock, RR11 118 showed high fungal and bacterial count. The beneficial bacteria like phosphobacteria and nitrogen fixers were also high in the rhizosphere of this clone. Among the assorted stock, RR11 203 showed

more bacterial population than RR11 118. The phosphobacteria solubilized tricalcium phosphate efficiently while solubilization of aluminium phosphate and ferric phosphate was poor. The endophytic bacterial population of plants raised on own stock and assorted stock did not show significant difference. The population ranged from 8×10^5 to 34×10^5 cfu/g root and the highest population was shown by RR11 203 on own stock. More mycorrhizal infection (97%) was recorded for RR11 105 on assorted stock than plants with their own stock (85%). The roots of GT 1 on assorted stock had less mycorrhizal infection (78%) than plants on own stock (97%). Out of the 71 rhizosphere and endophytic bacterial isolates, 19 were antagonistic to the growth of the root pathogen *Phellinus noxius*.

10. Waste management in rubber processing

A prototype 1.4 m³ High Rate Reactor was installed at Elavampadom Model RPS, Chittady, Palakkad and the performance was evaluated. The average COD reduction efficiency was 72.78 per cent. The total gas production was 25.0 L per h. initially and was maximum (81.3 L/h.) on the 24th day of treatment. Generally, the gas flow was more in the afternoon and evening hours. The CO₂ percentage was 34-36 per cent and the methane content was 60-66 per cent. The pH of the effluent ranged from 5.22 to 5.31 and the pH of the treated effluent from 5.90 to 6.08 on twentieth day of the treatment. The pH remained almost constant in the range of 5.91 to 6.01 during other days.

The physicochemical and biological characterization of the effluent samples from outlet and inlet of the treatment plant is given in Table Path.7. Among the total anaerobes, sulphate-reducing bacteria were found to be

Table Path. 7 Physicochemical and biological characterization of the rubber effluent after biomethanation

Parameter	Rubber effluent	Inlet	Outlet	Percentage of reduction
pH	5.14	5.12	5.95	-
EC (dS/m)	5.60	5.42	3.20	-
TDS (mg/L)	5800	6718	2380	64.50
TSS (mg/L)	1020	1060	980	7.50
TS (mg/L)	6820	7778	3360	56.80
COD (mg/L)	16800	15600	7400	52.50
BOD (mg/L)	5800	5300	2800	47.16
Total anaerobes (per ml)	6×10^4	8×10^4	13×10^4	-
Methanogens (per ml)	3×10^2	4×10^2	7×10^2	-
Methane content (%)	62	-	-	-

predominant in the roll tube compared to others.

The Upflow Anaerobic Sludge Blanket (UASB) reactor was operated at 24 h HRT up to 22 days including the start up period to study the COD and BOD removal efficiency and biogas production. The COD and BOD removal efficiency was around 88 per cent. The pH value of the treated effluent during the steady state period ranged between 6.49 to 6.51 on the 18th day to 22nd day. The EC level of the treated effluent varied between 2.27- 2.32 dS/m. During the steady state period, the total solids (TS) removal was 81.9 to 83.7 per cent. The biogas production ranged from 3.5-3.9 L/L efficient per day. During the steady state period, the methane content was around 60.4 to 61.0 per cent. In the steady state period the total anaerobic population increased in the range of 39.0 to 42.0×10^2 per ml. The methanogenic population also increased as that of the total anaerobes and it was 29×10^2 per ml on the twentieth day.

As the reactor reached steady state under 24 h HRT, it was allowed to run for four days. Then the HRT of the reactor was changed to 12 h with same COD concentration of the feed. The COD removal ranged from 54-79 per cent during the 10 days of operation between 23rd day to 32nd

day with maximum COD removal 67.0 per cent on the 30th day.

During the first few days of the reactor performance at 12 h HRT the BOD removal decreased gradually and ranged between 76 and 56 per cent on the 23rd day to 24th day. Thereafter the BOD removal increased and reached 65 per cent on the 30th day.

The pH value decreased and reached 6.51 on the 23rd day. Thereafter the pH value gradually increased and reached 6.05 at 32nd day of the reactor performance at 12 h HRT. The EC value of the treated effluent ranged between 2.63-3.86 dS/m on the 23rd day to 32nd day.

The TS was measured in the treated effluent at 12 h intervals. As there was a change in HRT, a reduction in removal of the TS was noticed. The TS reduction was 61.1 per cent on the 23rd day and gradually decreased to 54.2 per cent on the second half of the 24th day. Thereafter TS removal gradually increased and reached 62.6 per cent on the first half of the 30th day.

There was a reduction in biogas production when the HRT was changed. The methane content showed a slight decrease when the HRT was decreased to 12 h. The total anaerobic population decreased to 31×10^2 /ml on the 32nd day. The methanogenic population also showed similar trend.

The effect of formalin in effluent treatment was studied along with other commonly used antimicrobial agents *viz.* Dettol, Harpic and Lysol. Formalin did not support the microbial growth in effluent from composite and aeration tanks while Dettol and Lysol showed the growth in five per cent and one per cent concentration.

Study on the effect of antimicrobial agents on the soil microflora showed that bacteria, fungi and actinomycetes were inhibited at all concentrations of formalin. Dettol, Lysol and Harpic did not have any effect on soil fungi and bacteria.

10.1. Characterization of effluents in PCRf

The pollution load of the rubber effluents generated in the PCRf located inside the RRRII campus was assessed (Table Path.8). Rubber effluents collected from different stages of treatment were found to be acidic in nature. Various parameters like TS, TDS, TSS, COD, BOD, pH, sulphide, coliform and *Escherichia coli* and the total bacterial populations were estimated. The content of all these parameters were very high at the initial stages of treatment in milling, composite and scrap effluents samples. Thereafter, these contents got reduced drastically after the aeration and was within the safe limit in the treated and filtered water. The total bacterial counts were also very high in all the samples and reduced gradually as the treatment progressed. Similarly coliforms and *E. coli* were present in all these samples irrespective of treatment.

10.2. Development of an antimicrobial agent for NR latex processing

Five chemicals were tested to find out an effective antimicrobial agent as a substitute of formaldehyde solution in latex processing. Dettol, Lizol and Harpic at 2 per cent concentration were found to be effective in suppressing the bacterial growth, while

Table Path.8 Pollution load of effluents at different stages of treatment

Effluents	Parameters (mg/L)				
	TS	TDS	COD	BOD	Sulphide
Milling	1753	1458	1104	534	96
Composite	1414	980	1075	425	80
Scrap	1183	866	788	306	61
Aerated	293	207	147	84	51
Sediment	224	155	53	23	44
Filtered	158	76	38	17	30
Treated water	55	64	25	11	28
Tap water	40	50	20	8	16

0.5 per cent concentration of formalin was sufficient.

10.3. Biomethanation from sheet processing effluent and its CDM potential

The recovery of methane from the rubber processing waste water is eligible for earnings under CDM. Based on the methane recovery from the RSS processing effluent in the RPS, a CDM project on bundled methane recovery has been developed involving 25 group processing units. The project activity is to recover the methane from the waste water by substituting the aerobic waste water treatment with anaerobic system for methane recovery and combustion.

The project activity will result in saving electricity which can be used for aerobic waste water treatment and the replacement of firewood (non-renewable biomass) used for drying of rubber sheets.

The project will lead to mitigation of emission of CO₂ by avoiding the use of electricity (mainly generated by using fossil fuels) as the waste water treatment under the project will switch over to anaerobic treatment from aerobic and the gas thus generated and captured will partially replace the firewood used for drying of sheet rubber. The estimated amount of emission reduction per year would be 758 CER.

11. Post-harvest storage of sheet rubber

Forty one bacteria were isolated from different rubber surfaces viz., cup lumps, scrap rubber, dried sheet rubber and latex. Ten of them were antagonistic to the major contaminant moulds isolated from stored sheet rubber namely *Penicillium* spp., *Aspergillus* spp., and *Trichoderma* spp. Among these, two isolates showed high level of antagonism.

Talc-based bioformulation prepared with the two efficient isolates was evaluated by applying it on sheet bails stored in the godowns of Mundamchola Estate, Nilamboor and Mundamchola Estate, Meenangadi, Wynad, Kerala. No mould growth was observed on the treated sheets in these locations even after six months of storage and the quality of the sheet was not affected.

PLANT PHYSIOLOGY DIVISION

The major research areas in Crop Physiology Division include environmental physiology, physiology of growth and yield, stock-scion interaction, tapping panel dryness, secondary metabolites and ecological impact of NR cultivation.

1. Environmental Physiology

1.1. Physiological adaptation to high light and drought stress

The photosynthetic CO_2 assimilation rate (A) and effective quantum yield of PS II (Φ PSII) were studied in irrigated and drought-stressed plants (withholding irrigation for 21 days in polybags) of five clones (RRII 105, RRIM 600, GT 1, PR 255 and RRII 208) during the summer months at RRII, Kottayam. Drought reduced 'A' significantly in all these

five clones. The clone RRIM 600 was found to be relatively tolerant as the rate of reduction of A and Φ PSII was smaller (Table Phy.1). The clones GT 1 and RRII 208 were shown to be moderately drought tolerant. Both A and Φ PSII were drastically reduced in RRII 105 and it was proved to be a drought susceptible clone.

Chloroplasts were isolated from irrigated and drought-imposed plants belonging to five clones after imposing severe drought and high light stress during the month of March 2007 by withholding irrigation in polybags. SDS-PAGE analysis of chloroplast polypeptides revealed that a 23 kDa stress protein appeared only in the early drought phase (till 21 days of withholding irrigation). The drought

Table Phy. 1. CO_2 assimilation rate and effective quantum yield

Clone	Photosynthesis 'A' ($\mu\text{mole m}^{-2}\text{s}^{-1}$)		Quantum yield (Φ PSII)	
	Irrigated	Drought - stressed	Irrigated	Drought -stressed
RRII 105	8.9 \pm 0.46	1.5* \pm 0.11	0.45 \pm 0.025	0.25* \pm 0.01
RRIM 600	9.1 \pm 0.87	3.7* \pm 0.25	0.44 \pm 0.03	0.35 \pm .01
GT 1	9.0 \pm 0.55	3.1* \pm 0.12	0.44 \pm 0.034	0.28* \pm 0.02
PR 255	8.7 \pm 1.00	2.7* \pm 0.10	0.48 \pm 0.04	0.30* \pm 0.015
RRII 208	9.7 \pm 0.92	2.6* \pm 0.80	0.4 \pm 0.04	0.27* \pm 0.05

n = 6, * Significant at 0.05%

tolerant clones, RRIM 600 and RRII 208 showed a prominent appearance of this protein in the chloroplast. However, after an intensive drought imposition for 30 days, this stress protein disappeared along with other PS II proteins from the SDS-PAGE analysis. This result indicated the occurrence of typical defense mechanism against drought in chloroplasts.

1.2. Identification of the molecular basis for drought tolerance

Differential display analyses were made to study the expression of genes under drought stress through RT-PCR method. A total of 101 differentially expressed transcripts were PCR-amplified and cloned into PCR-TRAP cloning vector and the selected clones were sequenced. Out of the 101 transcripts sequenced, 32 showed homology with known genes. The remaining transcripts were novel and might be specific to *Hevea*. The sequence data of 18 transcripts have been submitted to Genbank database.

1.3. Gene expression under different physiological stress conditions

As genes which were not responding to drought stress were also needed for microarray analyses, 41 transcripts of non-responding genes were eluted, PCR-amplified, cloned, sequenced and analyzed. PCR amplification of 160 clones was carried out and sufficient quantities of amplified products from 66 clones could be purified for microarray analyses. An array comprising 84 target DNA sequences were prepared at the Institute of Biochemistry and Plant Pathology, Helmholtz Center, Munich, Germany. Of these, 40 were differentially expressed, 26 were NRG under drought stress. Total RNA and mRNA from leaves of water-stressed and irrigated plants of three *Hevea* clones were isolated and used as complex probe for microarray analyses.

When total RNA was used for the synthesis of complex probes, membranes hybridized with probes from water-stressed leaf samples gave high background signals. This may be due to non-specific binding of components accumulated due to drought stress. Further, mRNA was used as complex probe, where two genes were found up-regulated and five genes were found down-regulated in clone RRII 105 after drought imposition for 14 days. In clone Dap 35, a gene for NAC 1 protein has been found up-regulated and six genes were down-regulated while in clone RRII 208, two genes were up-regulated and only one gene found down-regulated. Further, investigations are required to study more candidate genes found expressed under different stress conditions.

1.4. Xylem sapflow measurements in mature rubber plants

Xylem sap flow rate in two clones was recorded daily, by using Granier's type thermal dissipation probes (TDP). The changes in flow rate during wintering and refoliation period were analyzed. During wintering period, starting from January 10th 2007, the flow rate was declining and started increasing slowly after 5th February concomitant with refoliation. There was a summer (March-April) decline in sap flow rate in mature trees (Table Phy.2). The sap flow rate was directly influenced, by the sunlight intensity and sunshine hours but

Table Phy. 2. Mean daily sapflow rate in two clones of mature rubber at RRII, Kottayam

Month	Mean sapflow rate (L/day)	
	RRII 5	PR 255
September 2006	35 ± 1.0	28 ± 0.9
October	36 ± 0.9	20.4 ± 1.0
November	27 ± 0.7	22 ± 0.5
December	41 ± 0.8	38 ± 0.4
January 2007	28.5 ± 1.0	30 ± 0.7
February	14 ± 0.6	5.4 ± 0.34
March	16.3 ± 0.7	12 ± 0.4

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October	36 ± 0.9	20.4 ± 1.0
November	27 ± 0.7	22 ± 0.5
December	41 ± 0.8	38 ± 0.4
January 2007	28.5 ± 1.0	30 ± 0.7
February	14 ± 0.6	5.4 ± 0.34
March	16.3 ± 0.7	12 ± 0.4

had no significant relationship with other environmental parameters like temperature and rainfall.

1.4.1. Irrigation requirement of mature rubber in North Konkan region

Studies were made at RRS Dapchari to examine whether irrigation can be reduced from the maximum level (1 Etc) to 0.25 Etc and even lesser than that in a mature plantation with no adverse effects on its physiology. Irrigation was provided during January-May 2007. During 2007 summer, the irrigation was provided in plot B (0.25 Etc level, deep soil) and plot C (1.0 Etc level, shallow soil) and plot A left as unirrigated. The dry rubber yield was recorded for the summer months and compared with previous year yield (Table Phy.3).

Compared to the previous year, the dry rubber yield in all the treatments significantly increased during the summer of 2007. In shallow soil, 1 Etc irrigation during this year did not give any yield improvement over the partially-irrigated (deep soil area) plot B. It was therefore reconfirmed that in grown up trees the soil depth is more important for crop productivity rather than the level of irrigation.

1.5. Cultivation of elite rubber clones in high altitude location in Kerala

The experiment was initiated at Haileyburia Tea Estate Ltd., Elappara located

Table Phy.4. Growth and net CO₂ assimilation rate of *Hevea* at Haileyburia Tea Estate, Elappara

Clone	Plant height (cm)	Net CO ₂ assimilation(A) $\mu\text{mol m}^{-2} \text{s}^{-1}$
PB 260	80.6 \pm 1.67	9.32 \pm 0.32
RRIM 600	83.9 \pm 1.75	9.11 \pm 0.33
PB 235	82.6 \pm 2.01	6.93 \pm 0.36
GT 1	63.4 \pm 1.82	6.32 \pm 0.22
PR 261	54.3 \pm 1.63	7.52 \pm 0.28

in Idukki District, at an altitude of 900 m above msl. Five potential low temperature tolerant clones viz. PB 260, RRIM 600, PB 235, GT 1 and PR 261 were planted in field during the year 2006 in RBD with five replications. Clones PB 260, RRIM 600 and PB 235 showed significant increase in plant height compared to the clone GT 1 (Table Phy.4). Net CO₂ assimilation rate was recorded during summer and found higher in clones PB 260 and RRIM 600.

1.5.1. Rapid and non-destructive estimation of chlorophyll content in *Hevea* leaves

An experiment was initiated to estimate the chlorophyll content using CCM-200 (Opti-Sciences, USA) and a correlation was worked out in comparison to the conventional spectrophotometric method. The chlorophyll content index (CCI index) was measured in young and mature leaves from three clones of *Hevea* using the chlorophyll content meter. The total

Table Phy. 3 Yield of RRIM 600 under different level of irrigation in Dapchari

Irrigation level	Projected yield kg/ha (Jan-May)		Yield (g/lt)	
	2006	2007	2006	2007
Unirrigated- A	316	546	19 ^a \pm 1.5	35 ^a \pm 3.7
0.25 Etc-B (deep soil)	435	826	27 ^b \pm 1.6	53 ^d \pm 5.4
1.0 Etc-C (shallow soil)	300	624	19 ^a \pm 1.0	40 ^c \pm 4.3

chlorophyll content, chlorophyll *a* and chlorophyll *b* were estimated simultaneously using the standard method (1949). A positive and significant correlation was established between these methods. This method appears to be suitable for non-destructive and rapid estimation of chlorophyll content from a large number of *Hevea* clones in the field.

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

Wild germplasm accessions were screened for drought tolerance in field. A total number of 806 wild germplasm accessions planted during 2006 at Central Experiment Station, Chethackal, was visually scored for drought tolerance traits like leaf senescence, yellowing, surface wax, nature and orientation of leaves and vigor during summer season of 2007. The results were compared with the performance of RRIM 600 known for its drought tolerance. Thirtytwo wild accessions were found superior to clone RRIM 600.

1.7. Photosynthesis and molecular mechanism of drought tolerance

Drought is one among the major abiotic stresses that limits the expansion of natural rubber cultivation in non-traditional areas. Since photosynthesis is the first physiological process that gets adversely affected under abiotic stresses, a better understanding of the adaptation mechanisms of the photosynthetic machinery system to drought is very much essential for developing drought tolerant clones. Therefore, a study was carried out to understand the response of photosynthesis under drought stress in natural rubber plants. Two clones varying in their response to drought were exposed to water deficit stress and the basic mechanisms of light capture and their utilization for

carbon dioxide assimilation were studied at the level of photosystems. The studies showed that the two photosystems were affected differently under drought stress. The alterations in the gene expression pattern in two popular *Hevea* clones were analyzed using the technique of DD-RT-PCR and the results showed a marked difference in their gene expressions. This study showed that changes in the photosynthesis were accompanied by changes in gene expression pattern in young plants.

2. Physiology of growth and yield

2.1. Yield and yield components

Growth and yield of 12 clones planted in 1982 were evaluated in a long-term experiment conducted at Central Experiment Station, Chethackal. Highest girth and biomass were obtained in clones PB 235 and RR11 118 and minimum in clones RRIM 501 and PR 107. Clones PB 235 and RR11 105 were the top among high yielders. Declining trend in yield was noticed in the BI-2 panel of the 25-year-old trees irrespective of the clones. In order to correct the declining trend in yield, a Controlled Upward Tapping (CUT) experiment was initiated during 2007. CUT during the initial three months time (1/3S d/2 6d/7 ET 5% 6/y) with stimulation showed a significant increase in rubber yield in all the clones (Table Phy.5). The rubber yield was increased two to three-fold in clones PB 235, RR11 118, RR11 105, RRIM 600, GT 1 and RR11 300 compared to that of normal tapping under 1/5S d/2 system.

2.2. Studies on biomass accumulation in untapped trees

Biomass accumulation was studied in untapped and tapped trees of 12 clones planted in 1989. Trunk girth and shoot biomass of tapped and untapped trees showed significant dif-

Table Phy 5 Rubber yield in BI- 2 and CUT panels

Clone	Mean yield (g/lt)				
	Normal tapping				CUT
	2000-01	2004-05	2005-06	2006-07	May-July 07
PB 235	85.3 ± 7.8	76.74 ± 6.6	55.08 ± 3.4	47.54 ± 3.6	191.35 ± 5.1
RRII 118	47.0 ± 3.2	43.76 ± 3.1	33.06 ± 1.3	38.21 ± 2.1	152.82 ± 15.2
GT 1	56.5 ± 5.4	48.21 ± 3.0	35.03 ± 1.8	29.87 ± 1.1	120.39 ± 11.4
RRII 105	64.8 ± 5.0	47.49 ± 5.3	39.12 ± 2.5	43.28 ± 2.8	105.17 ± 13.3
RRIM 600	53.1 ± 5.2	40.07 ± 4.3	28.23 ± 2.7	29.91 ± 2.8	105.30 ± 11.3
RRII 300	43.7 ± 3.7	30.77 ± 2.2	26.86 ± 1.3	26.76 ± 1.3	104.05 ± 11.1
Tjir 1	49.4 ± 6.1	29.51 ± 2.3	25.29 ± 1.7	34.55 ± 2.3	87.41 ± 11.6
RRIM 501	45.5 ± 5.4	39.90 ± 3.7	23.05 ± 2.6	21.76 ± 1.7	79.50 ± 3.1
RRIM 703	68.8 ± 7.8	38.03 ± 4.6	19.39 ± 2.1	25.78 ± 2.8	71.04 ± 6.6
GI 1	41.0 ± 3.5	38.07 ± 2.0	28.60 ± 1.3	32.69 ± 1.4	62.95 ± 6.6
PR 107	24.6 ± 1.2	23.67 ± 1.5	21.37 ± 0.9	24.06 ± 1.4	57.91 ± 7.2
RRIM 612	22.3 ± 2.1	16.89 ± 0.9	16.49 ± 0.9	18.25 ± 1.8	47.06 ± 12.3

ferences. The clone RRII 118 followed by clone PB 311 continued to maintain higher biomass in untapped trees whereas the biomass loss due to tapping was higher in PB 311 compared to RRII 118.

2.3. Investigations on the mechanism of tapping-induced loss of biomass

The annual biomass increment of tapped trees was smaller than untapped control trees in five clones studied. At the end of nine years of tapping, RRII 105 lost the maximum biomass (31.3%) under ½S d/ 2 6d/7 system of tapping. The standing biomass in ½S d/3 6d/7 tapped trees was higher than d/2 in RRII 105, RRII 300 and PB 235 indicating intensive tapping had a bearing on shoot biomass. The biochemical parameters like ATP, total sugar and soluble protein contents were analyzed in latex during summer and post-monsoon seasons of 2006. The trees tapped under d/3 system recorded more ATP than trees tapped under d/2 system in the same clone. The trees tapped at high frequency (d/2) recorded more sugars in latex than trees tapped with low frequency (d/3). Among the clones, the

trees of RRII 105 with d/2 tapping system drained significantly higher sugars and soluble protein in latex.

2.4. Rubber biosynthesis

Quantification of HMGR-1 protein, an important enzyme related to the initial phase of rubber biosynthesis was carried out through ELISA. When the healthy trees were stimulated with ethephon the HMGR protein content decreased during the days that followed stimulation. Similarly prenyl transferase enzyme activity was also studied in the latex using ¹⁴C-labelled IPP as the substrate. The enzyme activity increased due to stimulation effect.

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

The ATP concentration in latex was measured in two-year-old plants of 10 clones with different yield potentials during post-monsoon period of 2006 and summer season of 2007. The young plants of high yielding clones showed a higher latex ATP compared to low yielding clones. The young plants of

the medium yielding clone such as GT 1 also ranked within the high yielding group. A positive correlation was established between ATP content in latex and the mature tree yield in the field trial.

2.6. Influence of intra luitoid and membrane enzymes on latex production and flow characteristics

Biochemical characterization of new RRII 400 series clones (RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430) in comparison with the control clone, RRII 105, was carried out. Parameters associated with latex production (sucrose, Pi and ATP), thiol metabolism (reduced glutathione, glutathione reductase, oxidized glutathione), antioxidant enzymes (SOD, CAT, peroxidase), parameters associated with cessation of latex flow (N-acetyl glucosaminidase, total proteins and hevein) and membrane enzymes involved in pH regulation (ATPase and pyrophosphatase) were measured and the data were statistically analysed. Higher level of ATP, luitoid membrane ATPase and proteins were observed in clones RRII 422, 414 and RRII 430 than RRII 105. All other parameters related to latex flow and production was comparable to RRII 105. Highest ATP was observed in clone RRII 429 whereas the antioxidant and membrane enzyme activities were low. The clone, RRII 417 also showed a declining trend in these parameters. The hevein content was relatively more in clones RRII 417 and RRII 429. SDS-PAGE profile of C-serum proteins of all the RRII 400 series clones was found comparable with RRII 105.

2.7. Oxidative stress preceding senescence/leaf fall

Biochemical and physiological parameters were studied in the leaves of *H. brasiliensis* during annual seasonal leaf senescence (wintering) to find out the

relationship between oxidative stress and leaf senescence. Higher levels of hydrogen peroxide, superoxide dismutase and lower activities of peroxidase and catalase were observed in leaves undergoing senescence (50 and 75 per cent yellowing leaves) compared to the non-senescent mature leaves (0 percent yellowing). With the progression of senescence, net carbon dioxide assimilation rate (A) and effective PS II quantum yield declined. In contrast, the photosynthetic excitation energy increased with advancing leaf age. Hydrogen peroxide content increased with advancing age of the leaves indicating elevated levels of lipid hydroperoxides in the tissues. Decreased levels of antioxidant enzymes with advancement in the age of the leaves indicated by the susceptibility to oxidative stress. A decrease in PS II activity coupled with carbon dioxide assimilation rate and increased excitation energy in the leaf tissues indicated that excess electrons produced in the photo systems reacted with oxygen molecules that might lead to ROS production, which hasten the process of wintering.

3. Stock-scion interaction

3.1. Intraclonal variability

Considerable intraclonal variations were observed in 13 *Hevea* clones for latex yield, PI and drc. Girth analysis during the initial years and girth at opening of the trees for tapping established a correlation between yield and girth from young stage onwards. There was a strong positive correlation between girth during the initial years and girth during the mature period. The results were compared with the data collected from six-year-old RRII 105 clone (116 trees) from CES, Chethackal which also had a very strong positive correlation between the girth at second year and sixth year.

3.2. Air-layering studies

The annual growth rate of own-rooted, double-rooted and budded plants was studied. Growth pattern of roots in these three types of root systems was studied by excavating the soil core. The root system was found to be comparable in all these three different propagation systems. However, double rooted plants have more fine roots / lateral roots. There was no significant difference between the girth of the plants among these three groups.

3.3. Scion-scion communication

Differential display experiments carried out using total RNA from latex to find out the stock influenced expression of genes in the scion of *H. brasiliensis*. The results showed that there was no remarkable difference in the gene expression pattern between tissue cultured and normal bud-grafted plants.

4. Tapping panel dryness

4.1. Free radicals and scavenging systems

Previous studies clearly indicated a relation between TPD and oxidative stress. The higher peroxidase activity and hydrogen peroxide (H_2O_2) content in the TPD-affected tissue may be the result of an increased production of superoxide radical O_2^- which can induce oxidative stress in the tissue. Similarly there was an increase in the malon di aldehyde (MDA) content in TPD affected tissue compared to normal healthy tissue suggesting the enhanced lipid peroxidation. Therefore MDA accumulation in the TPD-affected tissue can be implicated with oxidative stress-induced peroxidative damage. High peroxidases activity in TPD trees is capable of involving directly or indirectly with several biological reactions including the regulation of plant growth regulators especially ethylene biosynthesis. Earlier studies showed that a higher

peroxidase enzyme activity, higher levels of ethylene production and accumulation of cyanide (CN) in the bark tissues of TPD-affected trees. The wounding stress in the bark tissue due to tapping may enhance the ethylene production and thereby liberating more cyanide in the tissue. The accumulation of cyanide may be toxic to the tissues unless scavenged successfully. The enzyme, β -cyanoalanine synthase (β -CAS), an enzyme mainly involved in the detoxification of CN in plants was studied. The involvement of CAS activity in the bark tissue during the development of TPD was also studied. The low CAS activity in the TPD-affected tissue resulted in accumulation of CN in the bark tissue and that may lead to the CN toxicity.

4.2. Ethylene and oxidative stress

The ethylene-induced products in different parts of the bark tissues of both normal and TPD-affected trees were analyzed. In normal trees, the peroxidase enzyme activity was high in the region above the tapping panel. Whereas in TPD-affected trees the enzyme activity was high in the region below the tapping panel. However, the peroxidase (PX) enzyme activity in general was very high in all the regions of TPD-affected trees compared to the normal trees. Increased levels of H_2O_2 content were noticed in the tissues above the tapping panel in normal trees and it was still more in the tapping panel of the TPD-affected trees. There was an antagonistic relation between H_2O_2 content and PX activity and CN content and β -CAS activity in both normal and TPD-affected trees. The effect of ethylene stimulation on healthy trees was studied by applying ethephon in tapped trees of the same age (24 years old). Bark samples from the tapping panel of the trees under regular tapping and tissues from the virgin trees were collected simultaneously

for biochemical and histological/ histochemical analysis.

The biochemical components such as PX, H_2O_2 , MDA, CN, β -CAS and HMGR protein were analyzed in the bark tissues of untapped and tapped trees before and after stimulation (in trees under regular tapping). HMGR protein and PX enzyme activity were high in untapped trees before stimulation. CAS enzyme activity, CN, H_2O_2 and MDA contents were more in trees with regular tapping. The normal trees which were stimulated with ethephon showed increased levels of MDA, H_2O_2 and CN. High β -CAS, PX enzyme activity and HMGR protein were noticed in unstimulated trees.

The data showed that stimulation with ethephon reduces the level of cyanide detoxifying enzyme and thereby result in the accumulation of cyanide in the soft bark tissue. H_2O_2 production in the stimulated bark tissues increased along with peroxidase enzyme activity. But the increase in peroxidase enzyme activity was not proportional to the increase in H_2O_2 levels. This indicates that the production of cyanide and H_2O_2 molecules at levels much higher than what can be scavenged by the detoxifying enzymes may result in abiotic stress leading to metabolic disorders like TPD in stimulated trees.

4.3. TPD management

Many compounds claimed to have effect in abating TPD were field-tested for ascertaining the efficacy. A compound of biodynamic origin was tested for controlling TPD intensity. The compound was applied on 50-100 per cent TPD and control trees after removing the outer hard bark from bottom to a height of 2.0 m. The treatments were repeated in an interval of 30-40 days for a period of 10 months. All the trees were under tapping rest during the treatment period.

Most of the TPD trees appeared normal during the initial tapping period and some of them again started showing the symptoms of TPD within three months. TPD scoring and latex volume were determined during the subsequent tapping days. Though the trees were yielding latex, some of them showed partial TPD symptoms and a very few showed complete TPD within six months.

Another commercial product, VITEX, was tested on a few TPD affected (50-80%) and normal trees at RRII farm for a period of six months. All trees showed increased latex yield after the VITEX treatment and the yield declined within three months. On analysis, it was found that VITEX contained 2-chloroethylphosphonic acid (4%), which is the active ingredient present in the commercially available ethephon. Therefore, the enhanced latex production noticed in the normal and TPD-affected trees after the VITEX application was due to stimulation.

A product known as AGROCARE, a homeopathic preparation, was tested in a few TPD-affected trees (100%) at Malankara Estate, Thodupuzha. The trees were under tapping rest during the treatment period. While tapping on the same tapping panel and tapping system, out of 10 trees, nine showed normal latex yield without showing any symptoms of TPD for a period of 12 tapping days. The trees are now under monitoring.

4.4. Molecular basis of TPD

As a continuation of the identification of differentially expressed genes in TPD by differential display RT-PCR technique, another set of 18 genes was cloned and sequenced. Among them, seven transcripts were up-regulated and 11 transcripts were

down-regulated. Only nine transcripts showed homology with reported genes and the other nine transcripts did not match with any of the reported genes. The nucleotide sequence information of all transcripts was submitted in the GenBank database (Genbank ID EC030748 to EC030780).

Direct mRNA isolation was standardized using the magnetic beads. Subtractive hybridization was performed with the mRNA isolated from latex and bark. Subtraction was done with mRNA isolated from latex samples of healthy as well as trees with 50 per cent TPD incidence. The transcripts obtained by these reactions were cloned into pGEM-T easy vector of Promega and transformed into GenHunter competent cells. The transformed colonies were selected by colony PCR method and the selected clones were later sequenced. Another round of subtraction was carried out using the bark samples from wet and dry portions of bark tissues from the same tree with 50 per cent TPD incidence. Subtraction was done in both forward and reverse directions and about 750 clones were obtained, out of which about 550 were short-listed and sequenced. The expression of some genes are very specific to TPD and healthy trees.

4.5. Molecular basis of ethylene in TPD

Coding region of genes such as ACC synthase and ACC oxidase were PCR-amplified from cDNA of mRNA isolated from *Hevea* bark tissues. The PCR-amplified products were cloned into pGEM-T easy vector and its identity confirmed by DNA sequencing and BLAST analysis. Further, to facilitate cloning these cDNAs into the pET 20b expression vector, specific primers flanking with restriction sites were

synthesized for further steps involving the expressions of specific proteins using suitable PET vectors.

5. Secondary metabolites

5.1. Extraction of L-quebrachitol and purification

Latex samples were processed as per the protocol developed and crystallized L-quebrachitol from the of eluate (isolated compound) obtained through from ion exchange chromatography. The ion exchange resin eluate was concentrated with alcohol-water solution in different ratios and the crystals formed were dissolved in Milli-Q-water and heated for two minutes at 60° C and allowed to stand at room temperature for few hours for crystal growth. The protocol for isolation of L-quebrachitol from *Hevea brasiliensis* latex developed was submitted for patenting the methodology.

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

C-serum was collected from latex samples and osmotic potential was determined. Yield-related parameters such as bursting index, plugging index, dry rubber content and yield were also recorded from the latex of different clones. Total lipid content and individual lipid components were estimated from bottom fraction and rubber cream. A considerable variation of mineral composition was observed in the latex. The status of phosphorus (P), magnesium (Mg^{2+}), and potassium (K^+) were found high in peak yielding period than stress period in all clones. The data showed clonal variation in mineral content during the peak yielding and stress season. The calcium (Ca^{2+}) content was found to be high in all clones during stress period. The amount of

individual lipid components like glycolipids and triglycerides was high in the bottom fraction of drought susceptible clones compared to drought tolerant clones. Rubber cream fraction also showed a similar trend. The sterol content was higher in the bottom fraction of drought tolerant clones whereas, it was higher in the rubber cream fraction in the case of drought susceptible clones. This study aimed to understand the protective role of minerals during stress period by modulating the water relations.

6. Ecological impact of NR plantation

6.1. Impact of climate change on Indian natural rubber sector

An investigation was initiated to find out the long-term effect in the NR plantation sector due to climate change. The temperature and rainfall data were collected from the rubber plantations of different rubber growing regions in Kerala and analyzed to understand the regionwise (northern, central and southern Kerala) changes in the weather parameters. A comparison of the long-term trends in the rainfall patterns of traditional and non-

traditional rubber growing regions in India were also carried out. For this, 136 years rainfall data was collected from the website of Indian Institute of Tropical Meteorology (IITM). These analyses may help to simulate different climatic scenarios and model how they will influence the natural rubber productivity in the country.

7. Gene expression studies

7.1. Over-expression of chitinase in endophytes

An expression vector pHCMC5 for transforming *Bacillus subtilis* was obtained from BGSC, Ohio and was used for over-expressing chitinase gene in endophytes in rubber. Chitinase gene was PCR amplified using primers containing specific restriction sites from cDNA synthesized using mRNA of *Hevea* leaves. PCR-amplified chitinase was cloned into restriction-digested pHCMC vector and transformed into *E. coli* cells. Transformation protocol for *B. subtilis* was standardized and the pHCMC vector with chitinase gene was transformed into bacteria. Transformed cells when cultured produced proteins at significantly higher level.

RUBBER TECHNOLOGY DIVISION

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

1. Primary processing

A trial was initiated on low temperature storage of Natural Rubber latex enabling the processing of the same into latex grades of ISNR. NR conforming to ISNR 3L could be

obtained from the low temperature stored latex. Studies to find out new preservation systems for NR latex were continued. Assisted biological coagulation was attempted for field latex for producing block rubber and the properties of the resultant rubber were compared with that of formic acid-coagulated rubber. Efforts have been initiated to modify the solar drying system using water as heat transfer medium for drying of NR sheets. Preliminary trials were conducted for

drying crumb rubber using radio frequency drier. Observations were made on parameters such as drying time, power consumption and quality of rubber.

Received an Indian Patent for designing a semi-automatic machine for the upgradation of low quality sheet rubber (Indian patent No. 20180 dated 8.8.06).

2. Latex technology

Standardized a method for quick determination of dry rubber content of fresh NR latex with an accuracy of $\pm 0.5\%$. Trials were conducted on accelerated ageing of fresh centrifuged latex to predict quality parameters such as ZOV and ZST. Initiated a study on fatty acid-sensitized coagulation of fresh NR latex. Compared to conventionally coagulated sheet rubber, NR of better cure characteristics and higher modulus could be produced by this technique. Trials to improve the processing conditions of *in situ*-formed NR-silica composite were continued. A method was standardized for production of gloves with low antigenic protein content (within the specified limit) using deproteinized latex concentrate. Trials were carried out for the production of

deproteinized NR and low Mooney viscosity grades of ISNR from fresh NR latex. Deproteinization of skim latex was conducted using anilozyme and papain. The vulcanizate properties of the resultant rubber were evaluated in comparison with conventional skim rubber. The deproteinized rubber has enhanced mechanical and ageing characteristics (Table Chem.1).

3. Blends

Laboratory-scale evaluation of NR/HDPE blends in cycle tyre and rice polishing pad formulations showed promising results. Adhesive solution using NR/CR blends was prepared and supplied to a footwear manufacturing unit for further evaluation.

4. Nanocomposites

Nanocomposites based on NR/NBR and NR/XNBR were prepared by latex stage incorporation followed by coagulation and further processing. With the addition of 5 phr of organoclay, improvement in mechanical properties was obtained compared to vulcanizates from gum blends. The effect of incorporation of up to 10 phr of layered silicates in radiation vulcanized NR latex was examined in comparison with

Table Chem. 1. Vulcanizate properties of deproteinized rubber

Parameter	Gum			HAF filled		
	Control	*Anilozyme	*Papain	Control	*Anilozyme	*Papain
300 % Modulus (MPa)	2.4	1.9	1.8	13.8	12.0	12.0
Tensile strength (MPa)	15.6	19.8	20.0	20.1	24.4	25.0
Elongation at break, %	663	690	720	425	492	520
Tear strength, N/mm	33.5	36.5	37.5	74.5	78.7	78.3
Heat build-up, $\Delta T^{\circ}C$	8.0	6.0	6.0	29.0	25.0	25.0
Resilience, %	72.0	75.0	75.0	61.0	65.0	65.0
Compression set, %	28.8	25.6	24.8	38.9	34.8	34.0
Hardness, Shore A	40.0	36.0	36.0	64.0	59.0	60.0
Retention after ageing at 70°C for 7 days						
300 % Modulus (%)	128	134	138	95	102	104
Tensile strength (%)	65	73	75	76	83	86
Elongation at break(%)	90	98	95	84	88	90

a- conventional skim rubber, b & c- skim rubber obtained by deproteinization using anilozyme and papain respectively

Table Chem. 2. Vulcanizate properties of ENR 50 nanocomposite

Property	Gum	Ordinary clay	Nanoclay1	Nanoclay2
Tensile strength, MPa	20.6	20.8	22.3	24.7
Modulus 300%, MPa	1.7	2.0	3.8	4.5
Tear strength, N/mm	30	31	35	39
DIN abrasion loss, mm ³	162	160	110	103
Hardness, Shore A	42	46	54	56

Table Chem. 3. Vulcanizate properties of ENR 25 nanocomposite

Property	Gum	Ordinary clay	Nanoclay 1	Nanoclay 2
Tensile strength, MPa	20.2	20.6	21.5	22.7
Modulus 300%, MPa	1.7	2.2	4.6	5.0
Tear strength, N/mm	26	27	33	42
DIN abrasion loss, mm ³	160	160	115	99
Hardness, Shore A	42	46	54	56

commercial clay. The layered silicate nanocomposites exhibited better mechanical properties compared to the reference material. The ribbon-shaped layers offer high aspect ratio which in turn imparts better mechanical properties to the composites.

ENR 25 and ENR 50 nanocomposites were prepared using 10 phr of organically-modified layered silicate. Improved mechanical properties were observed for the same. (Table Chem. 2 & 3)

5. ENR as reinforcement modifier

Effect of mixing temperature on ENR-modified NR/silica system was evaluated. Heat treatment during mixing showed im-

provement in vulcanizate properties. Cure systems which exhibited good mechanical properties were identified.

6. Evaluation of rubber from different clones

Evaluation of sheet rubber from Agartala for clonal/seasonal variation was continued.

7. Collaborative project

A collaborative research programme with M/s Schiefflin Leprosy Research and Training Centre, Tamil Nadu was initiated for the development of special types of footwear for leprosy and diabetic patients.

TECHNICAL CONSULTANCY DIVISION

The activities of the Division were focussed 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. Establishment of rubber based industries

Project reports were prepared for the following industries.

Product	Firm
i. Moulded goods	M/s Haikai Rubbers, Palakkad
ii. Surgical gloves	M/s Amrutha Trust
iii. Moulded and extruded goods	M/s Vinstar Rubber Industries, Irappuram Rubber Park

iv. Automobile rubber components	M/s. West coast Rubbers, Calicut	xv. Rubber bulb for liquid dropper (medicinal)
v. Condoms	M/s. Rati Latex Industries, Delhi	xvi. Rubber diaphragm for respiration unit
vi. Mould for designer tiles	M/s Scorpio Tiles, Adoor	xvii. Rubber pads used for electronic equipment
vii. Solution adhesive	M/s Innovative Synthetics, Palakkad	xviii. Rubber compound for photocopiers
viii. Schemes for rubber	KRMF, Cochin based units (10 Nos)	xix. Pady dehusking rolls
ix. UNDP Project Latex - Sand Technology	Vasthuvidya-gurukulam, Aranmula.	xx. Tread rubber (3 Nos)
x. TSR unit	TFDPC, Agartala	
xi. Precured tread rubber and retreading materials	M/s Malabar Tyres, Davangere, Karnataka	

2. Product development

The following products were developed as per request from the entrepreneurs

- i. Latex adhesive
- ii. Petrol engine diaphragm and gasket for radiator
- iii. Balloons
- iv. Floor mats for export
- v. Rubber ring
- vi. Sponge sheet
- vii. Automobile rubber
- viii. Industrial rubber components
- ix. Bitumen based wall fixing sheet
- x. Nitrile rubber gasket
- xi. Stable mat of special design
- xii. Diaphragms (2 types)
- xiii. Rubber moulds for designer tiles
- xiv. Rice polisher components

4. Advisory services

Advisory services were offered to six firms. Technical advice was given through letters for queries received from 350 firms. The Division has also been associated with rubber parks at various levels of establishment.

5. Scheme on diversified uses of Natural Rubber

- a. Rubberization of roads: Associated with the evaluation of NRMB roads by Central Road Research Institute, Delhi. The studies have been completed and the results are encouraging.
- b. Associated with another study by National Transportation Planning and Research Centre (NATPAC), Trivandrum. The interim report has been received and reports are encouraging.

c. Natural rubber seismic pads

i. Joint project with Structural Engineering Research Centre (SERC), Chennai.

ii. 36 Nos. of seismic pads developed and tested successfully.

iii. Construction of a model building on seismic isolators being planned for actual trial.

The Division collected a total amount of Rs.5,57,493.50 towards testing, development and consultancy.

ECONOMICS DIVISION

The Division continued its research activities in five major areas *viz.*, (i) farm management; (ii) primary processing and marketing of NR; (iii) rubber products manufacturing industry and foreign trade; (iv) inter-crops and by-products; and (v) inter-divisional collaborative projects. Two projects were completed and reported during the period under review.

1. Age composition of mature area under natural rubber in India

The specific objectives of this inter-departmental study were (i) to estimate the age profile of rubber plantations in India for the period from 1980-81 to 2014-15; (ii) to understand the influence of age composition on average productivity for the period from 2001-02 to 2007-08; (iii) to compare the official estimates of national average productivity with those based on age profile,

for the period from 2001-02 to 2007-08; (iv) to project the influence of changing age profile on average productivity for the period from 2008-09 to 2014-15, and (v) to develop a methodology for estimating the average productivity and production of natural rubber based on age profile.

The main observations of the study were:

- i. steady growth in the share of area under the yield declining phase till the year 2011-12 with important policy implications on NR yield and production;
- ii. tardy progress in replanting of the area under the yield declining phase;
- iii. observed contradictions between official data on NR yield/production and the estimated yield/production;

Table Eco.1. Estimates and projections on the composition of mature area

Phase/year	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15
Share of yield increasing phase(%)	11.6	10.6	10.9	12.0	11.8	10.4	8.8	8.2	8.8	9.2	10.6	12.8	15.9	18.5
Share of yield stabilizing phase (%)	61.1	58.2	54.6	50.0	46.7	44.6	43.4	41.3	38.8	37.5	36.1	35.1	34.9	35.5
Share of yield declining phase (%)	27.3	31.2	34.5	38.0	41.5	45.0	47.8	50.5	52.4	53.3	53.3	52.1	49.2	46.0

- iv. need for focusing on replanting with due emphasis on traditional rubber growing regions; and
 - v. reconstructing the baseline data on area, age profile *etc.* based on a census of area under rubber cultivation in the country.
2. **MFN tariffs and value of imports of rubber and rubber products under the WTO regime**

This project is the fourth in the series of research programmes initiated to comprehend the challenges posed by the

WTO Agreement to India's rubber sector from a policy perspective. The book is the first comprehensive compilation to define the basic trade-related nomenclature and to provide the international trade-related database on all rubber and rubber products as per HS 2002. The book consists of 87 chapters. Chapter 1 deals with the description of all the basic concepts used in the book. The remaining 86 chapters contain bound rates, average basic import duty, number of tariff lines and value of imports pertaining to all forms of rubber and rubber products in 160 countries.

EXPLOITATION TECHNOLOGY

Research work on different aspects of latex harvesting was continued by the Division. All the ongoing experiments, onfarm trials and lab to land programmes progressed well. Growers who have gained confidence from low frequency tapping (LFT) trials in their own plantation extended LFT to more areas. Two estates in Sullia (S. Karnataka) shifted all their fields to 1/2S d/4 6d/7

frequency. Other areas of study were evaluation of mini and reduced spiral tapping cuts, long term evaluation of rain guarding and crop loss due to rain, panel change, and low frequency controlled upward tapping (LFCUT). Moreover, advisory on various aspects of exploitation technology were continued. Many samples of rainguard adhesive and ethephon were also tested.

Table Exp. 1. Yield performance of LFT systems with stimulation in clone RRII 105

Treatment	Tapping system	Yield (kg/ha) *	drc (%)
T0	1/2S d/2 6d/7	2442 ab	38.4
T1	1/2S d/3 6d/7 ET2.5% Pa 3/y	1545 c	38.9
T2	1/2S d/3 6d/7 ET2.5% Pa 4/y	1592 c	39.3
T3	1/2S d/3 6d/7 ET2.5% Pa 5/y	1583 c	39.5
T4	1/2S d/4 6d/7 ET2.5% Pa 5/y	2371 ab	41.1
T5	1/2S d/4 6d/7 ET2.5% Pa 7/y	2092 b	40.6
T6	1/2S d/4 6d/7 ET2.5% Pa 9/y	2239 ab	41.1
T7	1/2S d/6 6d/7 ET2.5% Pa 10/y	2625 a	40.9
T8	1/2S d/6 6d/7 ET2.5% Pa 12/y	2507 ab	40.1
T9	1/2S d/6 6d/7 ET2.5% Pa 15/y	2277 ab	38.7
CD ($P=0.05$) = 476			

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

Table Exp. 2. Dry rubber yield in clone GT 1 under weekly tapping at Neria Estate

Treatment	Year	Yield (kg/400 trees)	Cumulative TPD % (Aug. '07)
T1- 1/2S d/6 6d/7 ET 2.5% Pa (15/y) Feb - Oct (once in 3 weeks) Nov-Jan (monthly)	2003-04	1331	3.6
	2004-05	1415	
	2005-06	1453	
	2006-07	1510	
T2- 1/2S d/6 6d/7 ET 2.5% Pa (12/y)	2003-04	1299	4.0
	2004-05	1433	
	2005-06	1369	
	2006-07	1353	
T3- 1/2S d/6 6d/7 ET 2.5% Pa (10/y) Feb and March - Nil April-Jan (monthly)	2003-04	1385	2.8
	2004-05	1380	
	2005-06	1282	
	2006-07	1252	

1. Low frequency tapping (LFT) systems

In all the basal panel LFT experiments, panel application method of ethephon at 2.5 per cent concentration (ET 2.5% Pa) was followed for yield stimulation. However, the schedule and number of rounds of application of stimulant varied in different treatments.

Experiment on the yield performance of LFT with different levels of stimulation in clone RR11 105 (from opening onwards) was continued. Yield under d/2, d/4 and d/6 frequencies of tapping were comparable (Table Exp.1). Since the tapping panel was near to the bud union, yield was lower under d/3 frequency.

In another CRD experiment, weekly tapping with monthly stimulation gave good response whereas yield under d/10 frequency was comparatively lower than d/6.

Onfarm experiment on 1/2S d/4 6d/7 system of tapping at Apella Estate and 1/2S d/6 6d/7 system at Neria Estate in South Karnataka in clone GT 1 also progressed well. By rescheduling the months of stimulation under d/4 frequency during the second half of the year, better yield was achieved. Among three levels of stimulation

on trees tapped under weekly frequency, optimum yield was recorded with 15 stimulations per year (Table Exp. 2).

The experiment to evaluate long term performance of clone RR11 105 under d/3 and d/4 frequencies from first year of tapping was concluded. Ten years' consolidated data (Table Exp.3) indicated that yield comparable to that of d/3 frequency of tapping with stimulation (ET 2.5% Pa 3/y) could be obtained under d/4 frequency of tapping (ET 2.5% Pa 6/y). Incidence of tapping panel dryness was only 6 per cent (cumulative) by tenth year.

Table Exp. 3: Yield performance of clone RR11 105 under d/3 and d/4 frequencies of tapping

Year	Yield (kg/400 trees)		t-test
	d/3 (ET 2.5% Pa 3/y)	d/4 (ET 2.5% Pa 6/y)	
1996-97	1769	1732	T1 = T2
1997-98	1704	1720	
1998-99	1659	1889	
1999-00	2062	2172	
2000-01	1541	1555	
2001-02	2114	2057	
2002-03	2834	2147	
2003-04	2729	2440	
2004-05	2457	2217	
2005-06	2647	2306	
Mean	2152	2024	

The LFT trials with rainguard in Kanthimathy Estate (d/7) and in Hariharaputhra Estate (d/4) in Kulasekharam region (Tamil Nadu) in clone RRII 105 were continued. Annual average dry rubber yield of 2203 kg/400 trees and an annual average per tap yield of 43.4 kg could be obtained under d/7 system of tapping. Similarly, annual average dry rubber yield of 1978 kg/400 trees and annual average per tap yield of 36.7 kg could be obtained under d/4 system of tapping over a period of 10 months. Dry rubber yield of both the estates for the last four years is presented in Table Exp. 4

Table Exp. 4. Dry rubber yield (kg/400 trees) of clone RRII 105 at Kulasekharam

Year	Hariharaputhra (d/4)	Kanthimathy (d/7)
2003-04	1961 [*]	2334 [*]
2004-05	1956 [*]	2503
2005-06	2337 [*]	2596
2006-07	1978 [*]	2203
Mean	2058	2409

^{*} 11 months crop + 10 months crop

Demonstration trial on 1/2S d/6 6d/7 ET 2.5% Pa 12/y (Panel BO-1) and exploratory trial on 1/2S d/10 6d/7 ET 2.5% Pa 18/y (Panel BO-2) in clone RRII 105 continued to give promising yield on at CES, Chethackal (Table Exp.5).

Table Exp. 5. Dry rubber yield of clone RRII 105 at CES, Chethackal

Year	Yield (kg/400 trees)	
	d/6	(d/6 + d/10)
2002-2003	2117 (10 months)	2405 [*] (10 months)
2003-2004	2917	4120 [*]
2004-2005	2770	2160
2005-2006	1997	1971
2006-2007	2695	2255
Mean	2499	2582
Yield/tree (kg)	6.2	6.4

^{*} Initial two years, trees were tapped under 1/2S d/6 6d/7, from third year onwards shifted to 1/2S d/10

The onfarm experiments on evaluation of stimulation schedule under d/3 frequency of tapping for clones RRII 105 and GT 1 were concluded by the end of the reporting period. The yield increase for the clone RRII 105 with three stimulations per year under LFT was 16 per cent in the agroclimatic conditions of S. Karnataka whereas it was 27 per cent in Kerala (d/3 for 6 years and d/6 for 2 years at Thrithala). The yield (kg/400 trees) improvement by four stimulations in clone GT 1 at S. Karnataka was 17 per cent under LFT (d/3 for 6 years). Yield data provided by a grower who adopted d/3 frequency with three stimulation per year in clone RRII 105 from Pinnakkanad, Pala (tapping during 8-10 months/year) also indicated good

Table Exp. 6. Dry rubber yield under LFT at Pinnakkanad, Pala

Year	Frequency	Yield (kg/400 trees)	Yield (kg/tree)	Tapping months/ year
2001-02	d/2	1340	3.35	8
2002-03	d/3	1904	4.76	8
2003-04	d/3	1852	4.63	7
2004-05	d/3	2004	5.01	9
2005-06	d/3	1624	4.06	10
2006-07	d/3	1800	4.50	10

performance (Table Exp.6). The trees shifted to weekly tapping from April 2005 at Thrithala, Palakkad, continued to give good yield during 2006-07 also with very low incidence of TPD.

Onfarm trials on weekly frequency tapping in clone RRII 105 at Tropical farm, Adivaram, Kulappadam Estate, Mannarkkad and Vijayadri Estate, Kottayam progressed well. The labour strike at Manickal Estate, Mundakkayam and Balanoor Estate, Perinthalmanna partly affected the yield performance in the trial area. In general, TPD incidence was in the range of 4-7 per cent. Yield of clones PB 311,

PB 260 and PB 235 also was good at Mannarkkad and TPD was around four per cent only.

2. Low Frequency Controlled Upward Tapping (LFCUT)

Experiments on LFCUT with rainguarding in clones RR11 105, RR11 600 and RR11 118 continued to give good yield. When CUT was practiced under weekly frequency, ethephon application by groove method gave optimum yield. In clone RR11 105, per tree yield of 20 kg/year was obtained in 1/4S d/4 CUT treatment (T5) with once in three weeks stimulation and rainguarding (Table Exp. 7).

In clone RR11 118, under 1/3S d/6 CUT with once in three weeks stimulation (T4), yield comparable to that of 1/4S d/3

with once in six weeks stimulation (T1) and 1/4S d/4 CUT with once in three weeks stimulation (T2) could be obtained with rainguarding (Table Exp. 8).

3. Other experiments

The experiment on long term evaluation of rainguard was continued at Experimental Farm Unit, Rajiv Gandhi Institute of Technology, Pampady. Under d/2 system of tapping with out rainguard, crop loss was estimated to be 339 kg/400 trees. In the absence of rainguard 50 tapping days were lost under d/2 frequency whereas it was 33 days under d/3.

The experiment on effect of panel change on yield performance under different frequencies of tapping in clone RR11 105 indicated highest yield under d/4 system of tapping. This was followed by d/3 and d/2

Table Exp. 7. Yield response of clone RR11 105 to LFCUT at CES, Chethakkal

Treatment	Tapping system	Yield* (kg/400 trees)
T1	1/2S ↓ d/3 6d/7 ET 2.5% Pa 3/y	4612 cd
T2	1/4S ↑ d/3 6d/7 ET 5% La 12/y	6947 ab
T3	1/2S ↓ d/4 6d/7 ET 2.5% Pa 6/y	4827 bcd
T4	1/2S ↓ d/4 6d/7 ET 2.5% Pa 6/y	4307 cd
T5	1/4S ↑ d/4 6d/7 ET 5% La 23/y	8006 a
T6	1/2S ↓ d/6 6d/7 ET 2.5% Pa 12/y	5863 abcd
T7	1/3S ↑ d/6 6d/7 ET 5% Ga 24/y	6414 abc
T8	1/2S ↓ d/6 6d/7 ET 2.5% Pa 12/y	3846 d

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

Table Exp. 8. Yield response of RR11 118 to Low Frequency Controlled Upward Tapping at CES, Chethakkal

Treatment	Tapping system	Yield* (kg/400 trees)	No. of tappings
T1	1/4S ↑ d/3 6d/7 ET 5% La 9/y	5028 a	105
T2	1/4S ↑ d/4 6d/7 ET 5% La 16/y	5122 a	78
T3	1/3S ↑ d/4 6d/7 ET 5% La 8/y	5733 a	78
T4	1/3S ↑ d/6 6d/7 ET 5% Ga 16/y	5162 a	52
T5	1/3S ↑ d/6 6d/7 ET 5% Ga 12/y	4023 b	52

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

system of tapping. Under d/4 frequency of tapping, highest yield was observed with seven or nine stimulations with out panel change which is comparable to seven stimulations with panel change.

In the trial on evaluation of mini and reduced spiral tapping cuts at Experimental

Farm Unit, Rajiv Gandhi Institute of Technology, Pampady, trees under 1/4S d/3 system continued better performance. In an estate trial with six tapping blocks (all trees above 43 cm girth) tapped under 1/4S d/4 with stimulation (ET 2.5% once in 3 weeks), per tree yield was 2.8 kg and all the trees crossed 50 cm girth within one year.

GENOME ANALYSIS LABORATORY

The research activities of the Genome Analysis Laboratory are organized around three major areas which encompass (i) the development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping, (ii) development of genetic markers for biotic and abiotic stress tolerance and understanding the stress adaptation processes through transcriptome analysis and (iii) cloning and characterization of agronomically important genes.

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

Different marker technologies have been adopted and successfully established to characterize rubber genome.

1.1. Microsatellites for characterization of *Hevea* germplasm

Development of microsatellite markers was continued with the isolation and characterization of *Hevea* genomic clones containing microsatellite/ simple sequence repeats (SSR). Earlier 97 microsatellite primer-pairs were developed based on

dinucleotide repeats (CT/GA and AC/TG), three of which are gene-derived. Allelic diversity based on SSR polymorphisms at the locus encoding HMG-CoA reductase in rubber was reported. A microsatellite marker (hglu) based on the repeat sequences existing in the intron of β -1,3-glucanase gene sequence in rubber had also been developed, which appeared to be highly polymorphic as seven alleles could be detected in the survey with 40 cultivated clones. These seven alleles of the locus ' β -1,3-glucanase' formed 15 different allelic combinations/ genotypes existing in the popular/cultivated clones.

SSR-enriched genomic DNA library for trinucleotide repeats was constructed. Although most of the SSR libraries are enriched for a single type of SSR marker, three trinucleotide SSR motifs: (AAG), (AAT) and (GTG) were used for enrichment to achieve unbiased and complete coverage of the genome. Enriched genomic libraries for trinucleotide repeats were made both in lambda as well as in plasmid vector. More than 350 colonies from the plasmid library were picked up and grown individually. Out of them, 231 recombinant colonies were PCR amplified with vector-directed primer-pairs to check the presence of the inserts. Amplified products were subjected to Southern hybridization against DIG-labelled

trinucleotide repeat probe to identify the positive clones having tri-nucleotide repeats in them. Consequently 40 clones were identified as positive, bearing trinucleotide repeats. So far 20 positive clones have been sequenced and six of them are having the following trinucleotide motifs: AAG/CTT, GGT/ACC, GTG/CAC. Plaque lifts of the phage-library were performed for hybridization with the radiolabelled synthetic tri-nucleotide repeats oligos [AAG, GTG and AAT] as the probes. More than 300 primary positive plaques were identified through the first round of hybridization. Forty primary positive plaques were subjected to secondary screening.

1.2. Single nucleotide polymorphisms (SNP)

Identification of SNP in the gene/loci of interest is very important as they contribute directly to a phenotype or can be associated with a phenotype. Earlier the presence of SNP in seven genes from popular clones of *Hevea brasiliensis* was reported. Since the haplotypes (a group of neighboring SNP in the locus) are highly informative compared to single SNP, the haplotypes were determined through the sequencing of the cloned fragment containing heterozygous SNP. Details of haplotypes detected in three major genes involved in latex production in rubber are as follows: (i) a fragment of 1.5 kb at the 3' end of the Farnesyl diphosphate synthase gene contained four haplotypes with 11 SNP, (ii) Geranylgeranyl diphosphate synthase gene contained three haplotypes with five SNP in 500 bp sequence at the 3' end and (iii) Mevalonate kinase gene was identified with 11 SNP comprising five haplotypes within 800 bp fragment at the 3' end. Haplotype frequencies were also analyzed.

1.3. Genetic linkage map in rubber

Selected markers (RAPD and AFLP) developed in rubber were used to generate

banding profiles in a progeny population including parents. The work was continued with screening of a new set of 80 arbitrary primers (OPO, OPX, OPR and OPAL series) for polymorphisms using two parental clones along with their representative progenies. Fourteen microsatellite markers were also tested for polymorphisms between two parents. Out of the 14 (hglu, hmac4, hmac5, hmac13, hmac14, hmac17, hmct1, hmct2, hmct5, hmct9, hmct11, hmct16, hmct19 and hmct19A), 10 markers (hglu, hmac4, hmac5, hmac14, hmac17, hmct1, hmct5, hmct9, hmct19 and hmct19A) were found to be polymorphic and three of them were used for segregation analysis among the progenies.

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

2.1. Resistance gene analogue (RGA)

RGA approach was adopted to identify disease resistance gene in *Hevea*. Nucleotide sequences of 22 unique RGA clones isolated from rubber in the earlier studies were submitted to the NCBI GenBank. Efforts were made to develop more numbers of RGA clones from *Hevea benthamiana* and more than 100 putative RGA clones could be generated. Southern-blot analysis was applied to investigate the genomic organization of the confirmed RGA sequences. In most of the cases hybridization signals appeared as smear with varied intensities in different species of *Hevea* indicating the presence of a gene family.

An interesting observation was made while working with resistance gene analogues (RGAs) in *Hevea*. A primer-pair based on NBS conserved motifs was used to amplify

resistance gene analogues. Sequences of 16 putative RGAs clearly showed homology with retro-sequences. However, association of retrotransposons with disease resistance marker or insertion of retrotransposon within the gene itself had already been reported in several plant species.

An attempt was made to identify the functionally active RGAs. Reverse transcription (RT) polymerase chain reaction technique was adopted to amplify cDNA from RNA isolated from the *Corynespora* challenged leaf samples of RRII 105. A degenerated primer-pair was used for amplification of functional RGAs (cDNA). A fragment of ~0.6 kb was gel-purified and cloned. A total of 64 clones were generated. Out of 64 RGA clones (RT-RGA clones), 14 were processed for sequencing to get an idea about the functional disease resistance gene in rubber.

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 initiated. 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 for functional genomic studies in relation to cold stress was continued. Identified the involvement of four genes in cold adaptation process in rubber i.e. catalase, phosphatidylinositol/phosphatidylcholine transfer protein, NADH dehydrogenase and chloroplast FtsH protease. Recently more number of

differentially expressed transcripts/bands (down or up-regulated) were reamplified and cloned for sequencing.

2.2.2. Rubber EST project and gene discovery

A directional cDNA library was constructed from cold stressed leaf samples of rubber in Lambda 'ZAP Express' vector for the expressed sequence tag (EST) sequencing project. The objective of the EST was sequencing project to establish and provide a well-characterized, non-redundant EST resource for genetic enhancement of this important crop. To identify cold responsive genes, around 873 clones were isolated from the library and checked for their insert size. Twenty clones containing large inserts (>2.0 kb) were excised *in vivo* to form a phagemid containing the cloned insert. All these 12 phagemids were isolated and sequenced.

A subtracted cDNA library was also constructed from the cold stressed leaf sample using PCR-Select cDNA Subtraction kit (Clontech). The cDNAs were cloned directly into 'InsT/Aclone' cloning vector pTZ57R/T. A total of 213 clones were generated from the forward subtraction of differentially expressed transcripts.

3. Cloning and characterization of agronomically important genes

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

Cinnamyl alcohol dehydrogenase (CAD) (EC 1.1.1.195) catalyzes the final step in lignin precursor synthesis reducing the cinnamyl aldehydes (*para*-coumaryl, coniferyl and sinapyl aldehydes) to the corresponding alcohols in the presence of NADPH. Reverse Transcription (RT) polymerase chain reaction technique was

adopted to amplify the gene from the bark specific RNA pool. An RT-PCR product of 0.7 kb was amplified with a degenerated primer-pair. The fragment was gel-purified, cloned and sequenced. Nucleotide sequences (734 bp) of the clones were subjected to homology search with the GenBank sequences. These clones showed significant homology with CAD gene sequences from several plant species. Maximum sequence homology (85%) was detected with *Populus deltoids* cinnamyl alcohol dehydrogenase. Cloning of cDNA ends of cinnamyl alcohol dehydrogenase (*HevCAD*) gene from rubber was carried out using RACE technique. The 5' and 3' RACE products were sequenced in both directions and finally full-length gene sequence was deduced. The identity of the clones was inferred by sequence data comparison with the existing CAD sequences in the GenBank from other plants. Sequence of the full-length cDNA (derived from overlapping core region) revealed 1074

bp long open reading frame (ORF) including the translation initiation codon ATG and stop codon TGA. The cDNA sequence was found to encode a protein of 357 amino acids residues having maximum homology with the CAD of *Citrus sinensis* (E value: 7e-150) followed by *Populus trichocarpa* (E value: 2e-146). At the carboxy-terminal region, a motif 'Ser-Lys-Lue' was identified, which appeared to be the signal for translocation of CAD into peroxisome like other CAD. Along with the coding sequences of *HevCAD* gene, 74 bp 5' untranslated region (5'UTR) at the upstream and 296 bp 3'UTR containing polyadenylation signal at the down-stream were identified. Based on the sequence data generated by 5' and 3' RACE, a set of primers were designed and synthesized to clone a full-length cDNA of *HevCAD* from bark mRNA. The primer set could successfully amplify the full-length gene of 1413 bp in size including both the 5' and 3' UTRs. The amplified product was cloned.

CENTRAL EXPERIMENT STATION, CHETHACKAL

The Central Experiment Station, Chethackal, established in 1966 and having 255 ha. is the largest experiment station of Rubber Research Institute of India. It has been the cradle for most of the clones developed by RRII and the results of various trials from this station formed part of Rubber Board's recommendations. The ongoing trials include evaluation of clones developed through traditional and molecular methods, maintenance and characterization of *Hevea* germplasm, intercropping, nutrition, exploitation techniques including LFT systems,

stimulation practices, disease impact assessment *etc.* Other activities include apiculture, training programmes for staff and growers *etc.*

The total crop realized during the year was 1,73,301 kg. The man-days engaged were 59,238. A total of 298 tapping days was possible during the year. The Station provides all amenities to the 209 regular workers under the Plantation Labour Act. The CES dispensary takes care of the needs of the workers and the total number of patients attended during the period under report were 5872.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

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

1. Crop improvement

1.1. Evaluation of clones

The 1985 clone trial with 10 clones showed the highest mean girth (cm) in RRII 118 (82) followed by RRII 203 (81.8), RRIM 600 (79.8) and the lowest in PB 5/51 (55.3). Clone RRII 118 also registered maximum girth increment (3.18 cm) followed by RRII 203 (2.61), RRIM 600 (2.3) while PB 5/51 recorded the minimum (0.48 cm). The annual mean yield (g/t/t) was maximum in RRII 203 (44.9) followed by CT 1 (43.2) and PB 235 (42.4) and the minimum in PB 5/51 (26.1) in the 13th year under 1/2 S d/2 6d/7 system with winter rest. In case of trees under continuous tapping (1/25 d/2 6d/7 without rest), the highest annual mean yield (g/t/t) was recorded in RRII 203 (33.2) and RRII 118 (33.2) and the lowest in GI 1 (22.8). Moisture content of soil was found to be between 19 to 24 per cent during October 2006 to March 2007 with maximum in October and minimum in December.

In the 1986 clone trial with 10 clones, the highest mean girth (cm) was noticed in RRIC 102 (84.3) followed by RRII 118 (81), PB 310 (77.6) and the lowest in RRII 105 (67). The clone PB 310 showed the highest girth increment (3.0) followed by RRIC 102 (2.5), PR 255 (2.3) and the lowest in RRII 105 (0.7). The annual mean yield recorded in the 12th year of tapping was highest in PR 255 (52.4) followed by PB 310 (48.9), RRII 208 (45.9) and the lowest in PB 260 (23.4) in normal system of tapping. In case of continuously

tapped trees, the highest annual mean yield was recorded in PB 310 (38) followed by RRII 208 (35.7), PB 311 (34.2) and the lowest in RRIC 102 (24.2). Moisture content of soil was between 23 to 29 per cent during October 2006 to March 2007 with a maximum in October and minimum in December.

1.2 Evaluation of polyclonal population

Evaluation of polyclonal population in terms of growth and yield was continued. Among the 10 promising polyclonal seedling trees, the highest girth (cm) over 19 years of planting was observed in selection S2 (122.7) followed by S10 (116.3) and the lowest in S8 (87.5). The selection S10 showed the highest girth increment (4.3 cm) followed by S2 (4) and S7 (3.9) while S5 recorded the lowest (0.8). The highest annual mean yield (g/t/t) in 12th year of tapping was observed in selection S2 (222.6) followed by S1 (155.8) and the lowest was in selection S3 (13.7) in which TPD was high. Out of 10 promising polyclonal selections, the selection S2 ranked first (104.7) in terms of mean yield (g/t/t) over the first 12 years of tapping followed by S1 (94.0), S9 (69.9) and S10 (64.81) and the lowest in S5 (51.1) under 1/2 S d/2 6d/7 system of tapping. The typical TPD syndrome was observed only in selection S3. Except selection S4, all other promising polyclonal seedling trees showed high degree of tolerance to powdery mildew disease.

2. Crop management

The experiment to study the effect of NPK fertilizer on growth and yield of rubber with clone RRII 105 at Nayekgaon,

Kokrajhar indicated significant increase in girth (63.46 cm) and yield (50.94 g/t/t) with application of 40:40:40 kg/ha compared to 20:20:20 kg/ha and control. However, highest dose of N (60 kg/ha) resulted in highest yield (57.38 g/t/t) and no fertilizer control.

From the experiments on interaction between K and Mg at Sorutari farm and Nayekgaon (Kokrajhar), it has been observed that highest dose of K_2O (40 kg/ha) with MgO (15 kg/ha) resulted in significantly higher yield over control. No significant effect was observed in terms of girth.

The experiments at Sorutari farm and Nayekgaon (Kokrajhar) to study the efficiency of phosphatic fertilizers with varying solubility and amounts have shown that the highest dose (35 kg P_2O_5 /ha) of insoluble phosphorus (rock phosphate) resulted in better yield compared to the same amount of water soluble phosphorus (single super phosphate). No significant difference in girth among the treated plants was observed. Build up of available phosphorus in soil was more in the plots where rock phosphate was applied compared to single super phosphate and the same trend has been observed for the last six years.

Polybag nursery experiment on length of cutting of budded stumps has shown that sprouting success (89.6%) and plant girth (7 cm) were the highest where slanting cut was given 5 cm above the bud patch.

Budded stumps planted in bamboo basket (55 x 25 cm) resulted in maximum sprouting (92.6%), plant girth (6.3 cm) and height (54.3 cm) among the treatments.

The intercropping trial has shown that the growth of rubber when grown as monocrop and intercropped with pineapple is on par, but when intercropped with banana, the growth was better.

3. Crop protection

3.1. Survey on diseases and pests

Survey on diseases and pests of rubber was carried out in 37 locations covering 20 different rubber growing tracts in Assam, Meghalaya, Tripura and northern part of West Bengal. Incidence and severity of various pests and diseases were assessed and disease-prone areas were identified.

The severity of powdery mildew disease was noticed in the infection grade between 2.0 to 3.0 in some pockets of Assam, Meghalaya, Tripura and northern part of West Bengal. The severity of powdery mildew disease was high in PB 235, PB 5/51, RR11 308, RR11 300, RR11 430, RR11 422 and RR11 51. Clones, viz., PB 86, SCATC 88-13, SCATC 93-114, RR11 429, RR11 417, RR11 414, RR11 600, RR11 208, RR11 203 and RR11 703 recorded less powdery mildew disease. Incidence of *Periconia* leaf blight disease was observed on tender leaves in nursery plants during December/January in Assam and Meghalaya. Minor incidence of brown root disease was observed on mature rubber plants in different smallholdings in South Tripura resulting in mortality of the affected trees. Minor incidence of purple root disease was noticed in seedling nursery at RRTC, Hahara and the incidence was confined only in few locations of Assam and Meghalaya. Minor infestation of scale insect, termites, slugs and snails was also noticed in most of the locations surveyed in this region.

Table Ghy.1. Enhancement of plant biomass in AMF-inoculated plants

Treatment	Root biomass (g)	Shoot biomass (g)	Total biomass (g)
<i>G. fasciculatum</i>	20.3	33.8	54.1 (85.7)
<i>G. mosseae</i>	18.3	28.7	47.0 (61.1)
<i>G. fasciculatum</i> + <i>G. mosseae</i>	23.7	39.6	63.3 (117.1)
<i>G. margarita</i>	13.5	22.7	36.3 (24.4)
Uninoculated control	12.3	16.9	29.1

Per cent increase in growth over control is given in parentheses

Table Ghy.2 Variation in growth and yield of rubber under different exploitation systems (clone RRIM 600)

Treatment	Exploitation System	Girth (cm)	Annual girth increment (cm)	DRC (%)	Yield (g/t/t)	No. of tapping days	Yield (kg/ tree/ year)
T1	1/2S d/2 6d/7 regular tapping	71.61	1.73	30.82	36.60	143	5.23
T2	1/2S d/2 6d/7 One month rest (Feb)	62.00	1.11	30.20	33.65	131	4.40
T3	1/2S d/2 6d/7 Two months rest (Feb & March)	66.41	1.16	29.60	32.25	120	3.87
T4	1/2S d/2 6d/7 Three months rest (Jan- March)	68.75	1.15	32.77	41.15	107	4.40
T5	1/2Sd/3-6d/7 Continuous tapping- 5 Stimulations	69.38	1.20	29.91	47.61	95	4.52
T6	1/2Sd/3 6d/7 One month rest - do-	72.03	1.15	32.20	50.55	88	4.44
T7	1/2Sd/3 6d/7 Two months rest - do-	74.42	1.56	31.80	71.18	81	5.76
T8	1/2Sd/3 6d/7 Three months rest-do-	71.06	1.14	31.62	59.55	72	4.29
T9	1/2Sd/4 6d/7 Continuous tapping- 7 Stimulations	71.65	1.17	31.70	62.92	73	4.59
T10	1/2Sd/4 6d/7 One month rest - do-	72.60	1.20	31.51	64.15	67	4.30
T11	1/2Sd/4 6d/7 Two months rest -do-	69.26	1.46	34.02	65.58	61	4.00
T12	1/2Sd/4 6d/7 Three months rest -do-	76.44	1.96	34.20	77.80	55	4.28
	F test	NS		Sig.			
	SEm	2.78		0.80	6.44		
	CD(1%0.05)	NS		2.46	18.84		

3.2 Isolation, identification and molecular characterization of fungal pathogens of rubber

Fungal pathogens viz., *Periconia heveae*, *Colletotrichum gloeosporioides*, *Phellinus noxius*, *Helicobasidium compactum* and *Corticium salmonicolor* were isolated from different locations of this region. Cultures of *Periconia heveae* causing leaf blight disease of *Hevea* were studied for strain differentiation.

3.3 Evaluation of wild germplasm

Incidence and severity of powdery mildew disease in different wild accessions of *Hevea* germplasm at Sorutari, Nagrakata and Taranagar Research Farms under the agro-climatic conditions of Assam, northern part of West Bengal and Tripura respectively were assessed. Forty one wild accessions out of 540 and 25 out of 246 accessions of *Hevea* germplasm conserved at Sorutari and Taranagar farms respectively showed high degree of tolerance to powdery mildew disease. Twelve out of 64 wild accessions of *Hevea* germplasm showed high degree of tolerance to powdery mildew disease.

3.4 Control of purple root disease

The purple root disease in seedling nursery caused by *Helicobasidium compactum* was completely under control at DDC, Jenggitchakgre in Meghalaya.

3.5 Improvement of P uptake of rubber through AM inoculation

To select an efficient AM fungi for enhancing growth of rubber, the germinated seeds were inoculated with different strains

of AM fungi in poly bags. The results indicated that a mixed inoculum of *Glomus fasciculatum* and *lomus mosseae* was very effective in enhancing the growth of rubber seedlings followed by those inoculated singly with *G. fasciculatum* and *G. mosseae*. The total biomass of seedlings was the highest (63.3 g) in the mixed inoculum followed by the seedlings of singly inoculated with *G. fasciculatum* (54.1 g) and *G. mosseae* (47.0g). The per cent increase in growth over control was also found to be 117.1 per cent in the plant inoculated with both *G. fasciculatum* and *G. mosseae*, while it was 85.7 per cent and 61.1 per cent increase in singly inoculated with *G. fasciculatum* and *G. mosseae* (Table Ghy.1). The per cent AM infection in roots was also high in mixed inoculation (91.5%) than single inoculation.

4. Exploitation technology

An experiment on tapping rest and frequency interaction studies in *Hevea* was initiated during 1999 with the clone RRIM 600. Normal tapping (1/2S d/2 6d/7) with and without rest was compared with other tapping systems viz., 1/2S d/3 6d/7 and 1/2S d/4 6d/7 where stimulation had been imposed. The data revealed that treatments were not significant with respect to girth and girth increment. Maximum girth (76.4 cm) was recorded in 1/2S d/4 6d/7 with seven months rest followed by 1/7 (1/2S d/3 6d/7 with five stimulations and two months rest and minimum in 1/2S d/2 6d/7. The yield and DRC were influenced significantly by the treatments (Table Ghy. 2).

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The Station continued its research activities on nutritional requirements of rubber, potassium dynamics in rubber growing soils, intercropping, clone evaluation, exploitation studies and latex technological property studies. The Station is also engaged in providing advisory services and training to the growers of this region.

1. Crop improvement

1.1. Evaluation of clones

In clone evaluation trial (1995), involving 10 clones, RR11 105 (27.5 g/t/t), PB 235, PB 260, PB 311, SCATC 88-13 and RRIM 600 gave higher yield. In on-farm clone trial involving six clones, PB 235 showed the highest initial yield followed by PB 260 (13.2 g/t/t), RRIM 600 (12.6 g/t/t), RR11 208 (11.4 g/t/t), RR11 203 (9.4 g/t/t) and Haiken 1 (3.9 g/t/t). In the clonal block trial with eight clones, RR11 118 exhibited highest girth (38.6 cm) followed by PB 235 (37.0 cm) and PB 260 (35.1 cm). In another on-farm trial RR11 430 (23.7 cm) showed higher girth compared to other 400 series clones.

In the recombination breeding programme (1999), recombinant no. 31 (RR11 105 x RR11 208) registered high yield (52 g/t/t) compared to other combinations.

Eleven ortets of selected polyclonal seedling population (2000) were evaluated with RRIM 600 as reference clone. Selections 315 (47.2 cm), 461 (44.7 cm), 98 (43.2 cm), and 89 (43.2 cm) were observed to have better girth compared to RRIM 600 (41.4 cm).

Pollen viability was studied in different popular clones at Agartala, Nagrakata and Guwahati. Maximum pollen germination was observed in RR11 118 (24.6%) at

Guwahati and RRIM 600 (24.2%) at Agartala. In both the locations PB 86 showed minimum pollen tube germination and maximum viability (91%).

1.2. Conservation and evaluation of Amazonian germplasm in Tripura

In the evaluation trial of Amazonian germplasm accessions, it was observed that Acre accessions showed the highest girth compared to Rondonia and Mato grosso in Trial I and Trial II. Yield (g/t/t) data for three years showed that accessions RO 5449 (27.8) and MT 4796 (14.2) and MT 4788 (19.6) were having higher yield compared to other wild accessions. However, the yield was much lower than the clone RRIM 600 (40.5).

2. Crop management

In tea intercropping trial, annual green tea leaf yield was 847 kg per ha. and the rubber yield in the second year of tapping was 1188 kg per ha. In organic and inorganic trial, maximum immature girth continued to be on application of recommended dose of fertilizer with 20 kg of FYM per plant per year.

Clone RR11 417 showed higher growth compared to other clones in response to high dose of fertilizer. In another experiment, no difference was observed in fourth year growth of RRIM 600 in response to different levels and time of fertilizer application.

Soil physico-chemical properties were analyzed in 110 soils under mature rubber plantations in Tripura. It was observed that 45 per cent of the soils were deficient in available K while 42 per cent soils were in medium and 13 per cent in higher category. Soils were predominantly sandy clay or sandy clay loam or clay loam in texture, low to medium in OC and poor in CEC values. Light-textured soil contained higher amount

Table Net. 1. Distribution of K-fractions (mg/kg) in different soil texture

Textural class	No of samples	WS K	Ex-K	Non-Ex K
Sandy	8	18.7	30.2	342.4
Sandy loam	30	14.6	38.8	368.2
Sandy clay loam	30	12.5	50.2	408.7
Silty clay	12	10.4	72.6	695.6
Clay loam	10	7.5	88.4	538.6
Clay	20	5.3	104.4	474.5

of water soluble K and heavy-textured soil contained higher amount of exchangeable K (Table Net.1). Activity ratio of potassium, (ARe^k) in these soils ranged from 3.1 to 8.2 (mol/l) with a mean value of 4.2. The loamy-textured soils showed higher values for ARe^k compared to sandy loam or clay loam soils. Potential buffering capacity (PBC^k) of these soils ranged from 38.6 to 50.2 (cmol.kg⁻¹.mol^{-1/2}.l^{1/2}) with a mean value of 45.3 and these values were higher under clay loam or sandy clay loam soil where sustainability of potassium appeared to be for longer periods.

3. Crop physiology and exploitation

RRII 105 continued to give high yield under ½ S d/2 6d/7 frequency of tapping system. Yield under ¼ S and ½ S d/2 systems of tapping with application of five and three

stimulations respectively was comparable with ½ S d/2 system without stimulation.

Seedlings with relatively higher leaf nitrogen content (1.95%) recorded higher photosynthetic rate of 7.2 (±0.42) $\mu\text{mole m}^{-2}\text{s}^{-1}$, where the lower photosynthetic rate of 5.9 (±0.81) $\mu\text{mole m}^{-2}\text{s}^{-1}$ was observed in seedlings with 1.18% leaf N. The reduction was more predominant in low N type seedlings where the leaf N content and photosynthetic rate were recorded as 0.51 percent and 1.48 $\mu\text{mole m}^{-2}\text{s}^{-1}$ respectively. Corresponding stomatal conductance (g_s) was also altered depending on the leaf N content and was 81.4 $\mu\text{moles m}^{-2}\text{s}^{-1}$ in high N leaves compared to 47.2 $\mu\text{moles m}^{-2}\text{s}^{-1}$ in low N leaves. Maximum potential of PSII quantum yield (dark adapted Fv/Fm) was 0.67 in high N leaves during the mid day where as it recorded 0.23 in low N leaves indicating that N deficient leaves exhibit a higher level of photoinhibitory damage at mid day during winter season.

Rubber seed availability in the agro climatic condition of Tripura was on an average 255 kg/ha in 25 year old plantation of clone RRIM 600. Maximum seed fall was observed during first fortnight of August.

Table Net. 2. The monthly average values of different latex parameters

Month	TSC (%)	DRC (%)	NRC (%)	Ash (%)	Nitrogen (%)	Magnesium (ppm)
April	46.89	42.70	3.99	0.63	0.54	112
May	45.05	41.09	3.97	0.60	0.54	121
June	44.72	40.72	4.00	0.59	0.52	124
July	44.15	39.98	4.18	0.56	0.50	136
August	41.33	38.46	2.87	0.53	0.50	137
September	39.97	36.87	3.10	0.42	0.40	139
October	36.73	33.14	3.16	0.51	0.47	139
November	34.41	30.45	3.97	0.47	0.44	150
December	26.48	23.35	3.21	0.44	0.39	151
January	26.08	22.95	3.23	0.47	0.39	140

4. Latex technology

The variations in dry rubber content, total solid content and magnesium content with season were statistically significant while non-rubber content, ash and nitrogen were not significant. Dry rubber content and total solid content showed drastic decrease from summer to winter while magnesium content increased towards winter season (Table Net.2).

Dry rubber analytical values showed that P_s Mooney viscosity and gel content

decreased from summer (March-April) to winter (December-January) and accelerated storage hardening showed an increasing trend from summer to winter.

5. Advisory work

Discriminatory fertilizer recommendation based on soil and leaf analysis were offered to 418 rubber growers. A total of 1196 soil and 69 leaf samples were analysed during the period. Latex samples (299) were also analysed for drc and other latex parameters.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

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

1. Crop improvement

Yield data were recorded from 1985 and 1986 clone trials. In the 1985 trial, yield (g/t) of the clones is 47.3 (RRIM 600), 47.7 (RRII 105), 45.0 (RRII 203), 42.8 (PB 235), 37.9 (RRII 118), 38.1 (PB 86), 35.9 (GT 1), 35.6 (PB 5/51), 34.2 (CI 1) and 33.1 (RRIM 605). In the 1986 trial, yield (g/t) of the clones is 45.0 (PB 311), 40.1 (RRII 105), 38.0 (PB 310), 36.9 (RRII 208), 36.3 (RRII 118), 35.9 (PB 260), 34.8 (RRII 5), 34.0 (RRIC 102), 32.8 (PR 255) and 31.0 (RRIC 105).

Yield (g/t) of the three selected trees from the polyclonal population is 53.4, 59.2 and 49.7 respectively.

2. Crop physiology and exploitation

Growth, yield components (total volume of latex, dry rubber yield and dry

rubber content) and defoliation and refoliation pattern of clone RRIM 600 were recorded at different seasons. It has been observed that during winter period (Dec - Feb) average minimum air temperature was below 10°C, which adversely affected the growth, total volume of latex, dry rubber yield and drc. During winter period, the lowest drc was 25 per cent. Maximum yield was recorded in the months of September to November. Prolonged low temperature also enhanced the early defoliation and plants remained without leaves for three weeks.

To assess the performance of clone RRIM 600 in South West South and North North East aspect of slope in Garo Hills of Meghalaya, the growth and yield data were recorded periodically. Results indicated that plants growing in NNE aspect of slope showed higher yield (2065 kg/ha/year) than SWS (1836).

Different tapping systems in combination with tapping rest during winter season was studied. Yield and TPD were recorded in 1/2S d/2 and 1/2S d/3 tapping

system in different temperature regimes of 10 - 10°C, 15 - 15°C and 20 - 20°C, with control. In 1/2S d/2 tapping system, maximum yield (kg/ha/yr) was recorded in control treatment (1904) followed by 10 - 10°C temperature regime (1864), 15 - 15°C (1358) and 20 - 20°C temperature regime (1121) while in 1/2S d/3 tapping system, maximum yield was recorded in control treatment (1418) followed by 10 - 10°C (1021), 15 - 15°C (854) and 20 - 20°C temperature regime (741 kg). 1/2S d/2 tapping system showed higher yield than 1/2S d/3 tapping system under Garo Hills conditions. TPD incidence was recorded in the months of Nov/Dec and maximum TPD (%) was recorded in 1/2S d/2 system than 1/2S d/3. Maximum TPD was noticed in control tapping system which was 17 and 14 per cent respectively in 1/2S d/2 and 1/2S d/3 tapping system. In both the tapping systems, minimum TPD incidence was in 20 - 20°C temperature regime. During winter period, drc was 25 - 26 per cent.

Under the agroclimatic condition of Garo Hills, 90 per cent viability was observed in locally collected seeds while it was only 52 per cent for the seeds collected from Tripura State.

3. Crop management

3.1. Nutritional studies (On farm trial at Borgang, Assam)

A combination $N_{60}P_{30}K_{45}$ kg/ha gave the highest girth (75.52 cm), girth increment (1.30 cm), yield (83.90 g/t/t), drc (36.96 %) and total volume of latex (235.9 ml/t/t) followed by $N_{60}P_{30}K_{30}$ kg/ha and the minimum was in control plot ($N_0P_0K_0$). Improvement of soil fertility status and leaf nutrient content with application of NPK fertilizers, significantly increased the organic carbon content, available P and K and the highest was recorded with the

combination of $N_{60}P_{30}K_{45}$ kg/ha and the minimum was in control plot ($N_0P_0K_0$). After 17 consecutive years since planting, the soil fertility status improved significantly over their initial values. Organic carbon content of the surface soil was found increased significantly due to high accumulation of litter and balanced fertilization. A building-up of soil available P and K was also observed.

3.2. Leaf nutrient concentration in different clones

For providing clone-specific fertilizer recommendation under the agroclimatic condition of West Garo hills of Meghalaya, leaf samples of different *Hevea* clones were analyzed for N, P and K. The N concentration in leaf showed low to medium ranges and the maximum N content was in the clone RRIM 600 (3.45%) followed by PB 311, RRIM 605 and minimum in clone GI 1 (3.08%). The leaf-P content showed medium to high range and the maximum P content was noticed in the clone RRIM 600 (0.33%) followed by PB 310, PB 311, GT 1 and minimum in clone GI 1 (0.26%). The leaf-K content also ranged from medium to high and the maximum K content was noticed in the clone RRIM 600 (1.62%) followed by RRIM 605, PB 310, PB 311 and minimum in clone PR 255 (1.24%).

3.3. Soil moisture retention characteristics

To study the soil moisture retention characteristic of rubber growing soils under the agroclimatic condition of Meghalaya, 40 soil samples from the 20 rubber growing areas of Meghalaya were analysed for physical properties of soil and available nutrients.

The results showed that bulk density of the soils was 1.32-1.57 g/cm³, particle density was 2.37-2.62 g/cm³, porosity was 37.34-46.55 per cent and clay loam was the textural class

Table Nem.1 Soil moisture during the stress period in Meghalaya

Soil depth (cm)	Soil moisture content (%)(2006-07)					
	October 2006	November	December	January 2007	February 2007	March 2007
0-15	24.62	23.45	24.92	22.89	27.5	27.3
15-30	26.22	24.97	26.73	25.83	28.41	28.8
30-60	26.69	25.54	27.04	27.33	29.17	30.03
						Mean for stress period
						25.11
						26.83
						27.63

for all the rubber growing areas of Garo hills. Organic carbon was in medium range (0.86-1.37 %) and the soil was acidic in nature in all the locations. Available phosphorus was very low (0.03 -0.66 mg/100 g soil) and available potassium was in low to medium range (4.5 to 11.7 mg/100g soil).

Soil moisture estimation was done at the depth of 0-15, 15-30 and 30-60 cm from RRS Ganolgre farm during the stress period. Soil moisture increased in tandem with depth (Table Nem. 1).

3.4. Effect of shifting cultivation on soil properties

Soil samples were collected from shifting cultivation sites in West and South Garo Hills of Meghalaya to compare physico-chemical properties of soil with that of rubber growing areas.

In general both the soil sites of shifting cultivation are clay loam in texture. Bulk density and particle density showed increasing trends with increasing depth of soil, whereas porosity decreased with the soil

Table Nem.2 Nutrient availability in soils influenced by shifting cultivation

Table 2. Nutrient available in soils influenced by shifting cultivation																
Soil depth (cm)	Land form (kg/ha)	Available nitrogen (kg/ha)			Available P O _s (kg/ha) ¹			Available K O ² (g/kg)			Organic Carbon			Soil pH		
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
WEST GARO HILLS																
0-30	Upper Hill	375	324	304	6.94	8.91	7.51	125	130	103	13.6	13.3	13	5.21	5	4.9
30-60		320	325	306	4.7	5.31	4.95	118	104	92	10.9	10.6	10.3	5.09	5.2	5
0-30	Mid Hill	349	326	307	4.03	5.12	4.95	145	125	122	12.9	12.6	12.3	5.1	5	5.1
30-60		318	308	291	3.36	4.51	3.92	150	124	113	10.5	10.3	10.1	5	5	4.9
0-30	Foot Hill	415	403	392	8.51	9.56	9.8	159	126	195	14.5	14.1	14.3	5.41	5	4.9
30-60		344	324	310	7.39	8.51	8.35	154	118	171	11.8	12	11.1	5.2	5	5.1
Mean		354	335	318	5.82	6.98	6.58	142	121	133	12.4	12.2	11.8	5.17	5	4.9
SOUTH GARO HILLS																
0-30	Upper Hill	366	328	305	1.34	1.2	2.21	118	103	113	14.2	13.9	13.6	4.9	4.7	4.8
30-60		318	320	296	1.12	2.5	2.31	116	102	98	12.4	11.8	12	4.7	4.9	4.9
0-30	Mid Hill	336	332	321	1.79	5.36	4.31	138	145	121	13.2	12.6	12.9	4.6	4.4	4.5
30-60		306	280	274	1.56	2.32	2.2	134	160	121	11.5	11	11.2	4.5	4.7	4.4
0-30	Foot Hill	402	404	374	3.14	4.26	3.84	143	245	109	14.8	12.9	13.9	5	4.8	4.8
30-60		331	335	330	2.24	3.56	2.92	136	204	126	12.6	11.6	12.1	4.7	5	4.9
Mean		343	333	317	1.86	3.2	2.96	131	160	115	13.1	12.3	12.6	4.75	4.8	4.7
Grand mean		348	334	317	3.84	5.09	4.77	136	141	124	12.7	12.3	12.2	4.96	4.9	4.9
I= 1st Year, II= 2nd year and III= 3 rd year																

I= 1st Year, II= 2nd year and III= 3rd year

Table Nem. 3. Available nutrients and soil pH of the rubber growing soils of Meghalaya

Location	Organic Carbon content	Available nutrients (kg ha ⁻¹)		Soil pH
	(g/ kg)	P O 2 5	K O 2	
West Garo Hills	12.4 (6.8-21.6)*	4.03 (0.67-26.88)	191.1(100.8-309.1)	4.83(3.97-5.41)
East Garo Hills	11.9 (6.9-18.7)	6.27 (0.89-14.56)	206.3(91.8-318.1)	5.01(4.37-5.27)
South Garo Hills	11.5 (5.9-15.9)	1.79 (0.45-3.58)	122.7(94.0-143.4)	4.94 (4.54-5.24)
Ribhoi District	11.4 (6.3-15.6)	3.58 (0.45-12.54)	172.0(96.0-302.4)	4.97(4.83-5.13)
Other district	11.2 (5.7-15.5)	2.69(0.67-11.42)	146.9(107.5-282.2)	4.96(4.45-5.20)
Mean of Meghalaya State	11.7(5.7-21.6)	3.58 (0.45-26.88)	167.8 (91.8-318.1)	4.94 (3.97-5.41)

* Range values of the nutrients in parenthesis

Table Nem.4. Nutrient index and fertility rating of the rubber growing soils of Meghalaya

Location	No. of soil samples	Nutrient index			Fertility rating		
		Organic Carbon	P O 2 5	K O 2	Organic Carbon	P	K
West Garo Hills	68	2.09	1.02	2.05	M	L	M
East Garo Hills	22	1.94	1	2.1	M	L	M
South Garo Hills	26	2	1	1.92	M	L	M
Ribhoi District	24	1.88	1	2	M	L	M
Other district	20	2.05	1	2	M	L	M
Mean of Meghalaya State	170	1.99	1.004	2.01	M	L	M

L= Low, M= Medium, H= High

depth. A decline in soil pH, organic C, CEC, exchangeable cations, available P and K was noted with shifting cultivation period from one to three years (Table Nem.2).

3.5. Analytical/ Advisory work for fertilizer recommendation

Collected 40 soil samples from the 20 rubber growing areas of Meghalaya and analyzed for physical properties and available nutrients and fertilizer recommendation was given to the growers of Meghalaya.

Soil fertility management study of all districts of Meghalaya had shown that organic C content and available K are in medium level. The soil pH ranged from very strongly acidic to moderately acidic but in majority of rubber growing areas it ranged

between 4.94 to 5.01 (Table Nem.3). Nutrient index value for organic C and available K for the State as a whole is 1.99 and 2.01 respectively and lies in the medium level of fertility rating for rubber. Majority of the samples from all districts have shown low available P and nutrient index values ranged from 1.00 to 1.02 and the fertility rating is low for the entire Meghalaya State (Table Nem.4).

3.6. Rubber-based cropping system.

The performance of rubber (RRIM 600) and tea at 600 m altitude was assessed in which the rubber growth (81.1 cm) and yield (53.9 g/t/t) was found satisfactory. During the year, total tea production was 422.6 kg from 0.25 ha area and fetched a net income of Rs.2,240/-. In Garo hills rubber cultivation is more profitable than tea.

REGIONAL EXPERIMENT STATION NAGRAKATA, WEST BENGAL

The Station continued its research activities to develop suitable agrotechnology and identify the clones suited to the region.

1. Crop Improvement

1.1. Evaluation of clone

Four clone trials were laid out to screen clones showing better performance in yield and tolerance to high-speed wind, cold and high-sunshine intensity, for this agroclimatic condition. During 2006-07, the tree girth showed variation in four clone trials. In trial I, Haiken 1 ranked first (72.57 cm) followed by RRIM 703 and SCATC 93-114 (71.03 and 70.67 cm). Highest girth was recorded by RRIM 612 (71.40 cm) followed by PB 86 (69.10), RRII 105 (67.60) and RRIM 605 (67.30). In Trial II and in Trial III, PB 235 showed the highest girth (70.37 cm) followed by Haiken 1 and SCATC 93-114 (67.00 and 66.47 cm respectively). In Trial IV, Haiken 1 was the best (65.03 cm).

In terms of yield (g/t), PB 235 (43.9) showed higher yield followed by SCATC 88-13 (42.8) in Trial I. In Trial II, RRII 208 showed the highest yield (44.8) followed by RRIM 605 (41.1). In Trial III, PB 235 and RRII 208 (42.1) recorded the highest yield followed by RRIM 600 (39.9). Clones PB 280 and RRII 208 were the high yielding clones (37.7 and 37.4 respectively) in Trial IV.

1.2. Evaluation of germplasm

Screening of 1981 wild *Hevea* germplasm for tolerance to biotic and

abiotic stresses is one of the main stream research activities in North Bengal. An experiment was conducted with 22 accessions along with three popular check clones. Among these, GT 1 showed highest girth followed by RO 2890, RO 5557, RO 5363, RO 5430, RO 2636, RO 5348, RO 3172, MT 2229, CA 68 and PB 260. RO 5363 showed significantly high yield followed by RRII 105. The accession AC 607 showed the lowest girth and yield.

1.3. Performance of polyclonal seedlings

In the experiment laid out to study the performance of polyclonal seedlings, about 25 per cent trees showed girth ranging from 51-60 cm; 22.5 per cent of plants with 61-70 cm girth; 17 per cent with 71-80 cm and 10 per cent with above 80 cm girth. Regarding yield, about 127 trees showed above 3 kg/t/year, of which 17 trees showed yield above 6 kg/t/year. Action has been taken to maintain the trees with promising characters in the budwood nursery for future use.

2. Crop management

2.1. Nutritional trials

In the experiment on nutrient management with different doses of NPK in clone RRIM 600, the highest girth (71.5 cm) was observed in treatment $N_{45}P_{30}K_0$ and highest yield (81.8 g/t) in treatment $N_{45}P_{30}K_{40}$.

In the experiment to study the effect of split application of fertilizers, minimum girth as well as yield was recorded in the

control plot while higher girth was recorded for the treatment with four splits and higher yield for treatment with two splits.

2.2. Cropping system

The feasibility study on intercropping revealed that tea as an intercrop can successfully be grown with rubber with suitable planting geometry. Out of the six treatments tea intercropped with paired-row planting of rubber performed well. The girth of rubber was higher in intercropped situation compared to the monocrop. Girth of tree was not significantly different among monocrop or intercrop treatments. Green tea leaf yield was significantly higher in monocrop compared to the inter crop situation.

Other intercrop trial on rubber with arecanut is also in progress. The results showed that out of five treatments including monocrop rubber and monocrop arecanut higher girth for rubber was recorded in intercropped treatments while higher girth of arecanut was recorded in arecanut monocrop situation.

2.3. Rubber bush architecture

In the experiment (2002) to study the lopping effect on growth and yield of rubber, no significant difference was observed among different treatments with respect to girth. But mean annual yield was found significantly affected and it was the highest in the treatment where 25 per cent lopping was done than the treatment with 75 per cent lopping.

3. Crop physiology and exploitation

3.1. Tapping systems vs. tapping rest

The trends in yield pattern in low temperature-based rest system and tapping rest based on low winter temperature regime of 18-18°C, 15-15°C, 12-12°C and control (0-0°C) under two systems of tapping viz. $\frac{1}{2}$ S d/2 6d/7 and $\frac{1}{2}$ S d/3 6d/7 was analyzed. All the weekly data from two tapping systems and four treatments having three replications were considered. It was observed that $\frac{1}{2}$ S d/2 6d/7 system with 12-12°C rest treatment was the best combination of tapping system for this region.

3.2. Selection of seedlings raised from seeds of local mature plantation for budding

One of the main constraints in the NE is the late arrival of seeds from Kanyakumari district which leads to poor viability of seeds. Low temperature during winter is also a major constraint prevalent in the NE region which is not being experienced in the traditional region. This low temperature has reportedly shown to delay the growth of seedlings.

To overcome such constraints a small-scale trial was undertaken by collecting seeds from four ongoing block trials with clones PB 235, RR11 105, RR11 300 and RR11 600. The seeds collected per hectare from each block trials were 2150, 14400, 15000 and 20600, respectively. The seeds were then subjected to germination in seed beds to observe the viability. The average germination was found to be 50 per cent during this year.

REGIONAL RESEARCH STATION, DAPCHARI, MAHARASHTRA

Major thrust areas of this Station are development of suitable planting materials and location-specific agrotechnology for this drought-prone region. Experiments to evaluate low frequency tapping systems, irrigation requirement, screening of wild *Hevea* accessions for drought, growth and yield potential of various clones/ polyclonal seedlings are being carried out. Five research projects, now undergoing in the Station can be broadly classified into two categories, viz., environmental physiology and crop improvement.

1. Environmental physiology

Emphasis was given to the evaluation of suitable clones with desired characters like tolerance to water and high temperature stress. Three irrigation-based experiments were conducted to study the effect of irrigation and irrigation system on yield of rubber.

The irrigation experiment with Etc-based basin (1.00 Etc, 0.75 Etc and 0.50 Etc) and drip (0.75 Etc, 0.50 Etc, 0.25 Etc) irrigation treatments in clone RR11 105 was continued. The objective was to standardize and evaluate the irrigation systems in terms of water saving and total economy. From February 2000, the 0.75 Etc basin and 0.50 Etc drip were reduced to 0.25 Etc. Periodic observations on yield and yield attributes were recorded. The trees from all levels of basin irrigation showed higher girth as compared to drip irrigation system. The basin-irrigated trees recorded higher yield in comparison to trees from drip and control trees. (Table Dap.1). The reduced irrigation did not affect the growth and yield of rubber.

Table Dap.1. Effect of irrigation on growth and yield

Treatment	Mean girth* (cm)	Mean yield (g/lt)
Control (No irrigation)	61.01	44.50
1.00 Etc basin	71.76	55.95
0.25 Etc basin (Earlier 0.75 Etc)	68.50	55.39
0.50 Etc basin	67.29	57.47
0.75 Etc drip	66.76	49.83
0.25 Etc drip (Earlier 0.50 Etc)	66.44	47.02
0.25 Etc drip	62.91	47.19
SE \pm		0.85
4.12		
CD (P=0.05)	2.64	12.70

* as on March 2007

In the trial to study the effect of different levels of irrigation (1.00 Etc, 0.75 changed to 0.25 Etc from February 2000 and 0.50 Etc) on growth, yield and yield components of two clones viz. RR11 105 and RR11 118, results indicated that clone RR11 118 performed better in terms of growth while clone RR11 105 recorded better yield (Table Dap.2).

In the cost evaluation trial, the expenses incurred towards various inputs, farm practices and irrigation were monitored in irrigated and unirrigated trees of RR11 600. Irrigated trees were divided into two with respect to soil depth, one being maintained under reduced irrigation to 0.5 Etc (deep soil) and 1.0 Etc (shallow soil) level of irrigation. Meteorological and physiological parameters i.e., block yield, total latex volume, DRC, PI, BI and girth were recorded. The restricted irrigation level was reduced to further minimum level from 0.25 to 0.05 Etc with the aim to find out the optimum water requirement for mature trees in good soil depth area.

and girth were recorded

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

Treatment	Mean girth (cm)*		Mean yield (g/t/t)	
	RRII 105	RRII 118	RRII 105	RRII 118
Control (No irrigation)	62.55	75.60	47.30	50.67
1.00 ETc	70.42	88.41	59.99	48.63
0.75 ETc**	70.03	88.13	62.40	45.13
0.50 ETc	69.78	82.74	49.24	41.10
For irrigation treatments				
SE \pm	2.36		4.30	
CD (P=0.05)	5.79		10.58	
For clones				
SE \pm	1.06		3.74	
CD (P=0.05)	2.45		8.63	

* as on March 2007 ** changed from 0.75 ETc to 0.25 ETc from February 2000

2. Crop improvement

In the clone evaluation trial, monthly girth, DRC and fortnightly yield recording was continued. Clone RRII 208 is continuing to perform better in terms of growth and yield (Table Dap.3).

A screening of 130 wild *Hevea* germplasm accessions for drought tolerance under Dapchari condition was carried out along with four clones viz., RRII 208, RRII 105, RRIM 600 and Tjir 1. 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 in growth than those from Rondonia and Acre provenances. Among the control clones, RRIM 600 and RRII 208 were superior to RRII 105. Twenty five potential drought tolerant accessions were identified for further detailed studies based on 3-4 years field performance.

Table Dap.3. Growth and yield of *Hevea* clones

Treatment	Mean girth (cm)*	Mean yield (g/t/t)
RRII 5	61.36	38.97
RRII 6	64.00	47.65
RRII 105	57.28	40.24
RRII 208	65.41	54.50
RRII 308	57.92	25.32
RRIM 605	58.76	25.65
PB 260	61.45	30.21
PB 310	63.70	33.39
PB 311	59.75	36.65
RRIC 52	56.90	23.74
RRIC 100	59.32	37.02
RRIC 102	59.61	35.73
RRIC 105	57.31	22.06
PR 255	58.51	33.39
PR 261	57.32	25.43
SE \pm	1.86	5.10
CD (P=0.05)	3.80	10.43

* as on March 2007

REGIONAL RESEARCH STATION, DHENKANAL, ORISSA

The Station represents dry sub humid climate and it continued its research activities on crop improvement, crop management and crop protection, with special emphasis on identifying clones suited to this region.

1. Crop improvement

There are five clone evaluation trials, including one on-farm trial.

1.1 Clone evaluation

In the 1987 clone trial, both GT 1 (67.1 cm) and RRIM 600 (65.8) have recorded significantly higher mean girth over RRII 105 (61.3 cm). RRIM 600 has recorded the highest mean yield of 30.1 g/t/t, followed by RRII 105 (28.0). The clone RRIM 600 is found to be superior in terms of both yield and growth with annual girth increment of 1.83 cm.

In the 1990 clone trial, no significant difference in girth among the clones was recorded. Highest growth performance in terms of girth was recorded in SCATC 93-114 (74.1 cm) and RRII 208 (69.7). In terms of productivity, RRII 208 has recorded the highest mean yield of 37.1 g/t/t, followed by SCATC 88-113 (31.2), while SCATC 93-114 recorded the lowest yield (21.0). Clone RRII 208 showed overall superior growth, yield and adaptability among the clones in this trial.

In the 1991 clone trial, the performance of *Hevea* clones and polyclonal seedlings was compared. Among the clones, GT 1 (74.3 cm) and RRIC 102 (71.0) recorded superior growth. However, polyclonal seedlings with a mean girth of 82.7 cm exhibited better growth and adaptability as compared to

clones. RRII 208 recorded higher mean yield (39.6 g/t/t) followed by RRII 105 (36.8). In this trial also, RRII 208 proved higher growth and yield in the region.

In the 1999 clone trial, highest mean girth was recorded in RRII 208 (40.2 cm) followed by RRII 105 (39.0). The lowest girth was recorded in RRII 51 (30.0 cm).

1.2 Polyclonal seedling evaluation

To evaluate the growth and yield performance and adaptability of polyclonal seedlings in Orissa conditions, a trial was laid out in 1989. The highest mean girth was recorded in tree no. 32 (124.0 cm), followed by tree no. 471 (119.0 cm). Highest annual mean yield was recorded in tree no 154 (70.0 g/t/t). Ten elite polyclonal seedlings were identified for further evaluation and selection.

2. Crop management

The trial laid out with RRIM 600 in 1999 to study the effect of water soluble and water insoluble form of P on growth of *Hevea* and the higher dose of NPK fertilizers with appropriate number of split applications needed for optimum growth of rubber in Orissa revealed that water soluble phosphate fertilizer and split applications were better than water insoluble source. Highest girth was observed in 60 kg N, 60 kg P_2O_5 and 24 kg K_2O per ha.

3. Crop protection

Disease survey on powdery mildew disease in rubber plantations of this non-traditional area indicated no incidence of powdery mildew.

REGIONAL RESEARCH STATION, PADIYOOR, KERALA

The Station continued with the long-term research programmes initiated with the objective of identifying clones suited to the region and evaluation of clonal tolerance to drought/disease incidence. The field trials laid out include evaluation of germplasm, screening of clones for timber/latex traits, investigations on Genotype \times Environment interaction, large-scale testing of potential hybrid clones, water requirement studies, disease evaluation of clones and study of cropping systems.

1. Crop management

1.1 Physico-chemical characterization of soil

Study of the morphological and physico-chemical characteristics of the soils along with the site characteristics (Table Pad.1) was completed.

1.2 Water requirement studies

The experiment initiated in immature rubber with irrigation levels at IW/CPE ratios

of 0.3, 0.6, 0.9, 1.2 and an unirrigated control was continued. Irrigation was given during the peak summer months.

Growth observations recorded at periodic intervals indicated that irrigated treatments maintained significantly higher girth of plants though the seasonal differences in girth increment were marginal and non-significant (Table Pad.2).

1.3. Rubber and cashew cropping system

The trial initiated in 2001 to assess the performance of rubber and cashew cropping system was continued. Girth of individual trees in the combination remained unaffected due to interplanting.

1.4. Response to 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 levels (30:30:20,

Table Pad.1. Morphological and physico-chemical characteristics of soil

Soil	Geo-morphology	Topography	ESD	Drainage class	Erosion class
P11	Hills and ridges		Mod deep	Well drained	Moderate
P9	Hills and elongated ridges	Steep	Mod deep	Well drained	Severe
P8	Residual hill	Steep	Mod deep	Well drained	Severe
P6	Residual hill with plateau	Steep	Mod deep	Well drained	Severe
P12	Plain with hummocks	Gently	Deep	Mod. well	Moderate
P2	Intervening basin	Very gently	Deep	Well drained	Slight
P3	Undulating plain/ lower ridges	Gently	Mod deep	Well drained	Slight

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

Treatment	Number of irrigations given	Girth* (cm)	Girth increment (cm)	
			Wet season	Summer season
IW/CPE 1.2	12	45.5	5.26	0.55
IW/CPE 0.9	9	44.5	5.58	0.76
IW/CPE 0.6	7	46.5	6.25	0.72
IW/CPE 0.3	3	41.6	6.43	0.46
Control	Nil	39.1	5.71	0.47
SE (\pm)		1.47	NS	NS
CD ($P=0.05$)				

* as on March 2007

60:30:20, 90:60:40 and 120:60:40 kg/ha of N, P₂O₅ and K₂O). Higher doses of applied

fertilizer did not affect the girth of the plants significantly.

HEVEA BREEDING SUB-STATION, NETTANA, KARNATAKA

The major thrust areas of research in the Station are to evaluate clones under different biotic and abiotic stress factors and to identify clones suitable for commercial cultivation. Experiments on crop exploitation and protection are also carried out.

1. Crop improvement

1.1 Small-scale clone trials

Three trials are in progress where a total of 57 ortet clones (promising selections from seedling plantations) are under evaluation. Performance of top yielding ortets from each of the trials is presented in Table Kar.1. In the first trial (1988A); 15 ortet clones and three control clones viz. GT 1, RRIM 600 and RRII 105 are under evaluation. During 2006, the test clone T 2 recorded a maximum yield of 66.7 g/t, followed by C 1/2 (53.1), C 42 (50.3) and GT 1 (46.7). Mean yield over five years of tapping indicated clone T 2 to be the highest yielder with 64.5 g/t closely followed by GT 1 (61.5), C 1/2 (60.7) and C 42 (60.6). Two control clones, viz. RRIM

600 and RRII 105, recorded average yields of 58.5 and 55.2 g/t/t respectively. In the second trial (1988 B), among the 16 ortet clones and three control clones, (RRII 105, RRIM 600 and GT 1) under evaluation, average yield observed after five years for GT 1 was 70.4 g/t/t, as against 68.8 for T1, 59.8 for RRII 105 and 59.5 for O 40. The third trial (1988C) has 14 ortet clones and three control clones under evaluation. After five years of tapping yield recorded was 76.0 g/t/t for C 140, 70.8 for GT 1 and 70.6 g/t/t for RRII 105. Yield of RRIM 600 was 35.9 g/t/t.

In the three trials planted in 1991, a total of 54 test clones and three control clones are under evaluation. Performance of top yielding clones from each of the trials is presented in Table Kar.2. In the experiment 1991A clone PB 314 was the leading clone with a three year average yield of 96.6 g/t/t followed by PB 235 (92.2), PB 280 (86.1) and PB 311 (78.8). In the trial 1991 B maximum yield was noted for the clone RRII 5 (65.2 g/t/t) followed by RRII 105 (51.4) and RRII 3 (49.1). In the third trial (1991C) where 13 clones are

Table Kar.1. Performance of top yielding ortets from the ortet trials

Trial	Clone	Girth (cm)	Mean yield during 2006 (g/t/t)	Mean yield over five years of tapping (g/t/t)
1988A	T 2	103.4	66.7	64.5
	GT 1	79.3	46.7	61.5
	C 1/2	85.3	53.1	60.7
	C 42	94.6	50.3	60.6
1988B	GT 1	86.5	63.2	70.4
	T 1	102.9	57.3	68.8
	RRII 105	69.1	50.4	59.8
	O 40	82.1	49.4	59.5
1988C	C 140	91.7	68.5	76.0
	GT 1	88.0	64.6	70.8
	RRII 105	71.3	68.9	70.6
	O 26	87.4	68.4	69.2
Control clones*	C 6	53.8	67.2	66.2
	RRII 105	70.4	53.0	61.9
	GT 1	84.6	58.2	67.6
	RRIM 600	70.6	46.1	49.0

*Mean of three trials

Table Kar.2. Performance of top yielding clones in the small scale trials

Trial	Clone	Girth (cm)	Meanyield during 2006 (g/t/t)	Mean yield over three years of tapping (g/t/t)
1991A	PB 314	66.3	92.2	96.6
	PB 235	83.4	85.0	92.2
	PB 280	69.1	84.1	86.1
	PB 311	70.2	71.5	78.8
1991B	RRII 5	73.4	65.7	65.2
	RRII 105	59.3	52.4	51.4
	RRII 3	68.0	49.1	49.1
1991C	HP 83/224	81.0	60.2	58.1
	HP 83/225	59.6	46.5	46.3
	PR 261	70.2	42.6	41.9
Control clones*	RRII 105	59.9	63.0	64.2
	GT 1	70.3	38.3	38.0
	RRIM 600	52.1	30.7	33.4

*Mean of three trials

under evaluation, the highest yielding clone was HP 83/224 (58.1 g/t/t) followed by HP 83/225 (46.3) and PR 261 (41.9).

1.2. Large-scale clone trials

Growth and yield performance of clones in the large scale clone trial (1989) is summarized in Table Kar. 3. Among the 14

clones under evaluation, maximum yield was noted in clone RRII 203 (64.2 g/t/t) followed by KRS 25 (56.6) and KRS 163 (53.0). Popular clones, RRII 105 recorded an average yield of 49.5 g/t/t and RRIM 600 yielded 31.3. RRII 300 and SCATC 93-114 were the lowest yielding clones with a yield of 28.6 and 11.5 g/t/t respectively.

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

Clone	Girth (cm) at age 18 years (Dec 2006)	Mean yield during 2006 (g/t/t)	Mean yield over five years of tapping (g/t/t)
RRII 203	91.2 a	52.3	64.2 a
KRS 25	83.5 b	50.9	56.6 a
KRS 163	79.9 b	44.2	53.0 a
KRS 128	80.0 b	39.7	50.5 a
RRII 105	72.3 c	35.3	49.5 b
PB 255	74.3 c	38.2	49.4 b
SCATC 88-13	65.3 d	33.9	42.7 b
RRII 308	81.5 b	34.2	42.1 b
PR 255	64.9 d	29.3	37.4 b
Haiken 1	59.8 e	33.2	36.3 b
PR 261	71.6 c	25.8	34.4 c
RRIM 600	70.1 c	24.8	31.3 c
RRII 300	73.6 c	22.1	28.6 c
SCATC 93-114	67.9 d	11.2	11.5 d
CV%	5.6	29.8	20.8
SE (\pm)	2.4	5.8	5.0

Levels not connected by same letter are significantly different.

Growth and yield performance of clones in the large-scale clone trial 1990A is summarized in Table Kar. 4. After four years of tapping PB 260, PB 235 and RRII 105 were the leading clones with a yield of 58.84, 52.35 and 49.64 g/t/t respectively. HP 372 showed highest mean girth of 85.16 cm and the lowest mean girth of 60.38 cm was recorded by Tjr 1.

The trial planted in 2000 has eight clones under evaluation where most of the clones are RRII 400 series clones. Growth performance of clones in the trial is presented in Table Kar.5. After seven years, maximum growth was recorded for clones RRII 414 (51.6 cm) and RRII 430 (51.4) followed by RRIC 100 (43.7). RRII 105 recorded a mean girth of 38.2 cm. RRII 422 was the least vigorous clone (33.9 cm).

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

Clone	Girth (cm) at age 17 years (Dec 2006)	Mean yield during 2006 (g/t/t)	Mean yield over four years of tapping (g/t/t)
PB 260	79.67 a	55.96	58.84 a
PB 235	84.31 a	49.51	52.35 b
RRII 105	69.37 c	47.60	49.64 b
PB 311	71.33 c	46.83	48.40 b
HP 372	85.16 a	44.16	45.67 b
PB 217	74.33 b	43.03	44.37 c
HP 223	79.10 b	41.16	42.91 c
GT 1	75.53 b	39.10	41.59 c
GI 1	64.71 d	30.11	30.90 d
HP 187	68.96 c	23.36	24.28 e
HP 185	70.03 c	23.05	23.83 e
Hil 28	69.07 c	22.54	23.40 e
Mil 3/2	73.28 b	21.72	22.17 e
HP 204	63.77 d	19.13	19.66 e
Tjr 1	60.38 d	17.29	17.99 f
CV (%)	10.26	9.78	4.85
SE (\pm)	2.03	1.97	2.16

Levels not connected by same letter are significantly different.

Trial on estimation of genetic parameters

The trial planted in 1990 aims to evaluate parents and their progenies for estimating genetic parameters that are essential for planning plant breeding activities. Twelve clones and their progenies are under evaluation. During 2006, parent clones PB 235, RRII 105 and RRII 203 were the leading in yield with a yield of 67.7, 51.8 and 47.8 g/t/t respectively. RRII 203 (110.6 cm) followed by PB 235 (89.7 cm) were leading in growth, while IAN 45/873 recorded lowest girth.

Among the progenies, half-sibs of RRII 203, PB 235 and GT 1 recorded a yield of 43.7, 40.7 and 40.7 g/t/t respectively. Trends in mean yield over four years of tapping were similar to that observed in the year 2006. In

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

Clone	Girth (cm) 6 year (Dec 2005)	Girth increment during 2006 (cm)	Girth (cm) 7 year (Dec 2006)
RRII 414	41.5	10.1	51.6 a
RRII 430	40.8	10.6	51.4 a
RRIC 100	30.4	13.4	43.7 b
RRII 429	35.8	7.6	43.4 b
RRII 407	32.3	7.1	39.5 b
RRII 105	31.9	6.3	38.2 c
RRII 403	28.6	7.2	35.8 c
RRII 422	26.0	7.9	33.9 c
CV (%)	7.85	24.92	7.31
SE (±)	1.51	1.26	1.78

Levels not connected by same letter are significantly different.

general yield of half-sibs of high yielding clones was higher and yield of half-sibs of low yielding clones was lower.

HEVEA BREEDING SUB-STATION, PARALIAR, TAMIL NADU

Hybridization and clonal selection, evaluation of clones under the prevailing agro-climate, generation of polycross planting materials and refinement of plant propagation techniques in *Hevea* are the major activities of the Station.

1. Crop improvement

In the large-scale clone trial (1994) at Keeriparai, pooled data (Table Par.1) for the first five years of tapping revealed that PB 255 (75.63 g/t/t) is the best yielder closely followed by IRCA 109 (75.33), PB 314 (72.64) and IRCA 111 (71.60) also exhibited significantly superior initial yield than the control clone RRII 105 (50.83). IRCA 111 and PB 255 recorded better growth.

In the block evaluation experiment (1994) at Keeriparai, PB 311 (59.37) continued

Exploitation technology

Two experiments are in progress. In both the trials, five clones each are under evaluation to study the effects of different exploitation systems on yield performance. In the 1987 trial, all the clones responded well to 1/2 Sd/4 6d/7 system of tapping with stimulation. Compared to yield of the clones in 1/2 Sd/2 6d/7 and 1/2 Sd/3 6d/7 system without stimulation, yield recorded under d/4 system with stimulation was significantly high. In the other trial (planted in 1988) yield observed under d/3 system without stimulation and d/4 with stimulation was comparable in clones RRII 118, PR 255, PR 261 and RRIC 36. The clone RRIC 45 gave good response to d/4 with stimulation compared to d/3 yield without stimulation.

to exhibit the maximum yield of 59.37 g up to the fifth year of tapping, followed by PB 235 (53.63 g/t/t). The control clone RRII 105 (51.83 g/t/t) exhibited an increasing yield trend from the fourth year of tapping and had produced highest yield during 2006-07 (Table Par. 2). In the large-scale trial on 'Evaluation of Clonal Composites' (1994), the yield obtained from different treatments was on par with the control plot planted with RRII 105 alone (55.19 g/t/t). The pooled data for the first five years of tapping clearly indicated the advantages of mixed planting of selected clones.

In the multilocal clone trial on G x E interaction of selected clones (1996), RRII 203 (58.22 g) registered better yield compared to RRII 105 (53.71 g) up to the fourth year of tapping. The hybrid clones

Table Par.1. Mean girth and yield of clones in large-scale trial at Keeriparai

Clone	Girth (cm)	Mean yield (g/t/t)	
		2006-07	1-5 years
IRCA 18	71.26	60.65	56.77
IRCA 109	73.12	79.75	75.33
IRCA 111	81.59	76.20	71.60
IRCA 130	69.75	69.57	65.90
IRCA 230	78.40	73.09	51.23
PB 255	79.88	84.53	75.63
PB 314	75.46	60.66	72.64
PB 330	74.06	62.00	51.81
PB 28/59	67.59	62.05	53.49
RRIM 703	75.02	81.36	62.49
RRII 105	70.59	73.05	50.83
Mean	74.25	71.17	62.52
CD(P=0.05)	5.71	5.84	6.28

Table Par.2. Mean girth and yield of clones in the block trial at Keeriparai

Clone	Girth (cm)	Mean yield (g/t/t)	
		2006-07	1-5 years
RRII 5	65.86	42.75	42.72
RRII 50	66.15	34.34	40.71
RRII 51	67.03	41.09	34.63
RRII 105	64.09	74.72	51.83
RRII 176	75.00	43.78	40.72
RRIC 102	72.36	53.13	44.17
PB 217	78.73	60.57	48.12
PB 235	70.90	55.37	53.63
PB 260	73.19	53.97	47.40
PB 311	68.48	63.44	59.37
PB 28/59	75.70	60.83	52.51
PR 255	65.34	51.81	49.48
PR 261	65.50	47.21	46.13
Mean	69.87	52.53	47.03
CD (P = 0.05)	2.17	3.29	2.56

belonging to 400 series except RRII 429 exhibited an initial yield trend on par with RRII 105. In the observational trial at Vaikundam Estate (2000) all the five hybrid clones of 400 series attained tappable girth at the sixth year of planting and exhibited

better initial yield trend. The block evaluation experiments initiated at Velimalai (2002), New Ambadi (2002), Bethany (2006) and Palazhi (2006) Estates are under close observation for juvenile growth and incidence of diseases.

2. Breeding orchard

The breeding orchard consisting of 51 modern clones as parents was well maintained in an area of 5.0 ha. With a view to generate new clones with high yield and promising secondary characters, hand pollination (HP) was attempted with different parental combinations during the flowering season in 2007 also. The hybrid seeds obtained from HP 2006 was raised in a nursery for preliminary evaluation and selection. Ten potential high yielders evolved through HP carried out during 2001 and 2002 were supplied to RRII for further evaluation in clonal nursery.

3. New generation polyclonal seed garden

The polyclonal seed garden raised in 2000 at New Ambadi Estate, in an area of 9.0 ha, with nine modern high yielding clones was well maintained. Floral biology and initial fruit set of the clones were closely monitored.

4. Evaluation of root trainer plants

The root trainer plants transplanted at Churulacode attained tappable girth in the sixth year of planting. Root trainer plants transplanted at Velimalai Estate also exhibited better growth than polybag plants. Quantitative studies on the root development of plants raised in root trainers in comparison with polybag plants are being initiated.

LIBRARY AND DOCUMENTATION CENTRE

During the reporting year, 146 books were added to the stock of the library. The library subscribed 63 foreign journals and 81 Indian journals. About 30 other journals were also received as gift/exchange. Literature searches from CDs of CABCD and RAPRA were carried out.

Four issues of *Documentation List*, four issues of *Rubber Alerts* and one issue of *New Additions List* were compiled and distributed. 585 numbers of Press Clippings and 20 numbers of other SDI bulletins were distributed.

Natural Rubber Research Vol.19 (1&2) 2006 was published and its distribution was arranged (Former title: *Indian Journal of Natural Rubber Research*). Arranged the sale of 32 copies of the book *Natural Rubber: Agromanagement and Crop Processing*, four copies of *Rubber Wood: Processing and Utilization*, 10 copies of *Plant and Soil Analysis*, two copies of *Global Competitiveness of Indian Rubber Plantation Industry* and three copies of *Soil Survey Report of Rubber Growing Soils of Kerala and Tamil Nadu*.

AGROMETEOROLOGY

1. Climate resource characteristics of rubber growing tracts

An attempt was made at working out the spatial conduciveness of rubber over the traditional and non-traditional belts of India by adopting a suitable water availability index. The cumulative number of years of water deficit (percentage) period could affect the growth and yield of rubber. The Standardized Precipitation Index (SPI) has been utilized to identify areas in the traditional rubber belt of Kerala and compare the same with that of North East (NE) India. As the length of dry period increases, the intensity of drought also increases and will be shown in terms of the SPI values. The percentage of water deficit years has been estimated for a total of 147 pointwise rain gauge stations for these two regions. A standard weather generating method has been employed for filling up gaps in the data set spanning from 30 to 90 years. Dry period percentages were then plotted on to baseline maps of the NE India (non-traditional region) and the traditional region of Kerala. The spatial data were

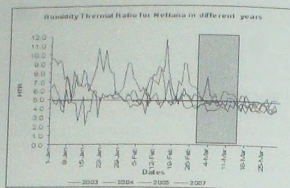
then subjected to point kriging for interpolation of points and contour structures were made which clearly differentiated areas with homogeneous amounts of dry periods. Different areas with the same spatial patterns have been discussed in terms of uniformity in distribution of rainfall which is much needed for rubber cultivation.

The results obtained for NE region showed agreement with an earlier study based on Precipitation Concentration Index (PCI). It is clear from the study that the rubber thrives in an area (traditional area) with comparatively lesser amount of dry period within its life span. While the worked out percentages varied from 9 to 22 per cent in Kerala, it varied from 10 - 32 per cent of dry period years in the case of the NE region. Parts of Wynaad, Malappuram, Alapuzha, Kollam and northern part of Idukki districts were found to have a good distribution of rainfall compared to the rest of Kerala, while similar such favourable areas were not seen in any regions of NE India. The major wet areas were more concentrated in the Western Ghats. The western and central parts of the

NE region were found to have an almost moderate distribution of water availability within the life span of the crop. The study can be completed if spatial profile on uniform yield, crop, age and agromanagement practices, are available for correlation.

2. Forewarning of pests and diseases

Previous incidences of *Corynespora* leaf disease were studied in relation to weather parameters which had a high degree of influence at Nettana, Dakshina Kanada region. From previous studies conducted, it was found that a maximum temperature of 34-36°C, a minimum of 17-20°C, morning relative humidity of 85 per cent, afternoon relative humidity of 40 per cent and sunshine duration of 8 h per day were the prerequisites to trigger off this disease in Nettana. On working out the Humidity Thermal Ratio (HTR) of the individual years, it was found that the value of HTR for the period spanning from the last week of February to second week of March was higher than 5.0 during the years in which high incidence of the disease was observed (Fig. Agromet.1). This



parameter was worked out for both morning and afternoon. While little relation with disease was found for the afternoon HTR, it was mainly the morning HTR which showed a strong relation with that of the disease incidence in the region.

3. Agromet database management

The agromet stations attached to Regional Research Stations are being maintained properly. Trainings and reinstallations were carried out for the maintenance of a uniform mode of data collection and presentation using standard meteorological forms.

Table Agromet. 1. Agrometeorological data from different research stations

Table Agri-met. 1. Agrometeorological data from different research stations							
Month	Temperature (°C)		Relative humidity (%)	Wind speed (km/h)	Sunshine (h)	Rainfall (mm)	Evaporation (mm)
	Maximum	Minimum					
RRII, KOTTAYAM, KERALA							
January	33.0	20.8	70	1.8	7.4	13.8	3.9
February	34.6	21.9	62	2.3	8.6	0.0	4.9
March	34.4	23.6	72	2.4	7.9	78.0	4.7
April	33.5	24.8	78	2.1	7.1	133.0	4.4
May	32.0	24.5	81	2.0	5.6	578.8	4.0
June	30.7	22.9	82	2.0	5.8	444.3	3.2
July	29.5	22.3	86	2.1	5.0	622.6	3.0
August	29.9	21.6	84	2.0	5.4	394.1	3.1
September	30.0	22.1	83	2.1	4.6	359.7	2.8
October	30.6	22.1	84	1.7	4.4	533.3	3.1
November	31.2	22.0	83	1.4	5.6	349.7	3.1
December	33.4	20.6	68	1.6	7.4	0.0	3.8

Month	Temperature (°C)		Relative humidity (%)	Wind speed (km/h)	Sunshine (h)	Rainfall (mm)	Evaporation (mm)
	Maximum	Minimum					
CES, CHETHACKAL, KERALA							
January	33.9	18.8	65	1.8	8.3	0.0	4.3
February	36.4	18.9	57	2.0	9.0	0.0	5.9
March	35.6	21.3	71	1.7	7.1	140.3	5.0
April	33.4	22.3	76	1.3	5.2	157.7	3.9
May	31.9	21.5	81	1.2	5.2	656.5	3.9
June	31.0	20.9	81	1.6	4.4	421.8	3.2
July	30.0	20.6	85	1.6	4.1	504.6	2.4
August	30.3	20.4	83	1.4	4.0	312.7	3.5
September	30.9	20.3	85	1.2	3.9	377.3	3.2
October	31.3	21.5	84	0.7	3.3	471.4	2.2
November	32.2	22.0	81	0.6	5.0	345.3	2.4
December	33.9	19.9	63	1.9	8.2	33.2	4.2
RRS, PADIYOOR, KERALA							
January	35.0	18.4	62	2.1	8.7	0.0	3.8
February	36.0	17.8	56	2.3	9.5	0.0	4.9
March	36.1	21.3	64	3.3	8.7	0.0	5.3
April	36.0	23.5	70	3.6	7.9	5.4	5.2
May	33.2	23.2	80	3.3	5.0	948.7	3.3
June	29.2	22.4	87	2.0	3.7	850.9	2.3
July	28.7	22.3	85	2.9	1.5	1001.7	2.6
August	29.6	22.3	87	2.6	3.2	669.0	2.7
September	29.9	22.3	84	2.3	3.6	437.9	2.4
October	31.9	22.3	82	1.8	4.9	322	2.9
November	33.1	21.9	80	1.3	5.6	67.6	2.8
December	34.0	18.8	62	1.7	7.9	0.0	3.4
HBSS, PARALIAR, TAMIL NADU							
January	32.3	20.9	74	-	6.5	20.4	4.5
February	34.8	21.0	74	-	7.3	0.0	5.5
March	34.3	22.7	81	-	5.2	235.8	4.5
April	34.6	23.9	81	-	6.1	36.5	4.6
May	30.9	24.0	83	-	4.6	240.0	3.9
June	32.6	23.6	81	-	5.4	173.2	3.6
July	30.9	24.1	84	-	3.4	159.7	2.3
August	31.1	23.7	79	-	5.1	74.4	3.4
September	31.2	23.0	86	-	2.5	443.8	2.4
October	30.9	23.2	89	-	3.4	271.4	2.9
November	31.1	23.2	89	-	4.0	0.0	2.6
December	33.1	21.6	71	-	5.7	0.0	4.1

Month	Temperature (°C)		Relative humidity (%)	Wind speed (km/h)	Sunshine (h)	Rainfall (mm)	Evaporation (mm)
	Maximum	Minimum					
HBSS, NETTANA, KARNATAKA							
January	35.7	13.6	88	-	8.2	0.0	4.2
February	37.0	12.6	85	-	8.5	0.0	5.4
March	36.3	16.8	87	-	8.3	11.3	5.4
April	36.1	19.8	87	-	7.7	39.2	5.1
May	34.4	19.9	90	-	5.9	532.6	3.8
June	30.1	18.8	92	-	5.3	993.1	2.3
July	28.5	19.0	90	-	1.9	1577.9	2.1
August	28.9	18.6	92	-	2.7	1034.2	2.5
September	30.8	18.8	92	-	5.0	560.6	2.4
October	32.3	18.4	91	-	5.4	430.0	2.4
November	33.2	17.9	87	-	6.0	166.3	2.6
December	34.5	12.9	81	-	7.9	0.0	3.2
RRS, DAPCHARI, MAHARASHTRA							
January	32.5	13.9	54	1.9	7.9	0.0	2.7
February	37.0	16.6	51	1.9	9.1	0.0	4.4
March	35.7	18.9	57	2.2	8.4	24.8	5.3
April	38.5	22.4	60	2.6	8.9	0.0	6.8
May	37.5	25.4	62	3.9	8.2	51.2	7.3
June	33.2	25.2	77	2.9	5.6	521.0	5.1
July	31.1	24.4	90	4.3	1.9	1130.2	3.3
August	28.7	23.7	90	3.6	2.5	1018.5	2.8
September	32.1	23.2	84	1.7	3.7	224.7	5.0
October	34.1	21.6	71	1.3	7.9	49.0	4.1
November	34.0	17.7	65	1.5	7.8	0.0	3.8
December	33.9	15.5	66	1.5	7.1	0.0	2.8
RRS, AGARTALA, TRIPURA							
January	25.2	11.0	74	0.7	6.8	0.0	1.8
February	31.1	17.6	71	3.0	7.3	0.0	2.5
March	34.1	19.3	68	2.5	7.9	0.0	3.7
April	34.0	22.8	72	3.5	8.0	64.2	3.4
May	33.1	23.9	79	3.0	6.5	484.8	3.4
June	31.9	25.2	87	4.5	4.7	459.0	1.9
July	32.2	25.9	83	3.4	4.7	172.2	2.4
August	32.8	25.4	83	2.3	5.6	319.4	2.5
September	31.6	24.8	84	2.0	5.3	248	2.3
October	31.9	23.3	83	0.8	6.3	61.0	2.2
November	29.2	18.2	77	0.9	6.0	3.0	1.8
December	27.3	12.0	73	0.9	6.3	0.0	1.7

Month	Temperature (°C)		Relative humidity (%)	Wind speed (km/h)	Sunshine (h)	Rainfall (mm)	Evaporation (mm)
	Maximum	Minimum					
RRS, GUWAHATI, ASSAM							
January	25.4	12.1	76	1.5	5.4	0.0	1.3
February	28.7	15.4	68	0.6	5.6	0.0	1.9
March	32.6	16.1	63	2.0	7.5	14.0	2.1
April	32.1	19.2	74	1.0	6.1	3.1	1.5
May	33.1	22.0	81	0.4	6.0	92.0	1.5
June	32.0	24.5	87	0.1	3.6	136.6	1.2
July	33.6	25.0	87	0.2	4.5	107.2	1.7
August	34.4	25.2	84	0.2	5.7	114.2	1.8
September	33.1	23.5	85	0.1	5.4	202.0	1.6
October	31.7	20.6	83	0.3	6.6	105.4	1.9
November	28.0	17.0	80	0.6	4.9	36.0	1.4
December	25.6	13.1	75	1.3	5.8	4.0	1.2
RRS, TURA, MEGHALAYA							
January	25.1	8.5	70	1.6	6.5	0.0	1.8
February	27.5	13.1	68	2.3	6.3	36.0	2.9
March	31.6	12.3	56	1.7	8.2	0.0	3.7
April	32.3	17.1	75	2.1	7.2	193	2.9
May	32.9	19.0	79	1.5	5.4	464	3.1
June	32.1	21.4	85	1.3	1.1	415	3.0
July	31.5	22.8	86	1.2	3.3	344	2.9
August	32.1	22.2	85	0.8	5.3	233	3.0
September	29.9	20.8	86	1.2	4.1	339	2.7
October	31.3	18.2	82	1.1	6.8	174	2.9
November	29.6	14.3	77	0.8	5.3	0.0	2.5
December							1.6
RES, NAGRAKATA, WEST BENGAL							
January	24.5	8.5	81	0.3	5.7	0	1.4
February	28.3	13.9	79	0.8	4.6	29.9	2
March	30.9	13.9	73	1.3	7.5	6.8	3
April	31.6	18.5	77	1.6	6.5	144.8	3.2
May	32.5	22	83	1.4	5.1	326.5	2.9
June	31.9	24.5	87	1.3	3.6	769.3	1.7
July	32.2	25.5	88	1	2.5	770.1	1.8
August	33.4	24.8	86	1.1	5.3	458.7	2.7
September	31.4	23.5	87	1	4.3	993.1	1.8
October	31.3	19.3	83	0.6	7.5	143.2	2.3
November	26.9	14.8	82	0.5	4.9	26.2	1.7
December	25.2	10.5	80	0.6	6	1.2	1.5

RRS, DHENKANAL, ORISSA

January	29.1	11.8	74	1.2	7.6	0	2.8
February	34.6	14.4	56	1.1	9.2	0	3.4
March	36.3	19.9	58	1.9	8.9	0	4
April	39.5	24.7	60	3	8.5	22	5.5
May	37.7	24.3	63	3.5	8	105.2	4.9
June	36.4	25.6	77	2	5.2	245.5	3.4
July	32.7	25.1	84	2.2	3.4	231.4	2.6
August	31.3	24.8	83	2.7	2.4	253	2
September	33.1	24.5	80	2.2	5.7	254	2.4
October	33.9	21.5	76	1.1	6.5	10.8	3.1
November	30.5	18.3	73	0.9	6.6	16.8	2.7
December	29.6	13.3	68	0.8	7.9	0	2.3

ANNUAL EXPENDITURE

Expenditure at a glance (2006-07)

Head of Account	Expenditure (Rs. in lakhs)
Non-Plan	
General charges	393.89
Projects (CES)	242.61
Total	636.50
Plan	
General charges	1256.10
NERDS Research Component	209.09
Total	1465.19
Gand Total	2101.69

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Scientist S3
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Rubber Research Institute of India

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Cover Photo

1. One hundred years old rubber trees growing in Narbong Tea Estate, Darjeeling, Himalayas.
Photograph: Dr. M. A. Nazeer; 2 & 3. Rubber and tea intercropping in the Regional Experiment Station, RRIL, Nagrakata, West Bengal.

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* With particulars of personnel as on 31.03.2007

Continued from inside front cover

Research divisions and functions

The major research divisions are Agronomy and Soils, Biotechnology, Botany, Germplasm, Mycology and Plant Pathology, Plant Physiology, Rubber Chemistry, Physics and Technology and Agricultural Economics. Studies on Latex Exploitation, Clone Evaluation, Genome Analysis and DRIS Fertilization are dealt separately.

The thrust areas of research of Agronomy and 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 Mycology and 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 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 establishments at Dapchari (Maharashtra), Dhenkanal (Orissa), Nagrakata (West Bengal), Paraliyar (Tamil Nadu), Nettana (Karnataka) and Padiyoor (Kerala).

Regional soil testing laboratories have been established at Taliparamba, Kozhikode, Thrissur, Muvattupuzha, Pala, Kanjirappally, Adoor and Nedumangadi. Mobile units for soil and leaf analysis are available at the 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).

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).

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