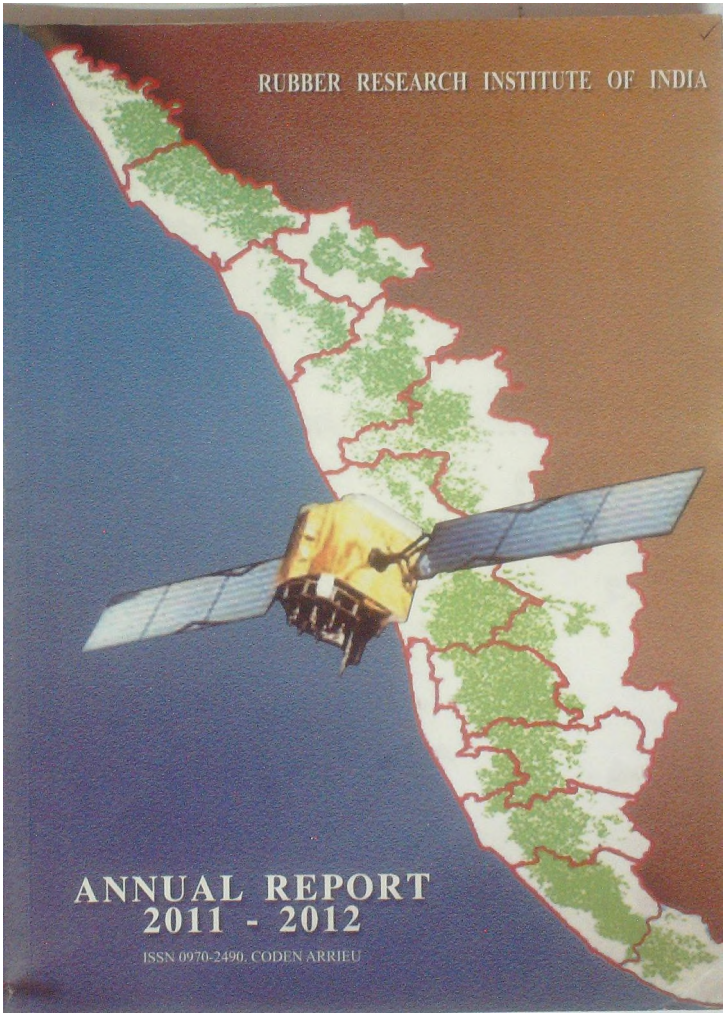

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Rubber Research Institute of India

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

Location

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

Organization

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

Continued on inside back cover

ANNUAL REPORT
2011-2012

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

Organization

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

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DIRECTOR'S REVIEW

The year 2011-12 saw marked progress in the various projects taken up by the Institute. A significant achievement was development of rubber distribution map of Kerala and Kanyakumari district of Tamil Nadu using satellite images with very good accuracy compared to the traditional land survey method. Another important development was that the breeding pool was expanded by adding seven more wild Amazonian accessions showing promising yield and girth characteristics. Accession AC 166 showed good yield although not comparable with RRII 105. The breeding team of RRII made a few hundred new hybrids by crossing between promising Wickham lines and Amazonian accessions.

RRII 400 series continued to find increasing acceptance among the growers.

A few clones showing promising drought tolerant traits were identified. Data from large-scale field evaluation trials continued to show superior yield and girth in PB 280. RRII 429 was the top yielder in Agartala and Nagrakata. Work for phase III of the participatory clone evaluation project was initiated. A mapping population for yield and *Phytophthora* resistance generated through interspecific cross between RRII 105 (*Hevea brasiliensis*) and F 4542 (*Hevea benthamiana*) are under nursery evaluation.

More number of genetic transformations using MnSOD gene was made and plants were generated. Several SSR and SNP markers were generated to enhance the genomic resources in rubber. A genetic linkage map was constructed with 224 markers. Cold-induced variations in methylation were detected in *cis* regulatory sites of a few genes associated with latex biosynthesis. Two drought-responsive genes were cloned and characterised.

A new study on evaluation of ortets selected from Regional Research Stations was initiated during 2011-12. RRII 430 showed relatively better intrinsic drought tolerant characteristics. 18% of the rubber growers resorted giving life saving irrigation to one year old rubber plants during summer in Central Kerala. This was 5 % in Southern region.

There were clear indications that secondary and micronutrient were fast getting depleted in the soil with long term rubber cultivation. Retaining natural weeds in mature rubber plants improved soil health. Using good quality planting materials and adopting good agricultural practices led to reducing the gestation period by over one year. Vetiver was a highly effective vegetative hedge for preventing soil erosion in sloppy terrain.

Low frequency tapping is the only immediate solution of scarcity of skilled tappers. Studies on low frequency tapping continued to give good yield with low

incidence of TPD, but timely stimulation was a prerequisite. Different clones behave differently to ethephon stimulation. Many experimental hybrids in the pipeline showed good response of ethephon stimulation. Low frequency tapping helps to address the issue of scarcity of skilled tappers. However, RPS network should be used for pooling tappers and other labours. Between 1980-2013, wage share of tappers decreased in real terms by 1.25%.

More farm mechanisation is becoming an imperative because of the acute labour shortage experienced in the agriculture sector. Three models of one man carrying mist blower were tested and recommended for spraying in rubber plantations. Our studies showed that minimum tillage should be practiced and indiscriminate use of heavy machinery for land preparation should be avoided to conserve soil from erosion.

Irradiating fresh latex with gamma rays gives better raw material for producing prevulcanised latex and deproteinised natural rubber with better processing characteristics. A new peroxide vulcanising system with excellent scorch control was developed. A new devulcanising agent was identified. A modified fast coagulation system was developed which allowed incorporation of fillers in latex stage. Processing aspects of a new polymeric filler were standardised which could replace carbon black. A protocol of recovering rubber from skim latex was also developed. Technical consultancy wing of RRII received samples from 711 firms which were tested for various parameters. 41 products were developed and queries from 1337 units were addressed during the reporting period. Rubber technology research and consultancy service for the industry will continue to get more priority in the coming years.

AGRONOMY/SOILS DIVISION

The research programmes of the Division are aimed at development and periodic refinement of agromanagement practices to improve growth and yield of rubber in different agro-climatic regions, to reduce cost of cultivation and to sustain soil quality. Various experiments on nutrient management, soil and water conservation, rubber based cropping systems, farm mechanization, ground-cover management and stress management are in progress. Experiments to develop an agronomic package for reducing the gestation period of rubber continued. Studies were also initiated to estimate the soil CO_2 flux from rubber plantations using respiration analyzer. Development of the rubber information system using remote sensing and GIS continued. The Division also functions as a centre for dissemination of knowledge on various soil and crop management techniques of rubber.

1. Nutrient management

The field experiment to study the effect of long term use of chemical fertilizers and organic manures on growth and yield of rubber and physico- chemical properties of soil continued. Significantly higher girth was noticed for the treatment with 25% of chemical fertilizer and 75% farmyard manure (Table Ag. 1). Different treatments with farmyard manure (FYM) alone or in combination with chemical fertilizers resulted in higher leaf N, P and Zn compared to the treatment with chemical fertilizer alone.

The experiment on sequential skipping of fertilizer application in mature rubber was continued. No significant difference among the different treatments was noticed for the last year annual yield and for the girth increment during the period 2002 to 2012.

Table Ag. 1. **Effect of long term (11 years) use of chemical and organic manures on growth of rubber**

Treatments	Girth (cm)
No fertilizer / No manure (Control)	54.3
Farmyard manure (FYM) alone	57.2
Chemical Fertilizers alone	56.3
25 % Fertilizers + 75 % FYM	60.3
50 % Fertilizers + 50 % FYM	56.9
75 % Fertilizers + 25% FYM	56.2
SE	0.66
CD (P =0.05)	1.99

Soil samples were collected from 110 replanting fields of 19 estates in the traditional rubber growing region of Kerala to assess the secondary and micronutrient status. Status of organic carbon and secondary and micronutrients showed wide variation (Table Ag. 2). Considerable variation was observed between regions also. Field experiments to study the effect of supplementing secondary and micronutrients on soil test basis were initiated at four estates *viz.* Thamarasery (Kozhikode), Palapilly (Thrissur), Cheruvally (Kottayam) and New Ambadi (Kanyakumari).

The field experiment to study the effect of composted coir pith (CPOM) as soil amendment in marginal soils, at Thanneermukkom, Cherthala continued. Girth of plants after four years did not show significant difference among the different treatments with FYM, CPOM and control.

Table Ag. 2. **Organic carbon, secondary and micronutrients status and pH of soils in the estate sector of traditional rubber growing regions**

Region	Mean/ Range	OC (%)	Ca	Mg	S	Zn	B	pH
					(ppm)			
North	Mean	2.2	205.2	19.2	2.4	0.7	0.1	4.56
	Range	1.55-3.5	129.5 – 398.2	3.6 - 49.0	104-4.1	0.5-140	Trace – 0.2	4.22 -4.93
North-central	Mean	1.3	300.8	47.1	6.8	1.11	0.4	4.47
	Range	0.5-1.9	116.1-611.8	14.7-106.5	2.7-11.1	0.3-2.1	Trace-0.8	4.20-4.86
Central	Mean	1.7	76.0	10.3	3.2	0.7	0.7	4.27
	Range	1.0-3.0	30.9-132.8	7.3-17.5	1.7-8.3	0.5-1.1	0.4-1.0	4.09-4.69
South	Mean	1.7	302.9	43.7	6.0	0.9	0.2	4.5
	Range	1.1-2.8	117.5-1029	8.7-167.3	2.0-16.6	0.4-1.7	Trace- 0.3	4.1- 4.9
General Critical Limit (GCL)	General	> 0.50 %	300 ppm	120 ppm	> 5 ppm	> 1.0 ppm (HCl)	> 0.5 ppm	-
	Rubber	> 0.75 %	-	10-25 ppm	-	-	-	-
% of field below GCL	General	Nil	84	95	63	68	68	-
	Rubber	2	-	21	-			

In the study on nutrient uptake by prominent clones of *Hevea*, it was observed that the micronutrient contents varied among the RRII 400 series clones. RRII 430 recorded highest iron content in trunk, branches and root.

2. Soil and water conservation

Experiment on evaluation of biological bunds for soil and water conservation in rubber plantations was in progress. Among the different vegetative hedges, planting of vetiver was found to be the best to control soil erosion in rubber plantation and was significantly superior to all other vegetative hedges (Table Ag. 3).

Table Ag. 3. **Quantity of soil deposited in trench 2 years after planting**

Treatments	Quantity of soil in the trench (tonnes ha^{-1})
1. Rubber + Vetiver	1.3
2. Rubber + Guinea grass	1.8
3. Rubber + Pineapple	1.8
4. Rubber + *Strobilanthes sp*	2.1
5. Rubber alone	2.6
SE	0.15
CD (P =0.05)	0.47

3. Intercropping and cropping systems

Experiment to evaluate the feasibility of growing perennial intercrops *viz.* coffee, vanilla, *Garcinia* and nutmeg under normal and paired row systems of planting of rubber continued. There was no significant difference between treatments with respect to growth of rubber plants (Table Ag. 4) in the paired row system of planting. In the normal system of planting, growth was significantly higher compared to control when mixed cropped with vanilla and *Garcinia*. Yield of rubber was not significantly influenced by mixed cropping with perennial crops. Coffee and vanilla continued to yield well while growth and yield of *Garcinia* were adversely affected by shade. Nutmeg had started bearing.

The experiment on evaluation of shade tolerant medicinal plants in mature rubber plantation was in progress. Among the various medicinal plants, Aratha (*Alpinia calcarata*) and Karimkurinji (*Strobilanthes cuspida*) performed well.

Table Ag. 4. **Mean girth and girth increment (cm) of rubber trees under paired row and normal systems of planting**

Treatments	Paired row system of planting		Normal system of planting	
	Girth 2012	Girth increment (2003-2012)	Girth 2012	Girth increment (2003-12)
Rubber alone	58.3	46.2	58.8	46.9
Rubber + Garcinia	61.2	48.3	63.6	51.4
Rubber + coffee	61.1	49.5	60.0	48.6
Rubber + vanilla	58.1	45.9	61.8	50.7
Rubber + nutmeg	59.4	47.9	61.1	49.9
SE	1.12	0.83	0.91	0.75
CD (P = 0.05)	NS	NS	2.81	2.25

Experiment on inter-planting of rubber with timber trees *viz.* teak, wild jack and mahogany continued. Growth and yield of rubber were not significantly influenced by row spacing, type of timber intercrops and their interactions. Wild jack performed better than teak and mahogany.

The field experiment on development of a multi-species rubber based cropping system for Tamil Nadu region (collaborative project with TNAU) was continued. The growth of rubber was not significantly influenced by the establishment of inter crops nine months after planting.

4. Ground cover management

The observational trial on establishment of *Mucuna* (cover crop) and fodder crops in the later immaturity phase of rubber, after the removal of pineapple intercrop was continued. It was observed that *Mucuna* spread in 75% area of the field (7th year). Among the different fodder crops, *Stenotophrum* continued to perform well in the 7th year of plantation.

Nitrogen fixation by legume cover crops, *Pueraria phaseoloides* and *Mucuna bracteata* were estimated using ^{15}N isotope dilution method. For *P. phaseoloides*, nitrogen fixation ranged from 79.8 kg N ha^{-1} during June-July to 39.3 kg N ha^{-1} during October-November and averaged 58.1 kg N ha^{-1}. For *M. bracteata*, nitrogen fixation ranged from 83.9 kg N ha^{-1} during June-July to 57.0 kg N ha^{-1} during October-November and averaged 69.7 kg N ha^{-1}.

The field experiment initiated at CES, Chethackal to study the impact of weeds on growth of rubber and to compare the effect of different covers on soil physico-chemical and biological properties as well as biomass and nutrient turnover was discontinued due to technical reasons and arrangements were made to replant the area in the coming season.

The project on comparison of rubber plantations with and without control of weed flora at Pathampuzha village in Kottayam District was continued. Soil samples from three depths *viz.* 0-15, 15-30 and 30-45 cm were collected and stock nutrients in 0-45 cm determined. Data on stock nutrients in 0-45 cm soil layer indicated that OC, N, Av.K, Av.Ca and Av.Mg were significantly higher in weeds not-controlled fields (Table Ag. 5).

It is to be noted that the significant increase in nutrient stock had taken place in

Table Ag. 5. **Nutrient stock in 0-45 cm soil layer per hectare under weeds controlled and not controlled rubber plantations**

Rubber fields	C	N	Av.P	Av.K	Av.Ca	Av.Mg	Av. Cu	Av.Zn	Av.Fe	Av.Mn
	tons ha^{-1}	tons ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Clean-weeded	70.3	5.5	12.1	223.5	275.0	80.9	21.7	4.9	245.9	139.7
Not-weeded	76.5	6.3	3.4	262.8	404.4	144.5	14.8	4.8	247.1	144.0
Significance	*	**	NS	*	**	**	*	NS	NS	NS

* $P < 0.05$; ** $P < 0.01$

weeds-not controlled rubber fields in spite of no fertilizer input for about 10 years. Measurements of soil CO_2 flux which is an indicator of soil microbial activity in these fields indicated that (Fig. Ag. 1) weeds not-controlled fields were significantly higher in microbial activity.

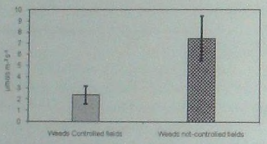


Fig. Ag. 1. **Soil CO_2 efflux in weeds controlled and not controlled rubber**

rubber growing soils was continued. Among the different land preparation methods evaluated, ploughing, pitting and terracing by earth mover (Hitachi) recorded significantly higher rate of soil erosion (11.6 t ha^{-1}) compared to all other methods (Table Ag. 6).

Table Ag. 6. **Quantity of soil deposited in trenches (2012)**

Treatments	Quantity (t ha^{-1})
Manual pitting and terracing (control)	7.2
Manual terracing and pitting by tractor- mounded hole digger	8.0
Pitting and terracing by Hitachi	8.4
Ploughing, pitting and terracing by Hitachi	11.6
SE	0.485
CD ($P = 0.05$)	1.55

5. Planting techniques

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

The field experiment to study the effect of mechanized land preparation on soil erosion and physico-chemical properties of

The experiment on effect of planting density on growth and yield of rubber was continued and the result indicated that, plants in the lowest density of 420 trees ha^{-1} recorded significantly higher yield (g t^{-1} t^{-1}) compared to all other treatments. The annual yield per hectare was significantly higher in the density 549 trees ha^{-1}.

The multi-locational trials on the effect of pit size on growth of plants were in progress. Significant difference in growth of rubber and development of lateral roots was not observed in all locations one year after planting.

With the objective to find out an alternative and cost effective germination media for rubber, an observation study was conducted twice. The experiments were laid out in complete randomized design with 15 treatments and two replications. The treatments included (1) germination bed with river sand (control) (2) raw coir pith (3) stain removed coir pith (4) new wood shavings (5) six months old wood shavings (6) raw saw dust (7) dried saw dust (6 months old) (8) rice husk (9) straw (10) coconut leaf with a layer of soil (11) dried litter (12) rock powder (13) directly on the seed bed (without medium) (14) soil rite and (15) heaping seeds on floor. In both experiments, germination bed with river sand, stain removed coir pith, dried saw dust (6 months old) and soil rite recorded significantly higher germination percent compared to all other germination media.

6. Development of agro management techniques for reducing the gestation period

The field experiment initiated to develop an agronomic package to reduce the immaturity period of *Hevea* at Malankara Estate, Thodupuzha was in progress. The girth of the plants under integrated management was significantly superior to all other treatments. Eighty per cent of the trees under integrated management attained tappable girth in six years compared to only 41.7% under the current recommended practice.

The similar experiment initiated at CES, Chethackal is being continued. There was significant difference in the performance of the two types of planting material used. The growth of direct seeded green - budded polybag plants was found to be superior to green - budded stumps raised in polybags. The girth of the plants under integrated management was found to be superior to that of respective type of planting material under standard practice (Table Ag. 7).

Table Ag. 7. **Effect of planting material and agro management practices on growth of rubber (December 2011)**

Treatments	Girth of rubber plants (cm)
Green - budded stumps raised in polybags + Std. practice	28.1
Green - budded stumps raised in polybags + Integrated management	30.4
Direct seeded green-budded plants + Std. practice	31.6
Direct seeded green-budded plants +Integrated management	34.1
SE	0.39
CD (P =0.05)	1.16

The experiment on the effect of different types of planting material (polybag - one whorl, two whorl and three whorl and root trainer - one whorl, two whorl and three whorl) on growth of rubber was continued. The results indicated that three whorl polybag plants were significantly superior to all others irrespective of the types of planting material and different stages of growth.

The experiment on age of rootstocks on quality of planting materials in rubber was continued. During the initial 18 months after planting, brown budded polybag plants (poly bag size 55 x 25 cm) and green budded polybag plants (retained for 16-18 months in poly bags of size 45x18 cm) had a significantly superior growth compared to the growth of all other planting materials. Twenty four months after planting,

significant difference in girth was not observed among different types of planting materials and same trend continued during 30 months after planting.

7. Stress management

The field experiment at Puthukkad estate, Thrichur, to develop agro--management techniques to mitigate the adverse effects of drought was continued. Growths of the plants were superior in the treatments with tillage, super absorbent polymer and direct seeding in polybags.

In the study on accumulation of potassium and drought tolerance in *Hevea* clones, significant difference was observed in leaf potassium content in different genotypes *viz.* germplasm accessions, HP clones and check clones (drought tolerant clones *viz.* RRII 414, RRII 430, RRIM 600 & RRII 208 and drought susceptible clones *viz.* RRII 105 and Tjir 1). Potassium content was significantly higher in clones MT 1627, MT 4788, MT 43MT 1649, HP 92, HP 53, HP 225 and RO 2153 compared to RRII 105 and Tjir 1. RRII 430 also recorded higher K content in leaves compared to RRII 105 and Tjir 1.

8. Rubber growing soils

The study on comparison of six soil ecosystems *viz.* mature rubber, rubber - *Mucuna* cover cropped, rubber - pineapple intercropped, cassava monocrop, teak mature plantation and forest in Kottayam district continued. Soil respiration measurements were carried out in these soil systems by measuring the CO_2 flux from soil surface which indicates the soil microbial activity and organic matter decomposition. The CO_2 flux data indicated distinct differences among the studied systems (Fig. Ag. 2). The soils under rubber systems and cassava mono-crop were similar and significantly lower in soil respiration than forest and teak soil systems.

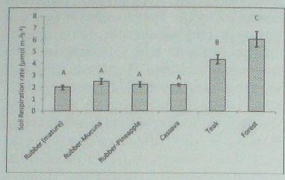


Fig. Ag. 2. CO_2 Flux in different soil systems

Also available status of Fe, Zn, Cu and Mn were estimated in these ecosystems. Zn was not significantly different among the systems studied. The Cu content was significantly higher in rubber based soil systems compared to teak and forest soils. The CO_2 flux (soil respiration) and Cu content in soils under the studied systems were significantly and negatively correlated. The Permanganate Oxidizable Soil Carbon (POSC) is reported to be a good index of active carbon which is labile in nature and closely associated with soil microbial activity. POSC contents were comparatively less in cassava and rubber based soil systems than multi-species teak and forest soil systems. However, rubber with *Mucuna* was an exception with relatively higher POSC content which is comparable to teak or forest systems.

The project on management of active and microbial carbon pools at Pottamkulam Estate, Yenthayar was continued. Girth of the rubber plants in different treatments did not differ significantly. Soil samples were collected from each plot and analyzed for OC, total nitrogen, pH, available Ca, Mg, P and K and POSC (Table Ag. 8). The POSC

Table Ag. 8. **Soil properties in different treatments on unit weight basis**

Treatments	N %	C %	POSC mg kg^{-1}	pH	Ca mg kg^{-1}	Mg mg kg^{-1}	P mg kg^{-1}	K mg kg^{-1}
NC	0.2	3.2	638.8	4.6	191.0	73.0	49.6	99.6
MC	0.2	3.1	592.1	4.5	109.7	27.5	27.4	83.5
NC+BF	0.2	3.2	636.8	5.0	327.8	104.0	168.7	210.5
MC+BF	0.2	3.4	694.3	4.5	148.7	34.2	59.0	91.1
NC+BF+G	0.3	4.0	867.7	5.3	675.2	266.3	127.8	308.6
MC+BF+G	0.3	3.8	746.8	4.5	150.7	36.9	22.9	99.1
Control	0.3	3.3	690.1	4.4	181.5	36.1	117.3	93.0
SE	0.0	0.1	19.7	0.1	5.0	2.1	-	2.7
CD (P= 0.05)	0.0	0.4	60.7	0.3	15.6	6.4	NS	8.3

(NC- Natural Cover, MC- Mucuna Cover, BF –Biofertilizer consortium, G- Glyricidia)

was more in the treatments with *Glyricidia* indicating that the labile carbon contents increased with *Gliricidia* mulchings. It was also noticed that the pH in general was more in treatments under natural cover than under *Mucuna* cover.

Measurement of soil CO_2 flux in a mature rubber plantation in RRII farm from four sites was carried out continuously on an hourly basis. The data recorded from June 2010 to December 2012 were classified to different seasons *viz.* monsoon (June to Sept), post monsoon (Oct – Dec), winter (Jan - Feb) and summer (Mar - May) and the hourly CO_2 flux values are shown in Fig. Ag. 3. The flux rates from 4 different sites, recorded separately, were averaged for each hour for a particular season. CO_2 flux rate was more during summer

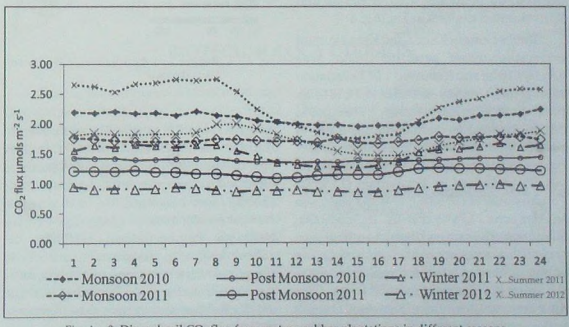


Fig. Ag. 3. **Diurnal soil CO_2 flux from mature rubber plantations in different seasons**

2011 among the studied seasons. The diurnal variations were more in summer 2011 and it could be noticed that during the day hours (09 – 20 hrs), CO_2 flux was lower than during the night hours (21- 08 hrs). Similar trend could be observed in winter 2011 also. Not much diurnal variations could be observed in soil CO_2 flux in other seasons.

An experiment was initiated to study the changes in soil nutrient status of rubber plantations in comparison to adjacent forest in non- traditional rubber growing tract of Andhra Pradesh.

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

Project on developing rubber based information system using remote sensing and GIS was continued. Preparation of rubber distribution maps and its statistics for Kerala and Kanyakumari district in Tamil Nadu has been completed. Spatial distribution of rubber area of Kerala and Kanyakumari is given in Fig. Ag. 4.

Fig. Ag. 4. **Rubber area distribution of Kerala and Kanyakumari**

Rubber area in Kerala and Kanyakumari estimated using satellite image was 5,19,909.10 ha and it showed 1.04 % variation from ground survey statistics (5,14,524 ha). Plantations with age less than 3 years could not be mapped as prominent signature of rubber was absent. Highest area under rubber was observed in Kottayam district (48.19%) followed by Ernakulam (23.58%), Pathanamthitta (20.54%), and the least was in Alapuzha (3.74%) district. In Alapuzha, Kannur and Kasaragod districts rubber area showed wide variations with ground survey statistics. No definite trend was observed in the extent of difference of district wise rubber area compared to ground survey. Overall accuracy of satellite image classification ranged from 75 to 96.68% with Kappa statistics varying from 0.63 to 0.95 %. Rubber area distribution under different classes of elevation, slope and soil management units (SMU) was assessed for Kerala and Kanyakumari district using GIS overlay technique. Results showed that 70% of the rubber area was distributed in 0-100 m elevation. Highest percentage of rubber area was distributed in slope categories 5-10 and 3-5 %. Rubber distribution was highest in SMU 3, 2 and 4 categories.

Spatio-temporal analysis of rubber area change during 1980-1992-2007 was attempted for Kanyakumari district. Area under rubber in 1980 was 10693.27 ha and it increased to 15886.71 ha in 1992 and to 20781.71 ha in 2007. Overlay analysis indicated that during 1980 - 1992 newly planted area was 10452.71 ha and same was 11269.02 ha during 1992-2007. Replanted area was high during 1992-2007 compared to 1980-1992.

FERTILIZER ADVISORY GROUP

Based on the analysis of soil and leaf samples discriminatory fertilizer recommendations were offered to large estates and small growers through the laboratories at RRII and regional stations. Two mobile soil testing laboratories are also functioning for the benefit of the small growers. The laboratories at regional stations also offer estimation of dry rubber content of latex samples. The details of sample analysed are given in the Table FAG. 1.

Table FAG. 1. **Details on soil, leaf and latex analysis and fertilizer recommendation offered**

Parameter	Number
Soil	11,100
Leaf	1,750
MST program	66
Fertilizer recommendation	6,500
DRC of latex samples	62,500

BIOTECHNOLOGY DIVISION

Genetic improvement of *Hevea brasiliensis* using modern tools is the major objective of Biotechnology research at RRII. The major ongoing research programmes in Biotechnology Division are: i) development of *in vitro* propagation methods for elite *Hevea* clones; ii) development of transgenic *Hevea* plants for better adaptation to environmental stressess and tapping panel dryness, latex yield and disease tolerance; iii) development of haploid plants, *in vitro* fertilization techniques and embryo rescue to compliment conventional breeding programmes; iv) study of molecular mechanism and characterization of genes controlling tolerance to diseases, abiotic stresses, latex biosynthesis and characterization of related genes; and v) study of laticifer cell specific gene expression and characterization of laticifer cell specific promoters.

1. Somatic embryogenesis and plant regeneration

Callus induction was obtained from newly initiated cultures using leaf explants collected from glasshouse grown budgrafted plants of *Hevea* clone, RRII 105. The optimized embryo induction medium supplemented with phytohormones such as

4.4 µM BA, 2.9 µM GA_3, 1.25 µM Kin, 0.45 µM 2,4-D and 1.08 µM NAA was used for embryogenesis. In order to improve the efficiency of somatic embryogenesis and plant regeneration different concentrations of poly ethylene glycol (0 - 10.0 g L^{-1}) and ABA (0-1.9 µM) were added directly in the medium. Maximum embryo induction frequency of 62.5% was obtained with a PEG concentration of 5.0 g L^{-1} and 0.75 µM ABA (Table Biotech. 1).

Table Biotech. 1. **Effect of ABA and PEG on embryo induction**

ABA	PEG (g L^{-1})				
(µM)	0	3.0	5.0	8.0	10.0
0	55.0	55.5	55.0	55.0	52.5
0.38	52.5	55.0	57.5	57.5	52.5
0.75	52.5	60.0	62.5	57.5	57.5
1.1	57.5	60.0	62.5	60.0	45.0
1.5	50.0	57.5	57.5	55.0	47.5
1.9	55.0	55.0	55.0	57.5	52.5
CD (P = 0.05) 1.8					

Mean % of embryo induction in 20 replicate samples of 100 mg callus. Experiments were repeated four times.

Experiments were also carried out for optimizing the concentration of sucrose for embryo maturation by supplementing the medium with sucrose ranging from 30-80 g L^{-1} and the results are given Table Biotech. 2.

Table Biotech. 2. **Effect of sucrose concentration on embryo maturation**

Sucrose (g L^{-1})	Embryo maturation (%)
30	65. 0
40	67. 5
50	70. 0
60	77. 5
70	75.0
80	75.0
CD (P = 0.05) 2.3	

Values given are mean % of replicate cultures and the experiment was repeated thrice

Maximum embryo maturation frequency of 77.5% was obtained with 60 g L^{-1} sucrose in WPM medium containing organic supplements CW (10%), malt extract (200 mg L^{-1}), casein hydrolysate (400 mg L^{-1}) and phytohormones, BA (0.5 mg L^{-1}), IBA (0.1 mg L^{-1}) and GA_3 (1.0 mg L^{-1}).

Refinement experiments were carried out with different media additives for somatic embryogenesis from immature inflorescence. Effect of amino acids *viz.* proline, arginine, asparagine, glycine, L-cystein and glutamine on embryo induction was evaluated. Four combinations with varying concentrations of the individual amino acids were incorporated in the embryo induction medium and the frequency of embryo induction was assessed in terms of the number of well developed embryos obtained after two subcultures. A combination of proline (200 mg L^{-1}), arginine (40 mg L^{-1}), asparagine (150 mg L^{-1}), glycine (20 mg L^{-1}), L-cysteine (50 mg L^{-1}) and glutamine (400 mg L^{-1}) could induce more number of normal embryos compared to the other combinations. Effect of phloroglucinol on embryo induction was also assessed by incorporating different levels of phloroglucinol (0.5- 5.0 mM) in the embryo induction medium. When the embryogenic calli were cultured over medium supplemented with 2-3 mM phloroglucinol along with the proven growth regulators, there was no callus proliferation. Instead more number of cotyledonary embryos could be obtained. These embryos were transferred to maturation medium for further development.

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

2.1. Embryo rescue and induction of polyembryony in *Hevea brasiliensis*

A protocol was developed for the rescue of immature embryos by adopting *in ovulo*

embryo culture, with successful plant regeneration in *Hevea brasiliensis*. By optimizing suitable culture media, the percentage of embryo recovery could be increased to 42%.

For the induction of multiple embryos, cultures were initiated with immature fruits (8-10 weeks old) of clone RRII 105. The growth regulator combination of GA_3 (2 mg L^{-1}) and Kin (3 mg L^{-1}), reported earlier for the induction of multiple embryos was kept constant and different levels of other growth regulators *viz.* zeatin, NAA, 2, 4-D and BA were tried for multiple embryo induction. Multiple embryos were induced in Nitsch basal medium supplemented with the growth regulators, zeatin (0.3 mg L^{-1}), GA_3 (2 mg L^{-1}) and Kin (3 mg L^{-1}). A maximum of 37 embryos could be obtained in presence of the above growth regulator combinations (Table Biotech. 3). In the same combination,

Table Biotech. 3. **Effect of various growth regulators on the development of cultured ovules**

Treatments	GA_3 (mg L^{-1})	Kin (mg L^{-1})	Zeatin (mg L^{-1})	Number of embryos (mean)
T1	3	2	0.1	13
T2	3	2	0.2	14
T3	3	2	0.3	37 ##
T4	3	2	0.4	2
T5	3	2	0.5	15
CD (P = 0.05) 1.0				

Embryos with embryogenic callus

along with multiple embryos, emergence of embryogenic calli also could be observed.

Three sets of polyembryonic plants obtained earlier were subjected to RAPD analysis for assessing the uniformity as well as origin. RAPD–PCR reactions were performed using two selected primers as per standard procedure. It was observed that the RAPD profile of all the polyembryony derived plants were different from the maternal parent RRII 105 for both the primers tested. Similarly all the plants from each set showed the same banding pattern with the tested primers which in turn proves single origin. Thereby uniformity as well as zygotic origin of the three sets of plants had been established.

2.2. Isolation and culture of pollen protoplasts for developing haploids in *Hevea brasiliensis*

The type and concentration of osmoticum as well as enzymes for digestion were standardized using pollen grains from mature male flowers prior to opening. Intact protoplasts were isolated in very high yield (80-90%) from the pollen grains of *Hevea*. Partial purification of the isolated protoplasts could also be achieved through sieving technique using sieves of 60-70 μ mesh size. Since complete removal of the debris was not possible, a mixture of pollen protoplasts with undigested pollen walls was used for protoplast culture. Effect of nurse culture on the plating efficiency of the protoplasts was also performed. Different nurse cultures tried in this experiment were a) embryogenic callus of *Hevea* induced from polyembryony derived material, b) callus derived from tobacco leaf cultures over MS medium with NAA and BA and c) callus raised from carrot segments cultured over modified MS with 2,4-D and BA. Partially purified protoplasts were cultured over the already standardized callus induction medium containing different nurse cultures. Simultaneously diploid protoplasts of *Hevea* were isolated from embryogenic cell suspension and purified according to the protocol already developed and cultured for callus induction in media with different nurse cultures. It was observed that in the case of pollen

protoplasts, division of protoplasts had not occurred in any of the media tried. Instead, most of the pollen protoplasts were found to be broken after a few days in culture. On the contrary, diploid protoplasts cultured over callus induction medium containing nurse cultures of *Hevea* started division resulting in the formation of micro colonies and micro calli.

2.3. Development of haploids through mature unfertilized ovule culture

Mature ovules were dissected from surface sterilized female flowers and were inoculated in N_6 medium supplemented with 10% sucrose for 5 days at 4°C. After the pretreatment, the ovules were transferred to the callus induction medium solidified with 0.4% phytagel. The ovules isolated from light yellow flower buds responded positively and swelled after 20 days of culture, during which the outer integument and the inner integuments could be clearly differentiated and a soft white tissue protruded out from the micropylar end of the ovule. This white tissue was separated and cultured in the callus induction medium containing 9% sucrose. Callus formation was achieved 25 days after inoculation with a frequency of 20%. Modified MS medium fortified with 6% sucrose, organic supplements (casein hydrolysate, malt extract, banana powder, yeast extract) and amino acids (serine, proline, aspartic acid and alanine) was identified as the best medium for embryogenic callus emergence. Effect of phytohormones on embryogenic callus induction was assessed using varying levels of NAA (0.5-2.5 mg L^{-1}) and zeatin (0.2- 1.0 mg L^{-1}) along with GA_3 (0.5 mg L^{-1}). Embryogenic callus formation with a frequency of 46% was obtained with NAA (1.0 mg L^{-1}), zeatin (0.6 mg L^{-1}) and GA_3 (0.5 mg L^{-1}) (Table Biotech. 4).

The embryogenic calli were proliferated in medium containing reduced levels of NAA (0.3 mg L^{-1}) and GA_3 (0.2 mg L^{-1}). For embryo induction, MS, modified MS and N_6 basal media supplemented with PEG (1-10%) and amino acids (arginine 100 mg L^{-1}, proline 200 mg L^{-1} and alanine 50 mg L^{-1}) along with growth regulators, Kin (0.25 - 0.6 mg L^{-1}) and GA_3 (0.2 - 1.0 mg L^{-1}) were tried. Somatic embryo induction was observed in N_6 basal medium containing PEG (3%) along with growth regulators Kin (0.4 mg L^{-1}) and GA_3 (0. 8 mg L^{-1}).

Table Biotech. 4. **Influence of Zeatin and NAA on embryogenic callus initiation from ovule explants**

Zeatin (mg L^{-1})	NAA (mg L^{-1})				
	0.5	1.0	1.5	2.0	2.5
0.2	4.0 (07.5)	10.0 (16.4)	14.0 (21.7)	4.0 (07.5)	(0.3)
0.4	20.0 (26.2)	22.0 (27.6)	16.0 (21.0)	6.0 (11.)	(0.3)
0.6	30.0 (32.9)	46.0 (42.6)	24.0 (29.2)	8.0 (12.8)	(0.3)
0.8	10.0 (16.4)	16.0 (23.3)	10.0 (16.4)	(0.3)	(0.3)
1.0	6.0 (11.2)	10.0 (16.4)	(0.3)	(0.3)	(0.3)

CD ($P = 0.05$) 11.8

The data was analyzed statistically using Arc sine transformation and the values given in parenthesis are the transformed ones.

3. Genetic transformation

Attempts were continued to develop transgenic plants with increased tolerance to abiotic stresses, tapping panel dryness and higher latex yield by *Agrobacterium* mediated genetic transformation using different gene constructs. Efforts were made to develop more transgenic plants incorporated with *MnSOD* gene under the control of CaMV35S promoter. Transgenic cell lines were produced from different transformation events and embryos were induced from one callus line. For enhancing embryo induction frequency, water stress was induced by adding phytagel ranging from 0.3 to 0.5%. Proliferated embryogenic callus was differentiated into small embryos by increasing the phytagel concentration and maximum embryo induction was obtained with 0.5% phytagel. By maintaining the embryos in the same fresh medium for 2 more months, they get matured, enlarged and many of them germinated. However, full plant regeneration was limited. Seventeen plantlets with full leaf development were obtained. Three plants survived after hardening and they were planted in large polybags (Fig. Biotech. 1).

The putatively transgenic callus lines integrated with *MnSOD* gene with FMV34S promoter were cultured individually over the callus proliferation medium. The proliferation medium was modified ½ MS basal medium fortified with 400 mg L^{-1} calcium nitrate, 10 mg L^{-1} silver nitrate, 0.4 mg L^{-1} 2,4-D, 0.4 mg L^{-1} BA and 0.2 mg L^{-1} NAA. Addition of 400 mg L^{-1} calcium nitrate and 10 mg L^{-1} silver nitrate in the proliferation medium facilitated the formation of soft friable callus. Effect of PEG (2%) along with 0.2 mg L^{-1} ABA on embryogenic callus induction was assessed. Three subcultures were made in this medium

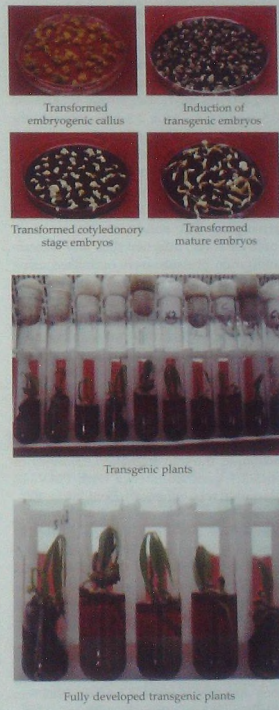


Fig. Biotech. 1. **Different stages of plant development from transgenic embryogenic callus integrated with *MnSOD* gene with CaMV35S promoter**

at six weeks intervals. After the third subculture, the yellow friable calli became brownish black with oily appearance. The calli were then cultured over embryogenic callus induction medium containing 0.1 mg L^{-1} 2,4-D, 0.3 mg L^{-1} BA, 0.5 mg L^{-1} Kin and 0.5 mg L^{-1} GA_3. After 2-3 subcultures in the same medium, some of the cell lines became embryogenic. The embryogenic calli were further cultured for embryo induction.

To improve the transformation efficiency, vacuum infiltration technique was attempted. The conditions for vacuum infiltration *viz.*, infection time and vacuum infiltration pressure were optimized using the *MnSOD* gene construct with FMV34S promoter. After optimizing the conditions for vacuum infiltration, transformation experiment was carried out with the gene constructs: 1) *MnSOD* with CaMV35S promoter, 2) antisense *ACC* synthase, and 3) sorbitol-6-phosphate dehydrogenase and *HMGR1*. Putatively transformed cell lines were selected after subjecting GUS histochemical staining. Employing this technique, the transformation efficiency of *MnSOD* gene construct with FMV34S promoter was enhanced from 14 to 40%. Similar results were obtained with sorbitol 6-phosphate dehydrogenase gene. *MnSOD* gene construct with CaMV35S promoter and antisense *ACC* synthase, the frequency was 30% but for *HMGR1* gene construct the frequency was 20%. The transformed cell lines were proliferated in medium containing the respective antibiotics. Five cell lines were selected randomly and PCR was performed with the DNA isolated, using gene specific primers. Amplification was obtained with gene specific primers in all the cell lines tested. The proliferated calli were further cultured for embryo induction on different media combinations along with control (untransformed) callus. Embryogenic calli were obtained from some of the cell lines.

Experiments were carried out to develop transgenic *Hevea* plants integrated with osmotin protein. Although, a good percentage of embryo induction was obtained from the transgenic cell lines, the embryo maturation and germination frequencies were low. Experiments were conducted for improving the frequency of embryo maturation and germination. For embryo maturation, hormone free medium supplemented with different levels of sucrose (30-150 g L^{-1}) were tried. Sucrose 75 g L^{-1} was found to be ideal for getting healthy cotyledonary embryos. The mature embryos with well developed and normal looking cotyledons were transferred to the germination medium. Full and ½ strength MS medium supplemented with different growth regulators, organic supplements, charcoal, sucrose (20-75 g L^{-1}) and different levels of mannitol (1-10 g L^{-1}) were used. The organic supplements tried were CW (5-20%), malt extracts (0-100 mg L^{-1}), casein hydrolysate (0-500 mg L^{-1}) and banana powder (100-500 mg L^{-1}). The growth regulators BA, GA_3, IBA and Kin were tried in combination. Combinations of TDZ along with BA, GA_3 and IBA were also tried.

It was observed that addition of 2.0 g L^{-1} mannitol was beneficial for the germination of transgenic embryos integrated with osmotin gene. Among the different growth regulators tried, 0.5 mg L^{-1} IBA along with 1.0 mg L^{-1} Kin and 1.0 mg L^{-1} GA_3 improved germination percentage from 2 to 10%. Three transgenic plantlets regenerated were transferred for hardening and they survived only up to 2-3 months. In order to rescue the transgenic plantlets, a few shoots were grafted to the seedlings developed from embryo rescue cultures. Out of 5 grafts tried,

one was successful and the shoot development was found to be normal. However, the growth was slow and after 6 months the graft also dried up.

Embryogenesis could be obtained from five of the newly developed transgenic cell lines. Embryos are under different stages of development. In order to study the expression of osmotin gene in *Hevea*, reverse transcription PCR was carried out using gene specific primers. Positive amplification was obtained which in turn confirms gene expression.

Agrobacterium mediated genetic transformation was carried out with the binary vectors containing the genes coding for *MnSOD*, *ipt* and TB antigen, using proliferated leaf callus as the target tissue. Embryogenic callus initiation and embryo induction were obtained using the standardized medium reported earlier for leaf explants. Four transgenic lines from *MnSOD*, five from *ipt* and seven from TB antigen have produced embryogenic callus. Embryogenic callus initiation and embryo induction was obtained from proliferated callus in modified MS basal medium containing 300 mg L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, B5 vitamins, amino acids, organic supplements such as coconut water (5%), casein hydrolysate (300 mg L^{-1}), sucrose (80 g L^{-1}) and phytohormones BA (4.4 µM), GA_3 (2.9 µM), Kin (2.3 µM), 2,4-D (1.8 µM) and NAA (1.08 µM). Rate of embryo induction from the transformed callus was less compared with non transgenic control. Maturation of the embryos from *ipt* transgenic callus could be obtained in the medium standardized earlier. About 15 transgenic plantlets regenerated were subjected to hardening, but they dried up gradually. Experiments are being continued for the regeneration of transgenic plants.

Effect of adding 50 mg L^{-1} α-lipoic acid in the modified infection and co-cultivation medium and 0-100 mg L^{-1} in the selection medium for improving transformation frequency and transgenic tissue regeneration was studied and the results are given in Table Biotech. 5.

Table Biotech. 5. **Effect of α-lipoic acid on transformation efficiency**

α-lipoic acid (mg L^{-1}) (selection medium)	Transformation efficiency (%)
0	11.1 (1.6)
25	13.3 (2.6)
50	16.4 (3.8)
75	15.5 (3.6)
100	14.8 (3.7)
CD (P = 0.05) 1.53	

**Data from four replicate experiments using 30 callus clumps per petri plate. Values in parentheses indicate transformed values*

Addition of 50 mg L^{-1} α-lipoic acid in the selection medium containing silver nitrate enhanced the transformation frequency to 16% with the *ipt* gene.

For developing transgenic *Hevea* plants integrated with *HMGR1* gene, the *Agrobacterium* strain, EHA 105 was used for infecting the cell suspensions raised from the embryogenic callus of the ovule. Twelve putative transgenic cell lines were generated and proliferated. PCR analysis using gene specific primers showed positive amplification in eleven cell lines and these cell lines were cultured for embryo induction. Influence of basal medium on somatic embryo induction was investigated employing MS, modified MS and WPM. The basal medium was supplemented with 30 g L^{-1} sucrose, proline and growth hormones. The modified MS medium contained 200 mg L^{-1} NH_4NO_3, 90 mg L^{-1} $MgSO_4$ and 2000 mg

L^{-1} KNO_3. Effect of polyamines on embryo induction was also evaluated by incorporating putrescine, spermine and spermidine (0-2.5 mg L^{-1}) individually in the embryo induction medium. Spermidine was found to be ideal for the induction of somatic embryos. The combined effect of spermidine and sucrose on embryo induction was assessed by incorporating different concentrations of spermidine (0-2.5 mg L^{-1}) and sucrose (50-90 g L^{-1}) in presence of growth regulators, Kin (0.7 mg L^{-1}) and NAA (1.0 mg L^{-1}) and the results are given in (Table Biotech. 6).

Addition of 1.5 mg L^{-1} spermidine and 80 g L^{-1} sucrose along with growth regulators enhanced the frequency of embryo induction to 72%. The transgenic embryos obtained from two cell lines were separated and cultured for maturation.

The embryogenic callus developed from zygotic embryos during the induction of polyembryony was also used as the target tissue for genetic transformation. Transformation experiments were done with three different binary vectors, containing the gene encoding osmotin protein, *HMGR1* and *MnSOD* with CaMV35S promoter. The putatively transgenic cell lines that emerged from these selection plates were separated individually and transferred to proliferation medium. When polyembryony derived embryogenic callus was used as the target tissue, putatively transformed cell lines could be obtained within 30 days of *Agrobacterium* infection, whereas in the case of clonal material, it took almost 60 days. Further the transformation frequency varied from 63-70% depending on the gene construct. Highest transformation frequency (70%) was obtained with osmotin gene followed by 68% with *MnSOD* and 63% for *HMGR1* genes. Transgenic cell lines from all the gene constructs were individually separated and proliferated in MS basal medium supplemented with growth regulators BA, GA_3 and Kin. The gene integration was confirmed in all the randomly selected callus lines by PCR analysis. The transgenic callus lines were cultured for embryo induction. High frequency embryo induction (62-65%) was achieved with three different gene constructs. Plant regeneration has been achieved with osmotin and *HMGR1* gene constructs and the frequency varied from 60-62%. Plants have been developed and hardened. Compared to explants of clonal origin, plant regeneration was faster and easier from this explant. The survival rate during hardening was much higher compared to clonal material.

Fifteen transgenic plantlets integrated with *HMGR1* gene were developed using polyembryony derived embryogenic callus and 10 of them were hardened and transferred to big polybags. Functional analysis of the transgene *HMGR1* was carried out with four HMGR1 transgenic plants developed through the above pathway. Leaf samples were

Table Biotech. 6. **Effect of spermidine and sucrose on embryo induction**

Sucrose (gL^{-1})	Spermidine (mg L^{-1})					
	0	0.5	1.0	1.5	2.0	2.5
50	32(5.6)	40(6.3)	55(7.3)	67(8.2)	51(7.1)	32(5.6)
60	37(6.1)	43(6.5)	60(7.7)	68 (8.2)	59(7.6)	40(6.3)
70	39(6.2)	49(7.0)	57(7.5)	61(7.8)	49(7.0)	28(5.2)
80	31(5.5)	36 (5.9)	60(7.7)	72(8.4)	48(6.9)	21(4.6)
90	29(5.4)	31(5.5)	47(6.8)	63(7.9)	28(5.2)	16(3.9)

CD (P = 0.05) 0.64

The experiment contained 25 callus clumps with four replications. Analysis was carried out using square root transformation and the values given in parenthesis are the transformed values.

collected from four transgenic plants along with non transgenic control plant and the total hmgr1 activity was measured using ELISA. Preliminary results indicated a higher enzyme activity in the transgenic plants compared with the control plants.

4. Molecular studies

4.1. Molecular mechanism of disease tolerance

Attempts were made for the functional characterization of the five β-1,3-glucanase gene isoforms and their promoters identified earlier from *Hevea* clone RRII 105. The promoter: GUS fusion binary vectors were used to transform *Agrobacterium* strain EHA105 and these cultures were used to transform tobacco leaf discs and *Hevea* callus by vacuum infiltration method. After vacuum infiltration, the explants were placed in the co-cultivation medium. After 48 hours, the explants were checked for the transient expression of GUS reporter gene. *Hevea* callus showed blue colour indicating GUS activity while the tobacco leaf discs showed no activity. Clear difference in GUS staining intensity was observed between calli

Table Biotech. 7. **Comparison of the putative *cis*-acting elements in the β -1,3- glucanase gene promoters isolated**

Putative *cis* element/ consensus	Function/ response	No. of elements found in the promoter containing					
		913 bp	847 bp	582 bp	553 bp	198 bp	Consensus sequence
Amy Box 1	Amylase box-responsible for gibberellic acid induced expression	1	1	1	-	-	TAACARA
CAAT Box		9	7	13	6	3	CAAT
DOF core	Signal responsive and or tissue specific gene expression	13	16	12	5	1	AAAG
E Box	Plant pathogen interaction	6	6	4	4	4	CANNTG
GATA Box	Light responsive element	13	13	9	2	1	GATA
GT 1 consensus	Cell type specific	17	19	11	4	-	GRWAAW
I Box core	Light regulated & tissue specific expression	13	10	7	-	-	GATAAGR
Myb core	Regulatory roles in developmental processes and defense responses in plants	4	3	2	2	-	CNGTTR
Myb consensus	Regulatory roles defense responses and dehydration stress in plants	2	1	-	1	-	YAACKG
Myb ST1	Dehydration stress	4	4	2	1	-	GGATA
Myc consensus	Dehydration induction	6	6	4	4	4	CANNTG
Root Motiff	Expression in root	6	7	4	7	2	ATATT
TATA box		9		8	12	5	TATAA
W box (TGACY) associated with WRKY (Zn finger)	Stress / pathogen defense regulated Expression	1	1	3	3	1	TTGAC
WRKY	Stress / pathogen induced expression	2	2	3	4	1	TGAC
Stress regulated	expression						

transformed with different binary vectors carrying different promoter sequences. The one inserted with 913 bp promoter showed the maximum GUS activity compared to all other promoter sequences. The transformed tobacco leaf discs were regenerated and the shoots emerged were also checked for GUS activity but no noticeable blue colour was observed. PCR amplification of the inserts from the DNA isolated from the transformed tobacco showed the presence of insert. Upon induction with 0.1% salicylic acid also no blue colour was observed. This may be due to the absence of transcription factors in the tobacco plant, corresponding to the major *cis*-elements of the promoter region isolated. Table Biotech. 7 shows the comparison of putative *cis*-acting elements and their frequency of occurrence, which was analysed with the PLACE software for plant promoter analysis.

4.1.1. The tissue specific expression of β-1, 3-glucanase gene isoform -Gln 4

Earlier a novel form of β-1, 3-glucanase gene (Gln 4) from the *Hevea* clone RRII 105 has been identified. The bioinformatics analysis and the tissue specific expression had been carried out in the reporting year. The full length (from the translation initiation codon to stop codon) genomic sequence of this novel form is single intronic with 1233 nucleotides. This form codes for a class I basic glucanase with 373 amino acids and designated as glucanase 4 (Gln 4) which varies with the other reported forms of *Hevea* in having a different stop codon, 'ochre' in comparison to the 'opal' codon in the other forms of β-1, 3-glucanase. To understand the expression pattern of the gene in different tissues, RNA was isolated from *Hevea* leaf, latex and callus. cDNA was synthesized and RT- PCR was performed using the specific primers for this form of β-1,3- glucanase. A high level gene expression was observed in the latex and in the callus but a low level expression was observed in the leaf. The results indicate that this novel isoform of β-1,3-glucanase is a basic glucanase localized in the vacuole and shows a constitutive high level expression in the latex and callus but showing a low level expression in the leaf.

In order to study the antifungal activity of the isolated novel forms of β-1,3-glucanase, Gln 2, Gln 3, Gln 4, Gln 5, Gln 6 and Gln 7 (isolated from FX clone), attempts were made to express the protein in the yeast, *Pichia pastoris*. The inserts were cloned in the non- secretory vector – pPICZ A and transformed to *Pichia*. The cells were grown at 30°C in a shaking incubator (280 rpm) until the culture reached an OD 600 = 4 (approximately 16–18 hours). Harvested the cells in the log phase of growth by centrifuging at 3000 X g and re-suspended the cell pellets in MM medium to get an OD 600 of 1.0 to induce expression. Methanol (100%) was added to a final concentration of 0.5%, every 24 hours to maintain induction. Transferred 1 mL of the expression culture to a 1.5 mL micro-centrifuge tube at each of the time points (hours): 0, 24, 48, 72 and 96 hrs. Centrifuged and the cells collected was washed with chilled PBS buffer and re-suspended in 500 µL of chilled PBS buffer. 300 µL aliquots of re-suspended yeast cells were transferred to 1.4 mL polyethylene tubes, kept in ice and subjected to sonication at an interval of 30 seconds at 100% power (H"12 watts/pin) for 3 times. The samples were then centrifuged at 8000 rpm for 15 minutes to pellet debris. The supernatant containing solubilized proteins was collected for analysis. Approximately a 43 kDa purified enzyme was observed in the gel (Molecular weight of the protein plus the weight of his-tag and C-myc epitope). The presence of the purified his tagged protein

was confirmed through western blot analysis with anti his tag antibody (M/S KPL, USA). The expressed recombinant protein was later subjected to antifungal analysis. For this antagonistic activity of each of the protein against the fungus *Phytophthora meadii* has been done in PDA plates. About 12 µg of the protein has been supplied in a filter paper disc placed in the actively growing fungal PDA plates. It was observed that among the different forms Gln 2 (with the 553 bp promoter sequence) showed maximum inhibitory activity. The activity can be represented as Gln 2> Gln 5> Gln 6> Gln 7 > Gln3 = Gln 4.

4.2. Tissue specific gene expression and characterization of promoters

4.2.1. Characterization of cis-prenyltransferase gene isoforms and promoters from Hevea

Cis-prenyltransferase is the enzyme responsible for the polymerization of isoprene units during rubber biosynthesis. Different isoforms of *cis*-prenyl transferase gene have been reported from *Hevea*. In our earlier studies a 5′ UTR intron was observed in the *cis*-prenyltransferase 2 gene. Attempt has been made to understand the role of 5′UTR intron in gene expression. *Cis*-prenyltransferase-2 gene was PCR amplified with its promoter from *Hevea* clone RRII 105 with promoter specific forward and gene specific reverse primers. The amplified fragment was cloned and sequenced. Sequence data showed that the amplified region upstream to the ATG codon was 500 bp in length. The newly isolated sequence of *cis*- prenyltransferase-2 promoter with gene is available in NCBI database under the accession no. JF926124.

The 5′ flanking region of *cis*-prenyltransferase-2 was analyzed for known motifs using the Plantcare and PLACE programmes. Two TATA core sequences were found at positions -213 to -220 and -390 to -397 with reference to the translational initiation site (ATG, +1), which matches the consensus sequence of a TATA core element, 5′-TATAWAW-3′. Another putative TATA box was found between position -138 to 146 (TATTTAAA). Putative CAAT box motifs were also located in *cis*-prenyltransferase-2 promoter.

Functional analysis of the *cis*-prenyltransferase-2 gene promoter was carried out to prove whether the isolated sequence is functionally intact and able to drive gene expression. *Cis*-prenyltransferase gene reported here is with one intron in the 5′ UTR region. To study the role of introns on gene expression, binary vector constructs containing promoter (with and without intron): GUS fusions were made and tested in a heterologous system, *Nicotiana tobaccum*.

By the manipulation of the restriction site *Pvu*I, the intronless version of *cis*-prenyltransferase promoter was constructed. With one forward primer designed from the upstream region of promoter and *Pvu*I restriction site anchored reverse primer designed from the 5′ UTR region, a 200 bp fragment was amplified from the genomic DNA. A 1.0 kb fragment was amplified from the cDNA using *Pvu*I restriction site anchored forward primer and a reverse primer from the coding region. Both fragments were digested using the restriction enzyme *Pvu*I and after purification, the fragments were ligated using DNA ligase. Using restriction enzyme site anchored (*EcoR*I and *Avr*II) forward and reverse primers, promoter region without intron (340 bp) was amplified.

To construct *cis*-prenyltransferase-2 Promoter: GUS fusion binary vector, *cis*-prenyltransferase-2 promoters (with and without intron), which were cloned in the

Strataclone™ sequencing vector, was subcloned into the binary vector pCAMBIA 1381-Z between *Eco*RI and *Avr*II sites upstream to the promoter less GUS reporter gene. Promoter fragments were amplified using *Eco*RI site anchored forward primer (PF) designed based on the promoter fragment obtained through inverse PCR and *Avr*II site anchored reverse primer (PR) designed based on the nucleotide sequence immediately upstream to the ATG codon in the 5′ untranslated region. Colonies obtained were screened through colony PCR. The isolated plasmids were subjected to sequencing and the right orientation of the inserted promoter fragments was confirmed. The resultant chimeric plasmid construct with introns contained a 500 bp fragment and with out introns contained a 340 bp fragment of *cis*-prenyltransferase-2 promoter.

The chimeric plasmids containing promoter: reporter gene fusions were introduced into competent *Agrobacterium* strain EHA105 by freeze thaw method. To evaluate the functioning of *cis*-prenyltransferase-2 promoters (with and without intron), a transgenic approach in *Hevea* and tobacco plants was attempted using the promoter: GUS fusion binary vector. This construct was introduced into tobacco by *Agrobacterium tumefaciens* mediated transformation of leaf discs and transgenic plants were developed. The expression of the GUS reporter gene was detected histo-chemically. The transgenic tobacco plants showed positive GUS activity and no GUS activity was detected in non-transgenic plants used as negative control. This study suggests that the promoter fragment of *cis*-prenyltransferase-2 analyzed here is functional as determined in terms of their ability to direct expression of the GUS gene in transgenic tobacco plants.

BOTANY DIVISION

The thrust areas of research of Botany Division this year were evaluation of promising pipeline clones breeding for drought tolerance, evolving high yielding clones for the traditional region, propagation studies and anatomical aspects pertaining to yield components and tapping panel dryness.

1. Evolving high yielding clones for the traditional area

1.1. Hybridization and clonal selection

The promising Wickham x Amazonian hybrids (WxA) were crossed with the RRII 400 series clones and RRII 105 with the objective of introgression of desirable genes from the wild germplasm. Over 85 hybrid seedlings and 450 half sibs generated from these parent clones were raised in a seedling nursery for further screening. The 42 promising selections from 200 hybrid clones resultant of the 1986 hybridization programme were studied for response to stimulation. All the clones responded to stimulation resulting in increase in yield, ranging between 47 and 376 %. Four of the hybrids exhibited

Table Bot. 1. **Yield components of hybrid clones during summer**

Sl. No	Clone	Total latex volume (ml)	DRC (%)	Summer season yield (g t^{-1} t^{-2})
1	93/2	71.0 ab	40.6 bcdef	28.3 ab
2	93/5	45.5 bcdef	40.3 bcdefg	18.0 bcdef
3	93/7	49.8 bcdef	44.2 abc	22.2 bcd
4	93/17	60.3 bcd	35.6 fg	22.7 bcd
5	93/22	16.6 g	41.6 bcd	06.9 f
6	93/27	56.0 bcde	41.3 bcd	22.9 bcd
7	93/37	92.0 a	38.3 defg	35.5 a
8	93/39	66.2 abc	40.3 bcdefg	26.9 abc
9	93/45	54.3 bcde	35.1 g	19.1 bcde
10	93/48	56.1 bcde	48.3 a	27.3 abc
11	93/172	50.8 bcdef	42.8 bcd	21.4 bcd
12	93/179	37.0 defg	39.0 cdefg	14.7 def
13	93/247	36.8 defg	41.2 bcde	15.1 def
14	93/248	38.2 defg	38.4 defg	14.7 def
15	93/250	41.6 cdefg	42.9 bcd	17.8 bcdef
16	93/263	39.7 cdefg	43.1 abcd	17.2 bcdef
17	AVT 73	23.3 fg	45.1 ab	09.8 ef
18	PB 252	30.6 efg	35.9 efg	11.5 def
19	RRIM 600	24.0 fg	40.1 bcdefg	09.6 ef
20	RRII 118	44.5 bcdefg	41.3 bcd	18.3 bcdef
21	RRII 105	39.0 cdefg	41.7 bcd	16.4 cdef
	GM	46.4	40.8	18.9
	CV%	36.4	7.9	36.9
	CD (P<0.05)	27.8**	5.3**	11.5**

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

better response to stimulation than clone RRII 105. Out of the 37 promising selections from 196 hybrid clones that were planted in eight SSTs in the year 1990, 20 clones responded better than RRII 105 when stimulated with ethephon.

The performance of W x W and W x A hybrids in the small scale trials laid out in 1995 was monitored. Clones such as 89/27 (W x W hybrid), 90/10 (W x A hybrid), 90/271 (W x A hybrid), 90/193 (W x A hybrid) and 90/109 (W x A hybrid) continued to exhibit superior yield over RRII 105. Source bushes of these clones were established and were multiplied for further participatory evaluations.

Out of the 21 clones and their parents under evaluation in two trials (planted in 1998), one clone was found superior and 13 clones were found on par with RRII 105 in

Table Bot. 2. **Yield components of hybrid clones during peak season**

Sl. No	Clone	Total latex volume (ml)	DRC (%)	Peak season yield ($g\ t^{-1}\ t^{-1}$)
1	93/2	169.1 abcde	36.1 bc	61.0 abcdef
2	93/5	120.8 cdef	38.9 ab	46.5 cdefg
3	93/7	183.3 abcd	39.2 ab	71.3 abcde
4	93/17	144.1 bcdef	36.3 bc	49.8 cdefg
5	93/22	71.6 ef	46.5 a	29.3 fg
6	93/27	146.2 bcdef	36.6 bc	53.2 cdefg
7	93/37	185.8 abcd	32.9 bc	62.8 abcdef
8	93/39	219.5 abc	36.5 bc	77.5 abcd
9	93/45	143.7 bcdef	39.6 ab	57.1 bcdefg
10	93/48	233.3 ab	41.8 ab	94.8 ab
11	93/172	263.3 a	37.6 abc	98.3 a
12	93/179	112.5 def	38.0 abc	42.5 defg
13	93/247	186.6 abcd	41.5 ab	77.8 abcd
14	93/248	50.0 f	41.9 ab	20.9 g
15	93/250	160.0 bcde	41.8 ab	67.1 abcdef
16	93/263	208.3 abcd	40.9 ab	81.9 abc
17	AVT 73	125.0 cdef	28.9 c	38.2 efg
18	PB 252	155.0 bcde	37.7 abc	60.7 abcdef
19	RRIM 600	135.8 bcdef	35.5 bc	48.2 cdefg
20	RRII 118	111.2 def	39.7 ab	44.2 cdefg
21	RRII 105	185.8 abcd	38.9 ab	70.3 abcde
	GM	157.7	38.5	59.7
	CV%	39.5	15.3	38.6
	CD (P<0.05)	102.8*	9.7ns	38.0*

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

terms of yield over a period of 6 years. Recording of yield components like latex volume and DRC during the summer as well as peak yielding season was continued in the sixth year of tapping too (Tables Bot. 1 and 2). The data indicated that volume of latex is more important in determining the yield than DRC. Clone 93/172 and clone 93/37 exhibited highest peak season yield and highest summer yield respectively.

When 17 hybrids and their parents were evaluated in trial 1999 A, clone 95/124 exhibited highest yield ($65.8\ g\ t^{-1}\ t^{-1}$) followed by clone 95/62 ($65.3\ g\ t^{-1}\ t^{-1}$) and clone 95/575 ($64.7\ g\ t^{-1}\ t^{-1}$) in the 6th year (Table Bot. 3). In another trial (1999 B), the hybrid clone 95/306 of parentage RRII 105 x RRII 118 exhibited the highest yield of 81.41 ($g\ t^{-1}\ t^{-1}$) followed by clone 95/455 of the same parentage. In SST 1999 C, clone 94/87 of parentage RRII 105 x RRIM 703 exhibited superior yield ($87.6\ g\ t^{-1}\ t^{-1}$) followed by clone 95/296 ($76.8\ g\ t^{-1}\ t^{-1}$) of parentage (RRII 105 x Mil 3/2).

Table Bot. 3. **Girth and yield of hybrid clones in the 6th year of tapping**

Clone	Parentage/Parent	Girth (cm)	Clone	Yield ($g\ t^{-1}\ t^{-1}$)
95/124	RRII 105 x PB 28/59	76.8 a	95/131	65.8 a
95/62	PB 242 x RRII 105	74.4 ab	95/7	65.3 a
95/575	RRII 105 x PB 235	72.1 abc	95/95	64.7 ab
95/242	RRIM 600 x RRII 203	69.9 abcd	PB 217	59.6 ab
95/95	RRII 105 x Mil 3/2	66.9 abcde	95/242	59.2 ab
95/131	RRII 105 x PB 235	65.1 bcde	RRII 105	58.7 ab
PB 217	Parent	64.8 bcde	95/184	57.7 ab
95/184	RRII 105 x Mil 3/2	64.3 bcdef	RRII 203	50.1 abc
95/104	RRII 105 x PB 217	64.0 bcdef	95/62	49.0 abc
95/552	RRIM 600 x RRII 203	64.0 bcdef	95/292	48.4 abc
95/129	RRII 105 x PB 235	63.7 bcdef	95/124	47.4 abc
Mil 3/2	Parent	63.1 cdefg	95/129	46.8 abc
95/118	RRII 105 x PB 28/59	62.8 cdefg	95/579	46.7 abc
95/4	RRII 105 x PB 217	62.7 cdefg	95/552	44.2 abc
RRII 203	Parent	62.3 cdefg	PB 235	43.2 abc
PB 235	Parent	62.1 cdefgh	95/243	42.6 abc
95/292	RRII 105 x PB 217	60.9 cdefgh	95/104	41.9 abc
RRII 105	Parent	58.9 defgh	95/575	40.9 abc
95/7	RRIM 600 x RRII 203	58.7 defgh	PB 28/59	40.0 abc
95/579	RRII 105 x PB 235	58.4 efgh	Mil 3/2	39.9 abc
95/243	RRIM 600 x RRII 203	58.2 efgh	PB 242	35.0 abc
PB 242	Parent	56.0 efgh	95/4	34.1 abc
95/106	PB 242 x PB 235	53.1 fgh	RRIM 600	33.5 abc
RRIM 600	Parent	52.0 gh	95/118	32.0 bc
PB 28/59	Parent	51.2 h	95/106	19.3 c
	General mean	62.7		46.6

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

1.2. Ortet selection

Ortets selected from Kodumon estate were evaluated in small scale trials laid out during 1993 and 1994. Clones OKn 39 and OKn 73 were found superior with an yield of 62.7 $gt^{-1}t^{-1}$ and 84.9 $gt^{-1}t^{-1}$ respectively over nine years of tapping. OKn 49, a potential latex timber clone continued to perform well with an yield of 71.0 $gt^{-1}t^{-1}$ and girth of 85.7 cm. In another small scale trial of ortet selections from smallholdings, O 73 (selection from a private estate at Kanjirappally) and O 72 (selection from the selfed progeny of RRII 105) exhibited superior yield (66.4 $gt^{-1}t^{-1}$ and 63.8 $gt^{-1}t^{-1}$ respectively) and girth (84.0 cm and 85.1 cm respectively) over RRII 105 which gave an yield of 50.5 $g\ t^{-1}\ t^{-1}$ and a girth of 68.3 cm). Data indicates that the ortets O 73 and O 72 could be selected as promising latex timber clones (Table Bot. 4).

Ortets selected from Konney, Mundakkayam and Cheruvally estates are being evaluated under large scale evaluation trials at CES, Chethackal. In terms of growth (fourth year of planting), clones such as MO28, Chy 035 and Chy 048 were found promising.

Table Bot. 4. **Yield and girth of ortet clones during 6th year of tapping**

	Yield over five years	Yield ($gt^{-1} t^{-1}$)	Girth (cm)
O 49 (PCK Kodumon)	26.5	48.4	78.1
O 77 (Erumely – EB1)	22.7	42.1	47.7
O 74 (Kanjirappally)	24.8	53.8	84.0
O 73 (Kanjirappally)	66.3	79.9	84.0
O 75 (Kanjirappally)	17.0	32.8	75.1
O 76 (Kanjirappally	13.2	25.7	61.1
O 21 (Kattappana)	45.0	46.4	64.3
O 72 (Progeny of RRII 105)	63.8	78.9	85.1
O 36 (Kothamangalam)	22.4	34.0	79.6
O 81 (CES, Chethackal)	26.8	41.3	98.8
O 79 (CES, Chethackal)	16.3	21.3	72.8
O 80 (CES, Chethackal)	23.9	44.2	66.0
O 78 (CES, Chethackal)	23.3	29.8	63.1
RRII 105	50.4	50.4	68.3
RRIM 600	34.5	32.3	63.5
CD (0.05)	-	23.4	17.2

2. Evaluation of clones

2.1. Large scale evaluation

Yield data of the 21 clones (planted in two LSTs of RRII 400 series clones during 1993) was collected at fortnightly intervals (11th year of tapping). Significant clonal variation in annual mean yield was found in both the trials. While in Trial 1, RRII 417 (79 g t^{-1} t^{-1}) and RRII 414 (72 g t^{-1} t^{-1}) were found better in terms of yield than RRII 105, clones such as RRII 430 (83 g t^{-1} t^{-1}), PB 330 (81 g t^{-1} t^{-1}) and RRII 422 (75 g t^{-1} t^{-1}) performed better than RRII 105 (73 g t^{-1} t^{-1}) in Trial 2. In the 1989 LST of introduced and indigenous clones at CES, PB 280 was found superior in terms of yield (136.5 $gt^{-1}t^{-1}$ in panel BI-1, (*i.e.*, the second year of renewed bark) as well as vigour (Table Bot. 5).

In another large scale trial at RRII farm, RRII 5 and RRII 118 were found superior in terms of yield over 13 years of tapping (Table Bot. 6). Broad sense heritability (H^2) yield was at 70%.

Table Bot. 5. **Yield of clones in the 14th year of tapping**

Clone	Yield (g t^{-1} t^{-1})		Girth (cm)
	Annual mean	Summer	(22nd year)
PB 235	67.9	43.4	94.6
PB 311	98.3	69.7	96.4
PB 280	136.4	108.0	105.0
PB 314	110.6	79.0	96.2
PB 312	81.2	44.4	91.9
PB 217	106.9	77.8	91.1
PB 260	84.9	55.3	90.4
PB 255	84.8	55.8	82.8
RRII 105	61.9	44.3	80.3
G. Mean	92.5	64.2	92.1
C.D.(0.05)	26.9	24.0	

Table Bot. 6. **Mean yield of clones**

Clone	Mean yield (g t^{-1} t^{-1})	
	13th year	Over 13 years
RRII 5	52.1	65.7
RRII 118	57.0	57.1
RRII 208	25.7	43.4
RRII 300	29.0	38.3
RRII 308	43.0	47.6
RRIM 600	32.2	40.0
RRIM 703	26.7	38.0
PCK 1 (PR 255)	40.7	44.8
PCK 2 (PR 261)	29.4	36.9
SCATC 88/13	19.9	41.9
SCATC 93/114	32.3	21.7
Haiken 1	13.3	24.7
RRII 105	53.1	49.7
G. Mean	34.94	42.27
CD	9.00	7.67
H^2	-	0.7

2.2. On-farm evaluation

Post release on-farm evaluation of RRII 400 series clones in smallholdings was undertaken in North, Central and South Kerala. RRII 400 series clones exhibited higher tappability in a holding at Kanjirappally, Central Kerala (Table Bot. 7). In another smallholding at Ayur, South

Table Bot. 7. **Performance of RRII 400 series clones in the 6th year**

Clone	Mean girth (cm)	Tappability (%)
RRII 414 (n=50)	55.2	86.0
RRII 417 (n=70)	54.3	91.0
RRII 422 (n=25)	53.2	96.0
RRII 429 (n=70)	51.4	65.0
RRII 430 (n=30)	54.8	90.3
RRII 105 (n=55)	47.6	39.3

Kerala, higher yield was recorded in RRII 422 (81.0 g $t^{-1}t^{-1}$) followed by RRII 429 and RRII 414 (Table Bot. 8).

Table Bot. 8. **Performance of RRII 400 series clones in the 10th year**

Clones	No. of trees	Girth (cm)	Mean yield g $t^{-1}t^{-1}$
RRII 105	50	55.4	41.2
RRII 414	70	61.4	68.0
RRII 417	50	60.2	67.1
RRII 422	55	58.4	81.0
RRII 429	115	55.4	68.8
RRII 430	16	56.7	55.9

Yield data of RRII 400 series clones planted in nine different locations indicated location wise variation. At Ponkunnam and Elenji, RRII 414 yielded better than RRII 105 while it was slightly inferior to control clone in two other locations. At Malayattoor, RRII 417, RRII 422 and RRII 429 exhibited higher yield than the control clone. RRII 430 was found the highest yielder in a trial at Oonnukal.

2.3. Genetic studies and investigations on genotype x environment interactions

The results of the G x E interaction study across various locations in India indicated that RRII 203 and RRII 422 are comparable to RRII 105 in Kanyakumari and RRII 429 as a superior clone in Agartala and Nagrakatta in terms of yield (Table Bot. 9). Recording of yield was continued at IMMT Bhubaneswar. The clones at RRS, Padiyur reached 5th year of tapping.

Table Bot. 9. **Mean yield (g t^{-1} t^{-1}) from 3 locations during the 8th year of tapping**

Clone	Location		
	Kanyakumari	Agartala	Nagrakatta
RRIM 600	54.9	58.8	51.5
RRII 429	46.5	62.2	83.5
RRII 203	75.5	46.4	46.5
PB 217	50.5	41.4	48.0
RRII 51	44.1	45.7	57.9
RRII 414	44.9	41.5	56.8
RRII 430	56.7	55.4	73.5
RRIC 100	51.2	41.1	73.0
RRII 422	59.3	59.3	77.6
RRII 105	75.5	46.7	67.2
RRII 417	64.4	61.4	88.0
RRII 176	70.5	45.7	44.4
CD	15.1	-	10.2

3. Participatory evaluation of rubber clones

Seven locations *viz.*, Kozhikode, Thrissur, Kumbazha, Cheruvally, Shaliacary and Kanyakumari were identified to initiate on-farm trials of phase 3 for which twelve clones were selected and polybag nurseries were established. The central researcher trial will be laid out at CES, Chethackal.

In the 14 field trials laid out in 2008 under phase 1 of the project, among the check clones, RRII 414 was found superior in girth in all the locations. Superior growth was recorded for RRII 430 in 11 out of the 13 trials. Pipeline clones via P010, P 021, P 026, P 067, P 072, and P 074 exhibited good growth in most of the locations. The trials laid out in 2010 in eight locations under Phase 2 of the project with 14 pipeline clones and three checks were maintained well after casualty replacements.

4. Breeding for other specific objectives

4.1. Breeding for drought tolerance

Results from the four small scale evaluations of drought tolerant ortets and hybrids evolved from drought tolerant parents over six years of tapping were collated and the most promising clones were shortlisted for further participatory trials. Yield components of these clones were also studied seasonally. Among the ortets selected (based on yield under rainfed cultivation in Dapchari), clone Dap 111, exhibited high volume of latex (280 ml tree^{-1} tap^{-1}) with a DRC of 36.18 %. Hybrid clones such as 93/214 (64.42 gt^{-1}t^{-1}), 93/58 (82.10 gt^{-1}t^{-1}), 94/90 (74.7 gt^{-1}t^{-1}) and 95/353 (66.41 gt^{-1}t^{-1}) were found superior among the clones being evaluated in various trials. Selected clones (27 nos) were multiplied and source bush nurseries were established at CES, Chethackal in order to generate sufficient budwood for multilocational trials.

In another set of two SSTs laid out in 2001 comprising 50 hybrids (fourth year of tapping), clone 95/98 (86.2 gt^{-1}t^{-1}; parentage RRII 105 x RRIC 104) and 95/279 (58 gt^{-1}t^{-1}; parentage RRII 105 x PB 260) were found promising.

4.2. Polycross progeny evaluation

A total of 29 promising high yielding clones were selected from 150 clones consisting of half sib progenies from 10 parent clones (evaluated in two field trials). Some of the selections are listed in Tables Bot. 10 and 11 below.

Table Bot. 10. **Growth and yield characteristics of latex – timber clones evolved by polycross breeding**

Parent	Clone	Rubber yield (g t^{-1}t^{-1})	Yield improvement (%)	Timber Volume (m^3 tree^{-1})	Girth at 11th year (cm)
RRII 105	106	57.5	28.2	0.10	64.2
PB 252	132	81.3	81.4	0.13	76.2
PB 5/51	147	55.9	24.6	0.08	60.4
Ch 26	161	80.4	79.3	0.08	63.7
Ch 26	199	53.1	18.4	0.08	64.7
PB 28/83	37	50.4	12.5	0.10	58.9
PB 215	151	55.3	23.2	0.08	61.8
PB 28/83	80	58.7	29.1	0.09	62.5
RRII 105	112	51.3	12.2	0.08	69.3

Table Bot. 11. **Growth and yield characteristics of latex clones evolved by polycross breeding**

Parent	Clone	Rubber yield (g t^{-1}t^{-1})	Yield improvement (%)	Timber Volume (m^3 tree^{-1})	Girth at 11th year (cm)
PB 28/83	81	65.9	47.0	0.05	50.7
PB 28/83	191	64.4	43.7	0.06	57.4
PB 215	93	59.4	32.6	0.05	54.2
PB 215	89	50.0	12.5	0.07	58.1
PB 5/51	82	62.8	40.0	0.06	56.8
Ch 26	162	56.5	25.1	0.05	51.1
PB 5/51	38	54.6	14.2	0.04	45.0
PB 28/83	140	55.3	23.1	0.06	60.7
PB 217	180	50.00	12.5	0.06	75.8

5. Anatomical investigations

In connection with the anatomical studies on tapping panel dryness in *Hevea*, in order to localize peroxidase activity in the bark tissue, both Guaiacol and o-dianisidine were employed using hydrogen peroxide as the substrate. Tangential longitudinal sections of bark when stained for peroxidase showed variation in its staining pattern with respect to seasons. Seasonal activity for this enzyme was noticed in the phloic rays but the activity could be seen throughout the year in the sieve tubes. The cell walls of sieve tubes in the inner soft bark, including those differentiated recently from the derivatives of cambium, stained reddish brown indicating its activity.

To study clonal variation with respect to sieve tubes in *Hevea brasiliensis*, parameters such as diameter, density and grouping pattern of sieve tubes were studied in clones *viz*. RRII 105, RRII 33, RRII 38, RRIM 600, RRII 118, RRII 208 and PB 260. Variations for these traits were observed.

6. Studies on propagation

In connection with the experiment on comparison of growth and yield of different forms of planting material though there was no significant variation among the treatments, the green budded plants performed well in terms of girth, dry rubber yield and volume of latex (Table Bot. 12). Results of another study on influence of different types of buds on field performance of plants raised from them showed the superiority of isolated semi green buds (Table Bot.13).

Table Bot. 12. **Girth and yield of trees after 7 years of growth in the field**

Treatments	Girth (cm)	Yield ($g\ t^{-1}\ t^{-1}$)	Yield (volume) (ml)
Brown budded stumps	56.9	47.4	178.3
Field budded plants	54.2	37.4	147.5
Green budded plants	57.0	51.3	182.5
Brown budded plants	56.9	46.8	156.6
Young budded plants – stock 42 days old	56.7	48.1	133.3
Young budded plants – stock 49 days old	56.9	42.2	154.0
Young budded plants – stock 56 days old	56.4	45.5	116.6
CD (0.05)	NS	NS	NS
SE	1.7	2.9	28.6
CV	5.5	32.6	11.2

Table Bot.13. **Scion height in polybags and girth of plants 4 year old after**

Treatments	Girth (cm)	CV	Girth (cm)	CV
	Polybags		Field plants	
Budded plants- Mature brown buds	24.8	13.22	24.9	16.6
Budded plants -Prominent semi green	24.4	16.23	26.5	17.0
Budded plants -Scale buds	24.6	16.48	22.6	22.6
Budded plants -Whorl buds	23.8	15.86	23.1	15.7
Budded plants -Light green buds	22.0	20.25	21.2	23.9
CD ($P = 0.05$)	NS		2.56	

GERMPLASM DIVISION

The germplasm collection being maintained at RRII comprises 3 main gene pools: the domesticated clones derived from the original Wickham collection, the 1981 IRRDB wild *Hevea brasiliensis* germplasm collection, and 5 other *Hevea* species. The conservation, agronomic evaluation, screening for diseases, abiotic (drought and cold) stress resistance and timber latex traits, and utilization of the useful wild germplasm in crop improvement, form the major activities of the Division. Formulation of Distinctiveness, Uniformity and Stability (DUS) testing norms for *Hevea*, and generation of a mapping population for *Hevea* are also being carried out.

1. Introduction, conservation and documentation

1.1. Domesticated genepool (Wickham collection) from secondary centers

The domesticated gene pool comprises 183 Wickham clones conserved in field gene banks in 1 clone museum at RRII Farm, Kottayam, and 2 germplasm gardens at CES, Chethackal.

Annual girth and monthly yield of the 5 IRCA clones in the Germplasm Garden IV planted in 1992 were recorded, and the data analysed statistically. Significant clonal differences were recorded for yield and girth. IRCA 130 continued to be far superior to all the other clones in terms of yield, while IRCA 111 and IRCA 109 were on par with RRII 105. IRCA 111 and IRCA 130 were superior to all others for girth (Table Ger. 1).

In the Germplasm Garden V, among the 20 clones, RRII 23, RRIC 100 and RRIM 609 continued to be the best clones in terms of yield (98.3-117.7 $gt^{-1}t^{-1}$), while the control clone RRII 105 had a yield of 72.4 $g\,t^{-1}t^{-1}$ (Table Ger. 2). The three clones also had higher girth ranging from 96.8 cm (RRIC 100) to 89.8 cm (RRIM 609), while the control clone RRII 105 had a girth of 72.5 cm.

Table Ger. 1. **Performance of IRCA clones in the 10th year of tapping**

Clone	Girth ** (cm)	Dry rubber yield ** ($gt^{-1}t^{-1}$)
IRCA 130	76.7	93.1
IRCA 109	67.1	52.5
IRCA 111	78.5	64.8
IRCA 18	65.7	39.6
IRCA 230	64.1	54.5
RRII 105	69.0	72.1
CD (P=0.05)	6.97	16.15

** *Clonal differences significant at P<0.01*

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

Clone	Yield** ($gt^{-1}t^{-1}$)	Girth** (cm)
PB 255	24.6	95.4
PR 255	41.0	60.8
RRIC 100	105.2	96.8
SCATC 88/13	23.0	77.0
RRIM 609	98.3	89.8
RRII 15	43.6	78.6
Haïken 1	35.8	77.9
RRII 27	51.9	65.1
RRIC 148	69.4	90.1
RRII 108	40.6	77.4
RRIM 618	20.7	56.0
RRII 20	46.2	73.2
RRII 12	16.0	77.8
SCATC 93/114	56.1	68.6
RRII 178	53.4	90.4
RRII 22	34.4	59.5
RRII 23	117.7	94.3
RRIC 102	73.8	78.1
RRIC 36	45.7	74.0
RRII 105	72.4	72.5
CD(P=0.05)	29.29	12.42

** *Clonal differences significant at P<0.01*

1.1.1. *Formulation of DUS testing norms in Hevea*

In order to formulate Distinctiveness, Uniformity and Stability (DUS) testing norms for *Hevea*, 49 Wickham clones were field planted in a replicated trial at CES, Chethackal, RRS, Dapchari and RRS, Agartala, for a multilocation project.

1.2. IRRDB 1981 wild gene pool

The wild germplasm comprising 3576 wild accessions is being maintained in different field conservation-cum-source bush nurseries (SBNs). Reestablishment of the conservation nurseries has been carried out. The first 3 re-established nurseries (SBNs 2003, 2004 and 2005) comprising of 550, 975 and 701 wild accessions respectively, were cut back, after ensuring proper identity. The 4th set of 806 accessions planted in conservation nursery SBN 2006 in an augmented RBD with 4 controls was maintained properly. The 2nd round of test tapping in 63 selected accessions of this nursery, with more than 50% test tap yield of that of RRII 105, was done for reconfirmation of their yield potential. SBN 2007 comprising 500 accessions was monitored for early growth and 25 potential accessions for yield were identified on the basis of testtapping. The accession AC 4130 recorded highest girth of 32.8 cm followed by RO 2608 (32.0 cm) and RO 857 (31.6 cm). The 1st round of testtapping carried out in 201 wild accessions in SBN 2008 identified 18 wild accessions with more than 50% of the yield of RRII 105, for a confirmatory round of testtapping next year.

1.3. Other *Hevea* species

Six species of *Hevea* and their hybrids available at RRII (*H. benthamiana, H. spruceana, H. nitida, H. camargoana* and 2 accessions of *H. pauciflora*, two *H. brasiliensis* clones, 5 natural putative interspecific hybrids and FX 516, an interspecific cross between *H. brasiliensis* and *H. benthamiana*), are being conserved in an arboretum planted at CES in 2006.

2. Characterisation and preliminary evaluation

In the preliminary evaluation trial PET (Ortets) 99, the best wild accessions were OM 1116 and OM 1109 with yield levels of 42.5 and 34.0 $gt^{-1}t^{-1}$ respectively, while the controls yielded an average of 91.8 $gt^{-1}t^{-1}$ (PB 260), 59.7 (RRII 105) and 44.0 (RRIM 600). Among the 46 wild accessions, OM 1105 had the highest girth (90.5 cm), followed by OM 1107 (83.8 cm). The girth of the controls ranged from 89.8 cm (PB 260) to 78.1 cm (RRIM 600) (Table Ger. 3).

Table Ger. 3. **Top wild accessions for yield and girth in Ortet Trial 99**

Accession	Yield ($gt^{-1}t^{-1}$) 3rd year of tapping		Accession	Girth (cm) 13th year of growth	
PB	260	91.8	OM	1105	90.5
RRII	105	59.7	PB	260	89.8
RRIM	600	44.0	OM	1107	83.8
OM	1116	42.5	RRII	105	79.1
OM	1109	34.0	RRIM	600	78.1
OR	1166	20.9	OR	1149	77.6
OR	1163	19.7	OM	1109	76.9
OM	1118	19.7	OM	1116	56.9
OM	1112	23.1	OR	1166	62.3
OR	1153	17.3			

Of the 47 wild accessions in preliminary evaluation trial PET 2000, nine had girth ranging from 62.0-80.2 cm, while that of the controls RRII 105 and RRIM 600 were 61.8 and 45.5 cm respectively. The two accessions RO 4599 and MT 4788, continued to show promising yield levels compared to RRII 105: 44.4 and 41.2 $g\ t^{-1}t^{-1}$ respectively.

In PET 2000A at RRS, Padiyoor, monthly yield and annual girth were recorded in 171 wild accessions. Accession AC 2537 recorded highest girth followed by AC 3609 and AC 3075, as compared to the control clone RRII

105. Monthly yield and annual girth were recorded in PET 2000B at RRS, Padiyoor. Among 166 wild accessions, accession RO 2883 recorded the highest girth of 63.3 cm followed by accession MT 39 (60.0 cm) whereas the check clones RRII 105, RRII 208 & RRIM 600 were having girth of 46.5 cm, 46.7 cm and 39.2 cm respectively. For mature yield, among the wild accessions, the highest yield was recorded by accession RO 341 (32.0 g) which is 85% of the yield of RRII 105, while among the 3 check clones RRII 105 recorded the highest yield (37.4 g). Accessions AC 567, AC 1964 and AC 824 recorded highest girth in PET 2002.

3. Further evaluation and selection

3.1. Clonal nursery evaluation

A clonal nursery planted last year at CES, comprising 15 selected wild accessions from SBN 2004 showing 50-80% test tap yield of RRII 105 was maintained for evaluating their yield potential.

3.2. Further evaluation trials

Annual girth and dry rubber yield were recorded and analyzed in the further evaluation trial FET 95 at the age of 17 years (8th year after tapping). Girth was maximum for the accession MT 1032 (80.2 cm) and minimum for MT 188 (41.5 cm). The mean girth of RRII 105 was 59.3 cm. Seven accessions from Mato Grosso provenance showed superiority for girth over RRII 105. The accession AC 166 consistently showed superiority for dry rubber yield (64.2 g $t^{-1}t^{-1}$) among the wild accessions, which is on par with the yield of RRII 105 (64.0 g $t^{-1}t^{-1}$).

Monthly yield and annual girth was recorded in the FET 2003 comprising 22 wild accessions and three controls. In the 9th year after planting, RO 2629 recorded the highest girth (61.2 cm) followed by AC 4149 (53.3 cm) and AC 626 (51.6 cm). RO 2629, AC 4149, AC 716 recorded the highest yields of 28.8, 26.2 and 26.1 g $t^{-1}t^{-1}$ respectively in the first year of tapping.

Significant clonal differences were seen for annual girth in FET 2005 comprising 22 wild accessions and 3 controls. Among the wild accessions, AC 2004, MT 4788 and MT 2217 had the highest girth in the 7th year of growth (48.1, 41.0 and 36.7 cm respectively), on par with the controls PB 235 (42.6 cm) and RRIM 600 (33.6 cm). Girth increment over the last five years showed that AC 2004 had the highest growth rate, followed by MT 2217 and MT 4788 (Table Ger. 4).

Table Ger. 4. **Growth of 22 potential wild accessions in FET 2005**

Accession		Girth 2012 (cm)**	GI 2012-2008 (cm) **
MT	43	34.6	24.6
MT	185	37.8	24.8
MT	1077	27.9	19.4
RO	1241	36.9	26.1
RO	1323	33.9	23.0
RO	1462	31.0	18.1
RO	1514	31.7	22.2
RO	1570	32.0	20.0
RO	1755	32.3	20.6
AC	2004	48.1	33.6
MT	2217	36.7	27.7
RO	2255	33.8	20.7
RRII	105	39.8	26.4
RO	3012	34.7	21.7
RO	3247	20.3	12.6
AC	3609	28.1	16.4
AC	3615	30.4	21.5
MT	4788	41.0	27.0
AC	4833	22.2	14.4
RO	4911	32.5	20.3
MT	182	34.6	22.9
MT	192	34.9	23.3
RO	2731	36.9	23.4
PB	235	42.6	26.7
RRIM	600	33.6	21.6
CD(P=0.05)		5.16	4.38

In FET 2008, accessions AC 176, MT 77 and RO 2846 recorded the highest girth out of the 26 wild accessions. 13 wild accessions in FET 2010A, selected from SBN 2004 having more than 80% test tap yield of RRII 105 and field planted at CES, Chethackal along with the check clones RRII 105, RRII 430 and RRII 414, were maintained properly.

3.3. On-farm trials

On-farm trials at 5 locations *viz.*, B.C. Cheruvally estate, Erumely, Malankara estate, Thodupuzha, Mooply estate, Trissur, Calicut estate, Kozhikode and Bethany estate, Kanyakumari for evaluating the performance of the 3 selected IRCA clones (IRCA 130, IRCA 111, IRCA 109) and 1 wild accession (AC 166) at multi locations are under evaluation.

4. Screening for stress tolerance

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

A clonal nursery comprising of 47 potential half-sibs of nine clones is being evaluated along with 3 checks *viz*, RRII 105, RRII 430, RRII 414 for their drought tolerance potential at RRS, Dapchari which is a drought prone area. Girth of the plants in the second year ranged from 3.5- 8.3 cm and the highest girth was recorded by a clone raised from the progeny of RRII 105. Among the four check clones, the highest girth was recorded by RRII 430 (6.5 cm). 22 clones recorded girth higher than RRII 430. Plant status on drying after experiencing their 1st summer (May 2011) was also recorded and is shown in Table Ger. 5.

Another clonal nursery evaluation trial comprising of 31 potential half-sibs planted last year at RRS, Padiyoor to study their drought tolerance potential, is being maintained.

A small scale trial comprising seven potential accessions out of 130 identified based on drought related growth parameters and rubber yield from the field screening trial 2003 at RRS, Dapchari along with check clones RRII 105, RRII 414, RRII 430, RRIM 600 and Tjir 1 was maintained properly for further evaluation of their drought tolerance potential in the drought prone area at RRS, Dapchari.

In the further field evaluation of selected *Hevea* clones at RRS, Dapchari in collaboration with Botany Division, the growth during the summer and peak period of growth in the 34 selected *Hevea* clones comprising 23 wild accessions, 5 HP clones and 6 check clones *viz.*, RRII 430, RRII 414, RRII 105, RRIM 600, RRII 208 and Tjir 1 was assessed. At the end of 4th year, out of 34 clones in this trial, 8 wild accessions and 1 hybrid clone showed girth superior to drought tolerant clone RRIM 600. Accession

Table Ger. 5. **Drying status of half-sib progenies and check clones after experiencing first summer (May-2011)**

0% drying	50-58% drying	Drying status of check clones
PB 242- 2 clones	AVT 73- 1	RRII 105- 83.3% (10/12)
PB 217- 2 clones	Ch 26- 1	RRII 414- 25.0% (3/12)
RRII 105- 2 clones	PB 28/83- 1	RRIM 600-16.7% (2/12)
PB 215- 1 clone	PB 242- 1	RRII 430- 8.3% (1/12)
Hybrid progeny-1 clone		

MT 4788 continued its superiority for girth after 4 years growth. Among the modern clones, RRII 430 showed significant growth difference from RRII 414 under Dapchari conditions (Table Ger. 6.). Based on data on visual scoring on leaf yellowing and chlorophyll content, accessions MT 1627, MT 1623, RO 2387, RO 2153 and hybrid clone 93/105 were identified as top rankers for a detailed study on drought related physiological parameters in polybag plants.

Table Ger. 6. **Summer and annual girth of potential test clones along with check clones**

Clone	Summer girth (cm) at 30 cm height May 2011	Clone	Annual girth (cm) at 150 cm height September 2011
MT 1681	19.8	93/92	18.2
93/225	20.0	MT 43	18.5
MT 5078	20.4	MT 5078	19.4
RO 1761	20.6	RO 1761	19.5
MT 40	20.7	MT 1616	19.5
MT 4856	20.8	93/105	20.0
MT 1616	20.9	MT 54	20.4
MT 54	21.4	MT 4856	21.2
93/270	21.4	93/270	21.4
MT 4788	23.2	MT 4788	21.8
RRII 430	23.0	RRII 430	21.8
RRII 105	19.0	RRII 105	19.1
RRIM 600	20.0	RRIM 600	17.9
RRII 208	18.8	RRII 208	16.8
RRII 414	16.6	RRII 414	15.5
Tjir 1	18.4	Tjir 1	16.7

4.2.2. Cold tolerance

A total of 64 wild accessions were evaluated for cold resistance in two trials at Regional Experiment Station, Nagrakata, West Bengal. Girth of the 12-year-old accessions recorded during pre- and post-winter period, showed significant variation. Monthly yield and canopy/branch characters were recorded in Trials 1 and 2. Accessions RO 2902, MT 5105 and RO 2387 recorded the highest annual girth as compared to the check clones SCATC 93/114 and RRIM 600 in Trial 1 where as in Trial 2, accessions MT 915, RO 2727 and MT 900 recorded the highest girth.

5. Screening for timber characteristics

5.1. Field screening

Annual girth, monthly yield and timber volume were estimated at the age of 11 years. Among the wild accessions, 2 accessions, AC 650 and MT 941 had timber volume higher than that of PB 260, RRII 33, RRII 118, RRII 105 and RRIM 600. Two other accessions showed dry rubber yield on par with RRII 105 and PB 235.

5.2. Screening for timber quality traits through lignin biosynthesis studies

Characterization of lignin monomer units in 1 wild accession (AC 4830) having high lignin content and 2 popular clones (RRII 105 & RRII 414) was done to understand the syringyl / guaiacyl monomer units (S/G ratio) using thioacidolysis & gas chromatography in collaboration with School of Biosciences, Sardar Patel University, Gujarat. The results indicated that the clones RRII 105 and AC 4830 were characterized by relatively high amount of syringyl lignin units in their monomeric composition (Table Ger. 7).

Table Ger. 7. **Klason lignin, guaiacyl (G) and syringyl (S) lignin monomer and S/G ratio of three clones of *Hevea brasiliensis***

Clone	Klason lignin (%)	Guaiacyl lignin unit	Syringyl lignin unit	S/G ratio
RRII 105	21.0	431.7	1126.0	2.61
RRII 414	16.0	529.5	649.0	1.22
AC 4830	21.4	526.0	1121.0	2.13

G, S, S+G: Yields of the Thioethylated guaiacyl (G), Syringyl (S) and total (S+G) expressed as µmoles/g of lignin.

6. Utilisation of *Hevea* germplasm

6.1. Hand pollination programmes

A hand pollination programme was conducted during 2009 at CES, Chethackal involving 3 wild accessions showing potential for yield, and 6 cultivated Wickham clones. The resultant 75 successful hybrid progenies are under nursery evaluation for early growth at CES, Chethackal. In another HP programme carried out at RRS, Padiyoor in 2009, 29 progenies derived from 2 crosses and 25 OP seedlings of RRII 105 planted in a seedling nursery were maintained. Morphological data was recorded at 2nd year of planting, to assess the growth and vigour of the progenies. Hybrid progenies from the cross RRII 105 X AC 675 recorded mean girth higher than the progenies derived from the cross RRII 105 X RO 368 but the per cent recovery of superior seedlings was more in the 2nd combination.

6.2. Generation of mapping population

The 96 seedlings generated from the interspecific cross between the popular *H brasiliensis* clone RRII 105 and *H benthamiana* clone F 4542 were raised in a seedling nursery and growth was monitored (Table Ger. 8). The cross was repeated this year to augment this collection.

Table Ger. 8. **Growth of interspecific hybrid progeny and budded controls (parents)**

Provenence	Number	Girth (cm)			CV
		Average	Min	Max	(%)
RRII 105	6	8.2	5.0	10.0	19.3
F 4542	8	7.6	5.0	10.0	19.6
Progeny	96	13.9	5.0	22.0	26.6

7. Other studies

7.1. Feasibility of ratooning in *Hevea*

Ratoons continued to be superior to their corresponding polybag grown counterparts in the eleventh year of growth in terms of girth, recording an average of 71.4 and 48 cm respectively. 134 ratoon plants (97.9%) have attained tappability, while the tappability of the interplanted polybag plants was only 93 (47.2%).

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

The collaborative project with ITDA, taken up in 2008 at RC Varam, by the Integrated Tribal Development Agency (ITDA), Govt. of Andhra Pradesh, with the objective of monitoring growth of new large scale plantings in the non-traditional area, was continued. During the reporting period, the growth of rubber plantations in 12 farmers' fields planted was monitored. In the 4th year of growth, Farm 7 recorded the highest mean girth of 20.1 cm and a mean height of 245.2 cm. Farm 12 recorded the lowest mean girth of 5.7 cm and mean height of 101.0 cm.

PLANT PATHOLOGY DIVISION

The division is mainly concentrating on the improvement of disease and pest management strategies using chemical and biological agents, assessment of yield loss due to diseases and evaluation of new clones for disease resistance. Studies on beneficial micro-organisms for plant growth, bee-keeping in rubber-plantations and treatment of sheet processing effluent were continued.

1. Leaf diseases

1.1. Abnormal leaf fall disease (ALF)

The experiment to improve the efficiency of ALF disease management in the clones RRII 105 and RRIM 600 at Chimony estate Thrissur was continued. The spraying was undertaken by Turblow mist blower during the second fortnight of May and 30 days thereafter for the second round. Two rounds of spraying @ 20 L /ha (1:5) was found to be superior in checking the ALF disease in clone RRII 105 and RRIM 600 (Table Path. 1).

Attempts were made to quantify the pathogenesis - related genes such as PR 1, β-1,3 glucanase and peroxidase during pathogenesis in clones tolerant (RRII 105) and susceptible (RRIM 600) to *Phytophthora*. The result indicated that the level of activity of these genes increased during initial hours (24^{th}/48^{th}) of inoculation in the leaves of both the clones. However, their activity either increased or remained constant in RRII 105 and decreased in RRIM 600 during later hours (Figs. Path. 1, 2 and 3).

The experiments to assess the impact of ALF disease in clones RRII 414, RRII 422, RRII 429 and PB 260 are in progress. The experimental trees were brought under tapping on attainment of recommended tappable girth. The clone RRII 414 recorded maximum girth increment (7.1 cm) with 86% tappability. Among the experimental clones, RRII 422 experienced moderate ALF

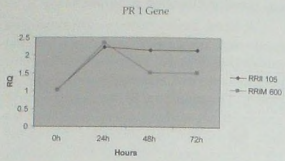


Fig. Path. 1. **Expression of PR1 gene**

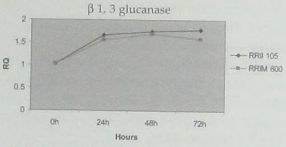


Fig. Path. 2. **Expression of β 1, 3 glucanase gene**

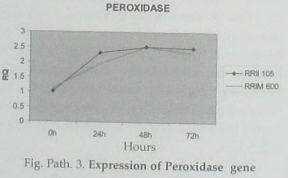


Fig. Path. 3. **Expression of Peroxidase gene**

Table Path. 1. **Per cent leaf retention in RRII 105 and RRIM 600**

Treatments	No. of rounds	Ratio	Leaf retention (%)	
			RRII 105	RRIM 600
COC(10 kg): spray oil (40 L)	1	1:4	60	40
COC(8 kg): spray oil (48 L)	1	1:6	70	50
COC(4 kg): spray oil (20 L)	2*	1:5	90	75
COC(8 kg): spray oil (40 L)	1	1:5	70	50
Unsprayed (control)		—	50	5

*Second round spraying 30 days after the first.

incidence and RRII 429 recorded very severe incidence of pink disease. Recording of yield, by dividing the area under each clone into two tapping blocks, to accommodate sprayed and unsprayed treatments, is being continued.

Two experiments on crown budded plants located at Malankara estate (clone PB 311) and Central Experiment Station, Chethackal (clone PB 260) are in progress. The control block (no crown budding) of PB 311 recorded very severe ALF disease, whereas crown-budded trees having crown clones Fx 516 and RRII 33 recorded <25% disease severity. Girth increment was higher in crown-budded trees. The experiment in clone PB 260, in which feasibility of crown budding at nursery stage was attempted, was brought under tapping in the seventh year of planting. Initial yield data indicated no significant difference between crown-budded and control blocks.

1.2. Powdery mildew disease

The trial initiated at New Ambadi estate Kanyakumari during 2008 to study crop loss due to powdery mildew disease in the clone RRII 105 was continued. Two blocks were demarcated and pre-treatment application of sulphur was undertaken at recommended dose at an interval of 10 days using micron duster during the initial year. In the subsequent years, one plot was maintained as dusted and other as undusted. The disease intensity was assessed on a 0 -5 scale (very light, light, moderate, severe and very severe) during each dusting. The block yield was recorded and the crop loss was calculated. A crop loss of 1.3%, 5.7 % and 5.5% was estimated during 2009, 2010 and 2011, respectively.

1.3. Corynespora leaf disease

A survey carried out during 2011 and 2012 disease season from January to April indicated that Corynespora disease was very much prevalent in Neyassery village in Thodupuzha (75%) and Pinavoorkudy area of Kothamangalam region (up to 90%). During 2012 the disease was very less compared to the year 2011. The disease was prominent in the immature phase of the clone RRII 105. The other clone particularly RRII 414 and RRII 430 exhibited the typical raised spot symptoms.

In vitro evaluation of various new generation fungicides for their efficacy against the growth and conidial germination of the pathogen revealed that the fungicides thiophanate methyl and ipridione + carbendazim were highly effective and comparable with the recommended fungicide carbendazim as they arrested the growth of the pathogen completely at 10 ppm. However, the conidial germination is completely inhibited by the fungicide ipridione + carbendazim at 50 ppm followed by thiophanate methyl.

Field evaluation of these fungicides on rubber seedlings was carried out at Ulickal nursery. It was observed that the fungicides pyraclostrobin + metiram and thiophanate methyl were effective with 7.2% disease severity each. In the unsprayed control the disease severity was 28%.

The chitinase gene cloned from GT 1, a clone tolerant to Corynespora leaf disease was effectively expressed in pET32a+ vector and recombinant protein was extracted. The antifungal activity of purified recombinant chitinase protein was confirmed by disc diffusion method on PDA plates using 10µg of recombinant protein. Polyclonal antibody was raised with the protein and expression of chitinase in the tolerant clone GT 1 was confirmed by Western Blot analysis

Attempts were also made to detect the chitinase activity by ELISA using the

polyclonal antibody. The results indicated that the *Corynespora cassiicola* induction elevated the chitinase activity in the tolerant clone GT 1 (Table Path. 2)

Table Path. 2. **Chitinase activity in tolerant and susceptible clone through ELISA**

	RRII 105 (Absorbance)		GT 1 (Absorbance)	
Sl. No.	Control	Induced	Control	Induced
1	0.8	1.1	1.1	2.0
2	0.9	1.1	1.1	2.0
3	1.0	1.2	1.1	2.2

1.4. Colletotrichum leaf disease (CLD)

A block trial was laid out at Vengathanam estate, Mundakkayam to study the efficacy of new generation fungicides against CLD on one-year-old RRII 105 plants with eight treatments. The results indicated that tebuconazole + trifloxystrobin was very effective with 8.8% disease severity followed by treatments involving tebuconazole (12.13%) and mancozeb (16.53%) compared to control (73.87%).

Survey carried out for assessing the intensity of CLD on immature plants of RRII 105 at Mundakkayam region showed that young plants (1st year) recorded very low disease intensity (11%) than the 2nd and 3rd year plants (50-93%).

In order to confirm the major cause of CLD in India, pathogen was isolated from diseased specimens collected from different locations representing traditional rubber growing regions having different symptoms like, typical raised spots, dark brown or black spots and dried margins . The emerging colonies from each bits were grouped based on the growth characters and conidial shape. *C. acutatum* was found to be more frequent with 69.39% of the colonies. Both the species could be isolated from all types of symptoms. Isolates producing perithecia in culture were obtained from 31% of the sampling locations and one representative isolate was identified by CABI as *Glomerella* sp. with more than 90% similarity to *C. gloeosporioides*.

Characterisation of the isolates of *Glomerella SP.* and representative isolates of *C.gloeosporioides* and *C.acutatum* was attempted. The isolates showed variation in colony colour , growth rate, conidial shape *etc.* within and among groups. The growth rate of *Glomerella* sp. and *C.gloeosporioides* varied from 1.4 to1.75cm and that of *C.acutatum* from 1 to 1.2 cm. Fungicide sensitivity test showed that *Glomerella* sp. and *C. gloeosporioides* were sensitive to carbendazim (2 µg ml^{-1}) and SAAF (50 µg ml^{-1}) but *C.acutatum* was less sensitive to these fungicides at the concentrations tested. Sensitivity to mancozeb (100 µg ml^{-1}) was almost same for the isolates though slight variation was observed among the isolates of the same species.

2. Stem diseases

2.1. Pink disease

New generation fungicides were tested for their efficacy against the growth (radial growth and biomass) of the pathogen *in vitro*. The results revealed that the fungicide tebuconazole was highly effective as it arrested the growth of the pathogen completely at 10 ppm. This was followed by the fungicide ipridione + carbendazim which inhibited the growth completely from 25 ppm onwards.

Field evaluation of new generation fungicides was carried out as prophylactic and curative treatment at Chittadi estate, Mundakkayam on 3-year-old plants of RRII 105. In prophylactic application, treatments

were imposed in the last week of May. The treatments were repeated after a gap of two months in case of traditional Bordeaux mixture spray and Kondody's Bordeaux paste. The results indicated that Kondody's Bordeaux paste was more effective with 85.9 per cent recovery of the plants followed by traditional Bordeaux paste (81.7%). The treatment involving traditional Bordeaux mixture spray was the least effective (56.7%). In curative application, the fungicides were mixed thoroughly in rubber kote and applied on the infected portion of the plants. The results showed that maximum recovery of plants (91%) was from the plot treated with Bordeaux paste followed by trifloxystrobin + tebuconazole with 88.3 per cent recovery.

3. Determination of the etiology of TPD of rubber

In the studies on transmission of TPD through budding, plants budded with scion taken from TPD affected trees as well as healthy trees on assorted root stocks planted at CES Chethackal were test-tapped to study the appearance of TPD symptoms. The trees were tapped daily and observed for TPD every month. Yield recording from all the tapped trees was carried out monthly by cup lump weighing. The results after one and a half years of tapping showed TPD in both groups of plants (Table Path. 3). This shows that stock is also playing a role in the development of TPD.

4. Pests of rubber

Field trial was laid out for the evaluation of eco-friendly formulations and insecticides against bark feeding caterpillar, *Aetherastis circulata* at PCK, Kodumon in completely randomized design with five treatments. Observations on the mean percentage reduction in the population at 10 and 20 days after the treatments were recorded. Among the treatments the combination of fenvalerate and carbaryl showed 96 per cent mortality after 20 days of spraying followed by neem oil 0.1 % and azadirachtin 0.03 % (Table Path. 4)

Table Path. 4. **Comparative evaluation of ecofriendly formulations and insecticides against bark feeding caterpillar, *A. circulata***

Treatment	% reduction in population	
	10 days	20 days
Beauveria bassiana (Mycojaal,10%)	10.1 (13.6)	17.7 (26.8)
Azadirachtin 0.03%	39.5 (36.5)	65.6 (58.3)
Neem oil 0.1% +carbaryl (0.1%)	57.1 (48.1)	79.3 (54.0)
Fenvalerate (0.02%)	93.8 (83.2)	96.0 (84.1)
Control	7.4 (6.8)	13.5 (14.3)
CD (P=0.05)	9.39	10.84

Figures in parentheses are arc sine transformed values

Laboratory studies on the bio-efficacy of larval parasitoids such as *Bracon brevicornis* and *Goniozus nephantidis* showed *B. brevicornis* as more effective in parasitizing *A. circulata*.

Field experiment was conducted to study the effect of neem-based insecticides on the mortality of both larvae and adults of mooply

Table Path. 3. **Incidence of TPD in plants budded with different scions**

Scion source	No. of TPD trees						
	June 2010	August 2010	October 2010	December 2010	February 2011	November 2011	January 2012
Healthy tree	0	4	2	3	9	10	13
TPD tree	0	2	5	5	7	9	9

beetle, *Luprops curticollis*. Adult beetles were attracted by putting semi-dried rubber leaves and treatments were applied. The results showed that all treatments were superior to control and at par in effectiveness on the mortality of larvae. Azadirachtin 0.03% showed 61.4 per cent control of adult beetles after 14 days followed by neem gold (58.14%), econeem (55.05%) and neem oil (43.80%).

5. Vector control

Surveillance on vector population dynamics was conducted in rubber plantations and coastal areas and evaluated different tools for IVM strategy. Studies were also carried out to find out the effect of rain guard on vector populations by collecting adult and larvae from rain guarded and non-rainguarded rubber plantations. Similarly the influence of inter crops on the population dynamics was also recorded by collecting adults and larvae from both intercropped and non-intercropped immature rubber plantations. The larvae collected were reared to adult mosquitoes and were identified in VCRC field station.

6. Bee keeping in rubber plantations

A field study was conducted on the effect of supplementary off-seasonal feeding on brood rearing activities of Indian honey bee, *Apis cerana indica*, in rubber plantations. Initial observations on the comb area, brood area and population of the colonies were recorded before giving various types of feedings. The observations were recorded after 15, 30 and 45 days after feeding. The results showed significant increase in the brood rearing and population of honey bees fed with both sugar syrup and pollen compared to sugar syrup alone and pollen alone fed colonies (Table Path. 5).

7. Microorganisms for improving growth of rubber and cover crops

The effect of inoculation of the 12 selected rhizobacterial isolates on growth of rubber seedlings was compared with a standard PGPR *Bacillus* sp. in polybags. The growth was generally better than the uninoculated control plants and was comparable with the plants inoculated with the standard (Table Path. 6).

Table. Path. 6. **Growth of plants (one year) inoculated with PGPR isolates**

Isolate no.	Girth (mm) diameter	Height (cm)
Std PGPR	10.7	106.8
Ri 25	12.6	111.4
K 43	12.3	124.4
K 52	12.1	123.7
RB 88	11.3	107.9
K 24	11.6	112.5
A 1	11.3	105.8
RH104	13.2	127.8
RH 34	13.7	121.4
3 pt	11.3	113.7
Ps 20	10.9	102.9
Ri 10	13.5	129.7
F 1	13.8	126.9
Control	10.3	103.8
CD(P=0.05)	0.69	06.47

Table Path. 5. **Effect of supplementary feedings on the comb area, brood building activities and population of *Apis cerana indica* during off-seasons**

Treatment (in cm^2) after	% increase of comb area rearing after			% increase of brood after			% increase of population		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Sugar feeding	15.9	15.9	28.3	23.4	37.4	69.6	33.8	48.0	62.2
Pollen feeding	17.0	17.0	25.3	20.2	38.2	61.0	31.2	36.7	48.3
Sugar & pollen	29.3	29.3	4101	26.3	49.78	81.6	35.3	53.9	75.9
CD (P=0.05)	10.6	10.6	10.5	9.2	9.15	8.44	5.46	9.11	10.62

The production of IAA by two selected *Azospirillum* isolates Azo 5 and Azo 12 from rubber plantations was studied and found to produce 1.8 μg m^{-1} and 12.4 μg m^{-1} of IAA production respectively. Population in soil based on IAR studies for four months showed the presence of the inoculated isolates.

Beneficial activities like phosphatase activity and IAA production of nine morphologically different phosphofungi from rubber plantation along with a standard phosphofungi were studied and many of them were found to be better than the standard (Table Path. 7).

Table Path. 7. **IAA production and acid phosphatase activity of phosphofungi**

Isolate	Acid phosphatase activity (μg PNP ml^{-1} broth)	IAA production (μg ml^{-1} broth)
PSF 1 (*Aspergillus* sp)	0.4	2.7
PSF 2 (*Penicillium* sp)	0.6	1.5
PSF 3 (*Penicillium* sp)	0.5	0.6
PSF 4 (*Aspergillus* sp)	0.5	0.5
PSF 5	0.8	4.0
PSF 6 (*Penicillium* sp)	1.0	6.8
PSF 7	1.2	1.4
PSF 8 (*Penicillium* sp)	1.0	-
PSF 9 (*Penicillium* sp)	1.7	0.7
PSF (std)	0.4	1.8

The efficiency of the isolates to promote plant growth was also studied by comparing with the standard isolate, *A. awamori* in polybags. The plants inoculated with PSF 5 had shown higher growth. Except three, all the isolates were comparable to the standard isolate in improving growth of plants upon inoculation (Table Path. 8).

In the biofarming trial, *Mucuna* seedlings raised in polybags inoculated with bacterial isolate NE 4 from North East India were field-planted. The plants were protected from shoot rot and Colletotrichum leaf fall disease during rainy season and assessed for various diseases periodically. The girth of plants was recorded and the plants in the integrated treatment were found better than other treatments.

8. Waste management in rubber processing

Evaluation of the the pilot reactor established at Elavamapadam RPS, Palakkad was continued. Hybrid reactor in its second season of operation could reduce COD by 94.65% and BOD by 94.4%. The pH after biomethanation was 7.0 - 7.2. The reactor could reduce total solids by 73.1% and dissolved solids by 75.3%. Hybrid reactor

Table Path. 8. **Effect of phosphofungi inoculation on growth of rubber seedlings**

Isolate	6 months		One year	
	Height (cm)	Girth (mm diameter)	Height (cm)	Girth (mm diameter)
PSF 1 (*Aspergillus* sp)	45.5	5.9	79.2	9.3
PSF 2 (*Penicillium* sp)	49.6	5.8	84.6	9.7
PSF 3 (*Penicillium* sp)	66.9	6.9	93.6	11.3
PSF 4 (*Aspergillus* sp)	70.3	7.3	113.8	12.8
PSF 5	71.7	7.3	130.5	14.0
PSF 6 (*Penicillium* sp)	68.7	7.0	116.9	13.1
PSF 7	74.0	7.0	118.8	13.4
PSF 8 (*Penicillium* sp)	70.1	6.9	128.3	13.6
PSF 9(*Penicillium* sp)	69.4	7.3	121.7	13.4
PSF(*A. awamori*)	66.9	6.8	122.8	13.0
Control	64.9	6.7	109.4	10.4
CD (P=0.05)	5.11	0.51	8.49	0.99

Table Path. 9. **Evaluation of integrated waste water treatment system with reed bed**

Parameter	Raw	Filtered	Anaerobic digester	Reed bed	Overall efficiency (%)
pH	5.3-5.7	5.3-5.8	6.9-7.1	7.1-7.4	
COD	11654.4	11021.5 (5.4)	582.57 (94.7)	159.88 (72.5)	98.6
BOD	5411	5020.66 (7.2)	262 (94.7)	26.43 (89.6)	99.5
TS	14166.7	12728.57 (10.1)	3618.18 (71.5)	2542.86 (29.7)	82.0
DS	12560	11633.33 (7.3)	3180 (72.6)	2300 (27.6)	81.6

All values are in mg L^{-1}, except pH. Values in parentheses are percentage reduction

yielded 8 m^3 biogas per day. The biogas from the reactor was used as fuel for burning in the smoke house for sheet drying and the utilization of biogas as an alternative fuel has reduced the use of firewood by around 35%.

In view of the drawbacks of the previous reed bed system, an improved system was made by converting the previous aeration tank treated water collection tank. The partially digested effluent from hybrid reactor after biomethanation was fed to the collection tank (feed tank) of the reed bed system. The flow was regulated to provide a uniform retention time. Effluent coming out after the treatment was analysed for the parameters like BOD, COD, solids and pH. The two major pollution parameters *viz.* COD and BOD of the treated water is well within the limit stipulated for safe disposal (Table Path. 9).

Though the new reed bed system was performing excellently, clogging of the system hindered its working. The flow rate of the effluent through the system was also very slow. Therefore further modification of the system is needed.

9. 'Distance diagnostic' information system (rubber clinic)

Updated the database of the 'clinic' based on the new developments in rubber research along with news on seminars, trainings *etc.* on aspects related to rubber disease control. Weather data at Kottayam was updated daily on the site. Disease alerts were given through the site as and when the chance of a disease was perceived. The clinic diagnosed 403 (39%) cases through 'assisted diagnosis' and 630 (61%) cases through 'self diagnosis' accounting for a total of 1033 during April 2011 to February 2012. The total number of visits to the site (site hits) were 7,532 with an average of 20 visitors/day during April 1, 2011 - March 31, 2012 (Source: Google Analytics). Visitors from 86 countries utilized the facilities in the clinic in which 81% of the visitors were from India of which 53.8% from Kerala.

10. Farm mechanization

A highly efficient mist blower with high capacity blower (12 HP) was designed, developed and introduced in collaboration with ASPEE, Mumbai. This was mounted on a mini tractor and field tested and found to deliver the spray fluid at a height above 85 ft. Three models of single man carrying mist blowers for the spraying operations in rubber plantations were experimented and introduced. All these new developments in spraying technology could economise the spraying operation in rubber plantations.

PLANT PHYSIOLOGY DIVISION

The major areas of research in the Plant Physiology Division are environmental physiology, physiology of growth and yield, tapping panel dryness, nutrition physiology, gene expression studies, secondary metabolites and ecological impact of natural rubber cultivation.

1. Environmental physiology

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

A 23 kDa chloroplast stress protein was shown to be prominently expressed in drought imposed young plants of *Hevea*. Measurement of Photosystem II activity (Φ PS II.) and analysis of stress protein expression in RRII 400 series clones were carried out. The magnitude of inhibition of PS II activity was less in clones RRII 430 and RRIM 600 as compared to other clones under drought condition. The present experiment indicated that RRII 414, RRII 417 and RRII 422 are relatively drought susceptible. The 23 kDa stress protein was purified and a polyclonal antibody against this protein was raised. Over expression of this stress protein in drought imposed plants was reconfirmed by western blot analysis. Further validation of this protein as a physiological marker is progressing.

1.2. Identification of molecular basis for drought tolerance in *Hevea*

Three year old field grown plants of clones RRII 105, RRIM 600, RRII 208 and RRII 430 at Regional Research Station, Dapchari, were subjected to drought stress for fifteen days during the summer period of 2011. Leaf samples were collected after confirming the magnitude of stress effect by measuring gas exchange parameters. This was followed by mRNA isolation, cDNA synthesis and qPCR analysis. Gene expression analyses were performed using ten stress responsive genes. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as endogenous control for normalization.

The relative gene expression analysis was performed using control plants of each clone as calibrator and the results are given in Table Phy. 1. Among the genes studied, the expression of peroxidase and WRKY transcription factor (WRKYtf) was significantly higher than their respective

Table Phy. 1. **Relative quantification of ten genes expressed under drought conditions in *Hevea* with reference to control plants of respective clone as calibrator**

Genes	Calibrator	RRII 105 D	RRIM 600 D	RRII 208 D	RRII 430 D
Peroxidase	1	1.7	3.9 *	6.5 *	6.8 *
LEA 5	1	0.9	4.5 *	1.2	3.3 *
CRT/DRE bf	1	1.9	1.1	2.5 *	1.1
WRKY tf	1	0.6	2.9 *	2.2 *	8.6 *
Tf MBF	1	1.1	0.6	1.5	1.2
GPX	1	1.2	1.2	1.3	1.1
ABCT	1	1.7	1.7	1.0	1.9
Hb333HP	1	0.6	1.3	2.6 *	1.0
Hb22HP	1	1.5	2.0 *	0.9	1.2
Hb20HP	1	1.3	2.3 *	1.1	2.2 *

* *Significant at 5% level*

controls in all the drought tolerant clones (RRIM 600, RRII 208 and RRII 430) while WRKYtf showed down regulation in the susceptible clone RRII 105 under drought conditions. Expression of LEA 5 protein and Hb20HP showed significant up-regulation in two of the drought tolerant clones (RRIM 600 and RRII 430). The expression of tfMBF, glutathione peroxidase and ATP binding cassette transport protein (ABCT) did not show any significant changes among the drought imposed plants of different clones. The present study reveals the existence of strong association of genes such as peroxidase, WRKYtf and LEA 5 protein with drought tolerance in *Hevea*.

1.3. Molecular studies on cold stress

Polybag plants of clone RRII 105 and RRIM 600 were exposed to low temperature in the growth chamber. Cold stress was assessed by measuring the photo-inhibition with gas exchange parameters. Quantitative PCR was performed for stress associated genes such as DnaJ protein, peroxidase, ETR1, ETR2, ACO2, HbHP20, HbHP22, cysteine protease, chitinase, annexin, SUMO activating protein, HbDRT5b, HbTPD24 and HbDRT50. When control of each clone was used as calibrator, out of the fourteen genes analyzed, peroxidase, HbDRT5b and HbTPD24 were highly up-regulated in both the RRII 105 and RRIM 600 clones. The expression of HbDRT5b and HbTPD24 (NAC transcription factors) were relatively higher in the tolerant clone RRIM 600. When RRII 105 was used as calibrator, the level of peroxidase in RRIM 600 was much higher. Levels of NAC transcription factors (both HbDRT5b and HbTPD24) were found higher in RRIM 600. The level of HbDRT50 and ACO2 was relatively higher in RRII 105 and RRIM 600, respectively.

1.3.1. Developing a microarray of stress responsive genes from Hevea brasiliensis

One set of *Hevea* plants (clone RRIM 600) grown in polybags at the nursery of RRII was exposed to drought stress by withholding irrigation for fifteen days while another set of plants was maintained with irrigation at field capacity. Leaf samples were collected on 5th and 10th day of drought imposition. Similarly another set of plants (clone RRIM 600) grown in polybags was exposed to low temperature conditions (minimum night temperature at 8°C and maximum day temperature at 20 °C) with 800 µE light during day time. Leaf samples were collected on 1st and 4th day of treatment. Isolation of mRNA was carried out from the leaf samples and sent for transcriptome sequencing using Illumina RNA-Seq technology and the results are awaited.

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

Hevea plants (clone RRIM 600 which is relatively drought tolerant) grown in poly bags were exposed to drought in the nursery of RRII, Kottayam. One set of plants (n=10) was subjected to water stress by withholding irrigation for 14 days and the other set of plants (n=10) was watered on alternate days to maintain field capacity. Leaf samples were harvested after assessing the drought status of the plants by measuring the net CO_2 assimilation rate (A) and stomatal conductance (gs). miRNA was isolated from frozen leaf samples and about 2 µg of small RNA with an equal volume gel loading buffer were loaded on a 12% denaturing (7M urea) polyacrylamide gel. The eluted miRNA fragments (21-25 bp) were ligated with a 3′ and a 5′ linker using miRCat cloning kit in two separate reactions followed by cDNA synthesis. The results

indicated the presence of miRNA bands in the range of 20-24 nucleotide (nt) size. The PCR amplified products when visualized on agarose gel indicated the presence of amplicons in the range of 62 bp size. These amplicons were further purified to proceed with cloning and sequencing.

1.4. Measurement of CO_2 and water vapour flux in rubber

Ecosystem level carbon dioxide (Fc) and water flux (LE) were measured during summer months of 2011 and 2012 by using the eddy covariance system installed inside a six year old immature rubber plantation. The mean NEE during summer season was 16g $CO_2 m^{-2} day^{-1}$. The evapotranspiration (ET) rate was calculated from the latent heat of vapourization (LE) and it was 4 mm day^{-1} in this plantation.

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

Under the current fluctuating climatic conditions, an understanding of the common mechanism of stress tolerance is very much essential to know the response of young plants of *Hevea* under different environmental conditions. A polybag nursery of *Hevea* clones (RRII 105, 208, 414, 429 & 430, RRIM 600, RRIC 100 and SCATC 88/13) was established at RRII. Drought induced reduction in dark Fv/Fm was observed in plants compared to saturation level irrigated plants. The effective PS II quantum yield (Φ PS II.) showed significant reduction upon exposure to drought. Leaf water potential showed a decrease in drought induced plants. The antioxidant enzymes like super oxide dismutase and ascorbate peroxidase were found high in drought imposed plants compared to control in all the clones studied. Cold treatment was given to RRII 430 and RRII 429. Observations were taken as in the case of drought imposition and noticed a similar trend.

1.6. Evaluation of modern *Hevea* clones for drought tolerance

1.6.1. Physiological evaluation of RRII 400 series clones for drought tolerance

Photosynthetic parameters in one year old plants in the field (CES, Chethackal) were better in clones RRII 430, RRII 414 and RRIM 600 compared to other 400 series clones. No variation was found in chlorophyll content and maximum and effective quantum yields of PS II in these clones under field condition during stress free season.

Gas exchange measurements in the polybag plants revealed a decline in photosynthetic activity in all the clones upon water stress. The 400 series clones showed better photosynthesis rate than the check clones (RRII 105 and RRIM 600) at 5th and 10th days of moisture stress. Clones RRII 422, RRII 429 maintained better stomatal conductance after 10 days water stress compared to other clones. While the maximum potential quantum yield of PS II did not vary among the clones, the light adapted effective quantum yield showed a slight decline in some clones.

Observations from RRS, Dapchari showed that there was more than 75% reduction in photosynthesis, stomatal conductance and transpiration in all the clones after 5 days of water stress and the gas exchange parameters were reduced to minimal levels at 10th days of stress. In terms of photosynthetic rate RRII 430 better adapted to moisture stress compared to all other clones in the North Konkan conditions. There was no significant decline in maximum quantum yield among the clones but effective quantum yield declined in all the clones after moisture stress.

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

1.7.1. Laboratory screening of germplasm accessions for intrinsic drought tolerance

The 37 top and 10 bottom ranking accessions from SBN 2004 and 14 top and 10 bottom ranking accessions from SBN 2005 were selected by field scoring and further subjected to laboratory screening for intrinsic drought tolerance traits at CES, Chethackal. The leaf discs punched from middle leaflets were incubated in 60% PEG solution and exposed to light (350 μmol $m^{-2}s^{-1}$) for four hours. Control leaf discs were incubated in water. The effective quantum yield of PS II (Φ PS II.) of treated and untreated samples were measured. On the basis of percent reduction in PS II quantum yield, the accessions were ranked for intrinsic tolerance. The percentage reduction varied from 5% in accession MT 1619 to 85% in accession RO 291. The accessions MT 1619, AC 2009, MT 196, RO 322, AC 1886, AC 494, AC 612, RO 2360, RO 855 and RO 2524 were ranked top in the list for intrinsic water deficit tolerance from SBN 2004. Similarly RO 2634, MT 2210, AC 173, RO 1406, RO 3184, MT 2210, RO 1425, MT 3702, RO 1421 and RO 2299 emerged as top ranking from SBN 2005.

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

Modern clones of RRII 400 series, *viz.* 414, 417, 422, 429 and 430 planted at CES, Chethackal were assessed for chlorophyll reduction in summer due to drought and high-light stress. Total chlorophyll content was estimated in the same plant during stress free period of October (2011) and stressful period of March (2012). The chlorophyll content in non-stressed period varied from 2.9 to 3.2 mg g^{-1} fw and it varied from 2.1 to 2.8 mg g^{-1}fw during stress period. The reduction in chlorophyll content was estimated and it ranged from 12.3% for clone RRII 430 to 30.2% for RRII 105. Clones were sorted and ranked for intrinsic tolerance based on chlorophyll reduction in summer. Altogether the clones were ranked in the order of RRII 430 >RRIM 600 >RRII 422 >RRII 429 >RRII 417 >RRII 414 >RRII 105 for relative tolerance based on chlorophyll pigment stability under drought and high light stresses.

1.8. Experimental cultivation of high yielding clones of rubber plants for establishment in higher elevation in Kerala

Though the overall growth performance of all the clones in a trial started during 2006 at a high altitude location - Elappara in Idukki district of Kerala was poor, PB 260 showed better girth (19.6 cm) followed by RRIM 600 (18.3 cm) whereas PR 261 (13.5 cm) recorded the least. In a trial started during 2007, clone RRIM 600 (15.4 cm) and RRII 208 performed better in girth than clones RRII 105 and RRII 414 (7 cm). The polyclonal seedlings planted in tea plantation showed the maximum girth (22.5 cm).

1.9. Evaluation of environmental stress tolerance and physiological adaptations of cold and drought tolerant ortet under varying agro-climatic conditions

A total of sixteen best performing ortets from drought/cold stress prone localities spread across five different agro-climatic area were selected and grown in three different locations. Ortets from drought/cold prone localities include NGK 1, 47, 69; GH

1, 3, 9, RRST 24, 37, 39, DAP 1, 34, 35, 36, RRSA 98, 315, 585 and clones from traditional belt include RRII 417, 414, 422, 429, 430, 105 and RRIM 600. The budded stumps were planted in polybags during September - October 2011 at CES, Chethackal. Percentage sprouting was found better in RRII clones compared to ortets from non-traditional areas. Field planting will be carried out during the present season at three different locations for comparative evaluation.

1.10. Drought survey

Drought survey was conducted during summer 2012 in one year old young plantations in Central and South Kerala. Information from each plantation was collected using a standard questionnaire by visiting the plot and visual scoring of the drought injury parameters. Nearly two hundred holdings were surveyed in random from both these regions. The major finding revealed that nearly 18% of the holdings resort to irrigating the young rubber plants in Central Kerala while only about 5% holdings irrigated their young plants in the Southern region (Table Phy. 2).

1.11. Physiological and proteome studies of cold stress in *Hevea brasiliensis*

Maximum potential chlorophyll fluorescence and effective PS II quantum yield (Table Phy. 3) were recorded in field grown polybag plants at RES, Nagrakatta during winter season, January 2012. Maximum Fv/Fm was found in RRIM 600 followed by Haiken 1, SCATC 88/13 and RRII 105. On the other hand, maximum PS II efficiency (Φ PS II.) was found in Haiken 1 followed by SCATC 88/13. Leaf sample from same clones were collected for proteome studies. The protocol for 2-D electrophoresis for proteome was standardized.

1.12. Studies on proteome of *Hevea brasiliensis* under drought stress

Seven clones of *Hevea viz.*, RRII 105, 414, 417, 422, 429, 430 and RRIM 600 were exposed to different levels of drought stress for 5, 10 and 15 days under field conditions in polybags. Better water potential was maintained in RRII 105 followed by RRII 429 on 10th day of drought. There were no significant differences in chlorophyll content of the clones in relation to drought imposition. Net photosynthesis was found

Table Phy. 2. **Extent of irrigation in young rubber plantations in central and south Kerala**

Region	No of holdings surveyed	Plots irrigated (%)
Central Kerala (*Palakkad, Mannarkad*)	100	18
South Kerala (*Nedumangad, Punalur, Kottarakkara*)	100	5

Table Phy. 3. **Chlorophyll fluorescence data of five *Hevea* clones exposed to cold temperature at RES, Nagrakatta**

Clone	Fv/Fm		Φ PS II	
	Control	Cold exposed	Control	Cold exposed
RRII 105	0.817 ± 0.008*	0.355 ± 0.016	0.611 ± 0.014	0.279 ± 0.020
Haiken 1	0.821 ± 0.006	0.432 ± 0.046	0.594 ± 0.014	0.350 ± 0.059
RRIM 600	0.839 ± 0.006	0.452 ± 0.047	0.573 ± 0.020	0.256 ± 0.092
SCATC 88/13	0.840 ± 0.006	0.413 ± 0.020	0.571 ± 0.026	0.329 ± 0.040

* *Indicates SE*

maximum in RRII 430 followed by RRII 417 on 10th day of drought imposition. Samples were collected and proteome studies of drought stressed plants are in progress.

2. Physiology of growth and yield

2.1. Studies on yield and yield components

Twelve clones planted in 1982 at CES, Chethackal were evaluated for clonal variation in biomass and yield. The trees were tapped for 18 years and the untapped trees were left without tapping throughout the study period. Before felling of trees all the tapped and untapped trees were subjected to slaughter tapping during 2011-12 and considerable high yield was observed in untapped trees. The hitherto untapped trees of clone RRII 105 recorded 296% increase in yield over the tapped trees followed by clone PB 235 (220%). It was very low in clone Tjir 1 (56%). However, the untapped trees exhibited low DRC content in latex (Table Phy. 4).

Table Phy. 4. **Clonal variation in yield of tapped and untapped trees subjected to slaughter tapping**

Clone	Mean yield (g $t^{-2}t^{-1}$)		
	Tapped trees	Untapped trees	% increase in untapped trees
RRII 300	71.3 ±4.9	167.8 ±28.7	135
PB 235	57.8 ±7.5	184.6 ±26.9	220
RRII 105	58.6 ±10.8	232.1 ±49.3	296
RRIM 600	63.5 ±8.1	143.4 ±13.7	126
GT 1	108.7 ±17.2	183.6 ±32.5	69
PR 107	44.6 ±7.2	94.8 ±13.6	113
Gl 1	57.7 ±15.3	169.8 ±20.5	194
RRIM 501	36.1 ±3.3	78.1 ±11.2	116
RRII 118	94.4 ±13.8	164.3 ±31.0	74
RRIM 703	78.2 ±10.3	146.3 ±29.6	87
Tjir 1	56.1 ±9.1	87.6 ±6.8	56
RRIM 612	60.1 ±10.5	160.5 ±33.0	167

± indicates SE

2.2. Intercropping with tree crops in rubber

An experiment was initiated at CES, Chethackal to evaluate the growth of rubber plants intercropped with tree species *viz.* mahogany and pathimugam between the rows of rubber (clone RRII 105) in two replications. Mahogany was planted both as single as well as three rows whereas, pathimugam was planted in single row between rubber. After 11 years of planting mahogany and pathimugam attained 39 cm and 19.9 cm girth, respectively whereas, the clone RRII 105 attained 57 cm girth. The yield of rubber trees will be monitored in terms of influence of tree intercrop.

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

The study continued during the year and similar trend as that of previous year was observed. PB clones recorded better shoot biomass increment than RRII clones upon tapping.

2.4. On farm trial for the selection of Latex-Timber clones

For identifying Latex-Timber clones for the traditional rubber growing region, four clones were selected in Malankara estate to find out the existence of any relationship between yield and annual girth increment. The clones were under tapping on S/2 d3 6d/7 systems. The annual girth increment and annual block yield during 2011-2012 were recorded. During the reporting period PB 235 was shown to be the highest yielding clone followed by RRII 105 (Table Phy. 5).

Table Phy. 5. **Annual shoot biomass (kg $tree^{-1}$) increment and rubber yield (kg ha^{-1}) in four clones under d3 tapping system in Malankara estate**

Clones	Shoot biomass increment 2011-12	Annual block yield 2011-12
RRII 105	14 ±3.2	1885 ± 91
PB 235	22.4 ±3.5	1922 ± 81
PB 260	19.8 ±4	1403 ± 48
PB 217	15.5 ±0.4	1869 ± 72

n= 75

The annual shoot biomass increment was also the highest in PB 235.

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

In continuation of the studies on relationship of latex ATP with rubber yield, the latex ATP data of young plants of different clones over five years was analyzed and correlated with the yield data of mature trees of these clones. A positive correlation was existing between young plant ATP and mature tree yield and was stable when the plants are getting matured. About 300 assorted seedlings were screened for latex ATP content and found some of them have very high latex ATP. Further studies are in progress.

2.6. Studies on rubber biosynthesis in *Hevea* clones

Prenyl transferase (RuT) enzyme activity in the whole latex was measured in mature and immature plants of ten *Hevea* clones of varying yield potential using ^{14}C-labelled IPP as the substrate. The clones selected were five high yielding clones (RRII 105, RRIM 600, PB 217, PB 235, PB 260), three medium yielding (GT 1, Tjir 1, PB 5/51) and two low yielding clones (RRII 33, RRII 38). The results indicated that rubber transferase activity in mature trees under tapping for 12 years and immature plants of the same clones were positively correlated with rubber yield. Among the clones studied, PB 217, PB 260, RRIM 600 and RRII 105 showed higher prenyl transferase activity.

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

The enzyme 3-hydroxy- 3-methyl glutaryl-CoA reductase (HMGR), which catalyses the synthesis of mevalonate from HMG-CoA is a key regulatory enzyme in the rubber biosynthetic pathway. Cloning and expression of *hmgr*1 was performed in order to obtain the HMGR1 protein. For this purpose, mRNA was isolated from latex of *Hevea* (clone RRII 105). cDNA was synthesized and PCR amplification of coding region of *hmgr*1 was performed using *hmgr*1 specific primers. The PCR amplified product (~1.8 kb) was cloned in to an expression vector (pRSET-A) and transformed in to *E. coli* (BL21DE3) cells. Protein expression in transformed cells when monitored by SDS-PAGE analysis indicated the presence of HMGR1 protein (61.6 kDa).

2.8. Molecular and biochemical basis of ethylene induced latex production in *Hevea brasiliensis* (ethylene receptors and signal transduction mechanism)

Hevea trees of clone RRII 105 of uniform girth and yield under S/2 d3 6d/7 tapping systems were selected and latex samples were collected before and after stimulating the trees with ethephon. Total RNA was isolated from control and stimulated trees of both clones. cDNA synthesis and quantitative expression analysis of ethylene receptors (ETR1 and ETR2) are in progress.

3. Tapping panel dryness

3.1. Location specific stimulant application on ethylene induced stress responses in the tapping panel of *Hevea* trees

An experiment was carried out at CES, Chethackal in clone RRII 105 under S/2 d3 6d/7 tapping system with an objective of reducing ethylene mediated stress responses in the tapping panel by applying the ethylene compounds away from the tapping area without compromising latex yield. In the present experiment bark application of 5% ethephon was made 1" wide around the tree trunk in the following regions: (a) 5% Ethephon at 150 cm above the bud union (1" wide ring); (b) 5% Ethephon just above the

bud union (1" wide half spiral) ; (c) 5% Ethephon at both above and below positions away from the tapping panel (1" wide ring); (d) 2.5% Ethephon above and below away from the tapping panel. Trees with panel application of 2.5% ethephon and unstimulated trees were selected as controls. Biochemical parameters such as ATP, sucrose and thiols in latex and stress indicators like phenol, proline, MDA and H_2O_2 in the bark were analysed. Significantly high ATP and very low sucrose was observed in trees applied with 5% ethephon above and below regions away from the tapping panel.

3.2. Molecular basis of TPD

3.2.1. *Investigations on the molecular physiology of tapping panel dryness syndrome (TPD) in Hevea brasiliensis: cloning and characterization of TPD responsive genes*

Validation of TPD specific transcripts was continued with a new set of 11 genes *viz*. Annexin, SUMO activating protein, ETR1, ETR2, ACO2, 20 HP, 22 HP, 33 HP, Cystein protease, Chitinase, DRT 5b and TPD 24 by quantitative PCR (qPCR) method. cDNA obtained for mRNA of bark samples from different trees from healthy, late dripping and trees with 10, 25, 50, 75 and 100% TPD were used as template for the qPCR analysis. Among the 11 genes tested by qPCR, expression of DRT 5b and TPD 24 was found higher at the stage of late dripping and in advanced stages of TPD. The hypothetical protein (33 HP) displayed an increasing level of expression from the onset of late dripping to 100% TPD stage.

3.2.2. *Involvement of ethylene in Hevea rubber biosynthesis and tapping panel dryness*

A new set of primers were used to PCR amplify the coding region of β-cyanoalanine synthase and ACC oxidase and the PCR amplicons were cloned into the cloning vector. Further the ACC oxidase was sequenced and was cloned into an expression vector for protein production. The protein was visualised on SDS PAGE gel. The proteins would be used for raising antiserum which would be necessary for the immune-analysis for its expression in different clones.

4. Gene expression studies

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

Chitinase coding region was cloned into a modified expression vector (pHT43 vector devoid of LacI repressor) after restriction digestion with appropriate restriction enzymes. This vector after transforming into *E.coli* was multiplied and was sequenced to confirm its orientation and identity. This vector plus chitinase construct was used to transform WB800N, a mutated strain of *Bacillus subtilis*, and 8LK strain isolated from *Hevea*. The efficiency of the recombinant vector (with chitinase) for over-expression of protein and its secretion was confirmed by transcript expression analysis, chitinase activity and by SDS PAGE. The results confirmed the presence of chitinase protein in the medium (as extracellular protein) during the log phase (without IPTG induction). But this vector was highly unstable when transformed into the 8LK cells, the endophytic *Bacillus* species of *Hevea*.

5. Secondary metabolites

5.1. Quantification and identification of inositols in *Hevea*

After getting a patent on L-quebrachitol production, further steps were initiated for licensing the technology and scaling up the production of L-quebrachitol from the latex serum. It requires few refinements and

further contacts are being established with some leading R& D companies in this regard.

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

Osmotic concentration was worked out for the biochemical components estimated from the latex. This was used to calculate the osmotic potential and to see the effect of these solutes in water relation of latex. It was found that the contribution of carbohydrates and free amino acid to osmotic potential of latex was on par.

RUBBER TECHNOLOGY DIVISION

During the reporting year, the activities of the Division were focused mainly on evolving improved techniques in primary processing, latex-filler master batch, reinforcement of NR using polymeric fillers, scorch control of peroxide vulcanisation and rubber nanosilver composites. In addition, refinement of rubber recycling process is taken up as a new thrust area.

1. Primary processing

1.1. Skim latex processing

Trials were conducted to use the effluent obtained during the processing of skim latex to generate bio gas. From the laboratory study it was found that at a particular concentration of coagulant, rubber and its serum got separated into two layers. The layer containing skim latex could be separated and used for recovery of skim rubber. The effluent was suitable for generation of bio gas.

2. Latex technology

2. 1. Radiation vulcanised natural rubber latex (RVNRL)

A method was standardised to improve the quality of RVNRL films using creamed latex. It was observed that exposure of fresh NR latex to low doses of gamma radiation caused chemical changes that favoured vulcanisation. The chemical changes included micro gel formation and partial removal of proteins to an extend which do not adversely affect the strength of the vulcanized films. Unlike centrifugal process, the number of smaller rubber particles in latex after creaming is higher in creamed fraction. All these factors were expected to contribute to enhanced mechanical properties of RVNRL as shown in Table Chem. 1

Table Chem. 1. **Mechanical properties of radiation vulcanised natural rubber latex**

Properties	Creamed preserved field latex (PFL)	Pre-irradiated creamed PFL
Gum strength (MPa)	1.5	3.0
Modulus 300% (MPa)	0.9	1.1
Modulus 500% (MPa)	1.1	1.6
Tensile strength (MPa)	22.0	25.3
Elongation at break (%)	1379	1283
Solvent swelling (%) (after 24 hours)	230	170
Tension set after 1 hour at 300% elongation (%)	10	6

3. Rubber Technology

3. 1. Reinforcement

3.1.1. NR/ polymeric filler system

To improve the abrasion resistance of NR/PF-system, small proportion of polybutadiene rubber (BR) was incorporated in the system. Addition of BR improved the abrasion resistance without adversely affecting other vulcanisate properties. The mechanical properties based on the formulation given in Table Chem. 2 are shown in Table Chem. 3.

Table Chem. 2. **Formulation of the mixes NR/ polymeric filler system**

Ingredients	Control	A1	A4
NR	65	100	70
PB	35	0	30
PF	0	10	10
Zinc oxide	5	5	5
Stearic acid	2	1	1
HSL* quinolin	1.5	1.5	1.5
ISAFblck (N220)	30	0	0
HAF black (N330)	25	0	0
MOR **	0.8	0.8	0.8
Sulphur	2.5	2.5	2.5

**2,2,4-trimethyl – 1,2 – dihydroquinoline*
** 2-(4-Morpholinothio) benzothiazole

Table Chem. 3. **Technological properties of the vulcanisates**

Properties	Control	A1	A4
Tensile strength (MPa)	23.3	26.5	24.21
Elongation at break (%)	549	610	586
Modulus, 100 % (MPa)	3.1	5.3	5.1
Modulus, 200 % (MPa)	6.9	9.1	9.1
Modulus, 300 % (MPa)	11.5	12.3	11.7
Tear strength (N/mm)	82	75	74
Hardness (Shore A)	62	62	62
Heat build up (DT °C)	27	9	9
DIN abrasion loss (mm^3)	76	96	73

3.1.2. Latex - filler master batch

Latex master batches containing mixed filler systems (carbon black/silica/clay) were also prepared. It was observed that there was almost no filler loss during the processing. Higher modulus along with better tear strength was observed for the mixed filler master batches as compared with mill mixed compounds.

3.1.3. Silica reinforcement of rubbers

Silica reinforcement of natural rubber and epoxidised natural rubber of varying epoxy content *viz.* 10, 20, 25 and 50 mole %, were compared with and without silane coupling agent and with that of carbon black filled natural rubber. Formulation of the mixes is given in Table Chem. 4. Important

Table Chem. 4. **Formulation of the mixes for silica reinforcement study**

Ingredients	1	2	3	4	5	6	7	8	9
NR (ISNR 5)	100	100	85	85	85	85	85	85	100
ENR 10	-	-	15	15	-	-	-	-	-
ENR 25	-	-	-	-	15	15	-	-	-
ENR 50	-	-	-	-	-	-	15	15	-
Silica (VN3)	50	50	50	50	50	50	50	50	-
Silane (Si69)	-	4	-	4	-	4	-	4	-
ISAF	-	-	-	-	-	-	-	-	50
CBS	1.5	1.5	1.5	1.5	1.5	1.5	1.5		

(Above compounds except compound 9 also contain Naphthenic oil 5, Zinc oxide 4, Stearic acid 2, Mernox 6c (N-1,3 dimethyl butyl) N'-Phenyl p-phenylene diamine) 2, sulphur 2 , DPG 1.5)

Table Chem. 5. **Technological properties**

Parameter	1	2	3	4	5	6	7	8	9
Modulus 300 % (MPa)	7.1	15	11	17.7	11.7	18.2	11.2	14.9	14.1
Tensile strength (MPa)	26.4	25.1	24.7	21.3	24.6	19.5	22.7	24.5	24.0
Tear strength (N/mm)	105	91	106	53	98	54	98	89	109
Hardness (Shore A)	60	62	62	67	63	68	68	64	60
Abrasion loss (mm^3)	174	109	143	96	149	105	157	114	106
Heat build up ($\Delta T°C$)	15	8	15	9	16	11	19	12	19

technological properties are given in Table Chem. 5.

It was found that properties like modulus and hardness increased while abrasion loss decreased with level of epoxidation of ENR. All the composites containing silane showed improved properties over the unmodified ones.

3.2. Recycled rubber

A new radical scavenger has been identified for devulcanisation in a mechano-chemical process. In addition, a new concept of "simultaneous devulcanisation-revulcanisation process" is under study to address the twin problem of low scorch and lower vulcanisate properties of the devulcanised rubber when it is revulcanised.

3.3. Nanocomposites

3.3.1. *Nanosilver based nanocomposites*

Stabilised nanosilver dispersion was prepared using polyvinyl pyrolidinone as stabiliser at 10 ppb (parts per billion). Nanosilver was found to impart anti microbial property. Attempts were made to prepare nanosilver doped nanosilica for use in rubber.

3.4. Peroxide vulcanisation

Reconfirmed the capability of DCP/ TEMPO/N, N'-m- dimaleimide cure system to achieve sulphur/accelerator like scorch control in peroxide vulcanisation. This system suffered from a major draw back of very poor mould release after vulcanisation. Therefore, a new peroxide cure system, di (t-butyl peroxy isopropyl) benzene/TEMPO/ timethyl propane trimethacrylate (TMPTMA) has been developed. The new cure system could achieve excellent scorch control (Table Chem. 6) with very good mould release property.

Table Chem. 6. **Effect of coagent on cure characteristics di (t-butyl peroxy isopropyl) benzene/ /TEMPO / timethyl propane trimethacrylate /TEMPO at 175° C**

Properties	1	2	3	4	5
TEMPO	0	0.8	0.8	1.2	1.2
TMPTMA	0	0	4	0	4
Minimum torque((M_L) dN.m	31.8	20.6	20.3	19.3	16.4
Maximum torque((M_H) dN.m	122.5	100.9	117.0	94.4	111.0
M_H-M_L	90.7	80.3	96.7	75.1	94.6
Time for one unit rise in torque, ts_1.min	1.4	2.9	2.7	3.1	3.2
Time for two unit rise in torque, ts_2.min	1.5	3.2	3.0	3.4	3.4
Optimum cure time (min)	11.3	13.5	13.6	15.6	16.0

4. Collaborative project

4.1. Development of Hurth Coupling for rail locomotive

In connection with the development of Hurth Coupling Membrane for Chithranjan Locomotive Works (CLW), West Bengal, 10 more samples were despatched for evaluation this year. Development of Spheri block is being attempted.

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

Prototypes of hard and soft microcellular soles suitable for leprosy patients were prepared at RRII laboratory in the presence of technical persons from SLR&TC, and trained him for the manufacture and testing of microcellular rubber soles.

5. Development/advisory work

5.1. Tested and report was given for the damage of 5 nos of tyres referred from various Consumer Disputes Redressal Forums

5.2. Tested and report was given for 31 no of polythene samples for the RP Department and tested 1 no of logo material for its suitability as sticker in rubber bales for marketing division.

TECHNICAL CONSULTANCY DIVISION

The main activity of the Division is technical consultancy services for the promotion of Indian Rubber Industry. The Division caters to the needs of new entrepreneurs as well as existing rubber goods manufacturers. The ranges of services offered to various rubber industries include product development, quality control and certification, technical problem solving, training, *etc.*

1. Product development

41 products were developed as per the requests from the clients and the know-how was transferred to the clients.

2. Quality control

Technical support was rendered to various rubber industries, mainly to the small scale sector, by testing rubber compounds, rubber products and evaluation of raw materials.

- Products/samples were received from 711 firms and tested 8364 parameters and collected Rs.787894/- towards testing/development charges.

3. Advisory services

Matters relating to various aspects like selection of raw material, dosage of a particular ingredient, temperature/pressure for vulcanization etc. were always a subject of concern for small scale product manufacturers.

- Queries on technical matters from 1337 units were addressed during the reporting period.

ECONOMICS DIVISION

The Division continued its research activities confining to the five thrust areas *viz.* (i) farm management; (ii) primary processing and marketing of NR; (iii) rubber products manufacturing industry and foreign trade; (iv) intercrops and by-products; and (v) inter-divisional collaborative projects. During the reporting period, five projects, all pertaining to the farm management, were completed and reported. The summaries of the results of the completed projects are given in the following sections.

1. Evolution of Rubber Propagation Policy in India (1949-86)

This documentation is an earnest attempt to explore and analyse the major milestones in the evolutionary dynamics of the rubber propagation policy in India during the 38 year period (1949-86) in the context of the multidisciplinary study on rubber nurseries.

Unlike in the case of other major NR producing countries evolution of India's propagation policy had been unique with four distinct phases, *viz.*, the experimental phase, (1902-48), the interventionist phase (1949-60), the phase of partial decontrol (1961-86) and decontrol (1986 onwards). Although India had to confront relatively less favourable agro-climatic conditions, its well conceived policy initiatives with the active support of an enlightened planting

Table Eco. 1. **Major colonial policy measures on rubber (1942-47)**

Policy measure	Objectives	Outcomes
The Indian Rubber Control Order, March, 1942	To revamp the existing control systems, prohibit NR exports and co-ordinate measures in the backdrop of the second world war.	The Indian Rubber Control Committee was constituted and started functioning from April, 1942
The Indian Rubber Control and Production Order, November, 1942	To promote NR production and to strengthen the monopoly purchase scheme of NR, operational since 27th May, 1942	The Indian Rubber Production Board was constituted under the Chairmanship of Sir C.P. Ramaswami Iyer. It also marked the beginning of a protected price policy regime
Imposition of protective import tariffs ranging from 30-60 per cent on value added rubber products (1942-46)	To protect the domestic rubber products manufacturing sector from external competition	It laid down the foundation for a protective policy regime and sustained inward oriented growth of the industry
Introduction of the bill on the Rubber (Production and Marketing) Act 1947 in the Legislative Assembly of the Government of India on 4th November, 1946, by Dr. John Mathai (Member in Charge of Industries and Supplies in Viceroy's Executive Council)	To set up a statutory organization to look after the interest of rubber growers in India consequent to the expiry of Rubber Control and Production Order (1942) on 30/09/1946	The bill set out a comprehensive agenda for the development of the rubber sector in the country. The Act came into force from 19th April, 1947

community provided the platform for sustained growth impulses from the very beginning. The regulatory regimes on the production and distribution of planting materials ensured the maximum utilization of available resources and accumulated wisdom. The statutory provisions related to rubber planting and planting materials legitimized through the Rubber Act had been the backbone of the interventionist approaches and the desired outcomes. Table Eco. 1 summarises the major policy initiatives during pre-independent phase.

Another important feature of the propagation policy has been the strategies adopted for the dissemination of scientific knowledge through publications and open discussion forums. In effect, India achieved not only the highest adoption of modern high yielding planting materials but also the highest reported national productivity among the major NR producing countries. However, since 1961-62 onwards controls on the propagation segment had been gradually diluted culminating to the full decontrol in 1986. This policy shift ushered in a new era of rubber propagation system in the country dominated by the private nurseries.

2. Tapping labour shortage in the rubber smallholder sector in Kerala

The study highlighted the pivotal role of crop harvesting operations in the perennial crop dominated agricultural sector of Kerala with its region-specific features segmenting the crop harvesting labour market. Among the crop harvesting labour engaged in the perennial crop sector of Kerala, the tappers employed in the rubber smallholder sector are unique for the organizational arrangements, socio-economic background, gender and demographic dimensions as well as the piece rate based wage payment system. The tapping labour shortage is primarily perceived as a supply side issue in relation to the popular alternate daily tapping system with its organizational arrangements rooted in the single grower dependence. Accordingly, the estimated annual tapping labour requirement of Kerala's rubber smallholder sector is more than 48 million man days during the year 2011. Technically, the labour shortage is affixed to a prefixed system of tapping without exploring the underlying factors sustaining the alternate daily tapping system and a highly segmented nature of the tapping labour market. The demographic, gender and social dimensions of the labour are characterized by a highly skewed distribution of hired male labour in the higher age-group with minimal participation of female and family labour. The cumulative impact of growth in area under the crop, sub-division and fragmentation of the holdings, the growing uncertainty of NR prices and employment reducing strategies in the context of price uncertainty led to a decline in the wage share from 24.18% during the pre-reforms phase to 18.11% in the post-reforms phase. The estimated annual average income of the tapper was also lower than wages of labourers in alternative sources of employment (Table Eco. 2).

The uncertainty enveloping the NR market during the post-reforms phase signaled an overhaul of the erstwhile arrangements in the labour market. The four resultant changes are: (i) prominence attained by multiple grower dependence; (ii) decoupling of tapping and sheet processing operations; (iii) weakening linkages of credit and labour markets; and (iv) declining wage share despite a steady growth in the wage

rates. The subsequent policy initiatives to attract migrant labour from N-E states and the responses of both growers and tappers are yet to provide clear signals for a consensual breakthrough in the labour market conundrum.

A two-pronged strategy of institutional interventions is suggested in the backdrop of the limited scope of the existing schemes to retain the labour as well as to attract new sources of labour. The two components of the interventions consist of institutional interventions to popularise LFTS through the RPS network by encouraging group processing and labour pool mechanism to speed up the shift towards multiple grower dependence. The adoption of LFTS has to be made mandatory for availing the production linked subsidies of the Rubber Board. It is also necessary to make concerted attempts to mobilize female labour through the SHGs. In order to improve the wage income and to prevent erosion in wage share flexible schemes of production incentive and annual compensatory allowance are suggested. The benefits from these two schemes are closely related to trends in yield and the prices. The proposals have to be subjected to critical debate at various stages before refinement and final implementation.

3. Trends in wage share in the context of labour shortage: The case of tapping wage share in rubber smallholdings sector in Kerala

The study was undertaken to analyse the trends in wages and wage share during pre and post-reforms phases, to evaluate the growth rate and instability of wage share; and to understand the comparative influence of farm income and wages on wage share. The database consists of documented field level time series data on wages of smallholdings from primary sources and published official data on productivity and price during the 30 year period from 1980-81 to 2009-10. The study period is divided into two phases *viz.* pre (1980-81 to 1990-91) and post- reforms (1991-92 to 2009-10) phases in order to assess the comparative influence of economic reforms on the selected variables. The trends in the rate of tapping wages in rubber smallholdings sector were considered for the analysis of wages. The tapping wage share is defined as the share of annual tapping wages in annual farm income of the smallholdings. In order to remove the inherent ambiguities of prices and wages and to contain the effect of inflation, the real values of price and wages

Table Eco. 2. Estimated annual average wage income of different categories of labourers in Kerala (2011)

Category of labour	Estimated number of average working days	Average wage rate (Rs day^{-1})	Estimated average annual wage income (Rs.)
Tappers (Smallholdings)	129 [a]	100 [b]	52,374
Tappers (Estates)	300 [c]	180 [d]	54,024
Agricultural labour	230	300	69,000
Helpers in the construction sector	230	350	80,500

(a) The estimated average number of tapping days under the alternate daily tapping system with raingarding is 129 days annum^{-1}. The average number of trees tapped by a tapper in the smallholder sector is estimated to be 406.

(b) The average wage rate for tapping 100 trees during the year.

(c) The permanent tappers in the estate sector normally get 300 tapping days.

(d) Estate wage rate includes only basic wage plus dearness allowance and excludes the fringe benefits.

were estimated by using appropriate deflators.

The long-term trends in the selected variables were analysed by estimating the growth rate by fitting exponential trend of the type

$Y=ab^t$ (Gulati, *et al.*, 1994)

The stability of the different variables was measured by using Instability Index (Cuddy-Della Valle index) which is used as a suitable measure of variability in time series data characterised by long-term trends. The degree of relationship between farm income, wages and wage share was estimated by employing Karl Pearson's Coefficient of Correlation.

The results of the analysis showed that the average tapping wage share was 19.6%. The tapping wage share was comparatively higher during the pre-reforms phase (24.2%) compared to the post-reforms phase (18.1%). The trends in comparative growth rates and instability indices of real values of the selected variables over the two phases are given in Table Eco. 3. During the pre-reforms phase except the price (-2.0%) and wage share (-0.5%), other variables showed positive growth rates. But during the post-reforms phase while price recorded a positive growth rate (2.3 %), wage share grew at a negative growth rate of (–) 0.2% even with a comparatively higher growth rate in wages (4.7%).

During the 30 year period also the growth rate of wage share showed a negative trend (–1.3%) despite the positive growth rate in real wage (2.3%). The instability indices estimated for different variables showed that during the pre-reforms phase wage share (9.0) was the most unstable variable. During post-reforms phase though wages were comparatively stable (7.5) wage share was highly unstable (30.9). The results of the correlation analysis showed a negative and significant coefficient of correlation (-0.7) between farm income and wage share during the 30 year period and post-reforms phase. The results of the study revealed that the tapping wage share in Kerala's rubber smallholding sector not only lagged behind the significant strides made in the real values of farm income and wages but also grew at a negative growth rate during the 30 year period.

4. Trends in the adoption of planting materials under the smallholder sector in traditional rubber growing regions in India

The study analysed the trends in the adoption of planting materials and their density across different size-classes and regions. It is based on the information regarding area under New Planting/ Replanting, type of clones planted, year of planting, density (stand ha^{-1}), type of

Table Eco. 3. **Comparative growth rates and instability indices 1980-81 to 2009-10**

Variables	Growth rate (%)			Instability index		
	Pre-reforms phase	Post – reforms phase	Total period	Pre- reforms phase	Post – reforms phase	Total period
Price	(-) 2.0	2.3	0.1	6.0	27.3	23.9
Productivity	3.2	2.7	3.5	2.0	4.8	5.8
Farm income	1.1	5.0	3.6	6.7	28.7	27.0
Wages	0.6	4.7	2.3	8.0	7.5	16.1
Wage share	(-) 0.6	(-) 0.2	(-) 1.3	9.0	30.9	23.6

intercrop planted etc., gathered from the Rubber Plantation Development (RPD) scheme files available from 26 Regional Offices of the Rubber Board located in the traditional rubber growing regions. The database of the study consisted of 130658 permits covering 59742 ha for the seven year period from 2004 to 2010.

The average holding size of permit area (both new planting and replanting) in the traditional rubber growing regions is 0.5 ha during the year 2010. It is three percentage points higher than the size in 2004. The lowest average permit size was witnessed in southern region (0.4 ha). Among the regions, the north-central region has the highest permit size (0.6 ha). The average sizes in Tamil Nadu, central, and northern regions are 0.4, 0.5, and 0.5 ha respectively. After two decades of promotional campaign the small growers are showing positive response to multi-clonal planting, especially since the official release of RRII 400 series clones in 2005 for commercial cultivation. As a result, the area under the popular high yielding clone of RRII 105 had declined drastically. Trends in the adoption planting material are evident from Table Eco. 4.

Adoption of multi-clones has increased substantially in 2010 as compared to 2004 in all the regions of the traditional belt except Tamil Nadu. The monoclonal status of RRII 105 was clearly challenged by increased adoption of multi-clones and new high yielding RRII 400 series clones.

While RRII 430 is more popular in central region, RRII 414 is the popular clone in all other regions. These clones are more popular in central region and relatively less in north central region. Multi-clonal planting is positively correlated to size of holdings. All the size classes reveal uniform preferences to new clones across the regions.

In the case of density, only 19% of the total area under the smallholders is planted

Table Eco. 4. **Clone-wise adoption across regions (% share)**

Clone	Tamil Nadu		South Kerala		Central Kerala		North Central Kerala		North Kerala		Traditional region average	
	2004	2010	2004	2010	2004	2010	2004	2010	2004	2010	2004	2010
RRII 105	21.3	53.2	96.7	51.0	91.3	41.3	99.4	77.4	98.4	65.1	95.1	55.7
RRII 414	0.0	12.5	0.1	28.1	0.1	19.6	0.0	6.6	0.0	12.9	0.1	17.1
RRII 417	0.0	0.0	0.0	0.7	1.5	1.8	0.1	0.5	1.1	0.4	0.8	0.9
RRII 422	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1
RRII 430	0.0	5.7	0.1	4.3	0.0	21.1	0.4	3.3	0.2	4.5	0.2	10.0
Multiclone	40.8	10.2	2.6	15.5	5.8	15.5	0.0	11.4	0.0	16.8	2.6	15.3
Others	37.9	18.4	0.5	0.4	1.3	0.6	0.1	0.8	0.3	0.3	1.3	0.9
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total Area(ha)	154.3	133.6	1398.1	944.4	1796.9	1763.4	1070.4	672.5	3337.1	1679.2	7756.8	5193.1

Note: Others include PB 217, PB 235, PB 260, GT 1, RRIM 600 and RRIV which are relatively popular in Tamil Nadu region.

Table Eco. 5. **Percentage share of density across density-classes and regions during 2010**

Density class (plants ha^{-1})	South Kerala	Central Kerala	North Central Kerala	North Kerala	Kanyakumari	Traditional region average
< 420	0.1	0.2	0.6	0.1	0.0	0.2
420-500	5.0	26.7	18.3	20.1	5.2	19.0
500-600	46.1	62.1	65.6	67.8	49.5	61.2
>600	48.9	11.1	15.6	11.9	45.3	19.7

as per the recommendation of the Rubber Board. Table Eco. 5 provides the density pattern across density-size-classes and regions.

It is evident that a density of more than 500 plants per hectare is adopted in more than 80 per cent of the area under study. Among the five regions, South Kerala registered the least adoption of the recommended density (5.0%). In the region, 48.9% of the area had a density of more than 600 plants per hectare. The density class of 500-600 plant per hectare was the choicest category among the smallholdings.

5. Availability of and access to infrastructural facilities to the rubber small growers in Tripura

The data on infrastructures from 161 households under nine block planting units (BPUs) and two group processing units (GPUs) were collected. Village level data on infrastructure were collected from the respective village offices of all the 11 units. Broad sector of infrastructures considered for the study are (i) transport facilities (ii) electricity (iii) drinking water (iv) educational facility (v) communication facility (vi) medical facility, and (vii) credit facility. The analysis followed the methodology adopted by the Centre for Monitoring of Indian Economy (CMIE) and Department of North Eastern Region (DONER) for infrastructure ranking of different districts of India (CMIE, 2000; DONER, 2009).

District-wise infrastructure indexing of Tripura by (DONER) showed that among the four districts, West Tripura performed better than North Tripura, South Tripura and Dhalai districts.

The index for villages covered under the study revealed that Barabhaiya (South Tripura), Dakshin Rani (South Tripura), Madhapur North (West Tripura) and Kamalasagar (West Tripura) are the toppers. However, infrastructure index of all the villages are much below the district-wise infrastructure index of DONER. This wide difference is indicative of the gaps in intra-regional availability of infrastructural facilities and the persistent backward status of rubber growing regions under the BPUs and GPUs. Table Eco. 6 provides the village wise index of infrastructures.

Table Eco. 6. Village-wise infrastructure ranking

Village	Score
P. Radhamohanpur	24
Amtali	37
Bhagwan Ch. P	39
Bagma	44
Laxmandepha	60
Devipur	66
Kaliram	70
Sipai Para	77
Madhapur S	77
Kamalasagar	78
Madhapur N	100
Dakshin Rani	103
Barabhaiya	116

The BPU/GPU-wise index showed that among the nine BPUs and two GPUs, Rajarshi GPU (South Tripura) was the topper followed by Dariabagma BPU (South Tripura), PS Para BPU (West Tripura) and Khamber Bari BPU (West Tripura) (Table Eco. 7). Though normally beneficiaries of a BPU/GPU is falling under the jurisdiction of one village office, beneficiaries of Khamber Bari BPU and PS Para BPU are from two villages each. An important

Table Eco. 7. **BPU/GPU-wise infrastructure ranking**

BPU/GPU	Score
Rambabu Para BPU	53
Kariyamura ii BPU	77
Laxmandepha BPU	78
Janmabhumi GPU	87
Rani BPU	88
RS Para BPU	94
Kamalasagar BPU	94
PS Para BPU	95
Khamberbari BPU	95
Dariabagma BPU	98
Rajarshi GPU	108

observation is the better infrastructure accessibility index of BPU/GPUs than the infrastructure availability index of the villages (Table Eco. 6 & 7), except Rajarshi GPU (Bharabhaiya Village) and Rani BPU (Dakshin Rani Village). Implicitly, it underlines the privileged status of the BPU/GPU households within the individual villages.

Table Eco. 8 shows the indicator-wise descriptive statistics of infrastructure availability in the villages and the BPU/GPU households. The table underlines the privileged status of BPU/GPU households with marked variations among the ten indicators selected.

Compared to the district infrastructure index of DONER performance of the villages and the BPUs/GPUs are poor. At the village level, rubber farmers in BPUs/GPUs have better accessibility in infrastructures except Rajarshi GPU and Rani BPU, irrespective of the location. However, the results highlight the need for proactive institutional interventions to build up necessary infrastructures to capitalize the potential linkage effects from a regional perspective.

Table Eco. 8. **Descriptive statistics on Village and BPU/GPU infrastructures**

Indicators	Village mean	Indicators	BPU/GPU mean	Correlation
Surfaced road as % of total road length	17.3	Surfaced road as % of total road length	80.4	0.63*
Percentage of villages electrified	66.9	Percentage of villages electrified	82.0	0.82**
Percentage of households having safe drinking water	54.4	Percentage of households having safe drinking water	89.4	NS
Schools (nos)	3.0	Schools (nos)	3.6	0.98**
Informal educational facility(nos)	1.8	Informal educational facility (nos)	2.0	0.98**
No. of doctors available in the locality	3.2	Doctor availabilty (%)	91.3	NS
Telephone (Land)%	3.9	Telephone (Land)%	5.0	0.83**
Telephone (Mobile) %	93.9	Telephone (Mobile) %	142.2	NS
Bank (nos)	0.2	Bank usage (%)	72.1	—
Non-Bank (nos)	10.3	Non Bank (nos)	2.2	—

**Correlation is significant at 0.01 level; **Correlation is significant at 0.05 level; NS - Not Significant*

LATEX HARVEST TECHNOLOGY DIVISION

The Division continued research and advisory services on various aspects of Latex Harvest Technology. The collaborative programme on popularising Low Frequency, d3, tapping among smallholdings initiated under all the Regional Offices of the Rubber Board during 2009-10 and 10-11, continued successfully. The new comprehensive trial on Low Frequency Tapping (LFT) initiated during November 2010 at Koney estate (in 8 ha. area) to demonstrate performance of d2 to d7 frequency was also continued. The Division initiated pilot studies on non-conventional harvesting methods such as vertical and micro-X tapping. The CUT demonstration trials initiated in various regional stations continued successfully in the third year also.

1. Low frequency tapping

Since Low Frequency Tapping is the need of the hour and the major thrust area, the Division continued various experiments, onfarm trials and advisory trials on LFT under different agro-climatic conditions. The comprehensive study initiated during November 2010 at Koney Estate of Harrisons Malayalam Limited to understand the performance of clone RRII 105 under d2 to d7 frequency coupled with various harvesting practices continued successfully. The programme also covered tolerance assessment of the lactiferous system under different tapping frequencies and stimulation based on biochemical indicators. Since, there was theft of field coagulam and late drip of latex during June and July, data analysis was carried out excluding these months. Under d7 frequency, it was essential to give fortnightly yield stimulation with 2.5% ethephon. Yield under different frequencies were comparable for the period from November 2010 to March, 2012. Lowest incidence of Tapping Panel Dryness was observed under d7 frequency with highest rounds of yield stimulation *i.e.* 24/y (Table LHT. 1). The biochemical studies also indicated healthy status of the trees.

Table LHT. 1. **Dry Rubber Yield (kg 400 trees^{-1}) and other attributes under d2 to d7 frequencies of tapping (November 2010-March 2012)**

Tapping system	Tapping days$^{\$}$	Yield	TPD %
S/2 (RG) d2 6d/7	189	2386	2.9
S/2 (RG) d3 6d/7	128	1983	3.2
S/2 (RG) d3 7d/7 ET 2/y*	148	2330	3.1
S/2 (RG) d3 6d/7 ET 3/y*	129	2248	2.3
S/2 (RG) d4 7d/7 ET 4/y*	111	2290	1.4
S/2 (RG) d4 6d/7 ET 6/y*	97	2054	1.9
S/2 (RG) d6 7d/7 ET 20/y*	74	2198	2.7
S/2 (RG) d7 6d/7 ET 24/y (2w)	65	2170	0.3

The participatory LFT programme with Rehabilitation Plantations Ltd, Punalur continued to give very good and comparable yield under d3 and d4 frequencies in the 5th year also with comparable incidence of TPD (6-7%). Dry rubber yield of 400 trees under d3 and d4 frequency at Kulathupuzha estate was 2052 kg and 2136 kg, whereas it was 2432 kg and 2568 kg at Ayiranallur estate. In another trial on LFT d3 at Mannarkkad, average yield of 400 trees during 2006-07 to 2011-12 was 2308 kg.

Experiment on Low Frequency Tapping (LFT) with various levels of yield stimulation in clone RRII 105 was continued at the Experimental Farm Unit (EFU) of Rubber Research Institute of India at Pampady. Yield under d2 frequency tapping was comparable

to d3, d4 and d6 frequencies of tapping. Under weekly tapping, tapping panel is closer to bud union. Renewed panel yield under d2, d3 and d4 frequencies of tapping also comparable to that of d6 frequency of tapping (Table LHT. 2).

The demonstration plot under weekly tapping with monthly stimulation at Central Experiment Station (CES), Chethackkal continued to give promising yield. With nine rounds of yield stimulation and 36 tapping days, the cumulative yield was 5.5 kg tree^{-1} and 59.6 kg (mean per tap yield) during July 2011 – March 2012. Incidence of tapping panel dryness was 5.6% and mean dry rubber content was 37.8%.

In the exploratory trial on LFT (d10) in clone RRII 105, high yield of 2000 kg 400 trees^{-1} in nine months (July 2011 – March 2012), was obtained during 1st year of BI-1 panel. Compared to previous year, yield was very high due to the panel change. TPD percentage is very low (1.9%) and annual average drc was 39.8%.

2. Collaborative programme of RRII and Regional Offices of Rubber Board in popularizing LFT d3 tapping with stimulation

The programme initiated during 2009-10 (phase I) and extended in 2010-11, continued successfully. Very good yield was obtained by the participants and there was no report of increase in TPD due to LFT and stimulation. Majority of the participants are utilising hired labour and follow d3 frequency without weekly one day regular rest, leading to higher number of tapping days per year. Hence stimulation rounds per year for them were modified, *i.e.*, reduced from 3 to 2 rounds per year. During the annual review meetings with growers, they were made aware of the pracices to be followed under LFT.

3. Controlled Upward Tapping

The survey conducted by Economics division indicated an alarming (above 40%)

Table LHT. 2. **Yield performance of LFT with stimulation in clone RRII 105**

Tapping system	Yield** (kg ha^{-1})	Tapping days	Panel
S/2 (RG) d2 6d/7 (control)	1193 b	147	BI-2(3)
S/2 (RG) d3 6d/7. ET2.5% Pa1 (1.5) 3/y*	1594 ab	103	BI-1(5)
S/2 (RG) d3 6d/7. ET2.5% Pa1 (1.5) 4/y*	1622 ab	102	BI-1(5)
S/2 (RG) d3 6d/7. ET2.5% Pa1 (1.5) 5/y*	1856 a	103	BI-1(5)
S/2 (RG) d4 6d/7. ET2.5% Pa1 (1.5) 5/y*	1881 a	76	BI-1(3)
S/2 (RG) d4 6d/7. ET2.5% Pa1 (1.5) 7/y*	1497 ab	78	BI-1(3)
S/2 (RG) d4 6d/7. ET2.5% Pa1 (1.5) 9/y*	1608 ab	76	BI-1(3)
S/2 (RG) d6 6d/7. ET2.5% Pa1 (1.5) 10/y*	1415 ab	52	BO-2(8)
S/2 (RG) d6 6d/7. ET2.5% Pa1 (1.5) 12/y	1441 ab	51	BO-2(8)
S/2 (RG) d6 6d/7. ET2.5% Pa1 (1.5) 16/y*	1140 b	52	BO-2(8)
LSD (0.05)	483.0	-	-

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

share of old and senile plantation in our country with low productivity. The past many years experience in traditional region and from the all India coordinated project on Controlled Upward Tapping (CUT), it is clear that CUT is a sure shot to enhance production and productivity of NR from old and senile trees.

4. Low Frequency Controlled Upward Tapping (LFCUT)

There are many plantations who have adopted various LFT systems during the past several years. Once these plantations are due for renewed panel tapping, introduction of Controlled Upward Tapping without changing the existing frequency is essential. Keeping this in view, the division is conducting various LFCUT experiments for the past several years to identify stimulation schedule for LFCUT.

Low Frequency Controlled Upward Tapping (LFCUT) with different harvesting practices was continued at Experimental Farm Unit (EFU). During the seventh year, yield under annual periodic panel change under d3 frequency and biennial panel change of high panel under d4 frequency was comparable. Yield of basal panel tapping under d6 frequency also was comparable to periodic panel change of d3, d4 and d6 frequencies of tapping (Table LHT. 3).

Another LFCUT trial with rainguarding was laid out at CES, in clone RRII 105 during 2004. The trees were planted during 1975. The statistical design was completely randomized single tree single plot consisting of 28 trees in each treatment. There were eight treatments comprising S/4 and S/3 spiral cuts under d3, d4 and d6 frequencies of tapping in combination with stimulation. In the first year of tapping, treatments T2,

Table LHT. 3. **Performances of low frequency tapping with rain guard under different harvesting practices in clone RRII 105**

Tapping system	Yield* (kg 400 trees^{-1})	No. of taps
S/2 d3 ET2.5% 2/y; S/4U d3 ET5.0% 6/y	3010 a	93
S/2 d4 ET2.5% 4/y; S/4U d4 ET5.0% 9/y	2088 c	71
S/4U d4 ET5.0% (3W)	2184 bc	71
S/4U d4 ET5.0% 18/y with annual panel change	2512 abc	73
S/4U d4 ET5.0% 18/y with biannual panel change	2941 a	73
S/2 d6 ET2.5% 6/y; S/4U d6 ET5.0% 12/y (2w)	1958 c	49
S/4U d6 ET5.0% (2W)	2741 ab	51
S/4U d6 ET5.0% 24/y (2w) - with annual panel change	2224 bc	51
S/4U d6 ET5.0% 24/y (2w)- with biannual panel change	2712 ab	51
S/2 d6 ET2.5% 6/y; S/3U d6 ET5.0% 9/y (3w)	2062 c	51
S/3U d6 ET5.0% 18/y (3w)	2452 abc	51
S/2 d6 ET2.5% 18/y (3w)	2482 abc	51
S/2 d6 ET2.5% 12/y (m)	2168 bc	51
LSD (0.05)	520	

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

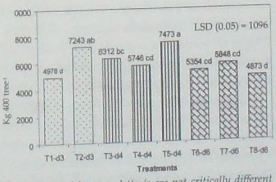
----------(kg 400 trees1)

Table LHT. 4. **Treatment details of LFCUT with rainguarding in clone RRII 105**

Treatment details	
T1 – S/4U d3 ET 5% 10/y (5 years);	S/2 d3 ET 2.5% 3/y (2 years)
T2 – S/4U d3 ET 5% 12/y (4 years) ;	S/2 d3 ET 2.5% 3/y (3 years)
T3 – S/4U d4 ET 5% 18/y (5 years) ;	S/2 d4 ET 2.5% 6/y (2 years)
T4 – S/4U d4 ET 5% 24/y (5 years) ;	S/2 d4 ET 2.5% 6/y (2 years)
T5 – S/4U d4 ET 5% 24/y (4 years) ;	S/2 d4 ET 2.5% 6/y (3 years)
T6 – S/3U d6 ET 5% 18/y (5 years) ;	S/2 d6 ET 2.5% 12/y (2 years)
T7 – S/3U d6 ET 5% 24/y (4 years) ;	S/2 d6 ET 2.5% 12/y (3 years)
T8 – S/3U d6 ET 5% 24/y (5 years) ;	S/2 d4 ET 2.5% 12/y (2 years)

T5 and T7 were rainguarded with tapping shade and others with polythene skirt. From third year onwards all the treatments were rainguarded with polythene skirt. Difficulty in lifting of wide polythene (90 cm) during tapping was overcome by placing cup hanger on the right side of the panel and supporting the rainguard on it.

During the first year, trees in all the treatments were tapped on the high panel, thereafter panel position and stimulation frequency varied according to treatments. Treatment details were given in Table LHT. 4. Trees were tapped with one day (Sunday) rest. All the data collected from 2004 to 2011 was compiled and statistically analysed.



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

Fig. LHT. 1. **Mean yield (kg 400 trees^{-1}) of LFCUT with rain guard in clone RRII 105**

Mean yield of seven years tapping were given in Fig. LHT. 1. S/4 d3 (T2) and S/4 d4 (T5) showed significantly higher yield than other treatments. Performance of CUT under annual panel change policy is better than biennial.

Among the weekly frequencies (T6, T7 & T8), S/3 d6 with annual panel change (T7) recorded highest yield. S/4 spiral cut under lower frequencies of tapping with appropriate stimulation is ideal for obtaining sustainable yield for more years. Tapping Panel Dryness (TPD) affected trees in the basal panel also gave good yield in the high panel.

In another experiment in clone RRII 118 planted during 1980 (the basal panel was not suitable for harvesting), continuous CUT for all the 12 months was adopted. There were five treatments comprising S/4 and S/3 spiral cuts under d3, d4 and d6 frequencies of tapping with varying levels of stimulation. During 2005-2010, stimulation frequency was once in 6 weeks under S/4 d3 frequency of tapping. During 2010-11, stimulation frequency was changed to once in three weeks under S/4 d4 and S/3 d4 stimulation frequency was once in 3 weeks and 6 weeks respectively. Under weekly tapping (S/3 d6) stimulation frequency was once in three weeks and monthly respectively. All the trees

Table LHT. 5. **Yield (kg 400 trees^{-1}) response of LFCUT with rain guard in clone RRII 118**

Tapping system	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	Mean *
S/4U d3 6d/7	4342 b	5028 a	4353 b	2285 b	2308 b	2480 bc	3466
S/4U d4 6d/7	4558 b	5122 a	5960 a	3506 a	3113 a	2605 bc	4144
S/3U d4 6d/7	4888 b	5733 a	6111 a	3394 a	2633 ab	2395 c	4192
S/3U d6 6d/7	6054 a	5162 a	6547 a	3626 a	2579 ab	3565 a	4589
S/3U d6 6d/7	4429 b	4023 b	5304 ab	2810 ab	2411 b	3139 ab	3686

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

* *NS*

were rainguarded with 90 cm polythene (skirt type). Ethepon 5% was applied on the lace (d3 & d4 frequency of tapping) and groove method for d6. Data collected from 2005 to 2011 was analyzed statistically and presented in Table LHT. 5. Under d6 frequency of tapping, HO-2 panel was tapped continuously for three years. Mean yield of six years under S/4 d3 frequency of tapping was comparable to S/4 d4, S/3 d4 and S/3 d6 frequencies of tapping. High panel tapping under CUT gives good yield even from trees of unsuitable basal panel. Performance of clone RRII 118 on high panel (CUT) is very good.

5. Other Experiments

5.1. Evaluation of mini and reduced spiral cut tapping

In the experiment at EFU, RIT, S/4 d3 continued to give good yield in the 13th year also. One farmer near Piravom had initiated S/4 d3 with yield stimulation on 1260 trees from January 2011. All the trees of 45cm and above were opened. Yield stimulation was with ethephon @2.5% concentration applied on panel at 45 days interval (excluding February, March & April). Dry rubber yield of first year was 3.41 kg tree^{-1} year^{-1} and has reported that there is reduction in time required for tapping. A commercial trial of reduced spiral tapping is proposed to start at B C Cheruvally estate during 2012-13.

In another location at Calicut, twenty trees of 46cm girth were divided into two groups. One set was tapped and the other was left untapped. At the end of one year yield of more than 2kg tree^{-1} was obtained. The girth of S/4 tapped trees was 53.7cm and untapped set was 54 cm, the difference was only 3mm.

5.2. Long term evaluation of rainguard: problem of production losses in the absence of rain guard and recovery through stimulation

Significant yield variation was observed among the treatments during 2011-12. Highest yield was observed under d2 frequency of tapping which was at par with d3 frequency of tapping with rain guarding. Though non rain-guarded trees in general showed higher per tap yield, annual yield was low. Crop loss could be partially compensated by higher levels of yield stimulation in non-rainguarded trees (Table LHT. 6). Total rainfall was 2955 mm and there were 131 rainy days during the year 2011-12. Tapping days were less during the year and has affected the

Table LHT. 6. **Effect of stimulation on recovery of yield loss under d2 and d3 frequencies of tapping in clone RRII 105 without rainguarding at EFU, RIT**

Treatments	Yield				DRC (%)	Tapping days
	kg 400 trees^{-1}	kg tap^{-1} 400 trees^{-1}	kg tree^{-1}	g t^{-1} t^{-1}		
S/2(RG) d2 6d/7	2488 ab	18.4	5.5	45.9	38.8 a	136
S/2 d2 6d/7	2615 ab	26.5	6.1	66.2	39.0 a	99
S/2 d2 6d/7 ET2.5% Pa. 3/y	2779 a	25.5	5.8	63.7	37.4 b	99
S/2 d2 6d/7 ET2.5% Pa. 5/y	2213 ab	22.7	5.2	56.8	37.5 b	97
S/2(RG) d3 6d/7 ET2.5% Pa.3/y	2058 ab	23.9	4.7	59.7	35.8 c	87
S/2 d3 6d/7 ET 2.5% Pa (3/y)	1471 ab	25.3	3.3	63.4	37.4 b	57
S/2 d3 6d/7 ET 2.5% Pa (5/y)	1386 b	23.6	3.3	59.0	37.4 b	59
S/2 d3 6d/7 ET 2.5% Pa (7/y)	1609 ab	27.4	3.7	68.5	36.5 c	59
LSD (0.05)	1362	NS	NS	NS	0.8978	-

crop performance particularly under d3 frequency of tapping.

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

In the experiment to evaluate "Mortex" in comparison with ethephon, stimulation treatments as per the schedule were imposed and data on dry rubber yield and other parameters were compiled. No significant difference on yield or DRC % could be observed between various treatments during the year 2011-12. With higher levels of Mortex application higher yield could not be obtained. At Experimental farm Unit, RIT also no significant difference in yield could be observed between ethephon (3/Y) and "Mortex" (6/Y).

5.4. Evaluation of non- conventional tapping methods : Evaluation of vertical tapping

In a preliminary observation on vertical tapping cut (22 cm) with stimulation on renewed panel (BI-1) indicated an average yield of 30 g $t^{-1}t^{-1}$ as against 34 g $t^{-1}t^{-1}$ in normal downward tapped trees (S2) under d3 frequency of tapping.

GENOME ANALYSIS LABORATORY

The research interests in the Genome Analysis laboratory encompass the following three areas: (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 molecular marker technologies have been adopted and

successfully used in the characterization of rubber genome during last year.

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

1.1.1. Marker development through GenBank data mining

Mining of rubber EST sequences for simple sequence repeats (SSR) resulted in identification of 31 novel repeat motifs (di, tri, tetra & penta) from 1556 EST sequences submitted in public domain during 02/05/2007 to 27/09/2011 and 29 SSR primers were designed for marker development.

Recently available next generation transcriptome sequencing data set (NCBI database - Acc. No. GSE26514) was analysed for large-scale SSR mining. The repeat number threshold was designated as more than five for dinucleotide, four for trinucleotide and three for tetranucleotide repeat motifs. Consequently, 698 dinucleotide, 867 trinucleotide and 72 tetranucleotide repeat sequences were identified from 48,768 unigenes. Flanking primers were designed for synthesis to develop SSR markers.

1.1.2. Genotyping of wild Hevea accessions

Genotyping of 60 wild accessions of *Hevea brasiliensis* and five other wild *Hevea species viz., H. benthamiana, H. spruceana, H. pauciflora, H. camargoana* and *H. nitida* was performed with 10 microsatellite markers generated in the lab. All these microsatellite primer-pairs showed cross-species amplification with five *Hevea* species, indicating their transferability. Several new alleles, mostly in homozygous state were observed in wild species.

1.2. Single nucleotide polymorphisms (SNPs) and haplotype structuring in the latex biosynthesis genes of *Hevea brasiliensis*

1.2.1. SNPs in farnesyl diphosphate synthase (FDPS) gene

SNP identification in entire 4.9 Kb *FDPS* genomic region of the clones RRII 105, RRII 118, RRIM 600, RRIC 52 and GT 1 was completed by sequencing the PCR product as well as the cloned fragments. In total 61 SNPs, 7 indels ranging from one to 64 bases and polymorphic CT repeats (CT8, CT9 and CT13) were identified among these five *Hevea* clones. Most of the sequence variations were identified with RRII 118. There was no base substitution in the 5′ 1379 bp of *FDPS* gene in RRII 105, RRIM 600, RRIC 52 and GT 1. But with the clone RRII 118, two distinct alleles were observed based on two major insertions and deletions in the first intron along with 35 base substitutions. From rest of the genomic region, a total of 25 SNPs were identified. RRII 105 was found to be completely homozygous for this region. RRIM 600 showed heterozygosity at 3 positions, whereas RRII 118, RRIC 52 and GT 1 were found to be highly heterozygous. Nonsynonymous SNPs were not detected in the entire gene sequence. Seven haplotypes were identified in this region of RRII 105, RRII 118, RRIM 600, RRIC 52 and GT 1 using the software DnaSPv.5.

The software also detected one recombination spot between the sites 1380 and 1585 (between the 1st and 2nd SNP in the haplotypes given above). The remaining 24 SNPs formed a single haplotype block.

Indels identified in *FDPS* were converted to STS marker and assessed in 40 popular clones. Four alleles were identified from the 40 clones.

Out of the 40 clones, 30 were homozygous. Homozygosity for allele-1 and allele-3 couldn't be observed among these 40 clones.

Sixty wild accessions (24 Acre, 18 Mato grosso and 18 Rondonia) and five *Hevea* species (*H. benthamiana, H. spruceana, H. pauciflora, H. nitida and H. camargoana*) were also genotyped using the same STS marker. Apart from the four alleles discovered in popular clones, new alleles were observed in the analyzed samples. Thirty-three accessions were found homozygous including five *Hevea* species while the rest showed heterozygous status.

1.2.2. *SNPs in hydroxymethylglutaryl-CoA synthase (HMGS) gene*

Sequencing of the 5.3 kb genomic region of *HMGS* was continued. Sequencing of this gene from all the 5 genotypes was completed except the 1800 bp region in the 5'-end of all the clones except RRII 118. The entire genomic region of *HMGS* was cloned from RRII-118 and sequenced. Complete genomic sequence of *HMGS* has been reported for the first time in *Hevea brasiliensis*. Intron-exon junctions were predicted using specific softwares and identified 12 exons and 11 introns.

1.2.3. *Rubber elongation factor (REF) gene*

Full-length *REF* gene (1.7 kb) was successfully amplified using three sets of primers and the products are being processed for sequencing.

1.3. Genetic linkage map in rubber

In linkage map construction, marker segregation data is being continuously integrated into the mapping data to saturate the linkage map of rubber. A total of 244 markers comprising of 100 RAPD markers, 86 AFLP markers, 52 SSR markers and five SNP based and one RGA markers were finally utilized for the construction of a genetic linkage map. The markers generated were analyzed using both the linkage map construction software MapMaker/EXP 3.0 and JoinMap 3.0. The dominant and co-dominant markers following a segregation ratio of 1:1 were analyzed initially using the linkage map construction software MapMaker/EXP 3.0. Marker groups were determined by using a maximum likelihood distance of 40 and a minimum LOD score of 2.0. Linkage groups were detected for each parent separately. In *H. brasiliensis*, the number of linkage groups for a saturated linkage map should be equal to the haploid chromosome number of 18. In this study, 23 linkage groups were formed both for RRII 105 and RRII 118, which could be due to the insufficient number of markers. All the 23 linkage groups in RRII 105 showed a cumulative genetic distance of 1384.7 cM, whereas RRII 118 had 768.5 cM from 23 linkage groups. Twenty-six markers were unlinked in RRII 105 out of 115 loci analyzed and 33 markers remained unlinked out of 99 markers analyzed in RRII 118.

Segregation analysis of all the markers developed was repeated using the software JoinMap 3.0 which could handle all types of segregation data. Marker groups were determined using a minimum LOD score of 3.0 and a recombination frequency of 0.4. Segregation distortion was noticed in 4.8% of markers in RRII 105 and 4% of markers in RRII 118, having a high chi-square value, and subsequently deleted from the analysis. Twenty-four linkage groups were identified both for RRII 105 and RRII 118. The total genetic distance covered was 762 cM in RRII 105 and 634 cM in RRII 118. Sixty-one markers were unlinked in RRII 105 out of 164 loci analyzed and 62 markers remained unlinked out of 145 markers analyzed in RRII 118. The distance calculated by JoinMap was

less compared to that calculated by MapMaker due to the differences in the computational procedures.

Two latex biosynthesis genes: farnesyl diphosphate synthase (*FDPS*) and mevalonate kinase (*MK*) were mapped using a co-dominant STS marker for *FDPS* and a SNP (F197C/T) marker for *MK* respectively.

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

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

2.1.1. *Resistance gene analogue (RGA) in rubber*

A full-length *R* gene, based on a functional resistance gene analogue (*RTRGA13*) in rubber was cloned from GT 1 and characterized for the first time. Length of the *R* gene was 3284 bp and the coding sequences was 2547 bp. Conceptual translation of the coding sequences revealed a protein of 849 aminoacids, which had characteristic NB-ARC domain and leucin-rich repeat (LLR) motif present in most of the *R* gene from other species and maximum homology was found with nbs-lrr resistance protein of *Populus tricocarpa* (E-value 0.0).

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

Nucleotide sequences of differentially expressed transcripts derived from *Corynespora* challenged leaf samples were analyzed to get an idea about genes involved during disease establishment in RRII 105. Altogether 135 up-regulated transcripts/ cDNAs were cloned and sequenced. These sequences were grouped into 13 contigs comprising 30 sequences and 105 singletons. Sixteen sequences showed significant homology with the known sequences from the GenBank and the rest were unique or having match with hypothetical proteins.

An effort was made to clone full-length gene encoding anthocyanidin 3-O-glucosyltransferase from GT 1. The full-length cDNA sequence (150 bp) encoding 470 amino acids was submitted to GenBank (JQ037843) and expression analysis is in progress. However, with the *GRAS* transcription factor (spanning over approximately 2100 bp encoding 688 amino acids in *Ricinus communis*), a portion of the gene is yet to be sequenced to obtain full-length sequence information.

2.2. Characterization of stress-tolerant clones of *H. brasiliensis* using molecular markers and gene regulation under abiotic stresses: Cold tolerance in rubber

Transcript profiling in two stress tolerant *Hevea* clones: PR 261 and RRII 208 in relation to cold stress were continued. Large number of differentially expressed transcripts (~800) was generated through DD-RT PCR for characterization. We characterized 160 transcripts/ clones. Earlier characterized 110 transcripts were annotated for submission to Genbank.

Glutathione peroxidase gene involved in stress regulation, showed clear discrimination among the wild accessions based on SNP haplotypes from three different provinces in Brazil. This gene was also identified in cold responsive ESTs. Therefore, transcript abundance of the gene was assessed in different tissues including leaf, bark (both young stem and mature tree), latex and root. Expression was found more in leaf followed by root, latex and bark.

3. Rubber EST project

3.1. EST generation - through sequencing

One hundred and twenty clones from a

bark cDNA library of RRII 118 were annotated and submitted to GenBank.

To generate cold responsive ESTs, 120 clones from leaf cDNA library of PB 260 grown in Munnar, were annotated and processed for submission to GenBank.

3.2. In silico analysis of rubber EST database for gene mining

The EST database of the NCBI holds 12385 *Hevea* sequences as of 24/08/2011. ESTs contain mostly partial coding and UTR regions associated with functional genes which hold lots of useful genetic information. An attempt was made to analyze the entire 12385 sequences together to find such hidden information on *Hevea* genome. All the existing sequences were downloaded and contig analysis was performed using the SeqMan module of the DNASTAR software. A total of 1490 contigs and 2237 singletons were obtained from the analysed sequences. The contigs were arranged based on their size and the consensus sequence for each contig was used for gene annotation.

4. Methylation dynamics of *Hevea brasiliensis*

4.1. Methylation specific PCR (MS-PCR)

MS-PCR was carried out with bisulfite treated genomic DNA of three popular clones, RRII 105, RRIM 600 and PB 260 before and after inducing cold stress in growth chamber. Both methylation sensitive (M) and in-sensitive (U) primers designed for a single locus were used in PCR amplification along with a reverse primer. Two sites were screened for the presence/absence of methylation in promoter sequence of *HMGR*. Amplification result using MsHMG1-M & U primer showed that the site was mostly un-methylated, since strong amplification was obtained only with the methylation insensitive (U) primer. MS-PCR using MsHMG2-M & U primer gave better amplification and clearly distinguished the methylated and un-methylated status of the clones.

4.2. Bisulfite sequencing of gene promoters

Bisulfite sequencing of a 229 bp region of the *HMGS* gene promoter region from poly-bag plants of the clones RRII 105, RRIM 600 & PB 260 before and after induction of cold stress was carried out. A 183 bp region of the *FDPS* gene promoter was also successfully amplified cloned and sequenced. The sequences were analyzed using the software CyMATE for identifying the methylation variations among the clones as well as within the same clone before and after cold stress.

In *HMGS* promoter, only Class II (CHG) and Class III (CHH) methylation patterns were observed. Actual CHG methylation accounted for 4.17% of the maximum probable methylation sites whereas CHH type added up to 3.49%. An interesting CHH type methylation pattern was observed at position 41 where RRII 105 plants appeared to be demethylated after cold treatment whereas PB 260 plants showed reverse trend of methylation, post treatment. In RRIM 600, one plant showed methylation after treatment while other remained un-methylated without any change. Putative stress induced class III methylation was observed at CAAT box and in close vicinity to TATA box of RRII 105 plants. A clone specific methylation pattern was observed at position 150 in RRIM 600 plants.

In *FDPS* promoter region all the three classes of methylation were observed. Actual CGN methylation accounted for 20.59% of the maximum probable methylation sites whereas CHG was 0.93% and CHH 1.80%. Putative stress induced DNA de-methylation

at a CGN site at position 3 was observed in all the plants except one RRIM 600 plant. On the contrary, a reverse pattern of stress-induced methylation was observed at an ATCT-motif (light responsive element) in RRII 105. Random methylations at other sites were also observed.

5. Cloning and characterization of agronomically important genes

5.1. Cloning and characterization of lignin biosynthesis gene(s) in *Hevea*

5.1.1. *Caffeic acid O-methyltransferase (COMT) gene*

Genes encoding caffeic acid O-methyltransferase involved in lignin biosynthesis were identified in rubber. Southern analysis of the genomic DNA using a partial fragment of *COMT* as probe revealed the presence of multiple forms of the gene and consecutively two *COMT* genes, designated as *HCOMT1* and *HCOMT2* were identified. Full-length cloning of *HCOMT2* was done from the bark tissue of RRII 118 and fully characterized. Full-length *HCOMT2* cDNA was 1347 bp including UTRs. Conceptual translation of the gene comprised of 368 amino acids. The sequence was submitted to GenBank (Acc. No. JQ037840).

5.2. Cloning and characterization of stress responsive genes

5.2.1. *Functional characterization of metallothionein (MT) isoforms*

The full-length putative *Hevea* metallothionein gene (leaf isoform) obtained from the subtraction library was analyzed using real time PCR to characterize its expression patterns in different tissues (leaf, bark and root). *Hevea* actin gene was used as the endogenous control. Relative quantification results showed high expression levels of this isoform in leaf tissue compared to bark and root. Expression rate was minimal in both bark and root with little difference between the two. The results are in agreement with the identified isoform *MT-3a*, a leaf abundant form based on its high similarity to other leaf metallothionein orthologs.

In order to study the heavy metal sequestration and detoxification properties of *Hevea* metallothionein gene (*HevMT-3a* isoform), a recombinant *E. coli* strain, engineered in our lab to express the *Hev MT-3a* gene was tested for its copper tolerance property. The recombinant bacteria containing *MT-3a* gene and a control *E.coli* cell harboring only the expression vector were grown in LB broth with different concentrations of $CuSO_4$. Similar growth was observed for both control and recombinant strain up to 6 mM $CuSO_4$ concentrations. At higher concentration of $CuSO_4$ (10 mM) colony growth was observed only with the recombinant clone bearing metallothionein gene. This result is an indication of the metal tolerance property of the recombinant clone conferred by the *Hevea* metallothionein gene it harbors.

5.2.2. *Drought responsive genes*

Full-length cloning and characterization of two more stress responsive genes: Myo-inositol-1 phosphate synthase (*MIPS*) and aquaporin PIP1 (*AQP1*) from rubber was carried out. The full-length cDNA of *MIPS* was 1950 bp encoding 510 amino acids, whereas, *AQP1* was found to be comparatively smaller in size (1254 bp) encoding 287 amino acids. Both these sequences were submitted to GenBank (Acc. Nos. JQ037841 and JQ037842 respectively). Functional characterization is in progress.

CENTRAL EXPERIMENT STATION, CHETHACKAL, KERALA

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

The Station meets the needs of the scientists of various disciplines of Crop Improvement, Crop Management, Crop Protection, Crop Physiology and Latex Harvest Technology. The station had two Divisions - A and B - of almost equal area. Apart from clone trials and bud wood nursery of pipeline clones, trials on low frequency tapping, CUT, Germplasm accessions, disease management and fertilizer dosages make up bulk of the experimental areas. Specialized trials like gas based tapping (G-Flex), intercropping and immaturity reduction etc. also make part of the experimental area. A three part tree crown budded area with canopy from FX 516 is laid to study disease resistance mechanisms. An Eddy- covariance tower gives micro-environmental data.

During the reporting period, the total crop realized was 210742 kg PFL, and 66267 kg scrap and 9171 kg coagulum. A total of 221 tapping days was possible in the year and 41 tappers (per day) were engaged for tapping. The CES Dispensary attends to the medical needs of the workers and the total number of visits of patients during the period under report was 3100.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

1. Crop improvement

1.1. Large scale trial for selection of location specific clones

Fifteen promising clones of *Hevea viz.* RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, RRIM 600, RRII 203, RRII 208, SCATC 88/13, IRCA 109, IRCA 111, IRCA 130, PB 280, PB 312 and PB 314 were planted in 2010 in RBD at RRTC Hahara for evaluation of location specific high yielding clones. Vacancy filling and planting of *Pueraria* cover crop was completed.

1.2. Evaluation of potential primary clones in clonal nursery

A nursery trial of eight potential primary clones of *Hevea* obtained from

Table Ghy. 1. **Juvenile girth and yield of potential primary clones of *Hevea* in clonal nursery**

Primary clones	Mean girth after 4 years of planting (cm)	Mean of juvenile yield	
		Yield (g t^{-1} 10 taps^{-1})	Rank based on yield potential
GH 1	16.3	38.8	3
GH 3	15.6	23.2	7
GH 4	21.3	32.9	5
GH 5	18.1	25.6	5
GH 6	21.8	33.5	4
GH 8	22.6	47.7	2
GH 9	25.6	88.1	1
GH 10	12.9	13.5	8
RRIM 600	16.5	22.4	Check clone
RRII 429	16.6	32.8	Check clone
CD (P = 0.05)	NS	44.1	

Sarutari Research Farm under RRS, Guwahati was started in 2008 (Table Ghy. 1) along with check clones, RRIM 600 and RRII 429. Girth was found highest in GH 9 and least in GH 10 after four years of planting in clonal nursery. Average of juvenile yield (g t^{-1} 10 taps $^{-1}$) over two years was maximum in GH 9 (88.1 g) and least in GH 10 (13.5 g) after four years of planting. Out of eight primary clones, GH 9 showed significantly higher yield than the check clones.

1.3. Large scale trial for evaluation of potential primary clones

10 potential primary clones (*viz. GH* 1, GH 2, GH 3, GH 4, GH 5, GH 6, GH 7, GH 8, GH 9 and GH 10) of Sarutari Research Farm were planted in LST along with two control clones (RRIM 600 and SCATC 88/13).

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

Pre-winter girth of all clones at 30 cm height was recorded from the on-farm trial at Umsiang. Among the RRII 400 series clones, the girth of RRII 430 was significantly higher than the control (RRIM 600) at the end of third year of planting followed by RRII 417 and RRII 429 (Table Ghy. 2).

Table Ghy. 2. **Mean girth of RRII 400 series clones at Umsiang**

Clone	Mean girth at 3rd year of planting (cm)
RRII 417	17.2
RRII 422	16.1
RRII 429	17.0
RRII 430	18.7
SCATC 88/13	16.0
RRIM 600	15.8
CD (P = 0.05)	1.8

2. Crop management

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

Experiment for development of an Integrated Nutrient Management (INM) system for young rubber with cover crop was initiated at RRTC, Hahara in clone RRIM 600 with seven treatments, with RBD. The treatments involved combination of different doses of inorganic fertilizers with and without biofertilisers and a biofertiliser alone treatment. The biofertilisers applied were *Azotobactor, Pseudomonas, Phosphobacteria* and *AMF*. Girth of rubber plants were not significantly different among the treatments (Table Ghy. 3).

Table Ghy. 3. **Girth of plants in different treatments**

Treatment	Diameter[1] (mm)			Girth [2](cm)		
	Dec. 08	June 09	Dec. 09	June 10	Dec. 10	Jan. 12
Control	17.0	20.6	7.3	7.5	10.5	17.7
Standard practice	16.8	20.0	7.7	8.0	11.5	20.9
25%N & P + BF *	16.9	20.2	7.4	7.8	10.2	19.0
50% N&P + BF *	17.3	21.4	8.1	8.8	12.3	24.3
75% N&P +BF *	17.1	20.3	7.7	7.6	11.0	20.0
Standard practice +BF	16.3	19.9	7.7	8.2	12.0	21.0
BF alone	17.5	21.8	8.0	8.5	11.7	20.8
SE	0.8	1.0	0.3	0.4	0.5	2.7
CD(P = 0.05)	NS	NS	NS	NS	NS	8.0

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

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

The experiment for conservation of soil using vegetative bunds was initiated at RRTC, Hahara, RRS, Guwahati. The clone was RRIM 600 with 7 treatments laid out in randomized block design. The treatments were rubber with vettiver, lemon grass, palmarosa, guinea grass, rice bean, cover crop and natural cover. The quantity of eroded soil collected in trench was found to be non-significant and the study was being continued.

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

An on-farm intercropping experiment was initiated at Morugdala, Kamrup district, Assam in a field with clone RRIM 600 with a spacing of 22' x 11'. The interspaces between rubbers were occupied by different crops with a view to find the possibility of generating income during the non yielding immature phase of rubber and also satisfy the food needs of the grower.

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

A project was taken up at the RRTC, Hahara farm, RRS, Guwahati during 2011 to find the possibility of reducing the pit size to reduce soil erosion and labour cost. The survival percentage for polybag plants (88%) under normal planting was found to be higher.

2.5. Development of locally viable and adoptable root trainer devices for propagation of rubber

The project was taken up at RRTC, Hahara during the year 2011 to compare the conventional plastic root trainer containers with locally produced bamboo and earthen root trainers. The sprouting percentage was found to be higher in bamboo containers (93%).

3. Crop protection

3.1. Survey on pests and diseases of rubber

Survey on pests and diseases of rubber was carried out in 37 sites covering 29 locations in Assam, Meghalaya and northern part of West Bengal (Table Ghy. 4). Powdery mildew (PM) disease was noticed in all the locations and the severity of the disease (PDI) was in the range of 25 to 65%. Incidence of Periconia leaf blight disease was noticed on tender leaves in nursery during December/ January and was ranging between 20–65%. Brown root disease (0.5 to 8.9%) caused by *Phellinus noxius* was noticed on three/four year old rubber plant in some private plantation in Assam and Meghalaya. Minor incidence of pink disease (0.5 to 1.8%) was observed in RRII 429 block plantation at RRTC, Hahara in Assam and also in two locations of private rubber plantation at Sahipara in Assam and Rangsol in Meghalaya.

Incidence of thread blight disease (35%) was observed in budwood nursery as well as in 25-year-old rubber plantation at Umling in Meghalaya. Incidence of Colletotrichum leaf spot disease (5%) was observed in nursery at Sarutari Research Farm and RRTC, Hahara during December/January. High infestation of white grubs (60%) with drying of plants was observed on one year old rubber plants in a private plantation at Kashipur under Udalguri district of Assam for the first time in North East region. Infestation of scale insect (5 to 30%) was noticed in most of the locations during the study period.

The reaction of three short-listed wild accessions of *Hevea* germplasm RO 1737, AC 587

Table Chy. 4. **Incidence of various pests and diseases of rubber in different locations**

State/Location	Altitude	Latitude/Longitude	PM	Incidence of Pests and Diseases (%)					
	(m)		(PDI)	BR	Pink	PLB	TB	SI	WG
Assam									
Sarutari*	74	26° 03′ N; 91° 53′ E	20.0	-	-		-	30.0	-
RRTC,Hahara*	172	26° 09′ N; 92° 01′ E	20.0	0.9	0.8	20.0	-	20	-
Rangdoloi*			50.0	0.8	-	-	-	10.0	-
Longham,Bichitore	456	26° 02′ N; 92° 25′ E	20.0	8.9	-	-	-	10.0	-
Padampur	70	26° 32′ N; 90° 28′ E	50.0	0.5	-	-	-	5.0	-
Kalajhar	187	26° 51′ N; 92° 05′ E	30.0	2.3	-	-	-	-	-
Khosurabari*	154	26° 48′ N; 92° 07′ E	40.0	1.5	-	-	-	-	-
Kashipur	145	26° 50′ N; 92° 07′ E	-	-	-	-	-	10.0	60.0
Kadampara	60.0	25° 54′ N; 91° 07′ E	30.0	1.6	-	-	-	10.0	-
Sahipara	55.0	25° 54′ N; 91° 10′ E	30.0	0.6	0.5	-	-	10.0	-
Mornai	40.0	26° 05′ N; 90° 45′ E	40.0	0.7	-	-	-	-	-
Rangphar	123.0	26° 03′ N; 91° 56′ E	40.0	0.5	-	-	-	10.0	-
Hatigaon	61.0	25° 59′ N; 90° 30′ E	40.0	3.5	-	-	-	-	-
Rewamahaswar	156	26° 09′ N; 92° 03′ E	35.0	1.3	-	-	-	-	-
Boko	44	25° 58′ N; 91° 12′ E	-	-	-	65.0	-	-	-
Manupara	35	25° 59′ N; 90° 50′ E	40.0	0.7	-	70.0	-	10.0	-
Neli*	68	26° 05′ N; 92° 17′ E	50.0	-	-	30.0	-	20.0	-
Barodoba	68	25° 54′ N; 91° 07′ E	30.0	-	-	-	-	10.0	-
Meghalaya									
Bajengdoba*	129.0	25° 59′ N; 90° 30′ E	50.0	0.9	-	-	-	-	-
Rangsol*	170.0	25° 54′ N; 90° 33′ E	50.0	2.5	1.8	-	-	10.0	-
Umling*	255	25° 57′ N; 91° 49′ E	65.0	-	-	30.0	35.0	20.0	-
Umsiang*	211	26° 03′ N; 92° 09′ E	60.0	-	-	50.0	-	20.0	-
Northern part of West Bengal									
RES,Nagrakata*	194	26° 05′ N; 88° 57′ E	20.0	0.5	-	-	-	20.0	-
Jiti*	363	26° 57′ N; 88° 56′ E	32.5	-	-	-	-	10.0	-
Birubari	62	26° 26′ N; 88° 40′ E	25.0	-	-	-	-	-	-

** = Susceptible pocket to Powdery Mildew disease; PM = Powdery mildew disease; BR = Brown root; PLB = Periconia leaf blight; TB = Thread blight; SI = Scale insect and WG = White grubs.*

and AC 5302 to powdery mildew disease was studied in field condition by artificially dusting the spores at Sarutari Farm in Assam. High level of tolerance to powdery mildew disease was observed in AC 587 as compared to other two accessions (RO 1737 and AC 5302).

An experiment was started in 2011 for management of purple root disease on 6-year-old rubber plants of RRIM 600 and RRII 105 at RRTC, Hahara in Assam with three replications (plot size: 7 plants plot^{-1}). Girth was recorded in treated and untreated blocks. Treatments with tilt (5 mL L^{-1}), calixin (6.25 mL L^{-1}) and contaf (6 mL L^{-1}) were imposed in soil at the tree base of all experimental plants.

Observation on treated and untreated block showed that the root system of all affected plants is healthy. However, fruiting body of the pathogen *Helicobasidium compactum* was not developed so far in the treated block.

The rate of decomposition of four types of forest litters (*viz.* rubber, sal, teak, bamboo) was studied by litter bag method under two ecosystems, *viz.* forest and rubber plantation. The rate of weight loss of bamboo leaf litter was faster followed by rubber, teak and sal litters in both the ecosystems. Rate of decomposition was rapid under the forest system covers as compared to mature rubber plantation.

The total microbial population associated with the decomposed litters was also higher which might have contributed for the rapid rate of decomposition. Populations of fungi, bacteria and actinomycetes were high in bamboo and rubber leaf litters as compared to teak and sal litters.

In the experiment for development of an integrated nutrient management system for rubber, different doses of inorganic as well as biofertlizers were applied singly and also in combination at the rhizosphere regions of young rubber plants. The total microbial build up in the soil and also the growth of rubber was assessed. An increase in the total microbial populations was observed after three months of application of fertilizers. The number of bacterial population was more and the number of populations of Pseudomonas, Azotobacter, actinomycetes and PSM were on par with control (Table Ghy. 5).

To study the dynamics of microbial status of rubber, tea, forest and barren soils in Assam and N. Bengal region soil samples were collected from forest, barren land, tea and rubber growing areas on seasonal basis and microbial analysis was carried out to understand the ecological impact of above ground vegetation as well as the chemicals used in the plantations. Maximum population was found harbouring on forest soils followed by rubber, tea and barren soil. Bacteria showed the highest population followed by actinomycetes and fungal population.

In order to exploit the naturally occurring antagonistic agents against diseases of rubber, soil microflora was isolated from the rhizosphere region of rubber. The antagonistic effect of few fungal species like *Penicillium* spp., *Aspergillus* spp., *Trichoderma* sp., *Fusarium* spp., *Cladosporium* spp. and a few bacterial species was assessed by dual culture technique. *Penicillium* spp., *Aspergillus* spp. and *Trichoderma* spp. showed antagonistic property towards the fungal pathogens.

Table Ghy. 5. **Quantitative distribution of microbial populations in the rhizosphere soils of young rubber plants (after 3 months of treatment)**

Treatment	Fungi (10^5)		Bacteria (10^8)		Actinomycetes (10^6)		Pseudomonas (10^6)		Azotobacter (10^6)		PSM (10^6)	
	0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20
T1	20.6	17.5	3.9	2.9	2.4	2.1	3.3	3.1	3.4	3.0	3.6	3.1
T2	20.8	16.1	2.9	2.4	2.3	2.0	2.5	2.0	2.9	2.2	2.7	2.1
T3	27.7	21.5	3.2	2.9	2.9	2.1	2.6	2.1	3.4	2.9	3.2	3.0
T4	23.6	19.7	3.1	2.8	2.4	1.9	2.7	1.8	3.0	2.5	3.2	2.9
T5	20.1	16.8	3.0	2.4	2.2	1.7	2.7	2.1	2.9	2.3	3.1	2.9
T6	18.4	14.3	2.7	2.1	2.1	1.6	2.1	1.8	2.4	2.0	2.9	2.1
T7	31.7	26.5	4.1	3.1	3.0	2.4	2.8	2.2	3.5	3.1	3.7	3.2

T1=Control, T2=IF, T3= 25% IF+ Bio F., T4=50% IF + Bio F; T5 = 75% IE +BioF.
T6 = 100% IF + BioF , T7 = Bio F.

4. Crop physiology

4.1. Effect of application of yield stimulant away from tapping cut

In order to understand the effect of stimulation on different areas of bark away from tapping cut, seven different treatments were considered along with unstimulated trees as control. The experiment was undertaken in clone RRIM 600 trees which were under S/2 d3 system of tapping. The first year yield data showed that yield in A, B, C and G were significantly higher (Table Ghy. 6) than that of the unstimulated (H) trees. The % of plants showing above 75% TPD in A, B, D and G were 5.2, 5.3, 5.9 and 7.7 respectively.

4.2. Experiment on shallow tapping at Guwahati

During low winter temperature period, the crop yield in rubber is not economic as the plants cannot ooze out sufficient latex. To avoid low winter temperature stress, tapping rest during this period is mandatory for North Eastern India. However, this long rest caters to loss of around 40-45 tapping days every year. It would be worth experimenting whether shallow tapping (tapping up to half of the bark thickness of normal tapping) during rest period can compensate the yield loss. The dry rubber yield, DRC and TPD in RRIM 600 clone at Sarutari Research Farm in Assam was recorded under different tapping systems. There was no significant difference between the yield and DRC in different tapping systems. 6% plants had shown above 75% TPD. Under normal tapping with winter rest and continuous S/2 d3 tapping without winter rest, the plants did not show TPD (103 tapping days) compared to shallow and continuous tapping (146 tapping days) (Table Ghy. 7).

Table Ghy. 6. **Effect of stimulation on yield of rubber**

Treatments	Yield ($g\ t^{-1}\ t^{-1}$)	Yield ($kg\ t^{-1}\ year^{-1}$)	% of plants showing above 75% TPD
A. Bark application of 5% Ethephon above 125cm from the bud union	49.7	3.7	5.2
B. Bark application of 5% Ethephon below the tapping panel	54.1	4.0	5.3
C. Bark application of 5% Ethephon on the bud union	53.4	3.9	-
D. Bark application of 5% Ethephon at both A and C Positions	47.6	3.5	5.9
E. Bark application of 5% Ethephon above 150 cm subsequent application below 150 cm up to 125 cm	41.2	3.1	-
F. Bark application of base material (diluents oil) at both A and C positions	48.0	3.6	-
G. Panel application of 2.5% Ethephon just above the tapping panel.	53.1	3.9	7.7
H. Unstimulated trees	43.3	3.2	-
CD ($P < 0.05$)	6.0	0.4	

Table Ghy. 7. **Pattern of yield in different tapping system**

Treatments	Yield ($kg\ ha^{-1}$)	DRC (%)	% plants showing above 75 % TPD	Number of tapping days
Shallow tapping during winter and normal tapping during other period	2706	31.6	6	146
Continuous S/2d2 tapping without winter rest	3213	31.6	6	146
Continuous S/2d3 tapping without winter rest	2721	32.0	0	103
Normal tapping with winter rest	2567	30.8	0	103
CD ($P = 0.05$)	NS	NS		

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The station carried out its research activities on cropping system models, nutritional requirement of rubber, clone evaluation, remote sensing study and latex harvest technology, etc. The other aspects of investigations were marketing of rubber and advisory services to rubber growers.

1. Crop improvement

1.1. Development of clones

Evaluation of 1262 hybrid progenies is in progress in four seedling nursery trials from which 25 hybrids have been selected for further evaluation. In the year 2011, 6962 hand pollination attempts were made out of which 825 seedlings were obtained. Nineteen selected hybrids are being evaluated in two clonal nursery trials along with check clone RRIM 600 (2008 & 2009). In the clonal nursery evaluation of selected hybrids (2009), HP 122 (12 cm) and 98/38 (12 cm) recorded higher girth than the check clone RRIM 600 (11.5 cm). One thousand two hundred and forty two half-sib progeny seedlings were evaluated in three seedling nurseries.

In the Small Scale Trial (2000) of ortets, the ortet 114 (46.5 g t^{-1} t^{-1}) exhibited highest mean yield over three years followed by ortet 98 (37.7 g t^{-1} t^{-1}) which were higher than RRIM 600 (37.4 g t^{-1} t^{-1}). Among the 13 ortets from four Regional Stations in the North Eastern region that are evaluated in clonal nursery (2008) along with three check clones, RRSG 248 (21 cm) recorded highest girth followed by the check clone RRII 208 (20.8 cm). Twelve ortets from the traditional area and two RRII 400 series clones (RRII 403 and RRII 407) along with the check clones, are being evaluated in clonal nursery (2009). Clone RRII 407 recorded highest girth (14 cm) and was found on par with check clone RRIM 600 (13 cm). Among the ortets, KO 27 recorded highest girth (13 cm).

1.2. Evaluation of clones

In the Large Scale Trial (LST) planted in 1995 with ten clones, mean yield over seven years showed that PB 311 (48.9 g t^{-1} t^{-1}) was the highest yielder followed by RRII 105 (48.1 g t^{-1} t^{-1}). In the LST planted in 1996 (GxE trial), consisting of 13 clones, clone RRIM 600 (58.11 g t^{-1} t^{-1}) was found significantly superior than all other clones tested.

Potential clones are being evaluated in four on-farm trials. In Killamura block plantation, clones such as PB 235 (1136 kg ha^{-1} $year^{-1}$) and RRIM 600 (1054 kg ha^{-1} $year^{-1}$) were found high yielders. At TFDPC plantation, Bagafa, South Tripura (2000), mean yield over five years from clone RRIM 600 was 19.3 g t^{-1} t^{-1}.

On farm trial at TRPC plantation, Pathalia (2005), consisting of RRII 400 series clones in the immature stage, clone RRII 429 exhibited maximum girth. At Hirapur Block plantation (2009), clone RRII 429 (8.6 cm) had the highest girth followed by PB 260 (8.2 cm), while RRII 422 (6.5 cm) recorded the lowest girth in the second year after planting.

In four clonal nursery evaluations involving 57 pipeline clones from traditional area, 11 popular clones and 19 potential clones are in progress. Clones BPM 24 and PB 311 recorded significantly higher girth compared to RRIM 600 in the clonal nursery trials.

Field trial involving 49 clones for the identification of reliable juvenile and mature characteristics for clone identification with the objective of standardising DUS testing norms is also in progress.

2. Crop management

Integrated nutrient management experiment with clone RRIM 600 with graded doses of inorganic fertilizers with and without bio-inoculum was continued. The highest girth (19.2 cm) was observed on combined application of 50% inorganic fertilizer with bio-inoculum in the third year. In another experiment, the growth of RRIM 600 (34.5 cm) plants during the fourth year was superior when 20 kg FYM along with 50% of the recommended dose of fertilizer was applied.

In the experiment on evaluation of a cropping system model, four intercrops were tested. The yield was 65 kg ha^{-1} for *Amorphophallus* for both models and 98 kg ha^{-1} and 75 kg ha^{-1} for *Colocasia* in model I and II respectively. Among the short duration crops, banana yielded 220 kg ha^{-1} and 270 kg ha^{-1} and pineapple yielded 1088 kg ha^{-1} and 1625 kg ha^{-1} respectively in Model I and II. It was also found that the mean girth of rubber was higher in intercropped area in both the models.

In the Rubber-Tea intercropping trial, during the mature stage of rubber, mean annual production of green tea leaf as intercrop was 192 kg ha^{-1} and 2160 kg ha^{-1} as monocrop. Tea intercrop yield was declining due to shading of rubber trees over the years. Mean rubber yield was 1319 kg ha^{-1}.

In the experiment on potting media for root trainers, young budding was done to one month old seedling in the root trainer with the selected potting media (top soil with cow dung in the ratio of 8:2) and it was observed that budding success rate was 55%.

In another experiment, there was no difference in growth of rubber after one year between the zero tillage and pit planting practices.

Five fodder crops were tested over a period of two years in the experiment on 'Yield and quality evaluation of forages under immature rubber'. It has been found that mean yield was highest for guinea grass (10.8 t ha^{-1}), followed by Stylo Signal grass (8.8 t ha^{-1}), fodder cowpea (7.5 t ha^{-1}), Maize (6.3 t ha^{-1}) and Napier Para grass (3.6 t ha^{-1}).

2.1. Remote sensing

In the remote sensing project, spatial mapping of existing rubber plantation of Tripura state has been accomplished using IRS-P6 LISS 3 and LISS IV satellite images. Three temporal images of LISS 3 were used to identify the current extent of NR plantation based on the temporal variation of Normalized Difference Vegetation Index (NDVI). Based on this estimation the total area under NR in Tripura state was found to be 45252 ha. Due to the usage of coarse resolution satellite imageries NR plantations of below 3 years of age could not be detected. Therefore, the spatial extent of NR plantations estimated based on this study was as on 2008. Waste land mapping was also attempted and waste land suitable for further expansion of NR cultivation was preliminarily estimated to be around 18500 ha. Ground truth observations were taken to ensure the accuracy levels of spatial mapping of NR plantations and waste lands.

3. Crop physiology

3.1. Latex harvest technology

In the experiment on different systems of tapping, the clone PB 235 continued to give the highest yield in S/2 d3 system of tapping compared to S/2 d4 and S/2 d6 systems of tapping. In another experiment, S/2 d3 system of tapping showed highest yield in clone RRIM 600 compared to S/2 d2 and S/2 d4 systems of tapping.

In another experiment, clone RRIM 600 showed higher yield under S/2 d2 system of tapping compared to shallow tapping in the same system. In the Controlled Upward Tapping (CUT) of the high panel (HO-1) the short cut (S/4 U d2 6d7) with stimulation ET 5% La 12 Y in clone RRIM 600 continued to give the highest yield compared to S/2 d2 system in BI panel.

4. Crop protection

Cross infectivity test of *Phytophthora sp* isolated from leaf rot of betel vine was tested in mature leaf of RRIM 600 clone under laboratory condition (at 22 ±1°C). The betel vine isolate was able to infect rubber leaf and produce black brown lesion. The mean diameter of lesions at 72 h of inoculation was 22.8 mm in betel vine leaf while it was only 14 mm in rubber leaf. However it was only moderately virulent to rubber leaf.

Growth of *Phellinus noxius*, the brown root disease causing pathogen was studied at 30, 34 and 38 °C. The fungus grows well at 30-34 °C. However, no growth of the fungus was observed at 38° C even after 7 days of incubation. The growth of the fungus was not observed to resume when it was again kept at room temperature.

5. Processing technology

Latex coagulation during winter season was studied. The study showed that when ambient temperature was around 10°C in the winter season, an average of 270 ml of 1% formic acid is required for complete coagulation where the normal recommended dose is 300 ml of 0.5% formic acid for four liters of latex with 12.5% DRC.

Experiments were conducted for developing smoke filters for improving colour of the smoke dried sheets. It was observed from different smoke filters that the filter with wire mesh could give the better colour for the sheets. However, the filter was found to be blocked and if the filter is fitted with GI pipes, it was found to give good colour to the sheets without any blockage to the filter.

Experiment to enhance the durability of mud wall was conducted and the results showed that the strength of the wall increased when latex was applied to it. High strength of the wall was observed when it was made with latex mixed with clay. However the wall coated with pre-vulcanized latex gave better finishing and least peeling off properties for the coating.

A survey was conducted to study the present status of biogas plants installed in different RPSs in Tripura state. The rubber processing unit effluents on anaerobic treatment produces the combustible gas, methane due to the activity of methano-bacteria and other micro-organisms. Preliminary results showed that the same gas can also be generated during low temperature period in winter season. One third of the fire wood could be saved by supplementing biogas for rubber sheet drying in addition to better colour of the sheets. It was observed that RPSs generally sell latex to the local heat resistant latex rubber thread manufacturing company available in this state. This practice generates insufficient effluent, which is one of the reasons for the non-production of Biogas in some plants. Lack of repair and maintenance of plant were other reasons. Most of the Biogas plants seem to be under utilized in this place.

6. Economics

A project entitled "An economic analysis of primary processing and marketing of Natural Rubber in Tripura" was undertaken covering 13 Block Planting Units, 15 RPS and 116 individual growers. Altogether, 305 small rubber growers were covered. It was found that processing of sheet rubber takes place only among the individual growers. The lower transaction costs of marketing and quicker price realization were the main reasons for processing sheet rubber. The individual farmers were mostly selling their produce as unsmoked sheets to village traders / subagents of dealers. The average deduction for selling unsmoked sheets to the subagents was 27.20 kg^{-1} from terminal market price of RSS 4 Kottayam prices. The 93% of BPUs and 80% of RPSs were processing/marketing their produce as preserved field latex (PFL). The channel of marketing of PFL has been dominated by the trading company (100% in BPU and 90% in RPS). The RPS/BPU process sheet rubber mainly during monsoon and peak season (at that time local heat resistance latex thread manufacturing company may not buy the entire quantity). The farm gate price realized by the beneficiaries/ members for one kg of dry rubber was Rs. 14.15 less than RSS 4 Kottayam prices.

7. Advisory work

Discriminatory fertilizer recommendation based on soil and leaf analysis was offered to 244 rubber growers of this region. A total of 1356 number of latex samples were analysed for DRC and other latex parameters. Total 4010 m of bud wood of high yielding clones were supplied to growers.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

The Regional Research Station, Tura continued its research activities on evaluation of clones/polyclonal population, latex harvest technology and crop management.

1. Crop improvement

1.1. Poly-cross progeny evaluation

The population attained an average girth of 8.7 cm ranging between 3.4 and 13.5 cm. Average test tap yield of 230 populations was 4.02 g $t^{-1}10$ $trees^{-1}$ with a range of 1.2-12.7 g $t^{-1}10$ $trees^{-1}$. On the basis of the test-tap yield and girth of the progenies, top 20% of the population was selected for further evaluation in clonal nursery trials. Selected seedlings were cut back to generate bud-woods. In 2011, a new nursery of 800 poly-cross progenies was set up at Ganolgre farm.

1.2. Clonal nursery evaluation

A clonal nursery was set up with three selections from Tura and four each from Agartala and Guwahati with RRIM 600 as the check. The experiment is in RBD design with 12 clones and three replications with a plot size of 6. Agartala selection RRSA 461 showed significantly higher height (2.48 m), girth (9.2 cm) and numbers of leaves (61.8)

Table Tura 1. **Growth parameters of clonal nursery evaluation trials at Ganolgre farm**

Location	Selections	Height (m)	Girth (cm)	No. of leaves	No. of whorls
Guwahati	X1	1.9±0.3	7.1±0.1	41.4±5.5	4.1±0.3
	X2	2.2±0.2	7.7±0.7	51.2±6.8	4.9±1.8
	X3	2.1±0.4	6.7±2.9	48.5±7.3	4.8±0.3
	X9	2.1±0.3	7.9±1.8	43.8±2.0	3.7±0.3
Tura	RRST 24	2.0±0.5	5.5±1.0	45.3±1.3	4.0±1.0
	RRST 27	2.3±0.4	8.2±1.3	49.7±5.2	4.0±0.0
	RRST 39	1.3±0.2	5.0±1.0	36.0±3.0	3.0±0.6
Agartala	RRSA 121	2.0±0.1	7.6±0.6	59.6±1.0	4.3±1.0
	RRSA 315	2.4±0.1	8.6±1.0	43.0±1.0	4.3±0.3
	RRSA 461	2.5±0.1	9.2±1.0	61.8±1.0	4.4±1.0
	RRSA 585	2.1±0.1	7.7±0.3	53.0±3.0	4.3±0.3
Check	RRIM 600	2.0±0.1	6.7±0.8	47.5±0.5	4.3±0.3
LSD*P<0.05		0.45	0.73	6.6	1.4
CV (%)		12.9	17.2	8.1	9.3

Table Tura 2. **Growth parameters of different clones in the on-farm trial at Mendipathar, East Garo hills of Meghalaya**

Clones	Height (m)			Girth (cm)			Branches		
	Momin	Rabha	Mean	Momin	Rabha	Mean	Momin	Rabha	Mean
PB 235	4.4	4.2	4.3	16.4	14.6	15.6	12.1	11.4	11.8
RRII 417	4.3	3.4	3.8	17.3	11.1	12.2	10.3	7.9	9.1
RRII 429	4.5	3.4	3.9	18.5	10.9	14.7	7.5	10.3	8.9
RRII 422	3.9	2.7	3.3	13.6	8.0	10.7	10.3	8.0	9.1
RRII 203	4.5	2.8	3.7	17.8	10.7	14.2	23.4	11.4	17.4
RRIM 600	4.7	3.4	4.1	17.3	12.9	15.2	9.9	11.1	10.5
LSD*P<0.05	0.2			1.1			2.3		
CV (%)	13.1			18.			8.2		

when compared to other selections, while Tura selection RRST 39 showed the lowest value for the above parameters (Table Tura 1).

1.3. Evaluation of clones (LST)

Establishment of LST with 18 clones is under progress (RRII 105, RRII 203, RRII 208, RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, IRCA 111, IRCA 130, REYAN 88/13, HAIKEN 1, RRIM 703, PB 235, PB 312, PB 314, PB 28/59 and RRIM 600 as check clone). Bud-wood nursery of the clones has been established at Ganolgre farm.

1.4. On-farm evaluation of selected clones

Three on-farm trials have been set up in the East & West Garo Hills of Meghalaya. 600 plants each of six clones *viz.* RRII 417, RRII 422, RRII 429, PB 235, RRII 203 and RRIM 600 were planted in two locations in the East Garo Hills and 400 plants each of four clones *viz.* RRII 417, RRII 422, RRII 429 and RRIM 600 were planted in the West Garo Hills of Meghalaya. In East Garo Hills, highest girth and height was found in PB 235 (15.6 cm and 4.3 m) followed by RRIM 600 (15.2 cm and 4.1 m). Maximum number of branches were obtained in RRII 203 (17.4) followed by PB 235 (11.8) and RRIM 600 (10.5) (Table Tura 2). In West Garo Hills, RRIM 600 was found fallen (2.31 m) but RRII 417 excelled in terms of mean girth (cm), number of leaves and number of whorls.

1.5. Half-sib progeny evaluation

On the basis of test tapping, yield and girth of the progenies selected in year 2008, 20% of top ranking population was selected for further evaluation in clonal nursery trials. In another experiment (2009), comprising of seven progeny clones (PB 86, Gl 1, GT 1, RRII 203, RRIM 600, RRII 105 and PB 260), progeny population of RRII 203 showed significantly higher height (3.25 m), girth (8.4 m) and number of whorls (4.9) when compared to other progenies. RRII 105 progenies were better in terms of test tap yield (2.18 g t^{-1}10 $trees^{-1}$). From this trial also, 20% of the top ranking progenies were selected for further evaluation in clonal nursery trials.

1.6. Development of poly-clonal seed garden

Eight hundred and fifty nine poly-bag plants belonging to 9 clones (RRII 118, RRII 203, RRII 208, RRII 422, RRII 429, Haiken 1, SCATC 88/13, PB 280 and RRIM 600) were planted in the field at Hahara, Guwahati.

1.7. Evaluation of poly-cross progenies from four stations of NE region

Polyclonal seeds (360 progenies) were collected from four locations in the NE region and planted (in RBD with 5 replications). Guwahati polyclonal progenies showed the maximum height (3.01 m), girth (8.2 cm), number of leaves (43.8) and number of whorls (5.4) when compared to other selections but statistically significant variation was not observed between the locations (Table Tura 3).

Table Tura 3. **Growth parameters of poly cross progenies collected from four stations of NE India**

Locations	Height (m)	Girth (cm)	Leaves	Whorls
Agartala	2.6±0.5	7.2±0.4	36.7±5.7	4.9±0.3
Tura	2.5±0.4	7.2±1.0	37.5±7.3	5.1±0.6
Nagrakatta	2.7±0.2	8.0±0.5	39.6±7.3	5.2±0.6
Guwahati	3.0±0.3	8.2±1.2	43.8±8.8	5.3±0.5
LSD(*P=0.05)	0.5	1.1	9.3	0.7
CV (%)	0.0	0.2	0.2	0.4

2. Crop physiology and latex harvest technology (LHT)

Low temperature (below 10 °C) during winter period is one of the main factor for depression of yield and dry rubber content in *Hevea* under the agro-climatic condition of Garo Hill. Yield and yield components were recorded and the results indicated that average total volume of latex was 186.1 ml t^{-1} t^{-1} yield 58.4 g t^{-1} t^{-1} and DRC was 33.8%. Complete defoliation occurred in 3rd week of February and lowest soil moisture was recorded in the month of January/ February.

Among the treatments, S/4 upward tapping system with once in three weeks stimulation in the upward cuts and periodic panel change of d/2 system showed highest yield compared to all other treatments (Table Tura 4).

Table Tura 4. **Annual yield (g t^{-1} t^{-1}) under controlled upward tapping systems during 2011-12**

Treatment	Yield (g t^{-1} t^{-1})	TPD (%)
T1 S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (3w)	91.6	14.4
T2 S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (m)	91.0	19.5
T3 S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (m)	85.5	17.6
T4 S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (6w)	76.5	12.2
CD (0.05)	5.31	NS

Shallow tapping – an option to stress alleviation in *Hevea* plantations during winter season in NE region, monthly yield and DRC (%) were recorded and presented in Table Tura. 5 and results indicated maximum total volume of latex in normal tapping system than shallow tapping system. DRC recorded in normal continuous tapping system was 1.2% lower than shallow tapping system. Normal continuous tapping system showed higher TPD than the shallow/ continuous tapping system and minimum was in normal tapping system.

An experiment on location specific stimulant application on ethylene induced stress responses in the tapping panel of the *Hevea* trees was initiated with the aim to reduce the ethylene mediated stress responses in tissues in the tapping panel by applying the ethylene compounds away from the tapping area without compromising the latex yield under the agro climatic conditions of Garo Hills. RRIM 600 clone was selected for the study with 8 treatments and with application of 5% Ethephon (Three times per year). Higher volume of latex (232.5 ml t^{-1} t^{-1}) with lower DRC (34.12%) was recorded in treatment with bark application of 5 % Ethephon above 125 cm from the bud union and near the bud union while lower volume

Table Tura 5. **Comparison of total volume of latex, DRC (%) and TPD (%) between normal and shallow tapping**

Months	Total volume of latex (ml t^{-1} t^{-1})			DRC (%)		
	Normal tapping	Shallow/normal tapping	Normal continuous tapping	Normal tapping	Shallow/normal tapping	Normal continuous tapping
February	**	113.3*	164.4	**	26.4*	25.4
March	**	93.1*	121.8	**	31.6*	29.5
April	**	88.5*	110.0	**	33.7*	32.1
May	90.0	70.0	95.0	33.3	34.7	33.2
June	106.3	95.0	108.3	34.5	35.3	34.0
July	165.0	158.3	166.6	37.6	38.5	37.2
August	92.3	88.3	93.3	37.5	37.9	37.4
September	138.0	135.0	140.0	37.8	37.8	37.4
October	162.0	150.0	160.0	35.9	37.0	35.9
November	207.0	195.0	205.0	33.9	35.4	33.8
December	199.6	188.3	196.6	31.9	33.1	31.9
January	165.3	152.5	158.3	27.9	28.8	27.7
Mean	147.7	132.1	143.3	34.5	35.3	34.1
LSD*P<0.05		10.56			0.05	
Yield (g t^{-1} t^{-1})	50.9	46.6	48.9			
Projected yield*** (kg ha^{-1} yr^{-1})	1782	2120	2225			
TPD (%)	11.2	14.5	19.5			

**Shallow tapping during rest period; ** Tap rest*

****Projected yield= [Yield (g $t^{-1}t^{-1}$)X Number of trees ha^{-1} X tapping days]/1000; Number of trees ha^{-1} =350; tapping days 100 for normal tapping and 130 for shallow/normal and normal continuous tapping.*

of latex (96.1 ml t^{-1} t^{-1}) and higher DRC (35.37 %) was observed in un-stimulated trees.

3. Crop management

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

Treatment-wise soil samples were collected and analyzed for available nutrients. Monthly cup lump yield (g t^{-1} t^{-1}), DRC (%) and total latex volume (ml t^{-1} t^{-1}) were recorded. Girth of the plants were recorded at different time intervals. Results indicated highest girth (90.5 cm), girth increment (2.98 cm), yield (56.4 t^{-1} t^{-1}), DRC (34.3 %) and latex volume (168.0 ml t^{-1} t^{-1}) for the treatment $N_{60}P_{30}K_{45}$ kg ha^{-1} and minimum for $N_0P_0K_0$ (Table Tura. 6). Application of NPK fertilizers had significantly improved available P and K contents in soil in the same treatment compared to the control.

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

Soil samples were collected at 0-15 cm, 15-30 cm and 30-60 cm soil depths and soil moisture determined. It was found that soil moisture content showed increasing trends with increasing depth of soil in all the months. Maximum soil moisture content was recorded during July-September period and minimum was in January-February period (Table Tura 7).

Table Tura 6. **Effect of N, P, and K combinations on yield, DRC, girth and total volume of latex under the Central Brahmaputra valley zone (CBVZ) of Assam during mature phase**

Treatment combination (N:P:K) kg ha^{-1}	Girth (cm)	Girth increment (cm)	Yield (g t^{-1} t^{-1})	DRC %	Total volume of latex (ml t^{-1} t^{-1})
T1 (0:0:0)	70.6	0.9	21.4	31.4	70.5
T2 (0:0:15)	71.9	1.1	22.2	31.7	74.2
T3 (0:15:0)	66.8	1.3	24.4	31.8	79.2
T4 (15:0:0)	73.1	1.4	27.3	32.1	87.5
T5 (0:0:30)	72.5	1.7	29.4	32.2	94.5
T6 (0:30:0)	75.7	1.8	31.2	32.5	99.2
T7 (30:0:0)	77.8	2.0	33.3	32.7	104.7
T8 (15:15:15)	76.7	2.1	34.8	32.9	112.16
T9 (30:15:30)	81.3	2.1	38.2	33.1	119.0
T10 (30:30:30)	81.7	2.3	41.2	33.2	127.2
T11 (45:15:30)	84.1	2.4	44.3	33.4	135.5
T12 (45:30:45)	83.9	2.5	56.9	33.6	142.8
T13 (60:15:30)	84.5	2.8	49.8	33.8	146.7
T14 (60:30:30)	85.5	2.9	53.0	34.1	159.3
T15 (60:30:45)	90.5	3.0	56.4	34.3	168.0
SEm ±	2.53	0.045	2.54	0.08	8.42
CD (P=0.05)	7.33	0.13	7.38	0.23	24.42

Table Tura 7. **Soil moisture content (%) under different depth at RRS, Ganolgre farm**

Months	Soil moisture content (%)		
	0-15 cm depth	15-30 cm depth	30-60 cm depth
April	20.5	21.5	22.9
May	22.7	23.9	24.5
June	23.7	24.9	25.7
July	25.7	26.4	27.2
August	27.0	27.9	28.1
September	27.3	28.1	28.7
October	23.4	24.5	25.0
November	21.0	21.5	21.9
December	20.5	21.4	21.9
January	19.2	19.5	20.1
February	18.2	18.9	19.3
March	18.3	19.2	19.4
Mean	22.7	23.5	24.1
SD	3.1	3.2	3.2
CV (%)	13.6	13.5	13.1

3.3. Analytical/ Advisory work for fertilizer recommendation

Fifty eight soil samples were collected from the rubber growing areas and made soil fertility assessment. Results indicated that in the top soil (0-30 cm) OC content was in the medium range (1.2-1.5%), available phosphorus was in the low range (0.14-0.38 mg $100g^{-1}$) and available K was in the medium range (7.1-8.2 mg $100\ g^{-1}$). The soil is acidic in nature with pH ranging from 4.5 to 5.6. Fertilizer recommendation was offered to the growers.

REGIONAL EXPERIMENT STATION NAGRAKATTA, WEST BENGAL

1. Crop improvement

1.1. Evaluation of clones

Experiments on multidisciplinary clone evaluation was initiated in 1990, 1991 and 1993 in the non-traditional area of Sub-Himalayan West Bengal with the objective of screening promising clones suitable for the area in terms of growth, yield and other attributes. The growth over twenty one years after planting, indicated (Table Nag. 1) that in trial I and II girth was highest in clone RRIM 612 (86.7 cm) followed by SCATC 88/13, RRII 118, Haiken 1 and RRIM 605. Here, girth of eight clones was superior to the check clone RRII 105. In trial III SCATC 93/114 showed significant higher girth than the check clone RRIM 600 and in trial IV none of the clones were superior to the check clone RRIM 600.

The mean yield (Table Nag. 2) of SCATC 88/13, RRII 300, PB 311, RRIM 703, RRIM 605, RRII 208, SCATC 93/114 and PB 235 were superior to the check clone RRII 105 in trial I and II. Yield of check clone RRIM 600 was appreciable in trial III, *i.e.*, none of the clones performed better than it. However, in trial IV the mean yield of PB 280 and Haiken 1 was higher than the check clone RRIM 600.

1.2. Evaluation of germplasm

Evaluation of germplasm in hot-spot areas like Nagrakatta, West Bengal was initiated in 1998 with 21 germplasms along with RRII 105 as check clone. Among the wild accessions maximum girth (Table Nag. 3) was found in RO 3172 followed by RO 2890, RO 5348 and RO 2635. Girth of eight clones were found superior to the

Table Nag. 1. **Pattern of girth (cm) in different clone trials**

Trial I and II	Girth (cm)	Trial III	Girth (cm)	Trial IV	Girth (cm)
GI 1	64.6	RRIM 612	71.8	PB 280	69.2 *
GT 1	70.4	RRII 208	64.1	RRII 308	60.6
Haiken 1	77.2 **	PR 107	66.8	RRII 208	64.1
PB 235	72.4 *	PB 310	66.2	PR 261	64.5
PB 311	68.3	SCATC 93/114	75.2 *	RRIC 104	60.0
PB 5/51	66.7	PB 260	63.0	SCATC 93/114	70.6 *
PB 86	70.5	RRIC 102	69.8	RRII 300	63.7
PR 107	70.1	PB 86	66.7	PB 235	65.6
RRII 118	78.9 **	Haiken 1	70.0	Haiken 1	68.1 *
RRII 203	67.3	PB 235	67.3	RRII 105	62.1
RRII 208	70.9	RRIM 600 (check)	67.7	RRIM 600 (check)	66.7
RRII 300	71.9 *	Collective mean	68.0	Collective mean	65.3
RRIM 605	75.9 **	CD (P = 0.05)	6.0	CD (P = 0.05)	NS
RRIM 612	86.7 **				
RRIM 703	71.9 *				
SCATC 88/13	70.9				
SCATC 93/114	79.5 **				
RRII 105 (check)	64.0				
Collective mean	68.4				
CD (P = 0.05)	7.1				

Table Nag. 2. **Pattern of yield in different clone trials**

Trial I and II	Yield (g $t^{-1}t^{-1}$)	Trial III	Yield (g $t^{-1}t^{-1}$)	Trial IV	Yield (g $t^{-1}t^{-1}$)
GI 1	27.3	RRIM 612	23.2	PB 280	40.7
GT 1	39.7	RRII 208	34.9	RRII 308	29.9
Haiken 1	30.3	PR 107	27.0	RRII 208	42.0
PB 235	40.9 *	PB 310	34.2	PR 261	32.2
PB 311	43.5 **	SCATC 93/114	28.8	RRIC 104	27.2
PB 5/51	32.7	PB 260	27.7	SCATC 93/114	26.7
PB 86	34.5	RRIC 102	29.1	RRII 300	36.3
PR 107	38.3	PB 86	29.4	PB 235	36.9
RRII 118	39.4	Haiken 1	35.6	Haiken 1	41.8
RRII 203	32.2	PB 235	35.0	RRII 105	30.5
RRII 208	42.2 *	RRIM 600	36.1	RRIM 600	37.8
RRII 300	43.8 **	Collective mean	31.0	Collective mean	35.2
RRIM 605	42.5 *	CD (P = 0.05)	NS	CD (P = 0.05)	NS
RRIM 612	39.3				
RRIM 703	43.3 **				
SCATC 88/13	47.7 **				
SCATC 93/114	41.2 *				
RRII 105	33.7				
Collective mean	34.7				
CD (P = 0.05)	6.8				

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

Accessions	Girth (cm)	Yield(g $t^{-1}t^{-1}$)	Accessions	Girth (cm)	Yield(g $t^{-1}t^{-1}$)
AC 1950	61.1	24.2	RO 2629	65.2	14.3
AC 607	36.8	20.0	RO 2635	70.1 **	5.1
AC 619	62.7	8.9	RO 2890	74.9 **	14.4
AC 623	46.3	2.7	RO 3172	76.0 **	12.2
AC 68	61.6	7.5	RO 5329	57.2	16.7
AC 763	53.4	30.2	RO 5348	72.8 **	20.3
MT 196	64.1	25.6	RO 5363	63.3	49.0
MT 2229	67.4 **	20.9	RO 5408	57.2	12.5
MT 2594	52.9	15.0	RO 5430	66.2 *	22.4
MT 44	66.3 *	14.4	RO 5557	69.2 **	17.5
RRII 105	60.1	47.2	RO 6139	53.6	11.1
CD (P = 0.05)	5.32 (girth) NS (yield)				

check clone RRII 105. In terms of yield, RO 5363 was found comparable to that of the check clone RRII 105. In general, the performance of Rondonia was better than Acre and Mato Grosso accessions.

1.3. Performance of polyclonal seedlings

Polycross seeds collected from seed garden in Kanyakumari, Tamilnadu were grown in the natural climatic conditions of North Bengal since 1990 with 240 trees in CRD (single tree single plot) with a 5x5 m distance. Mean girth of the population after 21 years of planting was 66.0 cm. The average block yield of the population was 39.2 g $t^{-1}t^{-1}$ during the reporting period. Seven per cent plants showed above 80 g $t^{-1}t^{-1}$ yield. Selected ortets were maintained at the nursery for further evaluation.

2. Crop management

2.1. Nutritional trial

For optimizing the fertilizer dose in the area of Sub-Himalayan West Bengal, an experiment on NPK in three factorial design was laid out. The girth data during this year (Table Nag. 4) did not show any regular pattern in different doses of fertilizer applied. Yield in $N_1P_{20}K_{20}$ was significantly higher (Table Nag. 5) than $N_0P_0K_0$ combination.

Table Nag. 4. **Girth (cm) of rubber clones in response to different doses of NPK fertilizers**

N Level	P_0			P_{20}			P_{40}		
(kg ha^{-1})	K_0	K_{20}	K_{40}	K_0	K_{20}	K_{40}	K_0	K_{20}	K_{40}
N_0	70.8	69.3	70.1	74.3*	70.1	69.8	71.9	68.9	71.4
N_{10}	65.2	69.5	70.7	66.2	74.7**	70.6	69.4	71.1	71.8
N_{20}	69.9	65.9	71.6	70.8	71.2	69.0	73.7*	69.6	71.0
N_{40}	68.9	75.2**	72.4	68.8	72.8	74.5*	74.4*	68.7	70.0
		NP	NK	PK	NPK				
CD (P = 0.05)		2.99	6.66	8.14	2.70				

* *Significant at 0.05% level;* ** *Significant at 0.01% level*

Table Nag. 5. Yield (g t^{-1} t^{-1}) distribution of rubber in response to NPK

N Level	P_0			P_{20}			P_{40}		
(kg ha^{-1})	K_0	K_{20}	K_{40}	K_0	K_{20}	K_{40}	K_0	K_{20}	K_{40}
N_0	38.2	30.9	37.9	43.7*	31.6	40.1	31.3	32.2	32.1
N_{15}	26.0	32.8	35.1	32.6	33.0	30.1	31.2	31.1	31.3
N_{30}	31.3	34.3	27.9	32.4	28.0	25.3	37.9	28.3	33.4
N_{45}	28.6	35.8	33.1	30.1	32.5	38.9	37.9	32.3	32.8
		NP	NK	PK	NPK				
CD (P = 0.05)		8.23	8.61	9.88	4.64				

2.2 Inter-planting trial

An inter-planting trial on rubber in tea was continuedin the Dooars area of West Bengal. Four different planting combinations of tea-rubber along with monoculture of tea and rubber as control were the treatments.

Green tea leaf yield in inter-planted plots (Table Nag. 6) was significantly lower than that of the pure plot due to heavy shade imposed by the mature rubber trees and also due to severe pest attack in inter-planted plots.

3. Crop physiology

3.1. Performance of polycross progeny raised from seeds of locally adapted mature rubber plantation

Rubber seeds procured from different regions of North East India (North Bengal, Assam, Meghalaya) and South India (HBSS, Kanyakumari) were cultivated in the Sub-Himalayan West Bengal in 2008 in order to understand whether there is any difference in performance of the plants while growing away from their native place. The data

Table Nag. 6. Growth and yield of rubber and tea

Treatments	Spacing	Tea yield (kg ha^{-1})	Rubber girth (cm)	Rubber yield (kg ha^{-1})
Pure Rubber	Rubber - 5 x 5 m	–	61.4	1448 (100%)
Rubber + Tea	Rubber - 10 x 2.5 m Tea – 10 x (1.0 x 0.6) m	1187 (70%)	56.8	873 (72%)
Rubber + Tea	Rubber - 12 x 2.5 m Tea – 12 x (1.0 x 0.6) m	1187 (70%)	56.8	873 (72%)
Rubber + Tea (paired row)	Rubber – 18 x (3 x 3) m; 2rows Tea – 18 x (1.0 x 0.6) m	1758 (72%)	62.3	813 (68%)
Rubber + Tea	Rubber – 10 x 5.0 m Tea – 10 x (1.0 x 0.6) m	2203 (70%)	64.6	354 (35%)
Pure Tea	Tea - 1.0 x 0.6 m	11174 (100%)		
CD (P = 0.05)		8161.77	6.12	870

Data in parentheses denote plant stand per treatment

during this year showed no significant difference between the girth of plants from different source of seeds.

3.2. Physiological evaluation of rubber clones in abandoned tea growing areas of Dooars belt of North Bengal

Attempt was made to introduce rubber in abandoned tea growing areas of sub-Himalayan West Bengal where the soil is either sodic or stony. Preliminary result showed that rubber can grow well in the alkaline pH of 8.3. For physiological evaluation of different clones grown in high pH soil, another experiment was initiated in soil pH of 7.3. Eight months old polybag plants of six clones *viz.* RRII 208, RRII 429, RRII 417, RRII 422, RRIM 605 and RRIM 600 were cultivated in blocks of 75 plants each in high pH soil along with control plants of same clone growing in soil pH of 5.5 at the research farm of RES, Nagrakatta. The success rate of the plants growing in high pH soil is 100 %. Almost all the plants showed cold injury in the form of leaf-tip drying and leaf fall. After two months of planting the girth of plants under high pH soil was better than that of normal soil in RRII 422 and RRIM 605. Among the clones growing in high pH soil, the girth of RRII 208 was significantly higher than that of RRIM 600.

3.3. Evaluation of environmental stress tolerance and physiological adaptations of cold and drought tolerant ortet selections under varying agro-climates in India

Field performance of cold tolerant clones when exposed to drought stress were studied by planting them in drought prone area and *vice-versa*, budgrafted plants of ortet selections from Nagrakatta, Tura, Agartala, Guwahati (cold) and Daphchari (drought) were raised at RES, Nagrakatta (Sub-Himalayan West Bengal) and maintained. The growth data of the polybag plants inside the polyhouse was collected. In general, the survival rate of Tura material was lower compared to that of the others.

3.4. Effect of application of yield stimulant away from tapping cut

In order to understand the effect of yield stimulation on different areas of bark away

Table Nag. 7. **Effect of stimulation on yield of rubber**

System of stimulation application	Average girth of the block (cm)	Yield (g $t^{-2}t^{-3}$)	Yield (kg $tree^{-1}year^{-1}$)	% of plants showing above 70% TPD
A. At 125 cm from the bud union	69.1	47.4	3.4	3
B. Below the tapping pane	169.6	52.7	3.8	15
C. On the bud union	68.4	50.0	3.5	5
D. At both A and C positions	67.8	52.5	3.7	8
E. Bark between 150 cm up to 125 cm	68.9	45.0	3.2	10
F. Bark application of diluents oil at both A and C positions	68.34	41.2	2.9	13
G. Panel application of 2.5% Ethephon	70.75	54.4	3.9	23
H. Unstimulated trees	70.2	43.4	3.1	5
CD ($P = 0.05$)	NS	6.09	0.56	

A to F : Bark application of 5% ethephon

from tapping cut, seven different treatments were considered along with unstimulated trees as control. The experiment was undertaken in BO-2 panel (4th year) of clone RRIM 600 under S/2 d3 system of tapping in blocks of 40 plants each.

Plants of all the treatments were of uniform girth. The first year yield data ($g\ t^{-1}\ t^{-1}$) showed that yield in treatments B, C, D and G were significantly higher and that on kg $tree^{-1}year^{-1}$ basis (Table Nag. 7) B, D and G was significantly higher than that of the unstimulated (H) trees. The % of plants showing above 70% TPD in B, C, D, were 15, 5 and 8 respectively whereas it was 23 in G and in unstimulated block it was only 5%.

3.5. Experiments in shallow tapping at RES Nagrakatta

Tapping rest is mandatory in North East region to avoid low winter temperature stress. Upon tapping rest, the crop productivity of the rubber plantations in North East is affected with an approximate loss of 30-40 tapping days in every year. To overcome the yield loss an alternate method is being tested by adopting shallow tapping without giving tapping rest during the winter months. Study was also initiated to understand the magnitude of stress induction in the tapping panel due to shallow tapping compared with normal tapping. Trees of uniform girth of clone RRII 105 with S/2 d2 tapping frequency in BI-1 panel (2nd year) were selected. Similar system was followed in RRIM 600 where trees were tapped in BI-1 panel (4th year). The tapping days obtained in shallow or continuous tapping system was 140 where in normal tapping system it was 103. The data showed that yield in shallow tapping was on par with the normal tapping system. The percentage of plants showing above 80% TPD was more in shallow/continuous tapping compared to normal tapping with rest (Table Nag. 8).

Table Nag. 8. **Yield pattern in different tapping systems**

Clone	Yield ($kg\ ha^{-1}$)		
	Normal tapping with rest	Shallow tapping during winter	Continuous tapping without rest
RRII 105	1259 (0)	1268 (4)	1234 (3)
RRIM 600	1300 (1)	1292 (2)	1253 (4)

Figures in parentheses is % of plants showing >80% TPD

REGIONAL RESEARCH STATION DAPCHARI, MAHARASHTRA

The mandates of this Station are to develop suitable clones and location specific agro-technology for drought condition. The experiments on crop improvement (screening of wild *Hevea* accessions, evaluation of clones, polyclone, pipeline clones, selected ortets and wild *Hevea* accessions for growth and yield performance under North Konkan condition), environmental physiology (irrigation requirement and irrigation methods, drought studies) and crop management (practices to mitigate the drought like soil moisture conservation) are being carried out.

1. Environmental physiology

1.1. Drip and basin method of irrigation

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

The irrigation experiment was started during 1987 with an objective to evaluate the advantage of drip irrigation over basin irrigation in terms of water saving and total economy towards the cost of irrigation. The treatment comprises of 1.00, 0.75 and 0.50 ETc in basin and 0.75, 0.50 and 0.25 ETc in drip methods of irrigation. From February 2000 onwards, the 0.75 ETc in the basin and 0.50 ETc in the drip were reduced to 0.25 ETc in order to find out whether irrigation requirement can be further reduced. The response of different irrigation levels by these methods was observed in terms of girth increment in clone RRII 105. A significantly higher girth was registered in basin irrigation at 1.0 ETc (77.4 cm) but it is on par for 0.25 (reduced irrigation from 0.75 ETc) and 0.50 ETc (74.75, 74.74 cm respectively) basin irrigation. It was found that the trees under different levels of basin irrigation (1.0, 0.25, 0.50 ETc) showed higher girth (77.4, 74.8, 74.2 cm) as compared to drip system and control (65.7 cm). The drip irrigation scheduling (0.75, 0.25*, 0.28 ETc) resulted in higher yield (36.3 g t^{-1} t^{-1}) as compared to basin irrigation (33.1, 36.3, 32.2 g t^{-1} t^{-1}). (Table Dap. 1).

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

Treatments		Yield (g $t^{-1}t^{-1}$)	Girth (cm)
Control		34.9	65.7
Basin	1.00 ETc	28.2	77.4
	0.25 ETc*	28.7	74.8
	0.50 ETc	33.5	74.2
Drip	0.75 ETc	33.1	73.5
	0.25 ETc**	36.3	71.9
	0.25 ETc	32.2	69.8
	SE+	3.8	1.2
	CD	NS	3.67

**Changed from 0.75 ETc to 0.25 ETc; ** Changed from 0.50 ETc to 0.25 ETc*

1.2. Cost evaluation trial

The cost evaluation trial was started during 1987 to find out the expenses incurred towards various inputs, farm practices and irrigation. The treatment contains irrigated and unirrigated trees of RRIM 600 and the irrigated trees were divided into two parts according to soil depth. Among them, one being maintained under reduced irrigation of 0.2 ETc (deep soil) and another under 1.0 ETc (shallow soil) level of irrigation. The block yield, total latex volume and DRC percentage were recorded. The reduced level of irrigation was further reduced from 1/4th to a minimum level of 1/5th with an aim to find out the optimum irrigation requirement for mature trees in good soil depth area. The

Table Dap. 2. **Effect of different depth of soil and different summer irrigation schedule on yield of rubber (g t^{-1} t^{-1})**

	Dry rubber yield 2011- 2012 (g $t^{-1}t^{-1}$)		
Months	A Control	B 1/5 ETc	C 1.0 ETc
April	29.5	28.8	22.4
May	20.5	20.5	16.8
June	25.3	24.6	20.1
July	27.6	28.3	26.5
August	26.0	30.5	22.6
September	28.8	28.9	20.2
October	30.0	31.7	23.0
November	49.2	27.6	19.5
December	57.6	38.2	21.2
January	64.4	32.7	22.7
February	30.6	30.3	17.2
Mean	35.4	29.3	21.1
SE+	4.4	1.4	0.8
SD	14.6	4.5	2.8

results (Table Dap. 2) showed a better summer as well as annual yield under the reduced level of irrigation (1/5th ETc) in deep soil area (31.5, 29.3 g $t^{-1}t^{-1}$) than the higher level of irrigation (1.0 ETc) (20.0, 21.1 g $t^{-1}t^{-1}$) in shallow soil area.

2. Latex harvest technology (LHT)

In order to study the optimum stimulation schedule for maximum latex harvest from low yielding regenerated bark, one demonstration trial on CUT (controlled upward tapping) was initiated. The study was initiated during 2009 in BI-1 panel (regenerated bark) of RRII 105 planted in 1983 with randomized block design. The objective is to identify the best CUT practice suitable to this region. Treatments comprised of S/4 and S/3 upward tapping cuts with periodic panel change of S/2 basal panel tapping (during monsoon months) under d3 frequency of tapping and different levels of stimulation. Yield recording of all tapping days, monthly DRC% (one week before stimulation), annual TPD % and recording of girth increment were also carried out.

Results show that S/3U d3 6d/7 system with periodic panel change recorded significantly highest yield both under upper and basal panels over all other treatments (Table Dap. 3).

3. Crop improvement

The thrust areas of the Station were development of drought tolerant clones, screening of wild *Hevea* accessions and RRII 400 series clones for drought tolerance, ortet selection and selection from half sib progeny of prepotent clones. The clone evaluation trial started in 1985 to evaluate growth and yield performance of 15 modern clones got completed.

3.1. Ortet selection

The results of the large scale evaluation of 14 ortets started in 2008 indicate OS 135 as the tallest and OS 36 as having higher girth among the ortets studied. The detailed growth characteristics are furnished in the Table (Dap. 5).

3.2. Germplasm screening

The experiment on screening of wild *Hevea* accessions for drought tolerance under Dapchari conditions was continued. The Mata Grosso accessions were found superior in growth characters than those from Randonia and Acre provinces. Based on their field performance over four years, about twenty five accessions have been identified to have drought tolerance.

Table Dap. 3. **Yield response of clone RRII 105 to Upward Tapping and basal tapping (April 2011 - March 2012)**

Treatments	CUT			Normal Tapping		
	g $t^{-1}t^{-1}$	kg $tree^{-1}$	kg ha^{-1}	g $t^{-1}t^{-1}$	kg $tree^{-1}$	kg ha^{-1}
T1- S/2 d3 6d/7, S/4U d3 6d/7 ET 5% La (3 w)	58.1	3.9	1557	44.1	1.5	598
T2- S/2 d3 6d/7, S/3U d3 6d/7 ET 5% La (3 w)	66.8	4.5	1792	44.1	1.5	600
T3 - S/2 d3 6d/7, S/4U d3 6d/7 ET 5% La (m)	53.6	3.6	1436	39.1	1.3	531
T4- S/2 d3 6d/7, S/3U d3 6d/7 ET 5% La (m)	55.1	3.7	1477	38.8	1.3	527
SE +		7.04		4.62		
CD (P=0.05)		NS		NS		

Table Dap. 5. **Growth performance of different ortets with control clones under Dapchari condion**

Treatments	Girth (cm) 2011-2012	Height (cm) 2011-2012	% yellowing February 2012
OS 1	12.5	436.8	4.6
OS 8	10.9	390.4	8.5
OS 34	14.2	484.4	5.5
OS 35	15.1	500.0	8.2
OS 36	15.8	485.4	13.8
OS 37	14.6	498.0	6.8
OS 42	13.8	419.4	10.1
OS 111	15.3	493.7	6.1
OS 135	14.6	480.7	4.1
OS 136	12.9	419.5	9.7
OS 173	15.3	483.0	3.9
OS 2016	15.0	487.7	3.8
OS 236	15.1	475.8	9.4
OS 317	14.7	469.7	5.3
RRII 105	11.3	304.8	9.0
RRII 208	15.2	448.7	4.3
RRII 430	13.7	475.3	9.0
RRIM 600	15.5	497.4	7.0
SE+	1.0	18.7	2.9
CD	2.1	53.7	NS

3.3. Evaluation of selected wild *Hevea* accessions

Three experiments comprising of 25, 47 and 11 selections are being evaluated. The 25 selections planted for the evaluation trial that was started in 2007 displayed wide variability for all the characters studied. The other two trials were started in 2010 with 47 and 11 wild accessions. Recording of growth parameters is being continued.

3.4. Clonal nursery evaluation

Four clonal nursery evaluation trials are in the initial stage to evaluate half sib progeny, polycross and half sib progeny of prepotent clones of pipeline clones. Experiment on identification of reliable juvenile and mature characteristics for clone identification in *Hevea* (50 divergent clones) is being continued. Standardization of distinctiveness, uniformity and stability (DUS) testing norms for evolving specific guidelines is being continued.

4. Crop Management

The experiment to find out the effect of vertical mulching and Kaoline 6% spray on growth and yield of clone RRII 105 with rainfed as control was in progress. The results of the experiment did not indicate any significant difference among the treatments in terms of growth.

REGIONAL RESEARCH STATION DHENKANAL, ORISSA

The Station in Dhenkanal district of Odisha, represents dry sub humid climate. The Station continued its research activities with the particular objective of identifying clones suited to drought prone conditions.

1. Crop improvement

Five clone evaluation trials, including evaluation of clones and of polyclonal population are in progress. The trials were laid out to identify most adapted and highly yielding clones under the dry sub humid climate.

1.1. Clone evaluation

In the 1987 experiment, RRIM 600 (74.4 cm) recorded significantly higher mean girth over

RRII 105 and GT 1. The elite clone RRIM 600 also recorded highest mean yield (37.3 g t^{-1} t^{-1}) while GT 1 recorded the lowest yield (30.6 g t^{-1} t^{-1}). The clone RRIM 600 was found superior in terms of both adaptability and yield.

In another clone trial (1990), clones such as SCATC 93/14 (84.2 cm), SCATC 88/13 (80.7 cm) and RRII 208 (78.5 cm) showed superior girth. RRII 208 showed overall superior performance especially in terms of yield (68.2 g t^{-1} t^{-1}) followed by SCATC 88/113 (67.6 g t^{-1} t^{-1}) and RRIM 600 (55.7 g t^{-1} t^{-1}), while SCATC 93/114 (33.6 g t^{-1} t^{-1}) and RRII 300 (44.9 g t^{-1} t^{-1}) recorded poor yield.

Table Ori. 1. **Mean girth and yield of elite clones**

Clone	Girth (cm)	Yield (g t^{-1} t^{-1})
RRII 105	69.5	34.6
RRIM 600	74.4	37.3
GT 1	76.5	30.6
Mean	73.4	34.0
CD(P=0.05)	9.61	5.58

In another experiment trial (1991), the performance of *Hevea* clones with polyclonal seedlings were compared. Clones GT 1(89.1 cm) and RRII 208 (85.1 cm) showed superior growth. However, polyclonal seedlings with a mean girth of 101.1 cm exhibited better growth and adaptability. RRII 208 recorded higher mean yield (81.4 gt^{-1} t^{-1}) followed by PR 255 (63.5 g t^{-1} t^{-1}) and RRIC 102 (61.4 g t^{-1} t^{-1}). In this trial also, RRII 208 exhibited higher growth, yield and adaptability in the region. GT 1 showed lowest yield of 44.3 g t^{-1} t^{-1} among the clones (Table Ori. 2).

In modern clones trial (1999-00), higher mean girth was recorded in RRII 300 (58.4 cm), while the IRCA 109 (32.7 cm) recorded the lowest. Highest initial mean annual yield was observed in RRII 351 (27.4 g t^{-1} t^{-1}) followed by RRIM 600 (22.3 g t^{-1} t^{-1}) and RRII 300 (22.2 g t^{-1} t^{-1}) (Table Ori. 3).

Table Ori. 2. **Performance of various clones**

Clone	Girth (cm)	Mean yield (g t^{-1} t^{-1})
RRII 5	80.6	48.3
RRII 105	78.9	57.5
RRII 208	85.1	81.4
RRII 300	83.7	47.1
RRIC 102	82.2	61.4
RRIM 600	76.0	53.8
GT 1	89.1	44.3
PR 255	83.2	63.5
PR 261	78.1	45.2
Polyclonal	101.1	55.8
Mean	83.8	55.9
CD(P=0.05)	8.66	16.28

Table Ori. 3. **Performance of various modern clones**

Clone	Girth (cm)	Mean yield (g t^{-1} t^{-1})
RRII 5	34.6	10.5
RRII 105	35.4	15.0
RRII 208	41.0	16.3
RRII 300	58.4	22.2
RRII 351	48.2	16.8
RRII 352	50.0	27.4
RRII 357	48.1	19.2
RRII 28/59	49.9	18.5
RRIM 600	52.8	22.3
IRCA 109	32.7	13.4
IRCA 111	42.0	18.1
Mean	44.9	18.2
CD (P=0.05)	7.12	4.10

1.2. Polyclonal trial

To evaluate the growth and yield performance and adaptability of polyclonal seedlings in Orissa conditions, a trial was laid out in 1989. Highest annual mean yield was recorded in O 4 (134.6 g t^{-1} t^{-1}) followed by O 6 (112.7 g t^{-1} t^{-1}). Ten elite polyclonal trees

were selected which were multiplied for further field evaluation and recommendation. Polyclonal population showed promising performance in the region.

1.3. Ortets evaluation

Ten ortets with few modern clones were field planted for further evaluation during 2008. Ortets such as OR 4 (23.4 cm), OR 8 (22.8 cm) and the clone SCATC 93/14 (20.5 cm) showed comparatively better preliminary growth.

2. Latex harvest technology (LHT)

2.1. Controlled upward tapping trial

Preliminary observations in clone RRIM 600 indicated that S/3 d2 upward tapping system with monthly stimulation of 5% and periodic panel change of basal panel with 2 rounds of stimulation per year showed maximum mean annual yield (Table Ori. 4)

Ori. 4. **Yield performance under controlled upward tapping**

Treatment	Mean yield (g $t^{-1}t^{-1}$)
1. S/2 d/2 ET 2.5%, S/4 d/2U ET 5% (3 w)	60.0
2. S/2 d/2 ET 2.5 % 2/y, S/4 d/2U ET 5% (m)	65.1
3. S/2 d/2 ET 2.5 % 2/y, S/3 d/2U ET 5% (m)	82.3
4. S/2 d/2 ET 2.5% 2/y, S/4 d/2U ET 5% (6w)	79.5
Mean	71.7
SE	3.79

REGIONAL RESEARCH STATION PADIYOOR, KERALA

The Station continued the research programs for identification of clones suited to the region and evaluation of clonal tolerance to drought and disease incidence. Field trials on agro-management practices for reduction of the gestation period in rubber are also in progress.

1. Crop management

1.1. Water requirement studies

The experiment on irrigation in immature rubber with irrigation levels at IW/CPE ratio of 0.3, 0.6, 0.9, 1.2 and an unirrigated control was continued into the mature phase. No significant increases in girth and girth increment between the treatments were observed. Observations recorded during the first year of tapping showed no significant difference with respect to summer yield.

1.2. Response to applied fertilizers in high yielding clones

The experiment laid out with three clones (RRII 105, RRII 414, RRII 429) and four fertilizer levels (30:30:20, 60:30:20, 90:60:40 and 120:60:40 kg ha^{-1} of N, P_2O_5 and K_2O) indicated no significant response in growth of rubber(Table Pad. 1).

Table Pad. 1. **Effect of fertilizer on growth of rubber**

Treatments	Girth (cm)		
	RRII 105	RRII 429	RRII 414
30:30:20	51.3	44.1	52.4
60:30:20	46.1	48.7	53.8
90:60:40	44.9	43.6	52.6
120:60:40	50.0	45.8	54.1
CD (P=0.05)	4.81		

1.3. Water consumption by rubber nurseries

Water consumption was observed to be higher for root trainer plants compared to polybag plants grown in polyhouses. Root trainer plants consumed 41.3 L per plant from seeding to two-whorl stage for a total duration of 282 days while the water use for a polybag plant was 32.3 L per plant for a total duration of 285 days. Polybag plants raised under open conditions consumed 35.9 L per plant through irrigation alone for total growth duration of 317 days.

2. Crop improvement

2.1. Large scale evaluation of clones

In the clone evaluation trial planted in 1996, PB 330 and IRCA 130 was found significantly superior to RRII 105 with respect to girth (Table Pad. 2). Mean annual yield and summer yield of IRCA 130 was significantly higher than that of RRII 105.

Table Pad. 2. **Growth performance of modern *Hevea* clones**

Clone	Girth (cm) 15th yr	Girth (cm) 16th yr	Mean annual yield (4th year g $t^{-2}t^{-1}$)	Summer yield (5th year g $t^{-2}t^{-1}$)
RRII 105	61.9	63.3	56.4	46.9
PB 314	62.5	63.9	45.1	37.5
IRCA 130	66.6	69.3	74.3	65.6
PB 28/59	61.7	63.3	50.2	43.2
IRCA 109	59.6	60.9	36.4	36.0
PB 330	68.0	69.8	49.7	37.8
IRCA 18	66.4	68.1	42.9	32.6
RRIM 703	55.3	56.9	37.7	30.8
IRCA 111	59.9	61.5	36.8	35.3
PB 255	62.5	63.5	63.5	52.5
IRCA 230	62.9	64.3	42.9	35.5
CD (P = 0.05)	4.79	4.72	12.3	14.2

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

In the 1996 field trial started under high altitude conditions (974 m MSL), significant superiority with respect to girth was shown by clones RRII 203 and RRIC 100 and the selections P 270, P 213 and Iritty over clone RRII 105. (Table Pad. 3). Yield of RRII 203 was found to be on par with that of PB 86, RRIC 100, and P 270 and was significantly superior to RRII 105.

Table Pad. 3. **Growth and yield in high altitude area**

Clone	Girth (cm)	Yield ($gt^{-1}t^{-1}$)
P 296	57.0	24.3
RRII 105	47.5	33.1
RRII 203	66.4	57.7
P 90	51.9	20.1
P 270	64.0	41.2
P 280	54.7	13.0
P 2	50.3	18.7
RRIC 102	47.2	15.2
PB 86	51.0	46.6
RRIC 100	64.1	41.5
P 121	48.8	16.6
P 213	60.2	32.0
P 1	57.0	23.4
P 155	46.6	18.4
Iritty	59.8	31.2
CD (P = 0.05)	12.7	23.1

HEVEA BREEDING SUB-STATION KADABA, KARNATAKA

The major thrust areas of research in the Station are to evaluate clones under different biotic and abiotic stress conditions and to identify clones suitable for commercial cultivation. Brief account of the progress of research projects is given below.

1. Small scale trials of selected ortet clones (1988A, 1988B and 1988C)

Three trials are in progress. All the trials were planted along with popular clones as controls in 1988 with the objective to evaluate selected ortet clones.

In trial 1988A, 15 ortet clones and three control clones are under evaluation. The ortets are: T 2, C 1/2, C 42, O 17, C 70, O 15, O 41, O 34, O 47, O 44, O 46, C 7/2, O 50, O 45 and O 19 and the control clones are: GT 1, RRIM 600 and RRII 105. Regular data collection on growth and other secondary characteristics was continued. Tapping was started in 2002. Mean yield data over nine years of tapping Table Kad. 1 indicated T2 to be the highest yielder (74.0 g t^{-1} t^{-1}) closely followed by the ortets *viz.* O 17 (68.0 g t^{-1} t^{-1}) and O 15 (63.8 g t^{-1} t^{-1}). Yield of control clones GT 1, RRII 105 and RRIM 600 were 64.6, 47.6 and 46.4 g t^{-1} t^{-1}respectively. With regard to growth, ortet O 47 recorded highest girth (142.2 cm) followed by T 2 (112.9 cm), O 17 (110.6 cm) and O 15 (102.9 cm). Though O 47 had highest girth (142.2 cm), its yield was only 54.4 g t^{-1} t^{-1}.

Among the 16 ortet clones and three control clones namely RRII 105, RRIM 600 and GT 1 under 1988B evaluation trial clones such as T1 (72.4 g t^{-1} t^{-1}) and GT 1 (69.5 g t^{-1} t^{-1}) were high yielders (nine years data).

Table Kad. 1. **Growth and yield performance of clones in the small scale ortet trial (1988A) over nine years of tapping (2002-2011)**

Clone	Girth (cm) (24 years)	Mean yield over nine years oftapping (g/t^{-1}/t^{-1})
T 2	112.9	74.0
O 17	110.6	68.0
GT 1	82.7	64.6
O 15	102.9	63.8
C 1/2	90.3	57.8
C 42	102.7	57.7
O 41	89.8	57.0
C 70	91.4	55.0
O 47	142.2	54.4
O 34	87.7	51.5
RRII 105	77.3	47.6
RRIM 600	74.3	46.4
O 44	78.1	37.4
C 7/2	79.1	35.6
O 50	68.1	32.0
O 46	87.4	30.3
O 45	82.6	27.1
O 19	72.1	24.2
CV (%)	11.6	16.4
SE (±)	6.1	4.6
CD (P = 0.05)	17.5	13.3

The 1988C trial consisting of 14 ortet clones and three control clones *viz.*, RRII 105, RRIM 600 and GT 1, GT 1 recorded maximum yield (84.0 g t^{-1} t^{-1}) followed by ortet O 55 (75.6 g t^{-1} t^{-1}) and C 140 (75.5 g t^{-1} t^{-1}). Control clones RRII 105 and RRIM 600 yielded 63.9 and 31.8 g t^{-1} t^{-1} respectively. Other ortets with notable yield are O 49 (69.6 g t^{-1} t^{-1}), O 26 (65.0 g t^{-1} t^{-1}) and O 11 (63.8 g t^{-1} t^{-1}).

2. Large scale clone trial 1989

This trial started in 1989 with 14 clones for evaluation. Maximum yield was found in clone RRII 203 (67.2 g t^{-1} t^{-1}) followed by KRS 25 (56.2 g t^{-1} t^{-1}). Check clones RRII 105 and RRRIM 600 yielded 46.3 and 32.7 g t^{-1} t^{-1} respectively.

3. Large scale clone trial 1990A

In this experiment started in 1990 with 15 clones, maximum yield was recorded in clone PB 260 (65.2 g t^{-1} t^{-1}) followed by PB 235 (62.3 g t^{-1} t^{-1}) and HP 372 (59.6 g t^{-1} t^{-1}). Tjir 1 was the lowest yielder (21.2 g t^{-1} t^{-1}) (Table Kad. 2).

Table Kad. 2. **Growth and yield performance of clones in the large scale clone trial (1990A)**

Clone	Girth (cm) 22 years	Mean yield over eight years of tapping (g t^{-1} t^{-1})
PB 260	88.6	65.2
PB 235	92.5	62.3
HP 372	98.3	59.6
PB 311	81.9	55.9
HP 223	94.4	55.4
PB 217	81.8	55.4
RRII 105	75.6	50.9
GT 1	81.9	48.9
HP 185	81.3	32.9
Mil 3/2	84.3	32.8
Gl 1	70.2	29.6
HP 187	78.1	27.8
Hil 28	76.7	26.3
HP 204	71.3	23.7
Tjir 1	71.6	21.2
CV (%)	4.1	9.9
SE (±)	1.9	2.5
CD (P<0.05)	5.5	7.2

4. Estimation of genetic parameters (1990B)

This trial was started in 1990 to evaluate the parents and their progenies for estimating genetic parameters that are essential for planning plant breeding activities. Twelve clones and their progenies are under evaluation. Up on completion of eight years of tapping, parent clones PB 235, RRII 203 and RRII 105 were found high yielding with an yield of 79.2, 55.7 and 50.1 g t^{-1} t^{-1} respectively. Among the progenies, half-sibs of RRII 203, GT 1 and PB 235 recorded 45.6, 43.7 and 43.5 g t^{-1} t^{-1} respectively. Progenies of PB 86, PB 213 and Tjir 1 were found low yielders (29.9, 26.9 and 23.6 g t^{-1} t^{-1} respectively).

5. Small scale clone trials of popular clones (1991A, 1991B and 1991C)

A total of 54 trial clones and three control clones are under evaluation in three trials to compare the growth and yield performance of trial clones *vis-à-vis* control clones *viz.*, RRII 105, GT 1 and RRIM 600. The trials have completed eight years of tapping.

In the trial 1991A containing 36 of both indigenous and exotic clones maximum yield was found in clones, PB 235 (77.6 g t^{-1} t^{-1}), PB 280 (76.8 g t^{-1} t^{-1}), PB 314 (76.7 g t^{-1} t^{-1}), PB 312 (72.6 g t^{-1} t^{-1}) and PB 311 (66.5 g t^{-1} t^{-1}). Clones, clones such as AVROS 352 (17.9 g t^{-1} t^{-1}) and CH 4 (13.8 g t^{-1} t^{-1}) were found low yielders.

Thirteen clones are under evaluation in trial 1991B. In this trial Clone RRII 5 gave maximum yield (69.5 g t^{-1} t^{-1}) followed by RRII 3 (60.5 g t^{-1} t^{-1}).

Table Kad. 3. **Growth and yield performance of clones in the large scale clone trial (2000)**

Clone	Girth (cm) 12 years	Mean yield over three years of tapping (g $t^{-1}t^{-1}$)
RRII 414	72.2	71.1
RRII 430	70.6	63.5
RRII 422	48.3	59.2
RRII 429	62.4	56.6
RRII 403	54.8	45.7
RRII 105	58.8	39.4
RRII 407	60.3	36.8
RRIC 100	66.4	33.5
CV (%)	6.8	16.3
SE(±)	2.4	4.8
CD (P<0.05)	7.4	14.5

In the third trial (1991C) containing 13 clones such as HP 83/224 (67.4 g t^{-1} t^{-1}) and PB 28/59 (56.6 g t^{-1} t^{-1}) were found high yielding during the first seven years of tapping.

6. Large scale clone trial 2000

Among the 8 clones under evaluation, in this trial, maximum yield was found in clones such as RRII 414 (71.8 g t^{-1} t^{-1}), RRII 430 (65.6 g t^{-1} t^{-1}) and RRII 422 (59.7 g t^{-1} t^{-1}). Parent clones RRII 105 and RRIC 100 yielded 41.3 g t^{-1} t^{-1}. Mean yield over three years of tapping is given in Table Kad. 3.

HEVEA BREEDING SUB-STATION THADIKARANKONAM, TAMIL NADU

Crop improvement activities like evaluation of modern popular clones under agro-climatic conditions of Kanyakumari, hybridization and clonal selection, generation of new cultivars by poly-cross approach and evaluation of new pipeline clones under the mega project 'Participatory Clone Evaluation' were given thrust at the Station. Improvement of plant propagation techniques in *Hevea* was also pursued actively during this period.

1. Crop improvement

1.1. Clone evaluation

Performance of 30 high yielding popular clones is being evaluated under nine large scale clone evaluation experiments. In the large-scale clone evaluation experiment initiated at Keeriparai (1994), the pooled data for 11 years of tapping is furnished in Table Par. 1.

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

Clone	Yield (g t^{-1} t^{-1})		
	2010-11	2011-12	Mean over 11 years
PB 314	94.3	94.2	77.4
IRCA 130	79.6	135.1	73.7
PB 28/59	63.8	78.8	59.7
IRCA 109	88.7	99.4	78.8
PB 330	88.7	92.7	61.9
IRCA 18	77.4	82.7	64.8
RRIM 703	47.1	121.7	67.0
IRCA 111	81.4	110.5	77.5
PB 255	90.3	131.6	86.2
IRCA 230	70.0	110.8	64.2
RRII 105	62.0	103.9	62.1
G. mean	76.7	105.6	70.3
CD (P≤ 0.05)	21.1	–	19.8

Evaluation of 11 clones (10 introduced from Malaysia and Cote d' Ivoire from 1963 to 1991) showed that PB 255 (86.2 g t^{-1} t^{-1}) continued to exhibit significantly higher yield than the control clone RRII 105 (62.1 g t^{-1} t^{-1}). Among the five clones introduced from Cote d' Ivoire in 1991, three clones *viz.*, IRCA 109 (78.8 g t^{-1} t^{-1}), IRCA 111 (77.5 g t^{-1} t^{-1}) and IRCA 130 (73.7 g t^{-1} t^{-1}) exhibited promising yield up to the eleventh year of tapping. The consistently high yield trend exhibited by PB 314 (77.4 g t^{-1} t^{-1}) was masked by the high incidence of TPD (52.0%). The mean yield for 11 years of tapping on the block evaluation experiment (1994) is presented in Table Par. 2.

Table Par. 2. **Mean yield of block trial (1994)**

Clone	Yield (g t^{-1} t^{-1})		
	2010-11	2011-12	Over 11 years
RRII 5	59.2	67.3	46.9
RRII 50	55.1	69.3	47.4
RRII 51	60.1	80.5	42.5
RRII 105	118.9	105.3	69.1
RRII 176	59.1	64.3	45.9
RRIC 102	63.9	93.5	55.6
PB 217	56.6	71.6	50.4
PB 235	51.2	76.6	56.1
PB 260	72.3	91.7	54.8
PB 311	64.3	71.4	60.0
PB 28/59	71.7	95.1	62.5
PR 255	74.7	80.7	53.7
PR 261	64.8	63.8	50.1
Mean	67.05	79.32	53.44
SE	3.71	3.86	4.04

Out of the 13 modern popular clones being evaluated in this trial, RRII 105 (69.1 g t^{-1} t^{-1}) continued to occupy the first position with respect to yield and the gap between RRII 105 and rest of the clones apparently widened as the age of tapping progressed. The regional popularity enjoyed by PB 28/59 (62.45 g t^{-1} t^{-1}) was fully justified by the high yield performance of this clone in comparison with some of the RRII clones like RRII 51 (42.5 g t^{-1} t^{-1}), RRII 176 (45.8 g t^{-1} t^{-1}), RRII 5 (46.9 g t^{-1} t^{-1}) and RRII 50 (47.4 g t^{-1} t^{-1}).

In the observational trial initiated at Vaikundam Estate (2000), all hybrid clones, except RRII 414 and RRII 427 exhibited better yield than RRII 105 (Table Par. 3).

Table Par. 3. **Mean yield of observational trial at Vaikundam (2000)**

Sl. No.	Clone	Yield (g t^{-1} t^{-1})		
		2010-11	2011-12	Mean over 7 years
1	RRII 422	95.8	69.1	62.5
2	RRII 414	67.4	53.6	52.5
3	RRII 105	89.1	75.4	56.2
4	RRII 417	96.1	65.5	60.1
5	RRII 430	85.1	54.9	57.7
6	RRII 429	67.2	61.7	53.0
7	RRII 427	63.6	65.7	49.5
	Mean	80.59	63.71	55.92

In the trial entitled *'GxE Interaction of selected Hevea clones'* initiated at New Ambadi Estate (1996), RRII 105 exhibited better yield than the hybrid clones (Table Par. 4). Among

Table Par. 4. **Mean yield of GxE interaction trial (1996)**

Clone	Yield (g t^{-1} t^{-1})		
	2010-11	2011-12	Mean over 10 years
RRII 414	59.9	34.5	46.1
RRII 417	73.5	37.2	50.3
RRII 422	75.9	43.3	51.2
RRII 429	57.2	44.6	41.9
RRII 430	75.0	37.6	53.0
RRII 51	59.5	26.7	37.5
RRII 176	75.2	62.1	45.6
RRII 203	88.2	54.8	59.5
RRIC 100	61.1	36.9	44.7
PB 217	65.8	48.8	39.4
RRIM 600	75.3	43.1	44.5
RRII 105	78.9	55.3	58.1
Mean	70.44	43.74	47.63
CD	NS	NS	NS

the hybrid clones, belonging to the 400 series, RRII 430 exhibited promising yield both at Ambadi and Vaikundam. The yield performance of RRII 414 was found not to commensurate with its performance reported from other traditional belt.

Yield performance of hybrid clones belonging to 400 series exhibited wide variations in two adjacent estates in the Kanyakumari region. So, in order to have an depth study on the performance of these clones, five on-farm trials were initiated representing five different micro-climates in this region. One block trial each initiated at Velimalai Estate (2002) and New Ambadi Estate (2003) were opened for regular tapping during 2011.

1.2. Hybridization and clonal selection

The breeding orchard at Paraliar was maintained by periodical pruning and pollarding of branches. The hybrids developed from hand pollination attempts (2011) were subjected to test tapping. Another batch of 14 promising selections from earlier experiments was field planted at Vellambimalai during the reporting period.

2. New generation polyclonal seed garden

A polyclonal seed garden consisting of nine modern clones as parents was established in an area of 9 ha. of land during 2000 at New Ambadi Estate, Maniankuzhy was well maintained and the polycross seeds collected during 2011 were raised at HBSS, Paraliar for preliminary evaluation. The plants raised at Paraliar (2010) were test tapped during 2011 and the potential high yielders were cut back for further evaluation. The polycross plants transplanted at Nagamalai (Isfield Estate) were monitored for high yield and promising secondary characters.

3. Participatory clone evaluation experiment

Observations on juvenile growth were recorded from the "On farm trial of pipeline clones" (2008) initiated at Tharuvaiyar and Vithura. Vacancy filling was carried out in two new OFTs at Bethany Estate during 2010. Planting materials were raised at Paraliar to initiate another OFT under the same project at Bethany Estate for planting during 2012.

4. Root trainer planting technique

In the field trial initiated at Churulacode (2002), root trainer plants continued to exhibit relatively better yield (54.1 g t^{-1} t^{-1}) than polybag plants (48.7 g t^{-1} t^{-1}). Root trainer plants at Kanjirappally (2005) exhibited significantly better growth than polybag plants and the trial was opened for regular tapping in January 2012. In connection with field experiment "Comparative evaluation of advanced planting materials produced by different propagation techniques" started at Bethany Estate during 2010, advanced planting materials were raised in root trainers in six regional nurseries of Rubber Board on experimental basis. Action has been initiated to establish three demonstration plots of root trainer plants in all the three zones in the traditional belt during 2012.

Training was imparted on root trainer planting technique to the officials and workers of six regional nurseries of Rubber Board, three estates in the private sector, five batches of small growers and nursery owners and one batch of delegates from Myanmar during the current reporting period.

LIBRARY AND DOCUMENTATION CENTRE

During the year, 112 books were added to the stock of the library. The library subscribed 45 foreign journals and 74 Indian journals. About 39 other journals were also received as gift/ exchange. Literature searches from databases of Books and Journals were carried out.

Four publications *viz., Rubber Board Publications: A Catalogue; Contributions of RRII on Rubber Diseases: An Annotated Bibliography; Theses and Dissertations on Rubber: A Catalogue; and Rubber Standards in RRII Library* were brought out. Compiled and distributed the *List of Current Periodicals 2011, New Additions List 2011* and two issues of *Documentation List*. Databases were updated by adding bibliographic details of 90 books and 403 journal articles. During the period library had arranged the distribution of 717 numbers of Press Clippings and 1646 numbers of other SDI bulletins.

Distribution of 428 copies of the RRII journal *Natural Rubber Research* and 574 numbers of RRII Annual Reports were also made by the library along with the sale and distribution of 462 numbers of RRII publications. Photocopies of about 52747 numbers of information materials were provided during this period.

AGROMETEOROLOGY

1. Climate resource characteristics of rubber growing tracts

Temporal and spatial variability in the long term precipitation series was studied using representative data from north to south of Kerala from 1901-1996. The study indicated consistency in the pattern and amount of rainfall received in northern districts, shift in the rainfall peaks in southern parts, increase in the summer rainfall across the State and decrease in the October rainfall in southern districts.

Initial (W) and conditional (W/W, W/D) probabilities of weekly rainfall of >10 mm, >20 mm, >30 mm, >40 mm and >50 mm at RRII for a period of 54 years (1957-2010) have been calculated using Markov Chain Model. Initial probability of getting >10 mm rainfall during 6th standard week (Feb 5-11) was only 11%. But if 6th week was wet the probability of becoming 7th week (Feb 12-18) also wet was 67%. Likewise the initial probability of receiving >10mm rainfall during 9th week (26th - 4th March) was 19 %. But if the 9th week was wet, the probability of 10th week also becoming wet was 60 %. Initial and conditional probabilities for more than 10mm rainfall per week was found to be more than 50% from 13th week (26 -1 April) and more than 75% from 16th week (April 16-22) to 22nd standard week (28-3 June). From 23rd standard week to 32nd week, W and W/W lies between 75 to 100 % for weekly rainfall of 10 mm to 50 mm. Mid season break in the rainfall series was observed from 36th week, up to 40th week. Thereafter the probability increases from 40th week to 45th week and further declines.

To find out the change in the reference crop evapotranspiration (ETo) with the established rise in temperature (T_{max}) of 2^{0} C

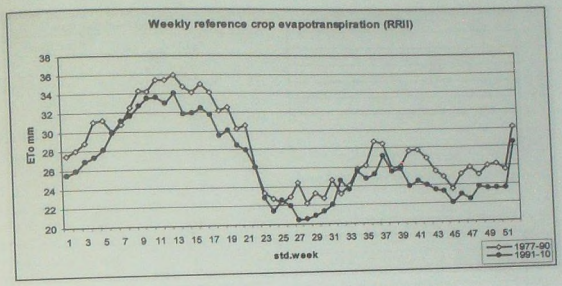


Fig. Agromet. 1. **Weekly reference crop evapotranspiration (RRII)**

during the recent years, weekly ETo at RRII from 1977-2010 has been calculated by modified Penman-Monteith equation. The study indicated evaporation paradox in humid region. Annual ETo declined from 1500 mm to 1350 mm at RRII and the rate of decline was 5 mm yr^{-1}. Pan evaporation declined at a rate of 9 mm yr^{-1}over the years.

2. Climate change studies

The minimum temperature regime during winter (December – February) at RRII for the period 1960-90 and 1991-2010 was analysed. Time series data of mean winter temperature during the base line period (1960-90) showed an upward trend at the rate of 0.02° C. A downward trend was observed during the later half which clearly indicated a shift in winter.

3. Climate resource characteristics of rubber growing tracts –NE region

The Potential Evapotranspiration (PET in mm day^{-1}) was calculated by the Modified Penman method (Frere and Popov, 1979) on a monthly basis by utilizing the climatic variables recorded in RRII, Kottayam and RRS, Agartala. The mean annual and monthly fluctuations in PET values (Fig. Agromet. 2a and 2b) were higher in Agartala than Kottayam. In Agartala, it was below 3.0 mm day^{-1} during the cold period of December and January. In Kottayam the mean monthly PET values were 4.0 mm day^{-1} during the monsoon season while it was above 4.0 mm day^{-1} for Agartala.

Significant negative trends were observed during the pre-monsoon, early monsoon months and in November for both Agartala and Kottayam (Table Agromet. 1). An annual decreasing rate of 19 and 11 mm day^{-1} in 100 years were noted in Agartala and Kottayam respectively. No significant changes were noted during the winter period of January and February for both locations.

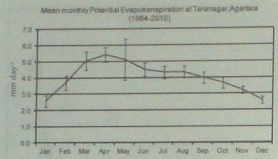


Fig. Agromet. 2a. **Annual variations in PET – Agartala, Tripura**

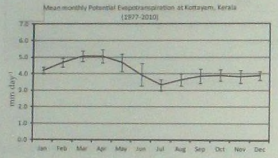


Fig. Agromet. 2b. **Annual variations in PET – Kottayam, Kerala**

4. Forewarning of pests and diseases (traditional region)

4.1. Weather and Abnormal Leaf Fall (ALF)

Analysis of the leaf fall data in Padiyoor, Kannur was carried out for the period 2009-11 for four clones RRII 105, RRIM 600, PB 235 and PB 5/51. During the infestation of ALF, it was observed that previous consecutive 5-day mean maximum temperature, minimum temperature, sunshine hours and rainfall were consistently related to the sudden onset of leaf fall for all the clones.

Table Agromet. 1. **Slope and R^2 of the PET trends in Agartala and Kottayam**

	Agartala			Kottayam	
	Slope	R^2		Slope	R^2
Jan	-0.033	0.01	Jan	0.002	0.01
Feb	-0.026	0.01	Feb	0.007	0.08
Mar	-0.039	0.08	Mar	0.009	0.08
Apr	-0.014	0.08	Apr	-0.026	0.41**
May	-0.014	0.40**	May	-0.043	0.65**
Jun	-0.013	0.65**	Jun	-0.046	0.47**
Jul	-0.008	0.47**	Jul	-0.011	0.10
Aug	-0.014	0.10	Aug	0.014	0.14
Sep	-0.011	0.14	Sep	0.006	0.02
Oct	-0.027	0.02	Oct	-0.015	0.20*
Nov	-0.013	0.20*	Nov	-0.025	0.41**
Dec	-0.017	0.41**	Dec	0.000	0.00
Annual	-0.019	0.37**	Annual	-0.011	0.47**

** Significant at 5% level; **Significant at 1% level*

The 5-day mean meteorological parameters for three individual years are shown in Table Agromet. 2. These prevailing conditions over five days coincided with the trigger of leaf fall on the sixth day in all the three years. When the favourable conditions remained for a longer duration, the leaf fall also was high throughout the period. However, the relationship with antecedent weather conditions as given in Jayaretnam *et al.*, (1987) with a lead of 9-15 days could not be clearly established in Padiyoor for the different clones.

Table Agromet. 2. **Five day mean values and range of meteorological parameters conducive to abnormal leaf fall for each year from 2009 to 2011**

	2009		2010		2011	
	Mean	Range	Mean	Range	Mean	Range
Maximum temperature (°C)	28.7	27.6-32.0	28.4	27.0-30.0	29.7	29.5-30.5
Minimum temperature (°C)	23.4	22.8-24.0	23.0	22.2-24.0	23.0	21.8-24.0
Rainfall (mm)	213.6	7.0-77.0	223.2	7.0-91.0	210.2	18.0-80.0
Bright sunshine hours	0.1	0.0-0.3	0.7	0.0-1.9	0.6	0.0-1.5

ANNUAL EXPENDITURE

Expenditure at a glance (2011-12)

Head of Account	Expenditure (₹ in lakhs)
Non-Plan*	
Non-Plan RRII	517.29
Projects (CES)	378.87
Total	**896.16**
Plan	
Research Scheme	2399.00
NERDS Research Component	438.20
Total	**2837.20**
Grand Total	**3733.36**

*Non-plan expense includes non-plan projects (CES)

SCIENTIFIC ADVISORY COMMITTEE RECOMMENDATIONS (2011-2012)

- Rainguard compound "Rubber stick" supplied by M/s. Unity Engineering, III/71, Kandanad, Thiruvamkulam (via), Ernakulam, for rainguarding rubber trees.
- New procedure for DRC determination through partial drying using microwave oven.
- Modified standards of planting pits for young rubber plants *viz.*,
  (a) In soils with a depth of 1 m or more, rubber can be planted in pits just sufficient to accommodate the poly bag plants.
  (b) In hard soils, larger pits will be beneficial.
- Chain saw models *viz.* Oleo Mac GS 650 C and Oleo Mac GS 44 supplied by M/s. Ratnagiri Impex Pvt. Ltd., Bengaluru, for tree felling/cutting operations in rubber plantations.
- 'Vitex' supplied by M/s. Mars Agencies Pvt. Ltd. Bengaluru as yield stimulant in rubber plantations.
- Integrated Waste Water Treatment System for the effective treatment for pollution abatement of RSS processing waste water.
- Three brush cutter models (i) Red Lands Model – RBC-35 (ii) Oleo Mac Model – 746 T and (iii) Stihl Model – FS 120, supplied by M/s. Red Lands Ashlyn Motors, Plc, Thrissur, M/s. Ratnagiri Impex Pvt. Ltd., Bengaluru and M/s. Andreas Stihl Pvt. Ltd., Pune, respectively for use as weed cutters in rubber plantations.
- Two models of mist blower *viz.* ASPEE AMB/85-H and STIHL-SR 420 supplied by M/s. Joseph Mathew Thomas & Co. Kochi for spraying oil-based COC for abnormal leaf fall and Corynespora leaf diseases in immature or mature rubber having a height up to 56 feet and 53 feet, respectively.

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GENBANK SEQUENCE SUBMISSION

Roy, C.B. and Saha, T. (2012). JQ037843: *Hevea brasiliensis* anthocyanidin 3-O-gluco-syltransferase (*ANGT1*) mRNA, complete cds

Saha, T. and Thomas, K.U. and Ravindran, M. (2012). JQ037840: *Hevea brasiliensis* caffeic acid 3- O-methyltransferase (*COMT*) mRNA, complete cds.

Saha, T., Thomas, K.U. and Ravindran, M. (2012). JQ037841: *Hevea brasiliensis* myo-inositol-1 phosphate synthase (*MIPS*) mRNA, complete cds.

Saha, T., Thomas, K.U. and Ravindran, M. (2012). JQ037842: *Hevea brasiliensis* aquaporin PIP1 (*AQP1*) mRNA, complete cds.

PATENTS

Alex, R., Sasidharan, K.K., and Jacob, J. (2011). Filed patent application for provisional specification titled "A novel process for the preparation of carbon black/silica/nanoclay master batch from fresh natural rubber latex" under application No 2564/CHE/2011(G602B) on 26/7/2011v through Ms Mohan Associates, Chennai.

Alex, R., George, K.M., George, B. and Jacob, J. (2011). Filed provisional specification titled "A new method for recovering skim rubber from skim latex" under application No 2565/CHE/2011 (G602A) on 26/7/2011 through Ms Mohan Associates, Chennai.

AWARDS

Kala, R.G., Abraham, V., Sobha, S., Suni, A.M. and Thulaseedharan, A. (2012). Influence of silver nitrate on somatic embryogenesis and *Agrobacterium* mediated genetic transformation in *Hevea brasiliensis: Second best poster award, International Conference on Advances in Biological Sciences*, 15-17 March, 2012, Kannur University, Kannur.

Kala, R.G., Anju, V., Sobha, S., Jayasree, P.K, Suni, A.M. and Thulaseedharan, A. (2012). Genetic transformation in *Hevea brasiliensis*: Effect of *Agrobacterium* strain, surfactant and antioxidants. *Best poster award National Seminar on Genomics and Genetic Engineering Strategies*, 27-28 February 2012, Periyar University, Salem.

Saha, T., Roy, C.B., Ravindran, M., Bini, K. and Thomas, K.U. (2011). Towards development of molecular markers for *Phytophthora* tolerance in rubber. *Best Poster Award, Phytophthora* 2011–*International Workshop, Seminar and Exhibition on Phytophthora Diseases of Plantation Crops and their Management* during 12-17 September, 2011, Rubber Research Institute of India, Kottayam.

Sasidharan, K.K., Alex, R., Jacob, J , Kurian, T. and Chandra, A.K. (2012). Carbon black/silica master batch from fresh natural rubber latex: Best paper presentation award. IRMRA 21st Rubber Conference on Emerging Trends in Developing Eco friendly and Energy Efficient Electrometric Material and Processing Technology, 20 -21, January, 2012, Mumbai.

TRAINING IMPARTED / RECEIVED

Das, G: Attended *Summer School*, 23 August - 12 September 2011, Department of Crop Physiology, Agriculture University, Jorhat, Assam.

Deka, H.K: Attended *Workshop, Seminar an Exhibition on Phytophthora* 2011, 12-17, September 2011, Kottayam

Mondal, G.C: Attended *HRD training Ethics and Values in Science* held at Indian School of Mines (ISM), 12 – 16, December 2011, Dhanbad, Jharkhand

Raj, A.N. *Attended Training on Project Planning, Research Methodology and Data Analysis* at RTI, Rubber Board, Kottayam from 2-4, November 2011.

Raj, A.N.: Attended *Training Programme on Commercialization Technological Innovations*, 13-17 February, 2012, Indian Institute of Plantation Management, Bangalore.

OTHERS

Varghese, S: Visited IRMRA laboratory and Tyre testing centre at Pune.

Varghese, S: Assisted JD (RT) for the preparation of curriculum Rubber Technology, ITI, Tripura.

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Agronomy / Soils Division	
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K. J. Joseph	Farm Officer
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P.M. Sebastian	Farm Officer
Rajen Mathew	Farm Officer

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P. Babuji	Farm Officer
T.V. Somaraj	Farm Officer

Rubber Technology Division

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Rosamma Alex, M.Sc., LPRI, M.Tech, Ph.D.	Deputy Director
Benny George, M.Sc., Ph.D.	Senior Scientist
Jacob K. Varkey, M.Sc., M.Tech., Ph.D	Senior Scientist
K. Mariamma George, M.Sc.	Scientist C (upto 31.08.2011)
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Tresa Cherian, M.Sc.	Junior Scientific Officer
M. Susamma Joseph, M.Sc.	Junior Scientific Officer

M.L. Geethakumariamma, M.Sc., Ph.D.	Junior Scientific Officer
C. Madheswaran, B.A.	Technical Assistant (Glass Blowing)(HG)
Technical Consultancy Division	
Siby Varghese, M.Sc., Ph.D.	Deputy Director
K.S. Mohandas, M.Sc., M.Tech.	Scientist B
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M.A. Fancy, M.Tech.	Junior Rubber Technologist (HG)
K.K. Sasidharan, M.Tech.	Junior Rubber Technologist
Reethamma Joseph MSc., Ph.D.	Junior Rubber Technologist
Mathew Joseph, B.Tech.	Junior Rubber Technologist
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K.I. Elizabeth, M.Sc., M.Tech., Ph.D.	Junior Scientific Officer
Tessy K. George	Junior Scientific Officer
Pilot Crumb Rubber Factory	
P.S. Sadeesh Babu, B. Tech., PGDBA	Scientist C
I. John Britto	Radiation Safety Officer
Economics Division	
K. Tharian George, M.A., Ph.D.	Joint Director
Toms Joseph, M.A.	Scientist C (working arrangement at H.O. w.e.f. 17.08.07)
Binni Chandy, M.A.	Scientist C
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T. Siju, M.Sc. (Ag.)	Scientist B
Joby Joseph, M.A.	Scientist B
K. Rajesh , M.A. Ph.D.	Scientist A
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M.A. Nazeer, M.Sc., Ph.D.	Joint Director
Library and Documentation Centre	
Accamma C. Korah, B.Sc., M.L.I.Sc.	Documentation Officer (HG)
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N. Latha, M.Sc., M.L.I.Sc.	Assistant Librarian (HG)
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P. Aneesh, M.Sc., P.G.D.C.A.	Statistical Inspector
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Thomas Baby, M.Sc., M.Phil., Ph.D.	Deputy Director
R. Rejikumar, M.Sc., M.Tech.	Instrumentation Officer (upto July 2011)
M.R. Anilkumar, Dip. in Inst. Tech.	Instrumentation Officer (wef 03-08-2011)
K.R. Suni	Assistant Instrumentation Officer (wef 30-12-2011)
Administration	
K. Raveendran Nair	Deputy Secretary (Admn.)
P.V. Raveendran Nair	Assistant Secretary
G. Asokan	Assistant Secretary
Sheela Peter	Section Officer

Accounts	
CA Zachariah Kurian, M.Com., FCA	Deputy Director (Finance)
Benoy Varghese, B.Sc., P.G.D.C.A., A.I.C.W.A.	Accounts Officer
R. Pushpakumari, M.Sc., I.C.W.A.	Accounts Officer
Aleyamma Varghese	Section Officer
Maintenance	
P. V. Vijayappan, Dipl. Civil Eng.	Estate Officer
T. Manoj, B.E.	Assistant Engineer (Civil)
Experiment Station at RRII	
P.J. George	Assistant Farm Manager
Thampy Andrews	Farm Officer
Security Wing	
P.V. Suresh Babu	Assistant Security Officer
Central Experiment Station, Chethackal, Kerala	
P. Mallinath Priyadarshan	Deputy Director
Mary Varghese (EOL)	Scientist S2
Jacob Abraham, B.Sc., M.B.B.S.	Medical Officer
N.K. Suresh	Medical Officer
Joseph Joseph	Assistant Director (Finance)
K.U. Saramma	Section Officer
T.R. Divakaran	Farm Manager
T.G. Sasi	Farm officer
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Front Cover Photo

Rubber area distribution of Kerala and Kanyakumari based on Satellite data

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1. Inaugural function of 24th Kerala Science Congress, 29-31 January 2012, Rubber Research Institute of India, Kottayam, India.
2. Inaugural function of International Workshop Seminar and Exhibition, on Phytophthora Diseases of Plantation Crops and their Management *Phytophthora 2011*, 12-17 September 2011, Rubber Research Institute of India, Kottayam

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Research divisions and functions

The major research divisions are Agronomy/ Soils, Biotechnology, Botany, Germplasm, Plant Pathology, Plant Physiology, Latex Harvest Technology, Rubber Technology and Economics. Studies on Clone Evaluation, Genome Analysis and DRIS Fertilisation are dealt separately.

The thrust areas of research of Agronomy/ Soils Division are investigations on the nutritional requirements of rubber, irrigation, intercropping, cover crop management, weed control and the study of the rubber growing soils. Development of tissue culture and anther culture systems for propagation and crop improvement of *Hevea* are the important areas in which the Biotechnology Division is engaged. The important fields of research of the Botany Division are breeding, evaluation and selection of new clones, propagation techniques, planting methods, anatomical studies and cytogenetic investigations. The Germplasm Division is concentrating on the introduction, conservation and evaluation of *Hevea* germplasm. The Plant Pathology Division is engaged in investigations on the diseases and pests of rubber and associated cover crops and their control. The Plant Physiology Division conducts studies on both fundamental and applied aspects of *Hevea* tree physiology. The Latex Harvest Technology Division is concentrating on all applied aspects of crop harvesting in rubber. The Rubber Technology Division concentrates on improvement in primary processing of rubber, its chemical modification, rubber product manufacture and quality control of processed rubber. The Technical Consultancy Division provides consultancy services for the promotion of the rubber industry. The Economics Division undertakes studies on economic aspects related to rubber plantations.

The research supporting sections includes Library and Documentation, Instrumentation, Statistics, Computer and Maintenance Wing. There is also a small experimental farm of 33 ha. at the headquarters of RRII.

Central Experiment Station

The 255 ha. Central Experiment Station at Chethackal (Ranni), 50 km away from Kottayam, was started in 1966. Field trials laid out by the research divisions cover almost the entire area.

Regional Research Stations

RRII has established a North-Eastern Research Complex with headquarters at Agartala having regional research stations at Agartala in Tripura, Guwahati in Assam and Tura in Meghalaya. The RRII has also set up regional research establishments at Dapchari (Maharashtra), Dhenkanal (Orissa), Nagrakatta (West Bengal), Thadikarankonam (Tamil Nadu), Kadaba (Karnataka) and Padiyoor (Kerala).

Regional soil testing laboratories have been established at Taliparamba, Kozhikode, Thrissur, Muvattupuzha, Pala, Kanjirappally, Adoor and Nedumangad. Mobile units for soil and leaf analysis are available at Kozhikode laboratory, apart from that at the headquarters.

National/International collaboration

RRII is a member of the International Rubber Research and Development Board (IRRDB), an association of national organizations devoted to research and development on natural rubber. Rubber Board is a member of the Association of Natural Rubber Producing Countries (ANRPC) and International Rubber Study Group (IRSG).

The RRII has research/ academic linkages with the Banaras Hindu University (Varanasi), Kerala Agricultural University (Thrissur), Kerala University (Thiruvananthapuram), Mahatma Gandhi University (Kottayam), Cochin University of Science and Technology (Kochi), Indian Agricultural Research Institute (New Delhi), Indian Institute of Sciences (Bangalore), Indian Institute of Technology (Kharagpur), National Chemical Laboratory (Pune), Sree Chitra Tirunal Institute of Medical Sciences and Technology (Thiruvananthapuram), Tamil Nadu Agricultural University (Coimbatore), University of Agricultural Sciences (Bangalore) and University of Goa (Goa).

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3. Rubber Technology

3. 1. Reinforcement

3.1.1. NR/ polymeric filler system

To improve the abrasion resistance of NR/PF-system, small proportion of polybutadiene rubber (BR) was incorporated in the system. Addition of BR improved the abrasion resistance without adversely affecting other vulcanisate properties. The mechanical properties based on the formulation given in Table Chem. 2 are shown in Table Chem. 3.

Table Chem. 2. **Formulation of the mixes NR/ polymeric filler system**

Ingredients	Control	A1	A4
NR	65	100	70
PB	35	0	30
PF	0	10	10
Zinc oxide	5	5	5
Stearic acid	2	1	1
HSL* quinolin	1.5	1.5	1.5
ISAFblck (N220)	30	0	0
HAF black (N330)	25	0	0
MOR **	0.8	0.8	0.8
Sulphur	2.5	2.5	2.5

**2,2,4-trimethyl – 1,2 – dihydroquinoline*
** 2-(4-Morpholinothio) benzothiazole

Table Chem. 3. **Technological properties of the vulcanisates**

Properties	Control	A1	A4
Tensile strength (MPa)	23.3	26.5	24.21
Elongation at break (%)	549	610	586
Modulus, 100 % (MPa)	3.1	5.3	5.1
Modulus, 200 % (MPa)	6.9	9.1	9.1
Modulus, 300 % (MPa)	11.5	12.3	11.7
Tear strength (N/mm)	82	75	74
Hardness (Shore A)	62	62	62
Heat build up (DT °C)	27	9	9
DIN abrasion loss (mm^3)	76	96	73

3.1.2. Latex - filler master batch

Latex master batches containing mixed filler systems (carbon black/silica/clay) were also prepared. It was observed that there was almost no filler loss during the processing. Higher modulus along with better tear strength was observed for the mixed filler master batches as compared with mill mixed compounds.

3.1.3. Silica reinforcement of rubbers

Silica reinforcement of natural rubber and epoxidised natural rubber of varying epoxy content *viz.* 10, 20, 25 and 50 mole %, were compared with and without silane coupling agent and with that of carbon black filled natural rubber. Formulation of the mixes is given in Table Chem. 4. Important

Table Chem. 4. **Formulation of the mixes for silica reinforcement study**

Ingredients	1	2	3	4	5	6	7	8	9
NR (ISNR 5)	100	100	85	85	85	85	85	85	100
ENR 10	-	-	15	15	-	-	-	-	-
ENR 25	-	-	-	-	15	15	-	-	-
ENR 50	-	-	-	-	-	-	15	15	-
Silica (VN3)	50	50	50	50	50	50	50	50	-
Silane (Si69)	-	4	-	4	-	4	-	4	-
ISAF	-	-	-	-	-	-	-	-	50
CBS	1.5	1.5	1.5	1.5	1.5	1.5	1.5		

(Above compounds except compound 9 also contain Naphthenic oil 5, Zinc oxide 4, Stearic acid 2, Mernox 6c (N-1,3 dimethyl butyl) N'-Phenyl p-phenylene diamine) 2, sulphur 2 , DPG 1.5)

Table Chem. 5. **Technological properties**

Parameter	1	2	3	4	5	6	7	8	9
Modulus 300 % (MPa)	7.1	15	11	17.7	11.7	18.2	11.2	14.9	14.1
Tensile strength (MPa)	26.4	25.1	24.7	21.3	24.6	19.5	22.7	24.5	24.0
Tear strength (N/mm)	105	91	106	53	98	54	98	89	109
Hardness (Shore A)	60	62	62	67	63	68	68	64	60
Abrasion loss (mm^3)	174	109	143	96	149	105	157	114	106
Heat build up ($\Delta T°C$)	15	8	15	9	16	11	19	12	19

technological properties are given in Table Chem. 5.

It was found that properties like modulus and hardness increased while abrasion loss decreased with level of epoxidation of ENR. All the composites containing silane showed improved properties over the unmodified ones.

3.2. Recycled rubber

A new radical scavenger has been identified for devulcanisation in a mechano-chemical process. In addition, a new concept of "simultaneous devulcanisation-revulcanisation process" is under study to address the twin problem of low scorch and lower vulcanisate properties of the devulcanised rubber when it is revulcanised.

3.3. Nanocomposites

3.3.1. *Nanosilver based nanocomposites*

Stabilised nanosilver dispersion was prepared using polyvinyl pyrolidinone as stabiliser at 10 ppb (parts per billion). Nanosilver was found to impart anti microbial property. Attempts were made to prepare nanosilver doped nanosilica for use in rubber.

3.4. Peroxide vulcanisation

Reconfirmed the capability of DCP/ TEMPO/N, N'-m- dimaleimide cure system to achieve sulphur/accelerator like scorch control in peroxide vulcanisation. This system suffered from a major draw back of very poor mould release after vulcanisation. Therefore, a new peroxide cure system, di (t-butyl peroxy isopropyl) benzene/TEMPO/ timethyl propane trimethacrylate (TMPTMA) has been developed. The new cure system could achieve excellent scorch control (Table Chem. 6) with very good mould release property.

Table Chem. 6. **Effect of coagent on cure characteristics di (t-butyl peroxy isopropyl) benzene/ /TEMPO / timethyl propane trimethacrylate /TEMPO at 175° C**

Properties	1	2	3	4	5
TEMPO	0	0.8	0.8	1.2	1.2
TMPTMA	0	0	4	0	4
Minimum torque((M_L) dN.m	31.8	20.6	20.3	19.3	16.4
Maximum torque((M_H) dN.m	122.5	100.9	117.0	94.4	111.0
M_H-M_L	90.7	80.3	96.7	75.1	94.6
Time for one unit rise in torque, ts_1.min	1.4	2.9	2.7	3.1	3.2
Time for two unit rise in torque, ts_2.min	1.5	3.2	3.0	3.4	3.4
Optimum cure time (min)	11.3	13.5	13.6	15.6	16.0