RUBBER RESEARCH INSTITUTE OF INDIA

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Rubber Research Institute of India

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

Location

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

Organization

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

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(Ministry of Commerce & Industry, Government 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

An important development during this year was setting up a mechanism in the Institute for transfer of technology to the rubber-goods processing industry in a transparent and competitive manner. The year saw negotiations for transfer of a couple of technologies to the industry. Another significant milestone was the development of a rubber component with excellent vibration damping characteristics for use in the submarines. There will be more concerted efforts to collaborate with the Indian defence and public sector establishments in their efforts to indigenise production of rubber products. A new chemical has been identified that can reduce the coagulation time of latex and impart resistance to fungal growth on the sheet.

Another significant development has been the initiation of a project on fertility mapping of rubber growing areas of Kerala, Tamil Naud and Karnataka in collaboration with the National Bureau of Soil Survey and Land Use Planning, Bangalore. Growth and yield of mature rubber trees were not influenced by withdrawing chemical fertilizers for a period of ten years. Allowing all types of natural flora to grow inside a mature rubber plantation in such a way that their canopy did not go above that of rubber had no adverse impact on growth and yield of rubber. In fact, this helped to improve the soil characteristics. These are findings that will have profound ecological impacts. Flux of CO_2 from rubber soils was measured for the first time.

More than 8000 crosses were made between popular Wichkam and promising Amazonian accessions. W x A hybrids from previous years' crosses showed promising yield and growth characteristics. Nine ortets were newly selected during the reporting year. In Phase III of the participatory clone evaluation programme twelve pipeline clones and three check clones were planted in on-farm trials in six locations. A new breeding programme for developing disease tolerant clones was initiated. About 2700 hand pollinations were carried out using RRII 400 series and RRII 105 and a few selected disease tolerant parents including germplasm accessions. A disease tolerant clone Fx 516 as male parent and RRII 414 and RRII 430 as female parents were used for more than 5000 hand pollinations for developing mapping population. In another project, more than 1800 the interspecific crosses were made for developing mapping population.

Among the various clones introduced from Cote d'Ivorie in 1992, the clone IRCA 130 consistently performed better than RRII 105 both in yield and vigour. About 1500 crosses wee made using wild accessions. MT 4856 and MT 4788 along with RRII 430 showed better drought tolerance. Adoption of RRII 400 series clones by growers continued to go up. RRII 430 also had better wood quality which was the least in RRII 105. Interestingly RRII 430 had high leaf

lignin content and this was related to better tolerance to fungal diseases. Earlier studies have shown that RRII 430 is also relatively better tolerant to drought. A few ortets selected from RRS, Guwahati showed better tolerance to cold as well as drought.

A project was initiated at RRS, Nagrakatta and RRS, Dapchari to measure the amount of water consumed by rubber trees in these diverse agro-climatic regions. A biodegradable polythene was developed in collaboration with private entrepreneurs which can be used as a rain guard. It was found that two rounds of spraying of COC in oil (1:5) @ 20L/ha. controlled abnormal leaf fall disease. It was found that many growers adopt unscientific intercropping of pineapple which is mostly done by contractors and not the growers. Banana and pineapple were the preferred intercrops with relatively larger holdings and subsistence crops such as tapioca, amorphophallus and colocasia were preferred by smaller holdings. Low frequency tapping was more popular in relatively larger holdings than smaller ones.

The 3-D structures of â-1,3-glucanase from *Hevea* and barley perfectly matched each other despite their significant amino acid variations indicating the highly conserved nature of this gene in plants. A chloroplast stress protein was validated as a physiological marker for identifying drought tolerance. Several transcrptome analyses were done for understanding the relevance of stress responsive genes and also developing reliable marker system for making linkage maps. Gene technology and rubber technology will be priority areas for research in RRII in the coming years. Steps will be taken to elucidate the whole genome sequence of rubber.

AGRONOMY AND SOILS DIVISION

The various research programmes of the Division are aimed at development and periodic refinement of agromanagement practices to improve growth and yield of rubber in different agroclimatic regions, reduce cost of cultivation and sustain soil quality. Experiments on nutrient management in nurseries, young and mature rubber were continued. Studies on species diversification in rubber plantations, mechanization of rubber plantations and stress management were in progress. The experiments to develop an agronomic package for reducing the gestation period of rubber were continued. Various projects on assessment of soil health were also continued. Development of the rubber information system using remote sensing and GIS is being continued. Estimation of area under rubber in Karnataka and rubber area distribution in relation to soil and landscape attributes was in progress. A project on soil fertility assessment and soil health monitoring of traditional rubber growing areas of Kerala, Tamil Nadu and Karnataka was initiated in collaboration with National Bureau of Soil Survey and Land Use Planning, ICAR. The Division also functions as a centre for dissemination of knowledge on various soil and crop management techniques.

1. Nutrient management

Nursery experiments were conducted at Central Nursery, Karikkattoor and at Regional Nursery, Kanhikulam, Palakkad to revise the current fertilizer recommendation for seedling nurseries. Growth of rubber seedlings in the treatment which received N and P @ 250 kg ha^{-1} was comparable with that of standard practice (NPKMg@ 500:250:100:37.5 kg ha^{-1}). Growth of seedlings was comparable when N and P @ 250 kg ha^{-1} was applied as urea and rock phosphate and as ammophos (20-20).

Experiments initiated during 2011, to study the effect of supplementing secondary and micronutrients in areas low in their status, on growth and yield of rubber were continued at four locations, *viz*, New Ambady estate in the southern region, Cheruvally estate in the central region, Palappilly estate in the north central region and Thamarassery estate in the north region. Soil analysis indicated that application of the secondary and micronutrients during first year of planting maintained the soil status in sufficiency level during second year also. At one location, integration of FYM with recommended dose of chemical fertilizers showed significantly higher girth while no significant difference between treatments was noticed at the other three locations.

The field experiment at CES, Chethackal to study the effect of long term application of inorganic and organic manures on the growth and yield of rubber and physico-chemical properties of soil was continued. Application of 25% recommended dose of chemical fertilizer and 75 % FYM recorded significantly higher girth than all other treatments.

The experiment on sequential skipping of fertilizer application in mature rubber was concluded. The results showed that the growth and yield of rubber were not significantly influenced by withdrawing the application of fertilizers for a period of ten years. The soil and leaf nutrient status nine years after the commencement of the experiment also did not indicate any significant difference among the treatments indicating that mature *Hevea* plantation can be considered as a partially self- sustaining

ecosystem with a constant cycle of uptake and return of nutrients from and to the soil.

The field experiment to study the effect of C POM as soil amendment in marginal soils, at Thanneermukkom, Cherthala was continued. Girth of plants did not showed significant difference among different treatments.

2. Soil and water conservation

The experiment on evaluation of vegetative hedges for soil and water conservation in rubber plantation was continued. The establishment of vegetative hedges *viz.*, vetiver, guinea grass, pineapple and *Strobilanthes sp* significantly reduced soil erosion. Among the different vegetative hedges, vetiver was found to be the better soil erosion control measure in rubber plantation which was significantly superior to all other vegetative hedges except guinea grass. Growth of rubber was not significantly influenced be establishment of vegetative hedges.

3. Intercropping and cropping systems

Experiment to evaluate the feasibility of growing perennial intercrops *viz.*, coffee, vanilla, *Garcinea* and nutmeg under normal and paired row systems of planting of rubber was continued. There was no significant difference between treatments with respect to growth and yield of rubber. Coffee continued to yield well, Garcinea has perished under shade in both experiments. One life cycle of vanilla was completed.

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

Experiment on interplanting of rubber with timber trees *viz.*, teak, wild jack and *mahogany* was continued. Growth and yield of rubber was not significantly influenced by row spacing, type of timber intercrops and their interactions (Table Ag. 1 and 2).

Table Ag. 1. **Mean girth (cm) of rubber during eleventh year (March 2012)**

Spacing	Intercrops				Mean
	Wild Jack	Teak	Mahogany	No intercrops	
Normal (6.7 x 3.7m)	53.3	57.4	57.5	55.5	55.9
Wide Row (10 x 2.4)	53.2	52.6	55.1	53.1	53.5
Mean	53.3	55.0	56.3	54.3	54.7
SE	Spacing	Intercrops		Interaction	
CD (P=0.05)	0.6	0.85		1.2	
	NS	NS		NS	

Table Ag. 2. **Mean rubber yield (g tree^{-1} tap^{-1}) during 2012-13**

Spacing	Intercrops				Mean
	Wild Jack	Teak	Mahogany	No intercrops	
Normal (6.7 x 3.7m)	54.4	51.0	42.4	44.8	48.2
Wide Row (10 x 2.4)	47.6	59.9	53.1	55.4	54.0
Mean	51.0	55.4	47.7	50.1	51.1
SE	Spacing	Intercrops		Interaction	
CD (P=0.05)	1.9	4.00		5.6	
	NS	NS		NS	

Among the performance of intercrops, *wild jack* performed better than teak and Mahogany (Table Ag.3).

Table Ag. 3. **Comparison of girth of timber crop under intercropped and monoculture situation**

Timber crop	Inter-cropped	Non-inter-cropped	't' statistics
wild jack	67.3	54.1	7.4*
Teak	33.4	31.8	0.54
Mahogany	25.8	38.0	6.9*

* Significant at 0.05

The field experiment initiated to develop a multi species rubber based cropping system for Tamil Nadu region was in progress. The establishment of intercrops *viz.* pine apple, three varieties of banana, cinnamon and cocoa did not influence the growth of rubber.

4. Ground Cover Management

The experiment 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 and biomass and nutrient turnover was continued. Preliminary observations on growth did not indicate any significant difference among treatments.

The field experiment to study the impact of manual, chemical and mechanical weed control methods on regeneration of weed flora was concluded. In manual and mechanical weed control methods, weeds attained 100 per cent of regeneration 75 and 90 days after treatment imposition respectively. In herbicide applied plots, regeneration of weeds started only after 60 days of treatment imposition. Weed regeneration was 23.22 and 28.3 per cent respectively, 75 and 90 days after treatment imposition

The project on comparison of rubber plantations with and without control of weed flora at Pathampuzha village in Kottayam District was continued. Above ground weed biomass in the sixteen fields in Pathampuzha region were collected and dry weight determined. N, P, K, Ca and Mg in the samples were determined and nutrients in weed biomass in each location estimated. There was significantly higher weed biomass in weeds-not-controlled fields and the carbon content in weed biomass was about eight times more than the weeds-controlled fields. Also the stock of N, P, K, Ca and Mg were significantly higher in weed flora under the weeds- not-controlled fields. The study was extended to two more locations in Punalur and Paika. Soil samples from these regions were collected and analysis is in progress.

An experiment was initiated to evaluate biodegradable plastic for weed control at CES, Chethackal with control and application of biodegradable plastic in the plant basins and *Glyricidea* in between plants.

The observational trial initiated at Malankara estate, Thodupuzha during 2009 to explore the feasibility of establishing *Mucuna* under partial shade indicated that *Mucuna* can be established in rubber plantations even after the removal of pineapple intercrop.

An experiment was started at CES, Chethackal to evaluate the cover crop *Calapogonium caeruleum* in rubber plantations.

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 of planting and twin system of planting continued to be superior to that of control (square system of planting). The canopies in altered planting systems started to exhibit asymmetrical pattern of growth.

The field experiment initiated during 2010 to assess the effect of mechanized land preparation on soil erosion and physical and chemical properties of soils was continued. The treatments with ploughing, pitting & terracing by Hitachi showed significantly lower bulk density and higher porosity compared to all other treatments. The no tilled treatments had significantly higher levels of SOC content in the surface 0-15 cm layers compared to the tilled treatment (pitting, terracing and tilling by Hitachi). Hydraulic conductivity of tilled plots showed significantly higher value of 5.73 x 10^{-3} cm sec^{-1} than no tilled plots and among the no tilled plots significant difference was not observed.

Soil erosion in different land preparation methods were significantly different (Table Ag. 4). The highest quantity of 5.16 t ha^{-1} of soil was eroded from the plot where pitting, terracing and tilling inter rows were done by Hitachi.

Table Ag. 4. **Quantity of soil deposited in trenches (2013)**

Treatments	Quantity ($t\ h^{-1}a$)
Manual pitting and terracing (control)	3.26
Manual terracing & pitting by tractor mounded hole digger	3.29
Pitting & terracing by Hitachi	3.66
Ploughing, pitting & terracing by Hitachi	5.16
SE	0.318
CD (P=0.05)	1.019

6. Development of agromanagement technique for reducing the gestation period

The field experiment to develop an agronomic package to reduce the immaturity period of *Hevea* at Malankara Estate was continued. Tapping commenced in the experimental field and yield recording was initiated.

The experiment initiated to develop an agronomic package to reduce the immaturity period of *Hevea* at CES, Chethackal is in progress. There was significant difference in the performance of the two types of planting material. The superiority of direct seeded green- budded polybag plants over green - budded stumps raised in polybags continued. The girth of the plants under integrated management was superior to that of the respective type of planting material under standard practice.

The experiment on the effect of different types of planting material (polybag - one whorl, two whorl and three whorl and root trainer - one whorl, two whorl and three whorl) on growth of rubber was continued. Three- whorl polybag plants were found significantly superior to others (Table Ag.5).

Table Ag. 5. **Effect of type of planting material and its stage on growth of rubber**

Treatment	Girth (cm) July, 12 (4 years)
Polybag plants (one whorl)	29.23
Polybag plants (two whorl)	29.13
Polybag plants (three whorl)	34.43
Root trainer plants (one whorl)	29.94
Root trainer plants (two whorl)	29.81
Root trainer plants (three whorl)	30.67
SE	0.72
CD (P=0.05)	2.13

The performance of all other planting materials *viz.*, polybag – one- whorl, two-whorl and root trainer – one- whorl, two-whorl and three- whorl were comparable.

The experiment initiated to evaluate the performance of rubber plants budded on stock plants of different age was continued. Significant difference in growth was not observed among different types of planting materials 3 years after planting.

7. Stress Management

The field experiment initiated at Puthukkad estate, Trichur, a comparatively drought prone area in the traditional rubber growing region to develop agromanagement techniques to mitigate the adverse effects of drought was continued. Tillage, super absorbent polymer and direct seeding in polybags mitigated adverse effect of drought stress (Table Ag. 6).

Table Ag. 6. **Growth of plants (cm) at 150 cm from bud union**

Treatment	June 2012
T1-Polybag plant raised from budded stump	9.76
T2-Root trainer plants	10.14
T3-Polybag plant raised by direct seeding	10.9
T4-T1+ tillage	11.54
T5-T1+ K supplement	9.57
T6-T1 + hydrogel	10.79
T7-T1+Life saving irrigation*	10.47
T8-T1+K+ tillage	10.08
T9-T1 + K+ tillage+ hydrogel	11.58
SE	0.29
CD (P=0.05)	0.91

* First year only

Another field experiment was initiated at Puthukkad estate to evaluate various types of mulches for soil moisture conservation.

8. Rubber growing soils

The study on characterization of soil organic matter in rubber plantation was continued. Among the four systems studied organic carbon was significantly higher in the *Pueraria* established immature field which was comparable with that of the mature plantation (Table Ag. 7). In all the systems, major portion of the carbon in the soil was found to be in the lower mineral associated (<53um) fraction. Water soluble carbon (WSC) in the *Pueraria* established field and banana intercropped field were comparable and it was significantly higher than the other two systems. Soil pH was significantly higher in the banana intercropped field followed by *Pueraria* established field and lowest in the pineapple intercropped field.

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

Table Ag. 7. **Soil chemical properties in different systems**

	OC (%)	WSC (ppm)	pH
Mature rubber plantation	2.15	412	4.88
Pueraria established immature field	2.3	601	4.91
Banana intercropped immature field	2.03	592	5.06
Pineapple intercropped immature field	1.65	392	4.79
SE	0.06	28.58	0.03
CD (P=0.05)	0.17	85.68	0.08

Table Ag. 8. **Carbon pool index and carbon liability indices of different systems**

System	OC	POSC*	WSOC*	HWEC*	Carbon Pool Index	Carbon Lability Indices with respect to		
						POSC	WSOC	HWEC
Rubber	2.41	817.79	35.40	76.95	0.46	0.69	0.29	0.38
Rubber + *Mucuna*	3.56	1031.54	70.00	168.76	0.67	0.87	0.57	0.84
Rubber + Pineapple	2.30	941.37	38.48	76.11	0.43	0.80	0.31	0.38
Cassava	1.99	777.47	44.74	65.53	0.38	0.66	0.36	0.33
Teak	3.23	1034.59	79.76	183.40	0.61	0.88	0.65	0.91
Forest	5.29	1179.39	123.60	201.06	1.00	1.00	1.00	1.00

*POSC - permanganate oxidizable organic carbon, WSOC -Water soluble organic carbon, HWEC - Hot water extractable carbon

district was concluded. The Carbon Management Index (CMI) of these six ecosystems were worked out based on the Carbon Pool Index (CPI) and Carbon Liability Index (CLI). The CPI represents the quantity of organic carbon and CLI represents the labile carbon quantity of an ecosystem with respect to the forest system. The organic carbon pool (CPI) decline was least in Rubber-*Mucuna* system and was most in the Cassava system. Rubber-*Mucuna* cover crop system had retained much larger organic carbon pool than Rubber-Pineapple intercrop system (Table Ag.8).

The decline in labile pool was the highest in cassava system and the least in teak system. Though the decline of total organic carbon pool was more in teak system than in *Mucuna* system, the decline in labile carbon pool was more in *Mucuna* system than teak system. This could be due to the multi-species system existing in teak soil system.

The Carbon Management Index (CMI) based on the three different carbon Liability Indices are shown in Table Ag.9.

The CMI calculated based on the POSC indicate that Rubber-*Mucuna* was the least deteriorated system closely followed by teak system. However, the CMI based on soluble carbon indicated that the teak soil system was almost similar or slightly less deteriorated system than Rubber-*Mucuna* system. Cassava was the highest deteriorated system and mature Rubber and Rubber-Pineapple systems were almost similar and had undergone more deterioration than Rubber-*Mucuna* and teak systems.

Table Ag.9. **Carbon management indices of different soil systems**

System	Carbon Management Indices		
	POSC	WSOC	HWEC
Rubber	0.32	0.13	0.17
Rubber + Mucuna	0.59	0.38	0.57
Rubber + Pineapple	0.35	0.14	0.16
Cassava	0.25	0.14	0.12
Teak	0.54	0.39	0.56
Forest	1.00	1.00	1.00

The project on management of active and microbial carbon pools at Pottamkulam Estate, Yendayar was continued to observe the long term effects of the treatments imposed in earlier periods. Girth of the plants was recorded and no significant difference could be noticed among the treatments.

The study on assessment of soil CO_2 flux from rubber plantations continued. *In situ* soil CO_2 flux, soil temperature and soil moisture were recorded on an hourly basis, continuously from June 2010 to May 2012 under a mature rubber plantation in RRII farm.

The average of two platform and two inter row sites to represent the field soil CO_2 flux,

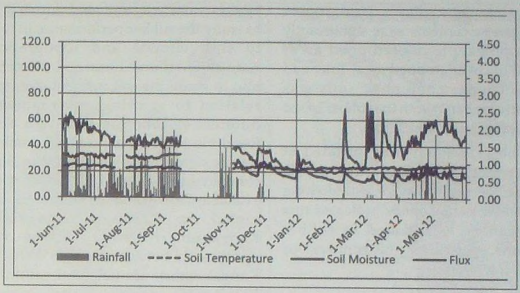


Fig. Ag.1. Field averages of soil CO2 flux rate (µmol/m2/s), soil temperature (oC) and soil moisture (%) from mature rubber field during the period 2011-12.

soil temperature and soil moisture in mature rubber plantation for the period 2011-12 were worked out and is shown in Figure Ag. 1.

During the monsoon period, soil respiration rate was more than during other seasons. In winter (Jan-Feb) and summer (Mar-May) flux rate (respiration rate) were comparatively less. During the summer months, the soil moisture clearly declined and CO_2 flux rates also declined.

In general soil CO_2 flux rate was slightly higher during night hours than during the day hours. The differences were more prominent during winter (Jan and Feb) and summer (Mar to May) period. The night soil temperature was slightly less than the day soil temperature throughout the period of observation. The daily soil CO_2 flux rate was influenced by soil temperature and soil moisture.

Heavy metal status of soils (0.1 N HCl extractable) under rubber-pineapple intercropping system in the central region of rubber cultivation was studied in comparison to soil under rubber-cover crop system and natural forest. Cadmium content was significantly higher in soil under rubber-pineapple intercropping system compared to rubber-cover crop system and forest (Table Ag.10). Total cadmium content of the soils was well

Table Ag. 10. **0.1 N HCl extractable heavy metals (mg/kg) in different systems**

System		Pb	Cd	Cr	Cu	Zn	Fe	Mn
1	Rubber+ Cover crop(n = 21)	3.38	0.03	0.28	26.17	1.43	36.39	32.96
2	Rubber + Pineapple(n = 82)	2.78	0.06	0.22	16.43	1.33	49.95	19.52
3	Natural forest (n = 15)	3.69	0.02	0.33	2.27	1.12	33.71	61.14
t stat	Rubber + cover crop Vs Rubber + Pineapple	**	**	NS	**	NS	**	**
	Rubber + cover crop Vs Natural forest	NS	NS	NS	**	*	NS	**
	Rubber + Pineapple Vs Natural Forest	**	**	*	**	NS	**	**

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

within the regulatory limits. Lead, copper and manganese contents were significantly lower in pineapple intercropped areas compared to cover cropped area. Total and available copper content of soils was significantly higher in both the rubber based systems compared to forest soil.

The observational trial initiated at Malankara estate, Thodupuzha during 2009 to explore the feasibility of establishing *Mucuna* under partial shade indicated that *Mucuna* can be established in rubber plantations even after the removal of pineapple intercrop.

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. Estimation of natural rubber growing areas in Karnataka for the year 2010 & 2012. Accuracy of satellite image classification was done using GPS readings and it ranged from 82.4% to 86.1%. Satellite based rubber area in Karnataka was 20,971.54 ha compared to ground survey statistics of 32,415 ha. indicating 11,443.46 ha lower than ground statistics. Highest rubber area was observed in Dakshin Kannada district followed by Udupi, Coorg, Shimoga and Chikmaglur. This difference is attributed to expansion of rubber cultivation to new areas in the region. Young plantations (< 3 year) were not mapped with this technique due to poor signature from these plantations. Vectorization of administrative boundaries, road network, locations and soil map unit of rubber growing areas has also been completed.

A subproject on "Geospatial analysis and soil nutrient dynamics of rubber plantations in relation to growing environment" was initiated during 2011-12 to study the rubber performance in relation to soil, climate and topography in Kanyakumari and Kasargod districts and also to study the soil nutrient dynamics in relation to growing environments at different elevation in Kottayam district. Results indicated that mean soil available P, K were significantly higher in Kanyakumari compared to Kasargod. However, soil OC, available Ca, Mg and pH were significantly higher in Kasargod compared to Kanyakumari district. Climate varied significantly between the two districts. In Kanyakumari, annual total rainfall was 1228.1mm against the potential evapotranspiration (PET) demand of 1749.5 mm while in Kasargod district, annual rainfall was 3461.7 mm against the PET of 1770.1 mm. In Kanyakumari even during the December to March period, central portion of the district showed good moisture adequacy indicating no moisture stress (Fig Ag. 2). Rest of the region showed poor moisture adequacy. On the other hand, throughout in Kasargod district during the same period, moisture status was poor.

With respect to performance of rubber, girth and yield of rubber in Kanyakumari were significantly higher than in Kasargod district. In Kanyakumari district soil OC, available Ca and Mg were identified as dominant factors influencing the performance of rubber. On the other hand in Kasargod district topographic factors (elevation and slope) were found as the dominant factors. In Kasargod high elevation favoured the performance of rubber due to modification in climate associated with elevation gradient. Because of this reason more rubber area distribution was seen at high elevation in Kasargod.

Phenology of rubber varied along the elevation gradient in Kottayam. Rubber at

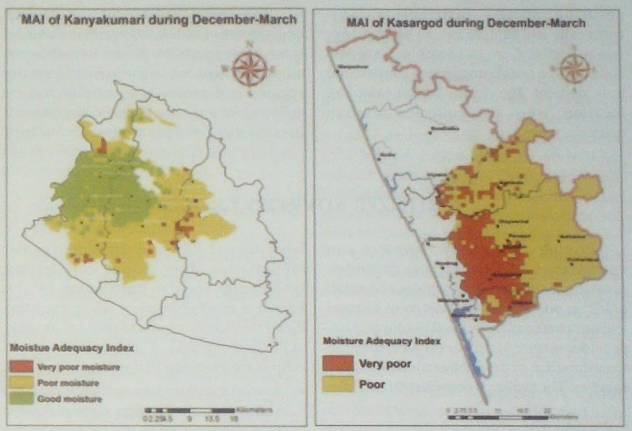


Fig. Ag. 2. **MAI map of Kanaykumari and Kasargod at different period**

high elevation showed early wintering and refoliation compared to low elevation. Number of new flushes were more at low elevation compared to high elevation. Climate was the major factor influencing the rubber yield at high elevation rubber ecosystem. In medium elevation ecosystems, nitrogen mineralization was the major factor influencing the rubber yield. At lower elevations, climate or nitrogen mineralization did not influenced rubber yield.

10. Soil fertility assessment and soil health monitoring of traditional rubber growing areas of Kerala, Tamil Nadu and Karnataka

A project was initiated in collaboration with National Bureau of Soil Survey and Land Use Planning, ICAR to document major, secondary and micronutrient status of rubber growing areas of Kerala, Tamil Nadu and Karnataka and mapping soil fertility. The project also envisages to establish and characterize soil health monitoring sites representative of the varied agro-climatic situations in the rubber growing regions. The first phase of the project envisages soil sampling from the region and analyses of soil samples for organic carbon, soil pH, plant available nutrients and exchangeable aluminium. For soil sample collection, area under rubber in each panchayat was estimated based on satellite images. With the help of officers of the Rubber Production Department spatially distributed holdings were identified for soil sample collection. GPS was also used as a guideline for

ensuring spatial distribution. One composite soil sample per 50 ha of rubber was collected. Core soil samples were also collected for estimating gravel content and bulk density. The details of the sampling location were also collected through questionnaires.

The second phase of the project envisages to establish soil health monitoring sites representative of the varied agro-climatic situations in the rubber growing regions and profile soil samples will be collected for analyses. Already, 57 sites were identified and soil samples were collected.

FERTILIZER ADVISORY GROUP

Provides advisory services on optimization of fertilizer use in rubber plantations for both large estates and small holdings on the basis of analysis of soil and leaf samples from individual holdings. Also, provides service to rubber growers through the estimation of dry rubber content of latex samples. The facility is provided through the laboratory at RRII and the nine regional laboratories. Two mobile soil testing laboratories are also functioning for the benefit of small growers by offering soil test based fertilizer recommendation by camping at the farmer's field. The details on sample output are provided in the table FAG 1.

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

Parameter	Number
Soil	7474
Leaf	845
MST program	45
Fertilizer recommendation	4000
DRC of latex samples	53267

- A new laboratory was opened at Kadaba, South Kanara district, Karnataka for providing service to the growers of the region.
- The regional laboratory, Taliparampa was renovated and reopened during the year.
- Inaugurated the issue of soil health card at the public function of the closing ceremony of the silver jubilee celebrations of the Rubber Producers' Society.

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 the Biotechnology Division have been focused on 1) development of *in vitro* propagation techniques for elite *Hevea* clones through somatic embryogenesis 2) development of transgenic *Hevea* plants for better adaptation to abiotic stresses & tapping panel dryness, increased latex yield and disease tolerance 3) techniques to complement conventional breeding programmes such as development of

haploids and dihaploids, development of protocols for embryo rescue and induction of polyembryony 4) study of molecular mechanisms and characterization of genes related to tolerance to diseases, abiotic stresses, latex biosynthesis and 5) study of laticifer cell specific gene expression and characterization of laticifer cell specific promoters.

1. Somatic embryogenesis and plant regeneration

Experiments were continued to refine the somatic embryogenesis and plant regeneration protocols developed earlier from immature anther and leaf explants. Different experiments were carried out with the aim of shortening the regeneration pathway using immature anther explants. The synergistic effect of three auxins 1) 2,4-D (0.5 mg L^{-1}), NAA (1.0 mg L^{-1}), IAA (0.5 mg L^{-1}); 2) 2,4-D (1.0 mg L^{-1}), NAA (0.5 mg L^{-1}), IAA (0.5 mg L^{-1}), and 3) 2,4-D (1.5 mg L^{-1}), NAA (0.25 mg L^{-1}), IAA (0.25 mg L^{-1}) along with 1.0 mg L^{-1} BA was tested for callus induction. The percentage of callus induction was highest in medium fortified with 2,4-D (1.5 mg L^{-1}), along with NAA (0.25 mg L^{-1}), IAA (0.25 mg L^{-1}). Replacement of Kin with BA in the earlier standardized medium improved the callus induction, growth, as well as friability of the callus. The friable callus induced was cultured for 2 weeks in ½ strength liquid medium. It was noted that two weeks culture in liquid medium was very beneficial for proliferation of the friable callus. The proliferated callus, upon subculture over embryo induction medium, globular to heart shaped embryos were obtained.

Leaf explants collected from glass house grown bud grafted polybag plants of clone RRII 105 and from *in vitro* developed somatic plants of clone RRII 105 were used for initiating viable and contamination free cultures. Callus induction was obtained after one month in leaf explants collected from glass house grown bud grafted plants and after twenty days in explants collected from somatic embryo derived *in vitro* plants. Proliferated callus obtained after three subcultures was friable. Embryogenic calli was obtained from the proliferated callus clumps within 3 - 4 months and each line were maintained separately and subcultured for embryo induction. Embryo induction (50-70%) was obtained in modified MS basal medium containing myoinositol (100 mg L^{-1}), adenine sulphate (50 mg L^{-1}), B_5 vitamins, amino acids, organic supplements, silver nitrate (10 mg L^{-1}), sucrose (60 g L^{-1}) and optimized level of phytohormones. The medium was also supplemented with activated charcoal (0.2%) and solidified with phytagel (0.5%). The primary callus clumps continuously produced new embryogenic callus on subculture to fresh medium for about six months.

The influence of different amino acids on embryo induction was studied. Three combinations were tried 1) - Glutamine (300 mg L^{-1}), proline (100 mg L^{-1}), arginine (40 mg L^{-1}), L- cysteine (50 mg L^{-1}); 2) Glutamine (300 mg L^{-1}), arginine (40 mg L^{-1}), L- cysteine (50 mg L^{-1}); 3) Proline (100 mg L^{-1}), arginine (40 mg L^{-1}), L- cysteine (50 mg L^{-1}). Among these, the first combination was found to be more suitable and helped in enhancing the rate of embryo induction. The embryos enlarged with root - shoot apex induction in the maturation medium on dark incubation and started germination after two weeks. Plant regeneration was obtained from germinated embryos in MS medium containing organic supplements.

Callus induction with improved texture was obtained from the cut ends as well as from the leaf surface in the modified callus induction medium containing picloram (2.0 mg L^{-1}) and phytohormones; BA, 2,4-D and NAA. Callus proliferation was obtained in the modified callus induction medium fortified with adenine sulphate (50 mg L^{-1}) and L-cysteine hydrochloride (50 mg L^{-1}) along with reduced 2,4-D and increased sucrose. Embryogenic callus was obtained in medium containing phytohormones, picloram (2.0 mg L^{-1}) and the amino acids glutamine (300 mg L^{-1}) and proline (200 mg L^{-1}). Embryo induction was obtained from the proliferated embryogenic callus with 60-70% frequency in the earlier standardized embryo induction medium.

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

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

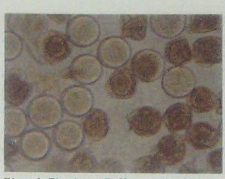
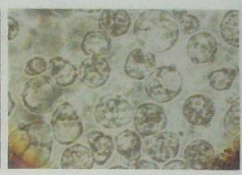
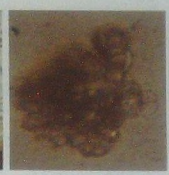
The growth regulator combination of GA_3 (2 mg L^{-1}) and Kin (3 mg L^{-1}) reported earlier for the induction of multiple embryos was kept constant and different levels of other growth regulators viz. zeatin, IAA, 2-iP, 2,4-D and BA were tried along with them for the induction of embryogenic callus and results are awaited.

Plants developed earlier through polyembryony were used for stock–scion interaction studies. DNA methylation studies were carried out in the polyembryony derived plants to understand stock- scion interaction, in collaboration with Genome analysis laboratory. Preliminary studies indicated no epigenetic variation among the polyembryony derived plants. Plants are being bud grafted on to assorted seedlings for further studies.

2.2. Generation of androgenic haploids in *Hevea brasiliensis*

2.2.1. Intact microspore culture

The influence of certain parameters on microspore division and subsequent micro callus formation such as different genotypes, various pre-treating agents, the stage of the microspores and γ-ray irradiation were assessed. Male flowers belonging to three genotypes namely clone RRII 105, RRII 414 and RRII 430 were separated from the inflorescence and surface sterilized with 0.1% $HgCl_2$ for 5 minutes. Anthers were dissected out aseptically and inoculated in the pre-treatment solution. Pre-treatment of the anthers were tried in sucrose solution (10 %), sucrose (5%) + mannitol (5%) and mannitol (10 %) alone. The pre-treatment was continued for a period of 5 to 10 days. The flower buds were also exposed to varying doses of gamma radiations (250-1000 Gy). The anthers were dissected, microspores squeezed out and cultured for callus induction. Observations showed the positive influence of the genotype on microspore division, where, the most responsive genotype was clone RRII 414 followed by clone RR11 430 and RR11 105. Among the two developmental stages of the pollen used for culture initiation, the early bi- nucleate stage responded positively towards culture conditions. A combination of mannitol (5%) with sucrose (5%) was ideal for microspore division and micro callus formation compared to other treatments. Maximum division of the microspores was achieved from the pollen grains isolated from irradiated flowers at 500 Gy. The division of the irradiated microspores was comparable to that obtained after sucrose + mannitol pre-treatment.

Biotech Fig. 1 a-c. **Pollen protoplasts** **Micro colony formation** **Micro callus**

2.2.2. *Pollen protoplast culture*

Pollen grains from mature male flower buds were separated and subjected to enzymatic digestion. Intact protoplasts were isolated in very high yield (80.90%). Partial purification of the isolated protoplasts could be achieved through sieving technique using sieves of 60-70 μ mesh size followed by centrifugation (Fig. 1 a). Two basal media viz. KPR and Chu N6 media, fortified with different levels of 2, 4-D (0.1-1.0 mg L^{-1}) and BA (0.1-1.0 mg L^{-1}) were enriched with *Hevea* nurse culture. Partially purified pollen protoplasts were cultured over membrane filters placed over these media and incubated in the dark at 28°C. Division of protoplasts leading to the formation of a few micro colonies was observed in N6 medium containing 0.8 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} BA (Fig. 1 b). In KPR basal medium, even though no micro colony formation could be detected, the protoplasts appeared intact and healthy. The cultures with micro colonies were dark incubated for further development. Addition of fresh medium, as drops, to the cultured protoplasts was done at fortnightly intervals. Micro callus formation from these micro colonies was obtained and are maintained in the same medium for further proliferation (Fig. 1 c).

2.2.3. *Culture of pollen tetrads and isolation of protoplasts*

The exine of mature microspore being hardy for protoplast isolation, another attempt was made from an early developmental stage of microspore namely tetrad. Young male flower buds of *Hevea* containing the tetrad stage of pollen, were collected and surface sterilized with 0.15% mercuric chloride for 3 minutes. Anther columns were dissected out and squeezed gently in the osmoticum. After removing the debri through filtration, the filtrate was centrifuged and the pellet was resuspended in a minimum volume of fresh osmoticum. When observed through an inverted microscope a mixture of tetrads and microspores with very little pollen mother cells (PMC) could be obtained which was suspended in the osmoticum. The suspension was centrifuged to pellet down the tetrad - microspore - PMC mixture which was cultured over different callus induction media and incubated in the dark. A portion of the tetrad - microspore - PMC mixture was subjected to enzymatic digestion with different levels of the enzyme cytohelicase alone (0.1-1.0 %), as well as with the enzyme cellulase (0.5%). When the isolated pollen tetrads were incubated with different levels

of the enzyme cytohelicase, cell wall digestion was observed with 0.3 -0.5% of the enzyme. Addition of 0.5% cellulase along with cytohelicase, could improve the digestion of the tetrads but the yield of protoplasts from the tetrads was very low.

2.2.4. *Generation of synogenic haploids in Hevea brasiliensis*

Unpollinated female flowers at various developmental stages, as evidenced by their size and colour, were collected from field grown plants of clone RRII 105 and surface sterilized. Intact ovules, ovules without outer integument and intact embryo sacs were isolated from these flowers and cultured for callus induction. About 80% of the intact ovules as well as ovules without outer integuments showed swelling within two weeks of culture followed by callus formation. Around 30% callus induction was obtained from the cultured embryo sacs within 3 months of incubation (Fig. 2 a). Since embryo sac originates from a haploid cell, callus induced from the cultured sacs is expected to be haploid (Fig. 2 b). Egg cell isolation from the intact ovules through enzymatic digestion was also attempted.

Biotech. Fig. 2. **Callus emergence**

Callus proliferation from embryo sac

For inducing callus from the haploid cells of the embryo sac, three sets of pre-treatments were given to the mature female flowers which include cold treatment of the flower buds, pre culture in the starvation medium, and irradiation. After pre-treatment they were inoculated in the callus induction medium and dark incubated at 25 ± 2°C. During cold treatment, the flower buds were maintained at 4° C for 10 to 15 days after which the ovules were isolated and cultured in the callus induction medium. Alternatively the isolated ovules were cultured in the starvation medium (KCl, 1.49 g L^{-1}; $MgSO_3.7H_2O$, 0.25 g L^{-1}; $CaCl_2$, 0.11 g L^{-1} and Mannitol (0.3 M) 54.63 g L^{-1} and 1 mM phosphate buffer of pH 7.0) for 7 days and subsequently transferred to the culture medium. Flower buds were also irradiated with γ-rays at three different dosages (50, 100, 200 krad) and the ovules with the inner integument were isolated and cultured for callus induction. The basal medium for callus induction include Murashige and Skoog, rubber tree medium and N_6 medium, supplemented with lower levels of growth regulators, 2,4 D (0.01- 0.2 mg L^{-1}), NAA (0.2-0.8 mg L^{-1}) and Kin (0.2 - 0.6 mg L^{-1}). Sub culturing of the ovules were carried out at monthly intervals and the responding ovules were scored after fifty days of culture. The explants subjected to cold treatment as well

as cultured in starvation medium failed to induce division. Irradiation with γ- rays triggered division in some of the ovules and the optimum dose was 100 krad. These cultures responded positively which was evident from the swelling of the ovules without any discoloration.

Embryogenesis was achieved at a low frequency from the embryogenic masses generated from the calli obtained earlier from the micropylar end of the mature ovules. The embryos obtained were separated from the embryogenic cultures and transferred for maturation. The embryo maturation medium was half strength MS basal medium supplemented with higher levels of KNO_3 organic supplements, amino acids (glutamine, proline and asparagine) and devoid of phytohormones. The matured embryos were cultured for plant regeneration.

2.2.5 *In vitro/in vivo pollinations for haploid production*

A new experiment was initiated to develop haploid plants by pollinating the female parent with irradiated pollen grains followed by rescue of embryos by *in vitro* culture. Pollen stimulates the division of the egg cell and thus induces parthenogenesis and leads to the development of gynogenic haploid embryo. Mature flower buds were collected from the field grown trees of clone RRII 105. They were subjected to different dozes of γ-rays ranging from 100 – 2000 GY and the pollen viability was studied by acetocarmine staining technique. The dosage of ã-rays for inhibiting 50% viability of pollen grains was found to be 1000 Gy.

2.3. Development of triploids in *Hevea brasiliensis* through endosperm culture

Seeds were collected from field grown *Hevea* trees. The hard seed coats were removed mechanically and the seeds were surface sterilized using 0.2% mercuric chloride for 5 minutes. Endosperm tissue were separated, cut into thin slices and cultured for callus induction in MS basal medium fortified with different levels of 2,4-D, NAA, BA and Kin. Callus induction at low frequency was obtained in the presence of 2.0 mg L^{-1} 2,4-D and 3.0 mg L^{-1} kinetin.

Isolation and culture of endosperm protoplasts were also attempted. Endosperm tissue from the sterilized seeds was cut into very thin slices and subjected for enzymatic digestion for protoplast release using different combinations of cellulase, macerozyme and pectinase at different concentrations and keeping the cultures in the dark with gentle shaking. A combination of 1% cellulase + 0.1% pectolyase was the most suitable one giving a maximum yield (70%) of protoplasts from the endosperm tissue. Protoplast release from the endosperm tissue initiated within half an hour of incubation and release of intact protoplasts continued up to 2 hrs. However, it was observed that with in 2 hrs the solution turned turbid and the released protoplasts could not be retrieved. It appears that the turbidity is due to the release of small oil granules present as stored material in the endosperm. Further experiments need to be carried out using endosperm tissue in the initial stages of its development from immature fruits.

2.4. Induction of polyploidy in diploid callus

Fresh calli induced from anther tissue of clone RRII 105 were proliferated in medium with reduced auxin. This proliferated callus was then used for chromosome doubling through colchicine treatment. Proliferated calli raised from immature inflorescence of clones, RRII 422 and 430 were also subjected to colchicine treatment. Colchicine at different

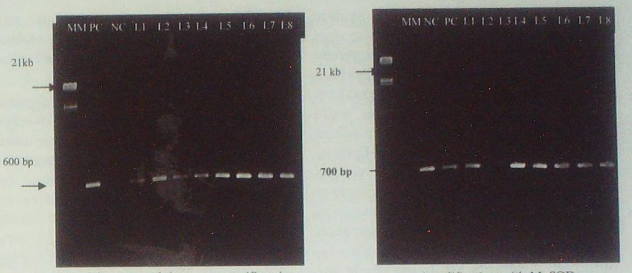
concentrations was incorporated in MS basal medium and calli were cultured over these media for different time intervals in two different experiments; 1.) colchicine (0.05, 0.1, 0.2, and 0.4%) for 24, 48 and 72 hours 2.) colchicine (0.01- 0.1%) for 2, 4, 6 and 8 hours. All the colchicine treated cultures were kept in the dark at 28°C and the results are awaited.

3. Genetic Transformation

All arrangements were made for the field trial of the earlier developed transgenic plants integrated with the MnSOD gene, at the Regional Research Station, Dapchari, Maharashtra State. These transgenic plants are expected to have increased tolerance to drought and other abiotic stresses as well as tapping panel dryness. However, since the 'No Objection Certificate" from the Maharashtra State Government for initiating the field trial is pending, the trial could not be started. The drought tolerant traits of these plants growing in polybags under containment conditions were evaluated in collaboration with Crop physiology Division. One year old plants of MnSOD transgenic *Hevea* lines (L1 and L2) and an untransformed line of clone RRII 105 were used in the present study to evaluate their physiological performance in a dry humid environment by withholding irrigation for six days and to see the recovery by re-watering for three days. The dry matter partitioning was relatively more towards the root in transgenic lines (55% and 60% in LI and L2, respectively) while, it was less in the untransformed RRII 105 (43%). After six days of moisture stress in polybags, pre-dawn leaf water potential and relative water content declined in all the lines, however, L1 showed higher tissue water content throughout the drought and following recovery periods. Chlorophyll content and effective quantum yield (ΦPSII) during drought period did not show a significant reduction, possibly due to the rapid development of stress. Net photosynthetic rate (P_N) declined rapidly and by the 3rd day of drought treatment, and reached near zero, barring L1, which showed slower decline in P_N. The decline in stomatal conductance (g_s) was more rapid than P_N in all the lines. On re-watering, recovery in both P_N and g_s was better in the transgenic lines than untransformed RRII 105, which did not recover fully from the drought impact. Antioxidant enzymes namely, superoxide dismustase and peroxidase did not show any consistency in their activities in the three lines. SOD activity was higher in transgenic line L2 whereas the other lines had lower activity under well watered and drought conditions. Lipid peroxidation was more in the transgenic lines possibly due to the excess H_2O_2 generated by SOD activity in the transgenic lines. However, it was found that line L1 had better drought tolerant capacity under the drought conditions in the North Konkan region of India.

The transgene integration in the transgenic plant developed in the previous year integrated with MnSOD gene using immature anther derived callus explants was confirmed by PCR. This transgenic plant from a new transgenic event has been fully acclimatized and ready for multiplication by bud grafting for further analysis of drought tolerant traits.

A new experiment was initiated to develop antibiotic marker free transgenic rubber plants for desirable traits. Cre/ loxP technique is used to remove the antibiotic marker gene once the transgene is integrated into *Hevea* tissues. A generic binary vector with Cre/loxP specific sequence was synthesized in collaboration with University



Amplification with hpt gene specific primer

Amplification with MnSOD gene specific primer

Fig. Biotech. 3. **Confirmation of multiple gene integration by PCR**

of Arkansas, USA. The generic binary vector was transferred to *Agrobacterium tumefaciens* strain EHA 105. For the functional validation of the generic binary vector, transformation experiment was carried out with tobacco callus. The transgenic callus lines obtained were cultured for proliferation. *Agrobacterium* infection was also carried out with *Hevea* friable anther callus and kanamycin was used for the selection of transgenic callus lines. Kanamycin resistant callus lines obtained were cultured for proliferation.

Experiments were also carried out for developing transgenic rubber plants for multiple traits by integrating two or more transgenes in *Hevea* tissue through repeated transformation. In this experiment attempts were made to incorporate MnSOD for abiotic stress tolerance and *hmgr*1 gene for increased rubber biosynthesis. MnSOD transgenic callus, under the control of FMV34S promoter were generated using zygotic embryo derived embryogenic callus. The MnSOD transgenic cell lines (T_1) were proliferated and the foreign gene integration was confirmed by PCR analysis with MnSOD gene specific primers. This MnSOD transgenic callus was used as the target tissue for *hmgr*1 gene integration employing vacuum infiltration technique. Since *hpt* was used as the selectable marker gene in the HMGR1 gene construct, hygromycin (40 mg/l) was used for the selection of transformed cell lines. Eleven hygromycin resistant callus lines were obtained and all the cell lines were cultured over callus proliferation medium fortified with hygromycin. Proliferated calli were cultured over embryo induction medium and embryo induction was obtained from four cell lines. The embryos obtained were transferred to embryo maturation medium and they are under different stages of development. A portion of the proliferated callus was used for PCR analysis with MnSOD and *hpt* gene specific primers. Amplification was obtained in all the cell lines tested with both set of primers, confirming the presence of MnSOD and *hmgr*1 transgenes in the transgenic callus (Fig. Biotech. 3).

The somatic embryogenesis system developed from leaf explants was also used for genetic transformation and development of transgenic plants. Embryogenic callus lines obtained from genetic transformation experiments carried out earlier were maintained viable by subculture in medium containing kanamycin (100 mg L^{-1}). Embryo induction was obtained from transgenic lines incorporated with all MnSOD and *ipt* genes with varying frequency. Maturation of the embryos, germination and plant regeneration from *ipt* transgenic lines could also be obtained. Attempts are being made for successful hardening of the plants. New *Agrobacterium* infections were also carried out with proliferated fresh leaf callus using *Agrobacterium* strain EHA 101 with two different binary vectors (1) carrying MnSOD gene and (2) carrying *ipt* gene. Callus induced in fresh leaf cultures initiated during 2012, using leaf explants taken from glass house grown bud grafted plants of clone RRII 105 were maintained in an actively proliferating stage were used as target tissue for *Agrobacterium* infection. The infection, co cultivation and selection medium were modified by inclusion of silver nitrate to prevent bacterial overgrowth, antioxidant α lipoic acid (50 mg/l) and surfactant pluronic F-68 (300 mg/l) to improve transformation efficiency. Transgenic calli harboring *ipt* and MnSOD genes were obtained and proliferated. Embryo induction was obtained from two lines each of the proliferated callus harboring Mn SOD and *ipt* genes.

Agrobacterium infection with leaf explants as target tissues was also attempted. Swelled leaf sections after two weeks culture in callus induction medium were used as target tissue for bacterial infection. The pre cultured leaf sections were transferred to petri plates and pretreatments given before bacterial infection. The infected leaf sections were blotted dry and placed in co cultivation medium containing silver nitrate and antioxidants. Selection medium containing silver nitrate and antioxidant along with the antibiotics carbenicillin (300 mg L^{-1}) and kanamycin (100 mg L^{-1}) was used. Infected leaf sections could be recovered free of bacterial overgrowth and callus induction was initiated in few explants proving the feasibility of using pre-cultured leaf sections as target tissues for *Agrobacterium* infection. Newly formed callus was subcultured for proliferation. Leaf sections in control plates gradually died.

Attempts were made for developing transgenic plants integrated with osmotin gene from new transgenic events. Transgenic cell lines were proliferated and subcultured for embryo induction in earlier reported medium. Embryos obtained were then transferred for maturation and germination. For embryo

Table. Biotech 1. **Effect of phytagel and ABA on maturation of transgenic embryos integrated with osmotin gene (%)**

ABA (mg L^{-1}) / Phytagel (%)	0.2	0.4	0.6	0.8	1.0
0.2	14.75(22.58)	17.75 (24.91)	21.75 (27.79)	27.25 (31.47)	30.75 (33.68) *
0.3	13.75 (21.75)	22.75 (28.48)	28.25 (32.105)	32.00 (34.443)	35.0 (36.27)
0.4	20.75 (27.08)	27.75 (31.78)	31.75 (34.29)	34.0(35.67)	35.75 (36.72)
0.5	28.0 (31.94)	35.0 (36.27)	41.25 (39.963)	46.0 (42.70)	38.0 (38.055)
0.6	29.5 (32.86)	24.0 (29.33)	24.5 (29.66)	22.5 (28.30)	20.25 (26.74)

CD (0.05) =1.16

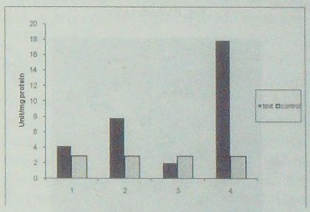
Data were subjected to arcsine transformation and transformed means are given in parenthesis.

maturation, effect of ABA along with different levels of phytagel was experimented and the results are given in Table. Biotech 1.

It was observed that the maturation of embryos improved with higher concentration of phytagel as well as ABA. Maximum maturation (46%) was observed in a combination of 0.5% phytagel along with 0.8 mg L^{-1} ABA. The embryos enlarged in size and the cotyledons became ivory coloured and opaque.

For germination, different levels of BA and GA_3 were tried. Maximum germination of 23.6 % was obtained in a combination of 1.5 mg L^{-1} BA and 1.5 mg L^{-1} GA_3. Higher concentrations had a negative effect on the germination of embryos (Table Biotech. 2). Few embryos germinated from the new transgenic lines were cultured for plant regeneration.

The transgene integration in the *hmgr*1 transgenic plants was confirmed through PCR and Southern blot analysis. The HMGR protein in the leaf tissues of the transgenic as well as the control plantlets was analyzed using indirect enzyme linked immunosorbant assay (ELISA) technique. Polyclonal antibodies raised in rabbit against the HMGR protein (*Arabidopsis* origin) were used as the primary antibody to study *Hevea* HMGR protein. The crude enzyme extract was prepared by grinding the leaf samples (250 mg) in liquid nitrogen and homogenizing in 4 ml 0.1 M phosphate buffer saline (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 minutes. The supernatant was collected and used as the crude protein extract. The crude extract obtained from the leaves of the transgenic and the control plants was coated over the ELISA plate and the absorbance was recorded at 450 nm. The experimental results showed that the transgenic plants had a higher HMGR activity compared to the control (Fig. Biotech. 4).



Biotech Fig. 4. **HMGR ELISA test**
Specific activity of HMGCoA reductase in the leaf samples of transgenic and control plants of *Hevea*

Table. Biotech 2. **Effect of BA and GA3 on germination of transgenic embryos BA (mgL^{-1})**

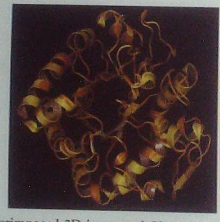
GA_3 (mgL^{-1})	0.5	1	1.5	2	5
0.5	10.40 (3.22)	12.40 (3.64)	12.8 (3.57)	14.40 (3.78)	10.80
(3.28)					
1.0	10.80 (3.27)	14.40 (3.79)	17.20 (4.14)	15.60 (3.94)	12.00
(3.46)					
1.5	14.40 (3.78)	18.40 (4.23)	23.6 (4.86)	17.20 (4.15)	14.40
(3.46)					
20	13.6 (3.78)	17.2 (3.68)	17.20 (4.14)	16.40 (4.05)	9.60 (3.08)
5.0	9.60 (3.08)	10.00 (3.15)	14.40 (3.78)	14.40 (3.78)	7.60 (2.74)
				CD (.05) = 0.89	

4. Molecular studies

4. 1. Molecular mechanism of disease tolerance

Six novel isoforms of the gene encoding β-1,3-glucanase (Gln) involved in abnormal leaf fall disease tolerance was characterized earlier from *Hevea*. Structure modelling studies were carried out with the deduced amino acid sequence of Gln2 isoform showing maximum antifungal activity in the *in vitro* system and compared with 3D structures of reported β-1,3-glucanase from *Hevea* and barley. There are 58 amino acid residues including the start and end glutamic acid residues, occupying the catalytic cleft of the six novel isoforms. It was observed that the residues are highly conserved except for very minor sequence variations (2-3 amino acids) in the catalytic cleft of these newly isolated forms. Amino acid sequence of the catalytic clefts of Gln 2 and a reported 3D structure of β-1,3-glucanase with ID #3EM5A is 100 % similar. The amino acid sequences in the catalytic clefts are similar in Gln 5 and Gln 6. The modelled 3D structure of Gln 2 isoform exhibited 100 % structural alignment with the reported crystallographic structure of the *Hevea* β-1,3-glucanase (PDB ID-3EM5A) upon superimposing (Fig. Biotech. 5 a). An interesting feature observed from the superimposed 3D structures of β-1,3-glucanase in barley (1GHS) and *Hevea* (3EM5A) was that, despite their significant amino acid sequence variations (an alignment score of fifty only), the structures of glucanases from both these distant groups were perfectly superimposing with each other showing the evolutionary structural conservation of β-1,3-glucanases in plants. The 3D models also showed similar pattern of the β-helices and â-sheets arranged in a regular pattern in both *Hevea* as well as in barley (Biotech. Fig. 5 b).

Fig. Biotech (a-b)



a. Superimposed 3D images of Gln 2 (yellow) and 3EM5A (orange)

b. Alignment of the catalytic clefts of Gln 2 (yellow) and 3EM5A (orange)

4.2. Gene expression of signal transducers related to abiotic stress tolerance in *Hevea brasiliensis*

A new project was initiated to study the expression of signal transducer related genes such as mitogen-activated protein kinase (MAP kinase) and calcium dependent protein kinase (CDPK) involved in multiple stress response. Attempts were made to amplify the MAP kinase gene, using primers designed based on consensus cDNA sequences of this gene from *Ricinus communis*, *Populus*, *Arabidopsis*, *Medicago*, *Vitis* and *Nicotiana* species. At optimum PCR

conditions a 0.75 kb band was amplified. Nucleotide sequencing revealed 734 bp showing 94% similarity with MAP kinase cDNA reported from *Ricinus communis* (XM_002534120.1). The sequence was aligned with the cDNA sequence of *Ricinus communis* and showed two gap regions in the *Ricinus* cDNA which were identified as GT-AG introns present in *Hevea* genomic sequence. The two introns are from region 350 – 422 bp comprising 74 nucleotides and from region 552 – 718 bp comprising 149 bp. Major differences are not seen except for a few random single nucleotide variations.

A 1671 bp cDNA encoding calcium dependent protein kinase (CDPK) was also amplified from the cDNA of *Hevea* clone RRII 105. Using the ClustalW tool, the alignment of obtained CDPK cDNA from *Hevea* clone RRII 105 with the reported *Hevea* cDNA sequence from RRIM 600 was analyzed and showed similarity at all nucleotide positions except at 13 sites. Using the ExPASY tool it was able to deduce the amino acid sequence for the CDPK protein containing 556 amino acids. Using pBLAST the amino acid sequence showed similarity with CDPK protein sequences. The protein domains were identified using the PROSITE tool. The first domain is the protein kinase domain (108-366) for binding the substrate. The remaining four domains are the EF-hands (421 - 431, 458 – 469, 494 – 505, and 529 - 540) that bind to the calcium ions. These five domains form the basic structure of CDPK protein.

4.3. Tissue specific gene expression and characterization of promoters

Characterization of *cis*-prenyl transferase gene isoforms and promoters from *Hevea*

Two forms of *cis*- prenyltransferase gene promoter of 716 & 582 bps upstream to the ATG codon were isolated through inverse PCR during previous years. The 582 bp promoter fragment was identified as the promoter of *cis*- prenyltransferase-2 (cis2) gene. Attempts were made to PCR amplify the 716 bp promoter fragment with its corresponding gene and a fragment of approximate size of 1.9 kb from the genomic DNA was amplified. The sequence data on alignment with the earlier isolated 716 bp promoter sequence showed that the PCR amplified fragment contains a new promoter. The gene portion of the promoter showed significant similarity to HRT2 mRNA (#ABO64661.2). The newly isolated promoter region contains 1088 bp upstream to the ATG codon. It contains an intron of 613 bp in the 5'UTR region.

The 5' flanking region of *cis*-prenyltransferase-3 was analyzed for known motifs using the Plantcare and PLACE programmes. One TATA core sequence was found at position -119 to -124 with reference to the transcription start site, which matches the consensus sequence of a TATA core, 5'-TATAWA-3' where W is either A or T. One TATA core sequence was found at position -127 to 132 (TATATA) with reference to the translational initiation site (ATG +1). Putative CAAT box motifs were also located in *cis*-prenyltransferase-3 promoter. Regions like wound-responsive element, MYB binding site involved in drought-inducibility, light responsive elements, heat stress responsiveness element, 5' UTR Py-rich stretch conferring high transcription levels etc are also present in *cis*- prenyltransferase-3 promoter.

Functional analysis of this novel *cis*-prenyltransferase-3 gene promoter was carried out to prove whether the isolated sequence is functional and able to drive gene expression. Binary vector constructs

containing promoter::GUS fusions were made and tested in a heterologous system, *Nicotiana tabaccum*. Transgenic tobacco plants containing chimeric promoter::GUS fusion in which a GUS reporter gene was placed under the control of *cis*- prenyltransferase-3 gene promoter from *H. brasiliensis* were generated. This construct was introduced into tobacco by *Agrobacterium tumefaciens* mediated transformation of leaf discs. Transgenic plants were developed and the expression of the GUS reporter gene was detected histochemically. The transgenic tobacco plants showed positive GUS activity. Presence of promoter-GUS fusions in the putative transgenic tobacco plants were confirmed through PCR using promoter, *hpt* and GUS gene specific primers. Presence of GUS gene transcripts in transgenic tobacco lines were confirmed through RT-PCR analysis. This study suggests that the promoter fragment of *cis*-prenyltransferase-3 analyzed here is functional in terms of their ability to direct expression of the GUS gene in transgenic tobacco plants.

BOTANY DIVISION

The Botany Division apart from continuing its research programmes on multilocational evaluation of promising pipeline clones, breeding for drought tolerance, evolving high yielding clones for the traditional region, propagation studies and investigations on anatomical aspects pertaining to yield components and tapping panel dryness, initiated new programmes on breeding for disease resistance, inheritance of tapping panel dryness, heritability of wood traits and modifications in the root trainer technology. It also launched fourth phase of the participatory clone evaluation project during the reporting period.

1. Evolving high yielding clones for the traditional area

1.1. Hybridization and clonal selection

In order to widen the genetic base of breeding populations and to develop improved clones by crossing promising Wickham x Amazonian hybrids (WxA) as male parents with high yielding clones (RRII 400 series clones and RRII 105) as female parents by attempting 4443 pollinations in seven cross combinations during the reporting period.

Among the 42 promising selections from four small scale trials (1989; 14th year of tapping), twenty six hybrid clones maintained higher annual mean yield and 36 clones maintained higher summer yield than RRII 105. Among the 37 selections from hybrid clones planted in eight small scale trials (1990), eighteen clones maintained

Table Bot 1. **Yield of clones in the 9th year of tapping**

Clone	Parentage	Yield (g t^{-1} t^{-1})*
RRII 105	Parent	81.9a
90/109	RRII 105XRO 26	73.7ab
90/274	RRII 105XMT 196	64.2ab
90/130	RRII 105XRO 24	52.6bc
Mean		35.4
CV (%)		45.3

*Values followed by the same letter do not differ statistically at P= 0.05

higher annual yield and 29 clones recorded higher girth than RRII 105 (13th year of tapping). In a small scale evaluation trial of W x A hybrids (9th year of tapping), yield of clones 90/109 and 90/274 was comparable to RRII 105 (Table Bot.1). In another small scale trial of W x A hybrids, 21 hybrids were found to yield better than RRII 105 (Table Bot.2). Hybrids 90/10, 90/271, 90/193, 90/170 and 90/174 performed better than RRII 105 (mean yield over nine years).

Table Bot. 2 Yield of W x A hybrids in the 9th year of tapping

Clone	Parentage	Mean yield ($g\ t^{-1}\ t^{-1}$)**	Mean yield over years ($g\ t^{-1}\ t^{-1}$)
90/10	RRII 105 x RO 142	106.82a	68.6
90/271	RRIM 600 x RO 87	97.94 ab	52.9
90/193	RRIM 600 x RO 87	88.63 abc	41.7
90/170	RRIM 600 x RO 87	77.68 bcd	45.7
90/174	RRIM 600 x RO 87	76.05 bcd	35.7
90/25	RRII 105 x RO 142	66.58 cde	46.7
90/34	RRII 105 x RO 142	62.05 defgh	47.9
90/29	RRII 105 x RO 142	59.70 defghi	44.5
90/241	RRII 105 x RO 142	49.55 efghijk	39.8
RRIM 600	Tjir 1 x PB 86	56.43 defghij	25.5
RRII 105	Tjir 1 x Gl 1	38.46 ghijk	37.3
RO 142	Rondônia accession	36.73 hijk	18.9
RO 87	Rondônia accession	35.16 ijk	20.3

*statistically significant at 5% level; **Means followed by a common letter are not significantly different by DMRT;

In another small scale evaluation trial (1995; W x W hybrids), 89/27 and 89/309 were identified as promising latex timber clones. In another small scale evaluation trial of hybrids (1998 and 1999), some superior clones like 93/37 (78.8 $g\ t^{-1}\ t^{-1}$) and 93/98 (66 $g\ t^{-1}\ t^{-1}$) and clones 95/242, 95/306, 95/346 and 95/19 with 73.4, 88.7, 87.6, and 86.5 $g\ t^{-1}\ t^{-1}$ respectively were identified. These clones with high yield and better secondary attributes were multiplied and planted in a source bush nursery at CES, Chethackal. In another small scale trial of 24 hybrids planted in 1999, one hybrid clone exhibited superior yield (76 $g\ t^{-1}\ t^{-1}$) over RRII 105 (34 $g\ t^{-1}\ t^{-1}$). In the three small scale trials of 54 hybrid clones (2001), 26 clones exhibited better yield than RRII 105. Clones *viz.* 95/425 (65.5 $g\ t^{-1}\ t^{-1}$), 95/519 (50.1 $g\ t^{-1}\ t^{-1}$) and 95/410 (54.6 $g\ t^{-1}\ t^{-1}$) showed superior yield over RRII 105 in their respective trials. In another small scale trial of 35 hybrid clones (2003), clones 96/135 and 96/422 attained better girth (58.4 and 58 cm respectively) than RRII 105 (50.7 cm).

In the clonal nursery trial (2007) of 22 hybrid clones generated from 2002 HP, clones 02/688 and 02/638 (PB 330 x RRII 414) showed superior girth (26 and 25 cm respectively) than RRII 105 (16.4 cm). Initial test tapping results indicate clone 02/638 (7.3 $g\ t^{-1}\ t^{-1}$) as high yielder. In another clonal nursery trial (2007) of half-sib progenies, progenies of LCB 1320 (37.7 cm), and Ch 153 (36.8 cm) were found vigorously growing (in the 4th year) when compared to check clones. Yield of PB 86 (15.5 $g\ t^{-1}\ t^{-1}$) was comparable to RRII 430 (16 $g\ t^{-1}\ t^{-1}$).

1.2 Ortet selection

In the small scale trial of ortets (Kodumon estate selection; 1993), clones*viz.* OKn 75 (74 $g\ t^{-1}\ t^{-1}$), OKn 28 (60 $g\ t^{-1}\ t^{-1}$) and OKn 39 (52.5 $g\ t^{-1}\ t^{-1}$) exhibited better yield, vigour and clear bole volume over RRII 105. In another small scale trial at same location (9th year of tapping), clone OKn 73 (84.5 $g\ t^{-1}\ t^{-1}$) was found superior. Similarly, clones *viz.* OKn 36 and OKn 49 continued to perform better (55.4 and 57.5 $g\ t^{-1}\ t^{-1}$ respectively) than RRII 105 (51.2 $g\ t^{-1}\ t^{-1}$) and with better mean girth of 78.9 and 85.9 cm respectively. In the small scale trial of ortet selections from small

holdings (7 years data), O 73 (76.2 g t^{-1} t^{-1}) and O 72 (73.8 g t^{-1} t^{-1}) continued to perform better with superior yield and girth compared to RRII 105 (52.6 g t^{-1} t^{-1}) indicating their latex-timber potential.

In a small scale trial of ortets (1998), clone OKr 48 recorded better yield over six years of tapping (35 g t^{-1} t^{-1}) and girth (72.2 cm) than RRII 105 (46.8 g t^{-1} t^{-1}; 64.2 cm). In another small scale trial of ortets (2000), clones *viz.* OKr 49, OKr 71 and OKGD 3 continued to perform better over four years of tapping. Clone OKGD 3 was found a potential latex timber clone (67.1 g t^{-1} t^{-1}; 67.3 cm). In the 2005 large scale trial of ortet selections, clones ChyO35 (46 cm) and ChyO48 (48 cm) registered better girth than RRII 105 (40 cm). A clonal nursery evaluation trial of ortets selected from one of the oldest seedling plantations (NRETC, south Andamans) comprised of GGI, GGII and GG III series of PBIG progenies was laid out at CES Chethackal with 13 clones.

2. Evaluation of clones

2.1. Large scale evaluation

In the two large scale trials of RRII 400 series clones (1993), clones *viz.* RRII 417 and RRII 403 in trial 1 and RRII 430 and PB 330 in trial 2 were comparable in yield to RRII 105 (11th year of tapping). Clone PB 330 (92.8 g t^{-1} t^{-1}; 94.7 cm) was found (12th year data) superior. In terms of mean yield over 12 years, RRII 417 in trial 1 and RRII 430 and RRII 422 in trial 2 were found superior to RRII 105.

In the multidisciplinary evaluation trial (1989A) of introduced and indigenous clones, RRII 5 and RRII 118 were found superior to RRII 105 (Table Bot. 3). In another large scale trial (1989 B) of introduced clones, except PB 310 all PB clones recorded superior yield in both virgin and renewed panels (Table Bot. 4). Yield over 14 years of tapping indicated clone PB 255 (80 g t^{-1} t^{-1}) and PB 280 (71.7 g t^{-1} t^{-1}) as superior clones. Among the Thai clones, KRS 163 was found better in yield.

Table Bot. 3. **Yield performance of clones**

Clone	Yield of BO.1 panel	Yield of BO.2 panel in	Mean Yield over in 4 years B1.1 panel
	(g t^{-1} t^{-1})	(g t^{-1} t^{-1})	(g t^{-1} t^{-1})
RRII 5	61.42	69.01	73.25
RRII 118	46.00	64.32	73.58
RRII 308	45.20	49.53	56.15
RRII 105	47.12	51.77	58.49
Mean	40.83	43.08	43.36
CD (P = 0.05)	6.52	8.17	13.08

Table Bot 4. **Yield performance of clones**

Clone	Yield of BO.1 panel (g t^{-1} t^{-1})	Yield of BO.2 panel (g t^{-1} t^{-1})	Mean yield over 4 years in B1.1 panel (g t^{-1} t^{-1})
PB 255	74.89	79.97	85.77
PB 260	60.46	66.62	60.45
PB 280	66.99	74.58	73.59
PB 310	48.14	64.00	57.75
PB 311	63.68	59.78	56.15
PB 312	68.14	71.85	57.24
PB 314	79.72	76.41	54.29
KRS 163	60.81	70.67	69.37
RRII 105	52.04	55.50	37.30
Mean	58.20	62.86	57.94
CD (P=0.05)	6.52	13.67	19.63

In the large scale evaluation of exotic and indigenous clones (1994; CES, Chethackal), two hybrids *viz.* 86/44 and 86/120 and RRIM 712 continued to maintain higher yield (9th year of tapping). Clone 86/120 with 91.1 cm girth was found a potential latex timber clone (Table Bot. 5). In the large scale trial of hybrid clones at Padiyoor (1996 A), hybrid 86/44 (57.6 g t^{-1} t^{-1}) was found

Table Bot. 5. **Yield and girth of clones during 9th year of tapping**

Clone	Yield ($g\ t^{-1}\ t^{-1}$)	Girth (cm)
86/ 44	88.53	74.63
86/ 120	81.60	91.06
RRIM 728	79.64	72.17
RRII 105	97.08	70.22
CD (P = 0.05)SE	18.236.62	6.442.20

superior over four years of tapping. Five hybrids *viz*. 86/428, 86/109, 86/300, 86/61 and 86/400 were found superior to RRII 105 (4th year of tapping). In another large scale trial (1996 b), hybrids 86/468 (66.5 $g\ t^{-1}\ t^{-1}$) and 86 / 613 (65.6 $g\ t^{-1}\ t^{-1}$) were found superior in yield (4th year of tapping).

2.2. On farm evaluation

In an on-farm evaluation trial for PB and PR clones at Shaliackary estate, Punalur (12th year of tapping), PB 280 (2162 kg ha^{-1}) performed better than RRII 105 (1896 kg ha^{-1}). Among PR clones, PR 261 exhibited better yield. Clone RRII 5 also yielded more than 2000 kg ha^{-1}. In another trial at the same location (1993; 14th year of tapping), yield of RRII 176 (4224 kg ha^{-1}) and RRII 50 (3898 kg ha^{-1}) was higher than clone RRII 105 (3087 kg ha^{-1}). The clone RRII 176 was found to perform well in Kanyakumari region also. In an on-farm trial at Ayiranallur estate (2003), yield of clone PR 261 (1693 kg ha^{-1}) was comparable to RRII 105 (1686 kg ha^{-1}) while clone PB 217 yielded about 1400 kg ha^{-1}.

Among the RRII 400 series clones (on-farm trial at Ayur; 2001), RRII 422 and RRII 430 (75.9 and 68.7 $g\ t^{-1}\ t^{-1}$) yielded more than RRII 105 (50.3 $g\ t^{-1}\ t^{-1}$). In the on-farm evaluation of RRII 400 series clones (Shaliakary estate; 2003), clone RRII 429 (59.7 $g\ t^{-1}\ t^{-1}$) continued to perform better than other clones. Clone RRII 414 (65.5 cm) and RRII 422 (66.3 cm) attained better girth than RRII 105 (55.5 cm). At Kanjirapally (2005), all the clones of RRII 400 series registered maximum tappability when compared to RRII 105. Initial yield of RRII 430 and RRII 414 was better than RRII 105. At Kulathupuzha (8th year of planting), RRII 414 (51.5 cm), RRII 429 (49.7 cm) and RRII 422 (48.2 cm) performed better than RRII 105 (48.5 cm).

Variation in yield was observed in RRII 400 series clones evaluated in small-holdings across Kerala. RRII 414 at Ponkunnam and Elenji, RRII 417, RRII 422 and RRII 429 at Malayattoor, RRII 430 at Oonnukal performed better than RRII 105. In Malabar region, while RRII 417 excelled in yield, bark thickness was almost similar to RRII 105 (4th year of tapping). While RRII 422 excelled at Tichur (4th year of tapping), RRII 414 excelled at Wandoor (Malappuram; 5th year of tapping). Maximum bark thickness was found in Clone RRII 422. Yield of RRII 429 (35.3 $g\ t^{-1}\ t^{-1}$; 51.07 cm) and RRII 414 (33.6 $g\ t^{-1}\ t^{-1}$) was comparable to RRII 105 (34.7 $g\ t^{-1}\ t^{-1}$; 52.6 cm) at Mannarkadu (2nd year of tapping). At 3rd year of tapping, clone RRII 414 showed maximum bark thickness (8.1 mm) and girth (53.9 cm).

2.3. Genetic studies and investigations on genotype x environment interactions

When narrow sense heritability (h^2) of TPD was computed using parent-offspring regression of 12th year data, high narrow sense heritability ($h^2 = 0.50$) for TPD was found. Progenies of PB 5/51 x RRII 208 showed minimum TPD incidence (3.6%) while those of RRIM 600 x PB 235 exhibited maximum incidence (29.6%). Progenies of RRIM 600 x RRII 33 did not exhibit TPD symptoms. Progenies produced from hybridization between clones with very low

(e.g., RRII 33) TPD incidences exhibited very low TPD incidences and vice versa in the clones with very high TPD (e.g., PB 235) incidences. TPD also was found to have genetic correlation with yield and girth (Table Bot. 6).

Table Bot. 6. Genetic parameters of tapping panel dryness

Regression estimates	Narrow sense heritability (h^2) for	
tapping panel dryness		
Offspring- 'mid-parent' mean	0.50	
Offspring - female parent	1.12	
Offspring - male parent	0.30	
	Genetic correlation	
	Yield	Girth
Tapping panel dryness (TPD)	-0.25	-0.41

Investigations carried out on 'genotype x environment' (G x E) revealed significant variation in yield among clones (Table Bot. 14). While RRII 176 (67.59 g t^{-1} t^{-1}) excelled at Kanyakumari, RRIM 600 (54.26 g t^{-1} t^{-1}) and RRII 422 (84.41 g t^{-1} t^{-1}) excelled at Agartala and Nagarkata respectively. Over nine years of tapping, clones RRII 203 and RRII 105 at Kanyakumari, RRII 429 and RRII 422 at Agartala and RRII 429 at Nagarkata were found top performers. At Padiyoor, clones RRII 430 (67.3 g t^{-1} t^{-1}), RRII 422 (60.5 g t^{-1} t^{-1}), RRII 417 (59.1 g t^{-1} t^{-1}) and PB 217 (57.2 g t^{-1} t^{-1}) exhibited better yield than RRII 105 (50.36 g t^{-1} t^{-1}) in the 6th year of tapping. At Bhubaneswar based on data of yield over three years and on third year of tapping, clones RRII 430 and RRII 429 were found best performers.

3. Participatory evaluation of rubber clones

Under Phase 1 of participatory clone evaluation (2008), 20 pipeline clones and three check clones *viz.*, RRII 105, RRII 414 and RRII 430 are being evaluated in 14 field trials. Clone RRII 414 from LST 1, P 070 and RRII 414 from LST 2 were found vigorous (Table Bot. 7). Among the check clones, RRII 414 and RRII 430 were superior in girth in

Table Bot. 7. Girth of pipeline clones at 4th year after planting of the Central LSTs

LST of clones under Batch 1		LST of clones under Batch 2	
Clone	Girth (4th yr)	Clone	Girth (4th yr)
P 074	22.4	P 044	23.9
P 068	18.7	P 064	19.1
P 067	19.2	P 065	18.6
P 061	19.7	P 062	14.1
P 060	13.9	P 069	15.6
P 084	17.9	P 063	15.8
P 010	19.0	P 066	14.5
P 015	18.8	P 087	16.1
P 076	18.6	P 072	21.6
P 021	22.8	P 027	16.7
P 088	16.7	P 078	21.7
P 053	21.5	P 070	24.2
RRII 105	19.1	P 026	22.6
RRII 430	21.5	RRII 105	17.3
RRII 414	23.3	RRII 430	21.3
G.M.	19.6	RRII 414	23.7
C.D.(0.05)	3.99	C.D.(0.05)	2.41

OFTs of all locations. Clones P 010, P 026, P 074, P 067, P 021, P 087 and P 072 exhibited better growth in most of the locations. Gokul Estate, Vithura was the best among locations of first batch of clones. The second batch of clones performed better at Be Be Estate, Punalur. In general, the growth of clones was better in the southern districts of Kerala. While in batch 1, girth of clones RRII 414, RRII 430 and P 021 were superior to RRII 105, P 026 and RRII 414 were superior in batch 2.

In Phase 2 of PCE project (2010), the central large scale trial was planted at CES and on-farm trials were laid out in eight

locations. Under Phase 3 of the project, trials were established in seven locations in 2012 with the central large scale trial at CES, Chethackal and OFTs at six locations involving twelve pipeline clones and three check clones.

4. Breeding for other specific objectives

4.1. Breeding for Drought tolerance

Growth and yield performance of hybrids developed for drought tolerance was assessed in the 7th year of tapping in two small scale trials (1998). In trial 1 (Table Bot. 8) while hybrid 93/214 exhibited maximum yield (94.9 g t^{-1} t^{-1}), clone 93/216 (58.8 g t^{-1} t^{-1}) topped in summer yield. In trial 2, hybrid 93/58 (85.87 g t^{-1} t^{-1}) was the highest yielder. In the small scale trial (1998) of ortets selected for drought tolerance from Dapchari, Dap 111 continued to record maximum yield (120 g t^{-1} t^{-1}) followed by Dap 236 (112 g t^{-1} t^{-1}) while RRII 105 yielded 87 g t^{-1} t^{-1}. In other two small scale trials (1999), seventy hybrid clones are under evaluation in drought prone conditions. Among the 14 clones evaluated in trial I, while clone 94/90 (76.5 cm) recorded highest girth, clones 94/23 (89.4 g t^{-1} t^{-1}) and 94/44 (88.9 g t^{-1} t^{-1}) recorded superior yield in the 6th year of tapping (Table Bot. 9). Among the 56 clones studied in trial 2, 16 clones performed better than RRII 105 (7th year of tapping). Clones 95/297 and 95/448 yielded 95 and 93 g t^{-1} t^{-1} respectively while RRII 105 yielded 57 g t^{-1} t^{-1}.

Table Bot. 8. **Yield performance of hybrids in the 7th year of tapping**

Clone	Parentage	Mean yield (g t^{-1} t^{-1})**	Summer yield (g t^{-1} t^{-1})**
93/214	RRII 105 x RRIC 52	94.9	40.2
93/88	RRII 105 x PB 86	70.7	37.9
93/216	RRII 105 x RRIC 52	68.9	58.8
93/270	RRII 105 x RRIC 52	61.3	35.7
93/105	RRIM 600 x RRIC 104	61.1	46.0
PB 217	PB 5/51 x PB 6/9	84.2	53.8
RRIC 104	RRIC 52 x Tjir 1	65.1	36.9
RRII 105	Tjir 1 x Gl 1	57.3	33.8
RRIM 600	Tjir 1 x PB 86	55.8	30.7
RRIC 52	Primary clone	39.6	34.2

**significant at p<0.01

Table Bot. 9. **Performance of clones in the 6th year of tapping**

Clone	Parentage	Yield (g t^{-1} t^{-1})	Girth (cm)
94/23	RRII 105XPB 86	89.4a	74.0ab
94/44	RRIM 600XRRIC 52	88.9a	74.6ab
94/50	RRIM 600XRRIC 52	74.1ab	67.6de
94/101	RRII 105XRRIC 52	63.5ab	61.3cd
PB 217	Parent	60.7ab	61.7bc
RRII 105	Parent	57.6bc	61.7bc
Mean		48.5	62.3
CV (%)		34.8	11.2

*Values followed by same letters are not statistically different

Table Bot. 10. **Performance of clones in the 4th year of tapping**

Clone	Parentage	Girth (cm)	Yield (g t^{-1} t^{-1})
95/98	RRII 105 x RRIC 104	58.9	98.7
95/3	RRII 105 x PB 217	62.9	79.2
95/518	RRII 105 x RRII 118	59.7	65.5
95/280	RRII 105 x PB 260	60.0	63.9
95/489	RRII 105 x AVT 73	64.3	57.1
95/108	RRII 105 x RRII 118	65.6	56.5
95/484	RRII 105 x PB 280	69.8	53.8
95/97	RRII 105 x RRIC 104	61.2	49.7
95/520	RRII 105 x PB 260	57.0	49.5
95/253	RRII 105 x AVT 73	55.1	48.0
RRII 105	RRII 105	55.4	45.6
CV		7.8	38.5
CD (0.05)		6.4	22.6

Among 36 clones (SST trial 1; 2001; fourth year), ten clones were found superior in yield and eighteen clones were found superior in girth to RRII 105 (Table Bot. 10). Clone 95/484 recorded highest girth (69.8 cm), clone 95/98 registered highest yield (98.7 g t^{-1} t^{-1}). Among the 14 clones evaluatedin trial 2, seven were found superior in growth. Maximum girth and yield was exhibited by clone 528 (67.8 cm) and clone 279 (75.4 g t^{-1} t^{-1}) respectively while RRII 105 attained 53.3 cm girth and yielded 52 g t^{-1} t^{-1}.

4.2 Breeding for disease tolerance

A seedling nursery evaluation trial of open pollinated progenies of FX 516 (a disease tolerant clone) was initiated by hybridizing RRII 400 series clones and RRII 105 with FX 516, WxW hybrid, WxA hybrid and wild germplasm accession RO 87 to develop disease resistant clones (Table Bot. 11).

Table Bot. 11. **Hybridization for disease tolerance**

Female parent	Male parent	Nos. of HP
FX 516	RRII 105	524
RRII 105	FX 516	68
RRII 105	341/90 (RRIM 600 x RO 142)	113
RRII 414	FX 516	1141
RRII 414	520/86 (RRIM 600 x RRII 33)	97
RRII 414	RO 87 (Rondônia accession)	53
RRII 414	193/90 (RRIM 600 x RO 87)	69
RRII 414	341/90 (RRIM 600 x RO 142)	353
RRII 417	520/86 (RRIM 600 x RRII 33)	172
RRII 422	520/86 (RRIM 600 x RRII 33)	37
PB 330	520/86 (RRIM 600 x RRII 33)	71
	Total nos. of HPs	2698

4.3 Molecular breeding

For developing a mapping population, hybridization was carried out by employing RRII 430 and RRII 414 as female and clone FX 516 as male parents. To overcome the asynchrony in flowering, the pollen samples collected from FX 516 were stored at various freezing conditions (4°C, -20°C, -80°C and -196°C) in 2011 and were subsequently used in 2012. Viability of the pollen was not affected in any of the freezing conditions attempted (Fig. Bot. 1).

4.4. Polycross progeny evaluation

In order to identify pre-potent clones, 150 half-sib progenies from ten parent clones are being evaluated in two field trials (1993). In trial 1, progeny of PB 242 (77.8 cm) and PB 28/83 (75 cm) recorded superior girth (11th and 12th year of tapping). Forty four clones were found superior to RRII 105 (Table Bot. 12). PB 242, PB 215 and Ch 26 produced maximum number of high yielding and uniform progenies indicating their prepotency.

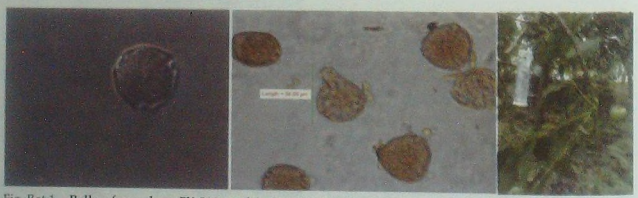


Fig. Bot 1. **Pollen from clone FX 516 used in hybridization programme and initial fruit set: Pollen stored under -196°C (left), pollen stored under -20°C (centre), initial fruit set recorded 56 days after HP (right)**

Table Bot. 12. **Performance of half-sibs of prepotent clones in trail 1**

Progeny	Yield (11^{th}yr.; g t^{-1} t^{-1})	V.R. within progenies	No. of clones superior to RRII 105
RRII 105	58.2	4.1**	3
PB 242	74.9	1.8	8
AVT 73	49.8	1.8	3
PB 252	54.6	1.7	3
PB 217	60.9	3.3*	5
PB 28/83	53.2	2.2	2
PB 5/51	51.6	6.3**	4
PB 215	63.9	1.7	7
Ch 26	67.9	1.9	6
PB 5/76	50.8	1.1	3
G.M.	58.6		
V. R.	3.5*		
Yield of RRII 105	58.4		

Table Bot. 13. **Performance of half-sibs of prepotent clones in trial 2**

Progeny	Yield (11^{th}yr.; g t^{-1} t^{-1})	V.R. within progenies	No. of clones better than RRII 105
RRII 105	78.67	3.95*	2
PB 242	62.73	3.40	1
AVT 73	55.11	5.77*	1
PB 252	67.22	1.13	2
PB 217	64.53	16.29**	1
PB 28/83	57.83	4.55*	1
PB 5/51	50.68	19.86**	1
PB 215	73.43	1.10	3
Ch 26	80.63	4.88*	4
PB 5/76	48.71	1.91	-
G.M.	63.95		
V. R.	4.51*		
Yield of RRII 105		75.49	

In trial 2 where mean yield of progenies ranged between 80.63 and 48.7 g t^{-1} t^{-1} (Table Bot. 13), 16 clones performed better than RRII 105 (11^{th} year of tapping). As in trial 1, the highest mean yield and recovery of high yielding clones was found in the progeny of Ch 26 and PB 215 reaffirming their superiority. Progeny of PB 252 was found superior in girth (87.6 cm; 19^{th} year of planting). In another trial (2005), trees of polyclonal seedling origin attained superior mean girth over RRII 105 seven years after planting. In clonal nursery trial of half sib progenies (2007), progenies of clone LCB 1320 (37.7 cm) and clone Ch 153 (36.8 cm), exhibited better growth that parents (in the 4^{th} year). Progenies of PB 86 (15.5 g t^{-1} t^{-1}) and check clone RRII 430 (16.0 g t^{-1} t^{-1}) registered superior yield.

Anatomical investigations

Studies on anatomical and histochemical changes were continued. Cell walls of sieve tubes in the inner soft bark including the recently derived ones from cambium got stained reddish brown with O-dianisidine while the remaining tissues were left unstained. Peroxidase activity was found to be seasonal in phloic rays and continuous in sieve tubes of bark tissues. Diameter, density and grouping pattern of sieve tubes varied among the clones studied (RRII 105, RRII 33, RRII 38, RRIM 600, RRII 118, RRII 208 and PB 260). Preliminary studies on chaotic and linear spatial display of movement of particles in the sieve tubes indicated the existence of almost uniform pattern in healthy and uneven pattern in TPD affected trees.

6. Propagation

The experiment on the influence of different forms of planting materials on growth and yield of rubber plants was continued. No significant change in growth and yield (12^{th} year) was found in trees raised with different planting materials (Table Bot 14).

Table Bot. 14. **Mean girth and yield (g t^{-1} t^{-1}) of trees raised from different planting materials**

Treatments	Girth (cm)	Yield (1^{st} yr)	Yield (2^{nd} yr)	Yield (3^{rd} yr)
Brown budded stumps	56.96	59.49	47.49	58.68
Field budded plants	54.27	54.93	37.46	46.35
Green budded plants	57.03	58.50	51.31	58.72
Brown budded plants	56.98	58.61	46.85	46.69
Young budded plants (stock 42 days old)	56.70	58.23	48.10	42.18
Young budded plants (stock 49 days old)	56.93	56.93	42.27	48.40
Young budded plants (stock 56 days old)	56.47	58.73	45.51	38.25
CD (0.05)	NS	NS	NS	NS
SE	1.79	2.32	2.96	3.11

In the experiment on influence of age of bud wood stock on growth and yield of rubber plants, plants raised from young and matured budwood attained a mean girth of 45.6 and 42.2 cm respectively (Table Bot. 15). Plants raised using buds from the mature trees were significantly inferior in girth (33.69 cm). Higher intraclonal variation in girth was found in plants raised with buds from mature budwood than from young budwood plants. Trees raised from young budwood source had better vigour and higher percentage of tappability (60.2%) than plants raised from mature budwood (39.1%). Plants developed using budwood from mature trees exhibited minimum tappability (11.1%).

In connection with the experiment on influence of various types of buds on growth, tappability and yield of rubber, trees raised from prominent semi green buds and whorl buds attained 45.6 and 42.1 cm respectively during 7^{th} year of planting (Table Bot. 15). Trees raised using light green buds recorded highest intra-clonal variation. Among the recommended buds, trees raised using prominent semi green buds possessed higher percentage of tappability in the 7^{th} year with an average of 61.9 % as compared to trees from whorl buds (37.5 %).

Table Bot. 15. **Tappability of trees using different types of budwoodin the 7^{th} year**

Treatments	Girth (cm)	Tappability (%)	CV
Trees - young budwood stock (Source A)	46.29	59.33	12.76
Trees - young budwood stock (Source B)	44.52	51.33	14.67
Trees - young budwood stock (source C)	46.62	70.67	14.79
Trees - young budwood stock (Source D)	45.14	59.33	14.70
Trees - old budwood stock (Source A)	42.01	39.66	19.68
Trees - old budwood stock (Source B)	42.48	38.50	15.47
Trees - budwood collected from old trees	33.69	11.11	23.11
CD (0.05)	4.91	6.98	

GERMPLASM DIVISION

The 1981 IRRDB wild *Hevea brasiliensis* germplasm collection, domesticated clones derived from the original Wickham collection, and 5 other *Hevea* species are being conserved at RRII. Management of this collection, formulation of DUS testing norms for *Hevea*, and generation of a mapping population, are the thrust areas of the Division. Alternative sources of natural rubber suitable for marginal lands are also being explored.

1. Introduction, conservation and documentation

1.1. Domesticated genepool (Wickham collection) from secondary centers

183 Wickham clones are being conserved in field gene banks with 1 clone museum at RRII Farm, Kottayam, and 2 germplasm gardens at CES, Chethackal. In the germplasm garden GG IV planted in 1992 and comprising the 5 IRCA clones, girth, dry rubber yield and incidence of TPD were analysed (Table Ger. 1).

Table Ger. 1. **Performance of IRCA clones in the 11th year of tapping**

Clones	Girth ** (cm)	Dry rubber yield ** ($gt^{-1}t^{-1}$)	Incidence of TPD (%)
IRCA 130	77.8	66.4	11.0
IRCA 111	79.3	53.5	6.0
IRCA 109	67.9	43.3	5.0
IRCA 18	68.5	35.5	8.0
IRCA 230	64.8	44.6	8.0
RRII 105	70.7	58.2	8.0
CD(P=0.05)	6.64	19.70	

** Clonal differences significant at P<0.01

During the 11th year of tapping, IRCA 130 and IRCA 111 were found to be very vigorous, with better performance for girth and dry rubber yield, indicating the potential of these clones as latex-timber clones. The clone IRCA 130 however also showed higher TPD than the other clones. Analysis of growth over years showed that IRCA 130 and IRCA 111 were consistently superior to all other clones for girth, while IRCA 130 was consistently the highest yielder followed by RRII 105 and IRCA 111 (Figs. 1 and 2).

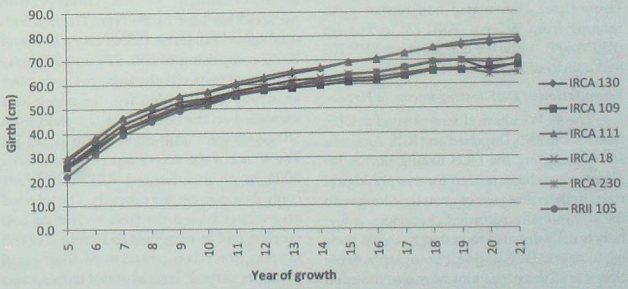


Fig. 1. Growth of IRCA clones over 21 years

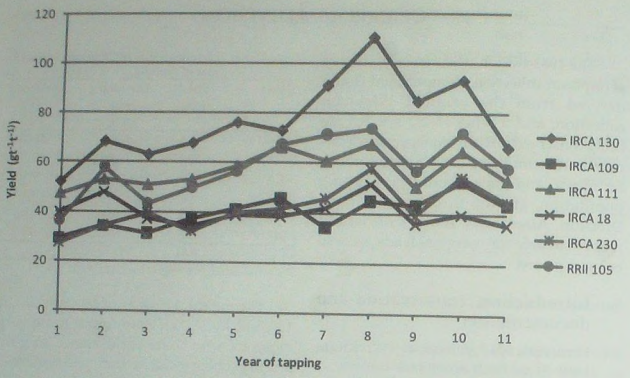


Fig. 2. Yield of IRCA clones over 11 years of tapping

In Germplasm Garden V, among the 20 clones, RRII 23, RRIC 100 and RRIM 609, continued to be the best clones in terms of yield (83.1 – 79.9 $gt^{-1}t^{-1}$), while the control clone RRII 105 had an yield of 62.7 $gt^{-1}t^{-1}$. These three clones also had higher girth ranging from 99.2 cm (RRII 23) to 93.8 cm (RRIM 609), while the control clone RRII 105 had a girth of 74.1 cm.

Formulation of DUS testing norms in Hevea

The 49 Wickham clones planted at CES, Chethackal, RRS, Dapchari and RRS, Agartala, for formulating the DUS testing norms for *Hevea*, were characterized for morphological traits in their first year of growth. Along with the traditionally recorded characters, a few new traits were also recorded to assess their usefulness in DUS testing. Photographs of the clones at all three locations were also recorded for a digital database.

1.2. IRRDB 1981 wild gene pool

3576 wild accessions are 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. 16 accessions identified on the basis of tetstap yield from SBN 2005 were multiplied for the next FET. The 4th set of 806 accessions planted in SBN 2006 in an augmented RBD with four controls is maintained properly. The selected accessions based on first round of test tapping will be subjected to 3rd round of test tapping for reconfirmation of their yield potential as the 2nd round of test tapping was affected by rain.

The SBN 2007 comprising of 500 accessions was monitored for early growth and test tap yield. Data were recorded on the traits yield ($gt^{-1}t^{-1}$) by test tapping in 3rd year, girth (cm) in the 2nd year to 5th year, girth increment (cm/yr) over 3 years, number of branches, crotch height (m), plant height (m) in the 2nd year and 5th year and height increment (m). 25 potential accessions for yield were identified and MT 4788, AC 226, AC 4155, MT 1007 were top yielders. Wide range of variability was observed for various yield and growth contributing traits (Table Ger. 2).

Of the 201 wild accessions in SBN 2008, 6 accessions were identified from the second round of testapping for promotion to the next FET for further detailed evaluation.

Table Ger. 2. **Range of variability for yield and growth characters in wild *Hevea* germplasm**

Characters	Wild accessions			Control clones				
	Minimum	Maximum	General mean	RRII 105	RRII 208	RRIM 600	PB 235	CV (%)
Test tap yield ($gt^{-1}t^{-1}$)	0.01 (MT5081)	3.52 (MT4788)	0.15	0.11	0.05	1.42	0.20	245.17
Girth (cm) 2nd year	3.0 (AC493, RO 1374, RO2367)	15.9 (AC1218)	7.8	5.7	4.8	6.8	5.0	42.83
Girth (cm) 3rd year	6.0 (MT4350)	21.0 (RO3792)	12.1	9.2	8.9	12.2	8.9	30.72
Girth (cm) 4th year	8.0 (AC3944)	28.3 (MT2226)	16.6	12.9	12.3	17.2	12.2	31.29
Girth (cm) 5th year	10.7 (RO2328)	37.5 (RO3792)	21.1	18.9	18.5	22.8	18.9	32.15
Girth increment (cm)	0.7 (RO319, AC3905)	10.0 (AC577, AC 734)	4.4	4.4	4.6	5.3	4.6	46.92
No of branches	1.7 (RO1754)	13.5 (MT1680, RO 4194)	4.9	3.8	4.1	4.6	3.9	53.76
Crotch height (m)	1.2 (RO4574)	9.6 (RO335)	3.4	2.4	2.6	3.0	2.9	26.00
Plant height (m) 2nd yr	0.9 (MT136)	7.3 (MT2226)	2.8	1.8	1.5	2.6	1.9	55.25
Plant height (m) 5th yr	1.5 (RO4574)	13.0(RO229)	6.5	4.3	4.3	6.5	4.6	41.2
Height increment (m)	0.5 (RO4574)	9.8 (AC989)	3.7	2.5	2.8	3.8	2.8	44.71

Note: Figures in parenthesis denotes the name of accession.

1.3. Other *Hevea* species

The collection of other species of *Hevea* available at RRII, comprising 6 species and their hybrids *(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. Characterization and preliminary evaluation

In the preliminary evaluation trial PET 2000, the 2 accessions RO 4599 and MT 4788 continued to show better yield levels than RRII 105 and were used for crop improvement through hybridization this year. At RRS, Padiyoor, monthly yield and annual girth was recorded in 171 wild accessions in PET 2000A. Accession AC 2537 (68.7 cm) recorded the highest girth followed by RO 2171 (61.7 cm) and AC 3609 (59.7 cm). Highest annual yield was recorded in accession RO 2786, AC 2670 and RO 2136. Monthly yield and annual girth were recorded in PET 2000B at RRS, Padiyoor. Among 166 wild accessions, AC 647 recorded the highest girth of 77.0 cm followed by accession RO 2883 (73.3 cm) whereas the check clones RRII 105, RRII 208 & RRIM 600 recorded girth of 53.2 cm, 46.1 cm and 44.0 cm respectively. 27 accessions had girth higher than RRII 105 showing their timber potential. For mature yield, among the 3 check clones, RRII 105 recorded the highest yield (44.2 g). Among the wild accessions, RO 341 recorded highest mature yield (43.0 g) on par with RRII 105, followed by accession AC 2004 (31.7 g).

3. Further evaluation and selection

3.1. Clonal nursery evaluation

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

3.2. Further evaluation trials

Annual girth, dry rubber yield and volumetric timber (bole) yield were recorded and analysed at the age of 18 years (9th year of tapping) in the further evaluation trial FET 1995. Five Mato Grosso accessions and 1 Acre accession showed significantly higher girth than that of the control clone RRII 105 The accession AC 166 had the highest dry rubber yield (65.3 $gt^{-1}t^{-1}$) which is statistically on par with the yield of RRII 105 (64.0 $gt^{-1}t^{-1}$) in the 8th year of tapping. Three accessions (RO 2385, AC 2004 and RO 2908) had 85% to 95 % of the yield of RRII 105, while 11 accessions showed 50% to 80% of the yield of RRII 105 (Table Ger. 3).

Table Ger. 3. **Potential accessions showing dry rubber yield**

Accessions	Dry rubber yield ($gt^{-1}t^{-1}$)	% of the yield of RRII 105
AC 166	65.3	101.9
RO 2385	61.1	95.5
AC 2004	54.9	85.7
RO 2908	54.6	85.2
AC 692	46.7	72.9
AC 650	46.7	73.0
AC 655	44.6	70.0
AC 635	44.0	68.7
MT 922	42.5	66.3
AC 638	41.5	64.6
RO 368	40.7	63.6
AC 668	36.3	63.3
MT 1020	35.1	54.8
AC 3013	32.7	51.0
RO 2976	32.3	50.0
RRII 105	64.0	
CD(P=0.05)	20.06	

Five accessions showed better performance for timber yield compared to RRII 105. Two Mato Grosso accessions (MT 999 & MT 922) showed resistance *Phytophthora* with 95% leaf retention. The trial was subjected to stimulated tapping this year.

Annual girth was recorded in the FET 2003 comprising 22 wild accessions and 3 controls. RO 2629 (68.4 cm) recorded the highest girth followed by AC 4149 (58.8 cm) and AC 626 (53.0 cm). RO 2629 and AC 4149 also recorded highest yield in the 2nd year of tapping, followed by AC 716.

Analysis of girth in FET 2005 comprising 22 wild accessions and 3 controls showed highly significant clonal differences. AC 2004 followed by MT 4788 had the highest girth (54.4 and 46.0 cm respectively), on par with the best check PB 260 (49.1 cm). Clonewise analysis of girth data showed that none of the clones, including controls, had attained 70 % tappability by the 8th year of growth. Accessions RO 1570 and RO 1755 showed the highest tappability percentage at 42.9 and 40.0 % respectively, while the best check PB 260 showed 30.8 %. Seven other accessions, including AC 2004 and MT 4788, showed tappability ranging from 15.4 - 26.7 %. In FET 2008, accessions AC 176, MT 77 and RO 2846 and 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 planted at CES, Chethackal along with the check clones RRII 105, RRII 430 and RRII 414 are under evaluation. Planting material for 25 selected wild accessions along with 3 control clones were multiplied and raised in poly bag nursery at CES, Chethackal for planting of FET 2013 in the ensuing planting season.

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 were maintained properly. Girth of the clones was recorded in all the 5 locations. At Malankara estate, Thodupuzha, among the IRCA clones, IRCA 111 showed good girth whereas at Mooply estate, Trissur, IRCA 130 was performing well. The wild accession AC 166 gave the highest girth among the 4 test clones at Mooply estate.

4. Screening for stress tolerance

4.1. Screening for biotic stress tolerance

As part of *Corynespora* disease tolerance screening of wild *Hevea* accessions, a set of 55 shorted listed accessions along with 2 control clones were multiplied for confirmation in a hotspot evaluation trial to be planted at Ulickal nursery, Iritty, a collaborative project with Plant Pathology division.

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

A clonal nursery comprising of 40 potential half-sibs of 9 clones and 7 hybrid seedlings is being evaluated along with 3 checks *viz*, RRII 105, RRII 430, RRII 414 for their drought tolerance potential at RRS, Dapchari, a drought prone area. Girth at 3rd year after planting ranged from 8.9- 18.5 cm among the 47 clones. The family of clone PB 5/51 recorded the highest mean girth. Among the 4 check clones, the highest girth was recorded by RRII 105

(19.1 cm). Family means of 9 clones on girth is shown in Table Ger. 4.

Table Ger. 4. **Family means of nine clones for girth**

Clone	Family mean (cm)
AVT 73	12.0
Ch 26	13.4
PB 215	13.4
PB 217	10.3
PB 242	13.3
PB 28/83	13.3
PB 5/51	16.9
RRII 105	13.5
RRII 203	13.7

Another clonal nursery comprising of 29 potential half-sibs and 2 hybrid progenies are under evaluation at RRS, Padiyoor, to study their drought tolerance potential at this location, which is a drought prone area in the traditional belt. Girth at 3rd year ranged from 6.8-14.4 cm among the 31 clones. Among the 4 check clones, the highest girth was recorded by RRII 430 (11.6 cm).

A small scale trial (normal spacing) comprising 7 potential accessions selected out of 130 wild accessions screened at RRS, Dapchari (close spacing) for their drought tolerance potential based on drought related growth parameters and rubber yield are under further evaluation at RRS, Dapchari for re-confirmation of their drought tolerance potential. Check clones are RRII 105, RRII 414, RRII 430, RRIM 600 and Tjir 1. Among the 7 accessions, accession RO 2976 recorded highest girth at 3rd year after planting.

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. Out of 34 clones in this trial, 9 wild accessions and 3 HP clones showed girth superior to drought tolerant clone RRIM 600 after completing 5 years growth. Accession MT 4856 recorded highest girth (27.7 cm) during the sixth year. Among the modern clones, RRII 430 showed significant growth difference with RRII 414 at Dapchari conditions (27.8 and 23.9 cm respectively). Top ranking accessions for girth during sixth year is shown in.

4.2.2. Cold tolerance

A total of 64 wild accessions were evaluated for cold resistance in 2 trials at Regional Experiment Station, Nagrakata, West Bengal. Girth of the 13 year-old accessions recorded during pre- and post-winter period, showed significant variation. Monthly yield, DRC, PI, CCI and leaf traits were recorded. Higher annual girth was observed in RO 2902, MT 5105 and RO 2387 as compared to check clones SCATC 93/114 and RRIM 600 in Trial 1. In Trial 2, accessions MT 915, RO 2727 and MT 900 recorded higher girth than that of the controls Haiken 1 and RRIM 600.

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, 14 accessions showed girth statistically on par with RRII 105; 4 accession on par with PB 235 & PB 260; 5 accessions on par with RRII 33; 2 accessions on par with RRII 118; and1 accession on par with RRIM 600. The timber (bole) volume was significantly higher in 2 accessions (MT 941 & AC 650) had than that of PB 260, RRII 33, RRII 118, RRII 105 and RRIM 600 (Table Ger. 5).

Table Ger. 6. **Tree girth and bole volume**

Wild Accessions	Girth (cm)	Bole volume (m^3)
MT 922	64.5	0.09
MT 941	61.4	0.1
MT 915	58.5	0.07
AC 650	57.5	0.13
RO 255	56.6	0.05
MT 919	56.0	0.07
AC 635	54.4	0.06
MT 1032	53.7	0.06
MT 999	51.7	0.06
AC 1021	51.1	0.05
Domesticated Clones		
RRII 118	68.0	0.08
PB 235	67.0	0.05
PB 260	65.4	0.08
RRII 33	63.7	0.06
RRII 105	56.9	0.06
RRIM 600	53.6	0.06
RO 322	51.3	0.08
AC 655	48.9	0.05
MT 935	48.4	0.04
MT 1020	48.1	0.04
AC 707	43.1	0.04
AC 685	42.0	0.04
AC 637	41.8	0.05
AC 651	41.2	0.06
RO 879	36.3	0.03
CD(P=0.05)	14.46	0.033

Two Acre accessions (AC 685 and AC 707) showed yield 28.3 and 30.3 $gt^{-1}t^{-1}$, which were statistically on par with that of RRII 105 & PB 235 (38.7 and 30.5 $gt^{-1}t^{-1}$).

5.2. Lignin biosynthesis studies

Wood lignin percentage of 6 *Hevea brasiliensis* clones and 1 *Hevea benthamiana* clone was estimated. Leaf lignin percentage was also estimated in these clones as well as in 4 other plant species grown as intercrops in rubber plantations *viz. Coffea arabica, Theobroma cocao, Tectona grandis* and *Artrocarpus hirsutus*. RRII 430 (24.3%) had the highest wood lignin percentage followed by RRII 422 (23.7%) , RRII 429 (22.0%) and *H. benthamiana* (21.8%) while the least was in RRII 105 (18.9%). *Artocarpus hirsutus* (31.8%) and *Hevea benthamiana* (31.5%) showed the highest leaf lignin percentage. Among *Hevea* clones, RRII 430 (26.6%) showed high leaf lignin (Fig. 3 & 4).

6. Utilisation of *Hevea* germplasm

6.3. Hand pollination programmes

A hand pollination programme conducted during 2009 at CES, Chethackal involving 3 wild accessions showing potential for yield, and 6 cultivated Wickham clones, resulted in 75 successful hybrid progenies which are under field evaluation for early growth and test tap yield. At RRS, Padiyoor 29 progenies derived from 2 crosses in 2009, along with 25 OP seedlings of RRII 105, are under evaluation in a seedling nursery. Morphological data in the 3rd year of planting was recorded. Hybrid progenies from the cross RRII 105 X AC 675 continued their superiority in girth compared to the progenies derived from the cross RRII 105 X RO 368 but the recovery of superior seedlings was 47 % in the second combination compared to 44% in the first combination.

Two wild accessions identified from PET 2000, RO 4599 and MT 4788, showing high mature yield supported by high latex vessel number, were crossed with RRII 105 this season. 538 and 949 crosses were made respectively with these two accessions. Initial fruit set was 9 and 28 (1.7 and 2.9%) respectively.

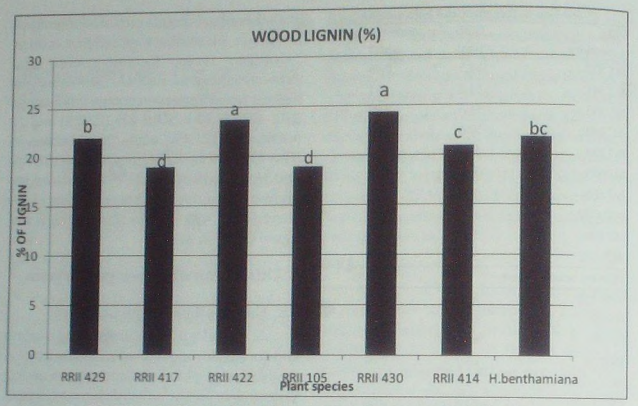


Fig. Ger. 1. Wood lignin (%) in 6 *Hevea brasiliensis* clones and *H. benthamiana*

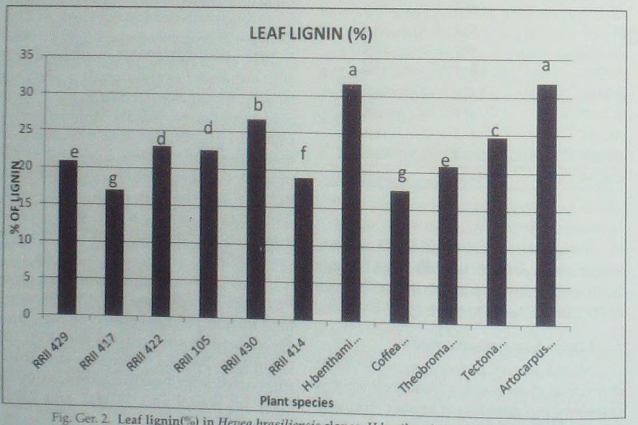


Fig. Ger. 2. Leaf lignin(%) in *Hevea brasiliensis* clones, *H.benthamiana* and four intercrops

6.4. Generation of mapping population

From the 96 seedlings generated in 2009 HP between *H brasiliensis* (RRII 105) and *H benthamiana* (F 4542), growth of the progeny established in the seedling nursery was monitored: girth ranged from 5.0- 30.5 cm, with an average of 18.8 cm. The seedlings were subjected to testtapping: HP 09/109 and HP 09/34 gave the highest testtap yield of 3.42 and 2.99 $gt^{-1}t^{-1}$ respectively. The cross was repeated this year too: 1829 crosses were made, and the initial fruit set was 44 (2.4%).

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

This collaborative project with ITDA was taken up with the objective of monitoring growth of new large scale plantings in 2008 at RC Varam, by the Integrated Tribal Development Agency (ITDA), Govt. of Andhra Pradesh. Growth of rubber plantations in 12 randomly selected farmers' fields was assessed. In the 5th year of growth, Farm 7 recorded the highest girth of 29.5 cm and Farm 10 recorded the lowest girth 9.5 cm.

Studies on Alternative Sources of Natural Rubber yielding plants

Initiated studies on the conservation and management the germplasm lines of guayule rubber (*Parthenium argentatum*) and Ceara rubber (*Manihot glaziovii*) as alternative sources of natural rubber with the objective of understanding the feasibility of cultivation of these potential drought resistant plant species in semi-arid stress prone areas and marginal lands in India. One scientist acquired overseas training on the germplasm conservation, cultivation and related R&D activities on guayule rubber from various USDA laboratories at USA. One indigenous accession of Ceara rubber was multiplied and established in the field at RRII.

8. Other studies

8.1. Feasibility of ratooning in *Hevea*

Girth was recorded in the 12th year of growth in the two sets of plants- the ratoons as well as the plants raised conventionally in polybags. Ratoons continued to be superior (Table Ger. 7). 98.5 % and 60.1 % of the ratoon and polybag plants respectively have attained more than 50 cm girth so far. High variation for yield in individual plants was observed for yield. 18 and 2 plants respectively recorded more than 50 $gt^{-1}t^{-1}$. Yield of the two existing RRII 105 ratoon plants (others are 50 different clones) was 49.0 and 59.1 $gt^{-1}t^{-1}$.

Table Ger. 12. **Growth and yield of ratoon plants**

	Girth (cm)		Yield $gt^{-1}t^{-1}$	
	Ratoons	Polybags	Ratoons	Polybags
Number	135	203	124	35
Max	111.0	85	107.5	103.7
Min	16.0	16.5	8.7	8.6
Avg	74.6	51.3	27.8	16.8
No.>50	133	122	18	2

PLANT PATHOLOGY DIVISION

1. Leaf diseases

1.1. Abnormal leaf fall disease

The experiment to improve the efficiency of ALF disease management in the clones RRII 105 and RRIM 600 at Chimony estate Thrissur was continued for the third year. The spraying was undertaken by Turblow mistblower during the second fortnight of May. Second round of spraying

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

Treatments	Ratio	Leaf retention (%)					
		2010		2011		2012	
		RRII 105	RRIM 600	RRII 105	RRIM 600	RRII 105	RRIM 600
COC(10kg): spray oil (40L)	1:4	90	60	60	40	95	60
COC(8kg): spray oil (48L)	1:6	82	82	70	50	95	55
COC(4kg) : spray oil (20L) (Two rounds)1st round - 0 day 2nd round – 30th day	1:5	99	99	90	75	95	75
COC(8kg): spray oil(40L)	1:5	94	54	70	50	95	50
Control	—	47	5	50	5	90	10
$CD_{(0.05)}$		11.4	5.6	5.8	5.5	NS	4.3

in the experiment was imposed 30 days after first spraying. Observation on the leaf retention, leaf fall from leaf collection baskets were recorded. Two rounds of spraying of COC in oil @20 L/ ha (1:5) was found to be superior in checking the ALF disease during the three years of study. (Table Path.1)

Bio-degradable oil supplied by Bharath Petroleum Company Ltd. was subjected to phytotoxiciy test and found non-phytotoxic. Block trials were carried out during 2012 season at three locations to evaluate the usefulness of bio-degradable oil as a carrier for COC in clone RRIM 600. Bio-degradable oil sprayed plots were found to retain more leaves indicating its better efficiency as a carrier for COC against ALF disease comparing the conventional mineral oil.

In the field trial to evaluate the yield loss due to powdery mildew and ALF at Padiyoor in the clones RRII 105, RRIM 600, PB 235 and PB 5/51, sulphur dusting was undertaken during January and February and spraying of COC in oil against ALF was under taken during last week of May in the respective plots. The monthly block yield was recorded and yield loss was calculated. The crop loss observed was shown in Table Path. 2. The girth increment was found to be less for untreated plants.

Isolates of *Phytophthora* spp. collected from 14 different regions from 2010-2012 were characterized by their mating type, and resistance to fungicides . Out of 690 isolates tested, 169 were of A1 mating type and 521 were A2 mating type, indicating predominance of A2 mating type in rubber plantations. Of the isolates screened for fungicide sensitivity to Fytran, 18% was sensitive, 58% were intermediate and 24% were resistant.

To find out the differentially expressed genes during Phytophthora induction, plants of *Phytophthora* tolerant clone FX 516 were challenge inoculated with zoospores of *Phytophthora* and subtracted cDNA library was developed. Subtracted cDNA showed homology with resistant related cDNA of other plants and hypothetical proteins

Attempts were made to evaluate the antagonistic potential of a bacterial endo-symboint against ALF disease causing pathogen *Phytophthora meadii*. The antagonistic compound in the culture supernatant of *Alcaligenes* sp. EIL-2 was extracted with diethyl ether and the extract

Table. Path. 2. **Powdery mildew and ALF disease- % leaf retention and yield loss in different clones**

Clones	Powdery mildew disease		Abnormal leaf fall disease		% yield loss
	Dusted	Undusted	Sprayed	Unsprayed	
RRII 105	95	90	90	40	6.45
RRIM 600	86	70	60	10	28.46
PB 235	85	75	80	65	13.73
PB 5/51	85	65	70	50	10.49

inhibited the growth of *P. meadi* under *in vitro* condition. In the crude extract, five components were identified through thin layer chromatography with Rf values of 0.58, 0.61, 0.66, 0.72 and 0.88 and each component was separated through flash column chromatography. Of these five, one specific component was found to inhibit the mycelial growth of *P.meadii*. The active fraction was purified in RP-HPLC and their chemical

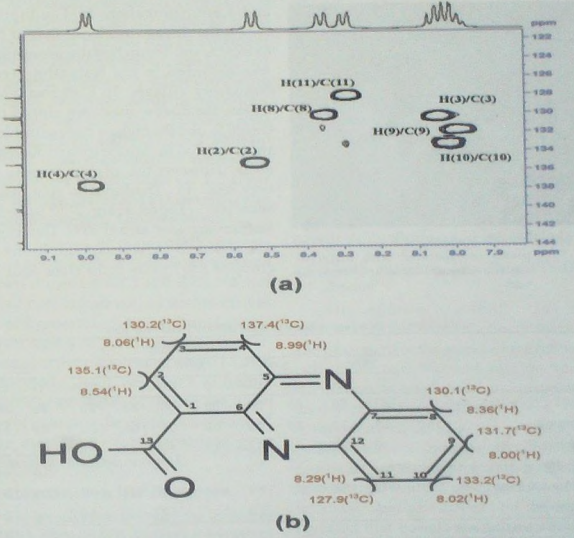


Fig. Path. 1 (a) **Two dimensional NMR-HSQC spectrum of antagonistic compound from *Alcaligenes* sp.(EIL-2) broth supernatant (b) predicted structure of the compound**

structure was elucidated by mass spectroscopy, FTIR, ^{1}H and ^{13}C NMR, DEPT NMR, 2D-NMR($^{1}H/^{1}H$-COSY, HSQC) analysis and was identified as phenazine-1-carboxylic acid(PCA) (Fig. Path. 1).

The purified phenazine-1-carboxylic acid showed antagonistic activity against *P. meadii*. The percentage inhibition of *P. meadii* mycelial growth increased with increase in the concentration of phenazine-1-carboxylic acid and the minimum inhibitory concentration of Phenazine-1-carboxylic acid against *P. meadii* was found to be 5 µg/ml (Fig. Path. 2)

Fig. Path. 2. **Growth inhibition of *P. meadii* on PDA plates incorporated with different concentrations of Phenazine-1-carboxylic acid**

1.2. Powdery mildew disease

Crop loss at New Ambadi estate Kanyakumari during 2008 to 2012 in the clone RRII 105 was continued. 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 as per the method reported by Jacob et al.(1992). The crop loss estimated were presented in Table Path.3.

1.3. Corynespora leaf disease

The survey was continued for the third year in the two localities *viz.*, Thodupuzha and Kothamangalam. In Thodupuzha region the disease incidence ranged from 68 to 90% and the highest disease incidence was recorded in the Nalpathu acre of Neyassery village. In Kothamangalam region, the disease incidence ranged from 80-100 % and the highest disease incidence was recorded in Varapetty and Kadavoor areas. However the disease severity was very less. In Thodupuzha area disease severity ranged from 2-20% and in Kothamangalam area 0-35%. The disease was observed to be maximum in the age group of 1 to 7 years in the clone RRII 105.

Table. Path. 3. **Powdery mildew disease intensity and crop loss**

	Disease intensity									
	2008*		2009		2010		2011		2012	
	D	UD	D	UD	D	UD	D	UD	D	UD
1st dusting	BB**	BB	BB	BB	BB	BB	BB	BB	BB	BB
2nd dusting	2.2	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd dusting	3.00	3.0	0.3	0.3	0.3	2.0	0.3	1.5	0.5	2.0
4th dusting	-	-	-	-	-	-	-	-	1.0	4.0
Yield(Kg/400 trees)	1798	1902	1827	1894	1663	1647	1409	1472	2218	2276
Crop loss(%)	-	-	-	1.3	-	5.7	-	5.5	-	3.0
Cumulative crop loss over 4 years										4.0

* Pre-treatment year D - Dusted

**BB = bud break UD - Undusted

The 400 series clones RRII 414 and RRII 430 exhibited less disease severity.

Evaluation of new generation fungicides was continued at Ulickal nursery on the polybag plants of the clone RRII 105. The results indicated that among the fungicides tested, pyraclostrobin+metiram, thiophanate methyl and ipridione + carbendazim were effective (9.6 % disease severity).

Transcriptome sequencing was adopted to gain a comprehensive overview of the *Hevea brasiliensis* transcriptome in response to Corynespora infection in two clones *viz.*, RRII 105 (susceptible) and GT 1 (tolerant). A comparison of the differential gene expression data revealed that an average of 397 and 649 genes were down regulated and upregulated respectively when comparing the control transcriptomes of the two clones. Upon challenge inoculation, 58 genes were down regulated and 574 genes were up regulated in the clone RRII 105. Whereas, in the case of the tolerant clone GT 1, 5 genes were down regulated and 817 genes were up regulated following pathogen infection.

Expression of major pathogenesis related genes against *C.cassiicola* infection in two clones *viz* GT 1 and RRII 105 was carried out. Enhanced expression of four major PR genes *viz.*, chitinase, Phenyl alanine ammonialyse (PAL) PR 5 and peroxidase was observed in the the tolerant clone GT 1 than in the clone RRII 105 which is highly susceptible to *C.cassicola*. During the initial hours of induction the expression was found to be maximum for peroxidase gene followed by chitinase and PAL.

1.4. Colletotrichum leaf disease

The experiment to study the influence of Colletotrichum leaf disease on growth of rubber at TR & T estate, Mundakayam on the clone RRII 105 was continued.. The disease severity in the protected block was in the range of 8 to 10 % whereas it was around 65 % in unprotected block. The average girth and height of the plants and the number of leaves in the second whorl were also observed to be high in the protected block.

The experiment to study the efficacy of new generation fungicides at TR & T estate, Mundakayam against colletotrichum leaf disease was continued for the second yer. The results indicated that the treatment involving tebuconazole + trifloxystrobin registered least disease severity (8.86 % and 9.83 %).

The study to confirm the major cause of the Colletotrichum leaf disease in India was continued for the second season. *C.acutatum* was found to be more frequent with 91.3% of the colonies. There were very few locations where either *C.acutatum* or *C.gloeosporioides* alone was isolated. Both the species could be isolated from all types of symptoms. Perfect stage or the ascigerous stage of the fungus *Colletotrichum gloeosporioides* (*Glomerella* sp) producing perithecia in culture could be isolated .

1.5. South American Leaf Blight (SALB) disease

The climate suitability for the occurrence of SALB in India, South East Asia and other parts of the world where rubber is cultivated was worked out using an environmental envelope or an expert-driven model using the ArcMap 9.3, the main component of ESRI's (Environmental Systems Research Institute) ArcGIS suite of geospatial processing programs. Individual maps for temperature, precipitation and RH for India, South East Asia and the world revealed that not all these three suitable environmental factors are present together during the refoliation period in India and

therefore, disease development does not take place in India. However, there is a threat for certain regions in South East Asia and Africa, which is a matter of concern.

2. Stem diseases

2.1 Pink disease

Evaluation of new generation fungicides as prophylactic and curative treatment was continued at Mundakayam estate. In the experiment with prophylactic application of various treatments maximum recovery of the plants was observed in case of traditional Bordeaux paste (98%) and Kondody's Bordeaux paste (97%). The treatments involving Kondody's Bordeaux mixture spray was comparable with untreated control in terms of per cent recovery of the plants. In the curative application, fungicides were mixed in rubberkote and applied on the infected portion of the plants. The results revealed that Kondody's paste was highly effective (100 % recovery of plants). Among the new generation fungicides the fungicide tebuconazole (90% recovery) was promising followed by copper hydroxide (Kocide 101) with 85 % recovery.

3. Pests of rubber

Conducted a preliminary study on the effect of *Metarhyzium anisopliae* on the larvae of mooply beetles by spraying the fungal suspension on the cluster of larvae. Three different concentrations of *Metarhyzium anisopliae* were sprayed and recorded mortality of larvae on the 3rd and 7th day of spraying. The results showed only 15 -38 % mortality of larvae after 7th day of spraying (Table Path. 4).

Conducted an extensive survey on the incidence of bark feeding caterpillar,

Table. Path. 4. **Effect of *Metarhyzium anisopliae* on the mortality of the larvae of mooply beetles, *Luprops curticollis***

Metarhyzium anisopliae in different concentrations	Percentage of mortality after	
	3rd day	7th day
Metarhyzium anisopliae (14x105)	10.90(19.29)	14.85(22.68)
M. anisopliae (28x105)	19.50 (26.22)	24.62(29.76)
M. anisopliae (55x 105)	31.33 (34.05)	37.76(37.93)
Control	7.96(16.40)	7.82(16.25)
CD at 5%	17.43	12.42

(Figures in parenthesis are arcsine transformed values)

Aetherastis circulata (Lepidoptera :Yponomeutidae), in rubber trees and observed Kodumon estate as the highly infested area. The pre pupal and pupal stages of bark feeding caterpillar from the infested trees were collected from January to April 2013 and kept in the laboratory condition for the emergence of adult lepidopteran or the parasitoids. The emergence of a parasitoid from the pupae was observed and was identified as *Braconid* sp (Braconidae). An entomopathogenic fungus was also isolated from the dead larvae.

The incidence of *Ptochoryctis rosaria* in rubber trees was recorded from different locations *viz*; Pathanamthitta, Punalur, Adoor and Kottayam. The attack was mainly noticed in clones PB260, PB235, RRII 105 and RRII 422 and was found confined to the outermost virgin bark areas.

4. Vector control

Carried out entomological surveillance on the vector population in different locations such as CES Chethackal, Aimcombu, Malankara, Ramapuram and Kumarakom. The biodiversity of mosquito species in rubber plantations was found less compared to non-rubber areas. *Aedes*

albopictus was recorded as the predominant species of mosquito (48%) in rubber plantations followed by *Armigerus subalbatus* (43%).

Conducted field experiments on the control of mosquitoes in three locations at Erumely, Mukkoottuthara and Mundakayam regions by spraying Bt formulations on pineapple axils. Observations were taken on 2nd ,5th and 7th day after spraying and also after two, three and four weeks. 100 percent mortality was noticed on 2nd day of spraying. Bt showed good control of mosquito breeding in pineapple axils up to three weeks.

5. Bee keeping in rubber plantations

Conducted a field study to find out the comparative efficacy of feeding of fruit juices on the brood rearing and foraging activity of Indian Honey bee, *Apis cerana indica* during periods of dearth in rubber plantations. Colonies were fed with juices of jack fruit, mango, pineapple (200 ml / colonies) and sugar syrup twice a week during off season. The percentage increase in the brood development and foraging activities of the colonies were found to be high in sugar syrup treated control colonies compared to fruit juice treated colonies.

Carried out a comparative study on the pollen content and bio chemical constituents of honey samples collected from different sources such as rubber, forest and other floral sources in collaboration with Environmental Resources Research Centre (ERRC), Trivandrum. As per the analytical report, the rubber honey appears to pass the recommended specifications.

6. Microorganisms for improving growth of rubber and cover crops

The role of 15 PGPR (plant growth promoting rhizobacteria) isolates in the germination of rubber seed was studied. The per cent germination, after 12 days and 30 days of planting was found high in the *Azotobacter* G inoculated seeds (76%) followed by RB 88 (72%), F1 (72%) , RH104 (71%) and in control 62%. The phosphofungi selected did not show any stimulating effect on seed germination.

About 1300 rhizobacterial cultures from 36 locations of South Karnataka and North Kerala, were isolated and studied their various beneficial activities for growth promotion. In the primary screening of 310 bacterial isolates, solubilisation of phosphate was shown by 67, ZnO by 45 and $ZnCo_3$ by 66 isolate. The production of IAA was shown by 20 isolates and siderophore by 19 isolates. Among 417 isolates of fungi collected, twenty isolates were selected based on their phosphate solubilising efficiency. Forty isolates solubilised ZnO and 33 isolates ZnCO3. Most of the phosphate solubilisers were also solubilizers of Zn compounds.

About 550 bacterial isolates collected during the summer season were tested for ACC deaminase activity and selected 80 isolates for further screening.

Eighty five actinomycetes collected from these locations were screened for antagonism against the six pathogens of rubber. Four isolates *viz*. 39, 45, 65 and 134 were antagonistic to all the five pathogens (Table Path. 5). Isolate 39 showed more zone of inhibition against *C. acutatum* and

Table. Path. 5. **Antagonism of actinomycetes against different pathogens**

Isolates	*P. noxius*	*C. salmonicolor*	*P. meadii*	*C. cassiicola*	*C. acutatum*	*C.gloeosporioides*
39	14mm	20mm	10mm	16mm	35mm	33mm
45	10mm	20mm	15mm	10mm	20mm	18mm
65	5mm	19mm	27mm	15mm	20mm	16mm
134	16mm	32mm	18mm	38mm	27mm	27mm

C.gloeosporioides, isolate 134 against *Corynespora*, *Corticium* and *P. noxius* and isolate 65 against *Phytophthora* and to *Corticium* with browning of mycelium.

Bacterial isolates (210 nos.) were also checked for antagonistic activity against all the pathogens. Four antagonists of *Phytophthora*, six each of *Corynespora* and *C. acutatum*, two of *Corticium*, three of *C. gloeosporioides* and two of *Phellinus* showing more zone of inhibition were selected.

In the biofarming trial the population of microorganisms in soil from the different treatments was estimated. The total bacterial population in the three treatments did not vary. Total fungal, phosphobacterial and *Pseudomonas* population was more in biological and integrated plots. Inoculated bacterial population in the rhizosphere soil was also more in these two plots.The plants in the integrated treatment three years after planting showed more growth (24.33 cm) followed by chemical (21.7 cm) and biological (20.6 cm) treatments which were statistically on par. Incidence of diseases was less in all treatments.

7. Waste management in rubber processing

The various treatment systems *viz.*, floor wash collection and coagulation tank, initial filtration unit, combined/equalization tank, biomethanation system (HRMR), diffused aeration system, clarification/sedimentation system, final filtration unit, treated water collection tank were integrated to obtain reduction in pollution load. This Integrated Waste Water Treatment System (IWWTS) was evaluated by analysing various samples collected with respect to various pollution parameters. The hybrid reactor-HRMR was founded to be effective in reducing the pollution load. It could reduce BOD by 94.57%, COD by 95.12%, total solids by 74.87%, and dissolved solids by76.50%. The pH also increased from the range of 4.8-6.3 to 6.8-7.2 (Table Path. 6).

The overall performance efficiency of the integrated waste water treatment system consisting of HRMR, diffused aeration, sedimentation and filtration was found to be highly effective. The system could achieve removal efficiency in BOD-99.48%, COD-98.63%, TS& DS- 81.50 %. The pH increased from the range of 4.7-6 (that of raw effluent) to 6.6-8 (treated final water). All the pollution parameter of the treated final water was well within specified safe limits for discharge to the environment.

The HRMR yielded 8m^{3}biogas /day. The utilization of biogas as an alternative fuel has reduced > 35% of firewood in the smoke house for sheet drying.

8. Farm Mechanization

The HDP pump used in the tractor mounted mist blower was replaced by a

Table. Path. 6. Efficiency of Integrated waste water treatment system

(Coir filter + HRMR+ diffused aeration + sedimentation + sand filtration)

Parameter	Raw effluent	Filtered Effluent	Discharge from HRMR	After diffused aeration and sedimentation	Final (After filtration)	Overall Efficiency of ETP (% reduction)	Safe limit
pH	4.7-6.0	4.8-6.3	6.8-7.2	6.5-7.7	6.6-8.0	—-	6-8.5
COD	10964	9037(17.57)	441(95.12)	183(58.50)	150(18.03)	98.63	250
BOD	5349	4859(9.16)	264(94.57)	30(88.64)	28(6.66)	99.48	30
TS	13119	11362(13.39)	2856(74.87)	2562(10.29)	2432(5.07)	81.46	2100
DS	12003	10388(13.45)	2437(76.50)	2371(2.71)	2219(6.41)	81.51	— —

Values are in Mg/l, except pH.

Values given in parenthesis are percentage reduction

centrifugal pump and field tested. Pumping of fungicide fluid was found interrupted frequently. The fungicide delivery pump, working through a pulley & belt system was also found ineffective during the operation in the field. The leaf retention in the plot where all the rows were sprayed with the tractor mounted sprayer was comparable with the conventional micron spraying.

In view of introducing single men operated mist blower, various units available in the market were evaluated for their performance. The efficacy of these sprayers were assessed by recording the ALF disease severity in terms of per cent leaf retention. All the single man mist blowers tested were found inefficient to protect the rubber plants from abnormal leaf fall disease where the height of the tree was around 70 feet.

PLANT PHYSIOLOGY DIVISION

The thrust areas of research in the Crop Physiology Division are environmental and stress 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

One year old plants of nine different clones of *Hevea* (*viz*., RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, RRII 105, RRIM 600, RRII 208 and PB 260) grown in big size poly bags were subjected to water deficit stress by withholding irrigation for ten days during summer season (March-April, 2012). Similarly one year old plants of RRII 105 and RRIM 600 raised in poly bags at RRS Dapchari, a severe drought prone area, were subjected to water deficit stress by withholding irrigation for six days during summer season. Chloroplast proteins prepared from these clones were subjected to western blotting using the polyclonal antibody raised against the stress protein. The result showed that the stress protein

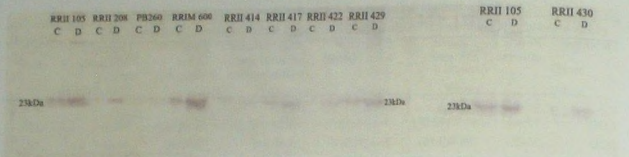


Fig. Phy. 1. **The expression of 23 kDa chloroplast small hsp in different clones of *Hevea*; Western blot showing the over-expression of the protein in drought imposed plants (C- irrigated control and D- drought imposed)**

expression was not visible in the clone PB260 and in all other clones protein with varied magnitude of expression (Fig.Phy.1). Drought tolerant clones such as RRIM 600, RRII 208 and RRII 430 showed prominent expression of this protein after water stress imposition.

Western blot analysis was performed for total leaf protein also. The result showed good profile with over-expression of stress protein in drought imposed plants. The stress protein differentially expressed among the clones were purified by repeated electro-elution and resolved by 2D gel electrophoresis to confirm its homogeneity and further sequencing of this protein was carried out by MALDI TOFF/TOFF method.

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

Six month old polybag plants of clones RRIM 600 and RRII 105 were subjected to drought stress and re-watering (two cycles of drought stress and re-watering). Leaf samples were collected after assessing the drought status of the plants by measuring water potential and gas exchange parameters. Quantitative gene expression analyses were performed in drought imposed plants for NAC transcription factor. GAPDH was used as endogenous control for normalization analysed with reference to control plants of and respective clones.

The gene expression analyses showed significant increase of NAC transcription factor expression after re-watering and subsequent drought stress. The increase in expression was more in a relatively drought tolerant clone RRIM 600 compared to the known susceptible clone RRII 105.

1.3. Molecular studies on cold stress in *Hevea*

Young polybag plants of clone RRIM 600 were acclimatized in a growth chamber and cold treatment was imposed by reducing the temperature to 8° C. Total RNA was isolated from both leaf and latex samples using the Spectrum Plant Total RNA Kit (Sigma-Aldrich). mRNA was isolated using magnetic beads (Dyna beads, Invitrogen, USA). Transcriptome library for sequencing was constructed according to the Illumina TruSeq RNA library protocol. Around 90735 transcripts were obtained, out of which about 961 transcripts were up-regulated under cold stress and about 109 transcripts were down-regulated. SSRs containing transcripts specifically found in cold responsive population were identified.

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

To develop ESTs of various stresses *viz.* drought, low temperature, ethylene and TPD, transcriptome sequencing has been

performed by outsourcing with M/s. Genotypic Technology, Bangalore. Data were analysed and the sequence informations have been submitted in the Genbank database. Further data compilation and arranging to get the microarray is being done.

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

Low molecular weight RNA was isolated from the leaf samples of drought imposed plants of clone RRIM 600 using mirVana miRNA isolation kit and separated on 12% denaturing (7M urea) polyacrylamide gel. The bands at the range of 20-24 nt were eluted using DTR columns. 3′ and 5′ linkers were ligated using miRCat cloning kit in two separate reactions. Reverse transcription was performed for the linkered small RNA and PCR amplification of cDNA was performed using linker specific primers. The amplicons were purified and proceeded with cloning and sequencing. A total of 50 clones were sequenced. By performing BLAST analysis against miRBase database v19.0, two sequences were found similar to miR166 family and one was found similar to miR482.

1.5. Ecosystem Flux measurements

1.5.1 *Sap-flow measurements in mature rubber plants*

With an objective of accounting the water use of rubber plants in different agro climatic regions sap flow measurement systems were commissioned in mature rubber trees at RES, Nagrakata, North Bengal and RRS, Dapchari, Maharashtra during September and December 2012, respectively.

The xylem sap flow rate (water L/ tree/ day) recorded in two rubber clones (RRII 417 and RRII 429) at RES, Nagrakata is given in Table Phy.1. The average water mining rate (sap flow) of mature rubber plantation at RES, Nagrakata during winter period was 13 L/tree/day. This rate was only around one third of the post monsoon season (30 L/tree/ day) indicated defoliation (wintering) and low temperature mediated reduction in transpiration.

Table Phy. 1 **Sap flow rate in two rubber clones at RES, Nagrakatta, West Bengal**

	Mean transpiration (T) water loss L/tree/day	
Month	RRII 417	RRII 429
Oct 2012	28±1.2	33±0.9
Nov 2012	29±0.7	30±1.2
Dec 2012	21±0.6	17±0.73
Jan 2013	14±0.7	11±0.6
Feb 2013	10±0.3	6±0.33
March 2013	19± 0.9	18.4±1.0

At RRS, Dapchari, Maharastra the sap flow system was installed in clone RRIM 600 with two different treatments; one set of trees (n=3) with irrigation during summer months and another set of trees (n=3) left without irrigation as rainfed trees. The average water mining rate of irrigated trees (L/tree/day) was significantly higher than unirrigated trees.

1.5.2. *Measurement of CO_2 and water vapour flux in rubber plantation*

The ecosystem level carbon dioxide and water flux and canopy level photosynthesis in a seven year old rubber plantation were continuously measured during the period (October 2012 to March 2013) using an eddy covariance system which was installed in a field standing tower at Central Experimental Station, Chethackal. On an average, the NEE was 11.6 g CO_2/m^2/day, which is equivalent to 42 tons CO_2/ha/year. The evapo-transpiration (ET) values obtained from the

latent heat of vapourization (LE) indicated 2.5 mm/day from this plantation.

The amount of carbon sequestered by the rubber trees was estimated during the same period by estimating the annual shoot biomass increment and the CO_2 sequestration realized from shoot biomass increment was 23 tons CO_2/ha/yr (which does not include root biomass, soil carbon, litter decomposition and sequestration by weeds and cover crops).

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

Studies were initiated to find out existence of any common mechanism of abiotic stress tolerance in *Hevea* clones under different environmental conditions. A polybag nursery of *Hevea* clones comprised of nine clones (RRII 414, RII 422, RRII 430, RRII 429, RRII 105, RRII 208, RRIM 600, and RRIC 100 & SCATC 88/13) was raised at RRII. At two whorl stage drought was imposed to RRII 422, RRII 430, RRII 208, RRIM 600 and RRII 105. Clones such as RRII 430 and RRII 422 maintained better photosynthesis and stomatal conductance under water stress. All the clones showed a reduction in ΦPSII and the magnitude of reduction was lesser in RRII 430 and RRII 208. These clones showed better photochemical quenching under drought. Leaf water content was well maintained in RRII 422 and RRII 208. A reduction in photosynthetic electron transport rate (ETR) occurred under drought treatment.

A) Diurnal variation in xanthophyll pigments

Diurnal variation in xanthophyll cycle pigment contents was analysed in poly bag plants of RRII 414. It was found that neoxanthin (Nx) content increased starting from 8.30 am to 2.30 pm and there after declined. Violaxanthin (Vx) and Lutein (L) contents were higher at morning time followed by a marked decrease till midday and showed an increment trend in the evening. Antheraxanthin (Ax) was not datectable at early morning however, there is a minor increase in the content after 10.30 am and stable till evening. Zeaxanthin (Zx) content was also not detectable at early morning but showed a significant increase in their level with increase of light intensity, recorded maximum at midday then decline during evening when sunlight falling. Xanthophyll cycle pigment pool size (V+A+Z) showed clear diurnal variation and found high at midday. High level of xanthophyll cycle activity at midday indicated a major role in dissipating excess light energy in mesophyll.

B) Sun/shade effects on xanthophyll cycle pigments

Polybag plants were grown under open sunlight and 50% shade conditions. Leaf samples were collected after 15 days of sun/shade treatment and analysed for xanthophyll cycle pigments. The data showed that neoxanthin (Nx) content reduced under open sunlight condition however, under shade condition it was found increased in RRII 208. A reduction in violoxanthin (Vx) pigment under open sunlight condition in all the clones was observed. Zeaxanthin (Zx) was not traceable under shade condition but higher levels were observed in sun exposed leaves. The xanthophyll pigment pool size was low in shade condition than the sun exposed leaves. Level of xanthophylls pigment pool size per unit of chlorophyll was found high in RRII 430 and RRIM 600 followed by RRII 422 and SCATC 88/13 (Table Phy.2). In all clones a reduction was observed in the level of total chlorophyll and α-carotene under open sunlight condition.

Table Phy. 2. **Variation in xanthophyll cycle pigment pool : total chlorophyll among the clones in sun exposed leaves**

Clone	V+A+Z / Chl. (%)
RRII 414	3.69
RRII 422	4.95
RRII 429	4.35
RRII 430	5.63
RRIC 100	4.96
SCATC 88/13	4.82
RRII 208	5.26
RRIM 600	5.55
RRII 105	3.89

1.6.2. Foliar application of potassium to mitigate drought

A study was conducted with an objective to find out the advantage of foliar application of potassium to tide over the adverse effect of drought in young plants of *Hevea*. Two clones were selected for this study (RRII 105 and RRII 414). After two weeks of drought imposition, RRII 105 plants showed significant reduction of photosynthesis. Drought plants showed drastic reduction in leaf water potential. But this reduction was lesser in plants with foliar application of potassium under water deficit. Under drought condition foliar application of K showed better rate of photosynthesis and stomatal conductance compared to without K application. Higher levels of carotenoid and ascorbic acid contents were observed in plants under drought with potassium application than control drought plants. It was found that potassium application protected the photosynthetic machinery from damaging effects of water stress with elevated levels of antioxidants and carotenoids.

1.7. 1 Physiological evaluation of transgenic Hevea plants in a dry environment

One year old bud grafted plants of untransformed RRII 105 and MnSOD transgenic lines (L1 and L2) were irrigated daily up to field capacity and another set of equal number of plants was kept un-irrigated for six days. After that the plants were irrigated daily to study the extent of drought recovery. Both the transgenic lines (L1 and L2) showed more dry matter partitioning towards the taproot. Partitioning to other parts of the plants was similar in all the lines. Pre-dawn water potential of leaf declined upon imposition of drought stress in all the lines. Though L2 had higher chlorophyll content throughout the study, there was no significant variation in chlorophyll index among the lines studied. A progressive decline was observed for CO_2 assimilation with water stress. The untransformed RRII 105 showed slow rate of recovery of P_N up to 27% after re-watering for three days (Fig. Phy. 2). Stomatal conductance also showed a decline under moisture stress and the decline was less in L1 compared to L2. The antioxidant enzymes activity did not show any trend in the transgenic and untransformed plants.

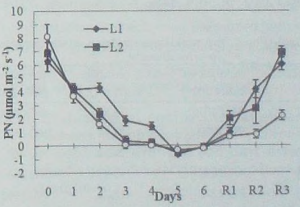


Fig. Phy.2. **Photosynthetic rate (P_N) of the transgenic lines (L1 and L2) and untransformed RRII 105 during six days of drought stress and recovery after re-watering for 3 days (R: re-watering)**

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

1.8.1. Laboratory screening of germplasm accessions for intrinsic drought tolerance

Ten top ranking and ten bottom ranking accessions of SBN 2006, twelve top ranking and ten bottom ranking accessions of SBN 2007 and seven top ranking and five bottom ranking accessions of SBN 2008, selected from field scoring were subjected to laboratory screening for intrinsic drought tolerance traits. Leaf discs punched from middle leaflets were incubated in 60% PEG (polyethylene glycol solution) and exposed to light. Control leaf discs were incubated in water. The effective quantum yield of PS II (ΦPSII) was measured using PAM 2100 chlorophyll fluorometer. The percent reduction in quantum yield of drought stressed leaf samples to that of control samples was calculated, sorted numerically and the accessions were ranked. The accessions that were exhibiting less reduction were ranked top as most intrinsic tolerant ones and *vice-versa*. Accessions AC 513, RO 2990, RO 1528, AC 967, RO 1398 and RO 2907 of SBN 2006, accessions AC 1914, RO 3672, AC 460, RO 366 and AC 1963 of SBN 2007 and accessions RO 3160, AC 1969, RO 2806, RO 1370 and RO 323 of SBN 2008, respectively, were selected as most tolerant ones. Percent reduction in ΦPSII of different accessions under *in-vitro* drought stress of various SBN are given in Table Phy. 3.

1.8.2. Nursery evaluation of selected germplasm accessions for drought tolerance traits.

Five tolerant accessions and three susceptible accessions selected by field scoring and known drought tolerant and drought susceptible check clones (RRIM 600 and RRII 105, respectively) were grown in polybags. Half of the plants were irrigated whereas the other half was kept un-irrigated for ten days before the measurements were made. Percentage leaf yellowing and leaf senescence was found less in accession MT 1623 followed by clone RRIM 600 and HP 105. Clone RRII 105 and accessions RO 85, MT 1616 showed severe drought injury. Similarly the clone HP 105, followed by RRIM 600 and accessions MT 1623, RO 2387 retained maximum number of leaves under drought compared to other clones.

Table Phy. 3. **Top ranking germplasm accessions selected from SBN 2006, 2007 and 2008 by lab screening for *in-vitro* drought**

Rank	Accessions (SBN 2006)	% reduction in PS II Q.Y.	Accessions (SBN 2007)	% reduction in PS II Q.Y.	Accessions (SBN 2008)	% reduction in PS II Q.Y.
1	AC 513	4.89	AC 1914	5.7	RO 3160	5.98
2	RO 2990	4.94	RO 3672	7.6	AC 1969	7.25
3	RO 1528	8.73	AC 460	13.7	RO 2806	9.85
4	AC 967	12.45	RO 366	21.6	RO 1370	13.60
5	RO 1328	18.71	AC 1963	22.5	RO 323	25.12
6	RO 2907	20.49	AC 4072	35.5	AC 3325	30.12
7	RO 2723	35.09	AC 4138	39.3	AC 1846	51.77
8	RO 2142	48.13	AC 3822	46.9		
9	RO 2863	50.14	AC 3567	49.7		
10	AC 3123	59.75	MT 1723	59.6		

Accessions RO 85, MT 41 and clone RRII 105 showed maximum reduction in leaves. The reduction in chlorophyll content was found less in accession MT 1623 (7.06%) followed by clone RRIM 600 (10.2%) and HP 105 (11.42%) and maximum reduction was noticed in clone RRII 105 (31.18%). Clone RRIM 600 followed by RO 2387, RO 2153, HP 105 and MT 1623 maintained higher leaf water status under drought whereas, in clone RRII 105 and accessions MT 1627 and RO 85 the mid- day leaf water potential was more negative indicating increased susceptibility to drought. Under drought stress the net photosynthetic rate was found more stable in accession MT 1623 (5.38 μ mol m^{-2} s^{-1}) followed by clone RRIM 600 (4.34 μ mol m^{-2} s^{-1}) and HP 105 (3.87 μ mol m^{-2} s^{-1}). The photosynthetic rate reduced drastically under severe stress in clone RRII 105 (0.07 μ mol m^{-2} s^{-1}). Under extreme drought (fifteen days drought) 13% of plants of RO 85 and 26% of plants in clone RRII 105 completely dried. In addition to this 100% leaf senescence was observed in few plants of clones RRII 105, RO 85 and MT 1627.

1.8.3. Physiological, biochemical and molecular factors associated with drought tolerance in Hevea germplasm accessions

Relatively drought tolerant (14 nos.) and susceptible (4 nos.) wild *Hevea* germplasm accessions, short listed based on extent of leaf yellowing, were raised in polybags. Six month old polybag plants were subjected to drought stress and assessed for drought tolerance by employing parameters such as photosynthesis (A), Fv/Fm, pigments and epicuticular wax contents.

Germplasm accessions RO 3157, RO 2432, RO 1406, RO 3261, AC 612 and RO 3184 showed higher wax content than the check clone RRIM 600. Five germplasm accessions showed higher total chlorophyll content than another check clone RRII 430. Six germplasm accessions showed higher carotenoid contents than clone RRII 430. The accession RO 3261 showed the highest photosynthesis rate whereas the accession RO 3242 showed the lowest rate. Two accessions RO406 and RO 3184 showed better photosystem II efficiency than the check clone RRIM 600 whereas RRII 414 showed the lowest Fv/Fm. The highest ranked accession for drought tolerance was RO 3261 followed by AC 612 and RO 3157.

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

To evaluate the performance of *Hevea* plants in high elevation in the traditional belt two field trials were initiated at Haileyburia Tea Estate, Elappara, Idukki Dist. Among the five clones planted in 2006 clones PB 260 and RRIM 600 showed higher girth followed by GT 1. Clone PR 261 continued as lowest performing clone at high altitude. In field trial 2007 clone RRIM 600 performed better than clones PB 235, GT 1, RRII 105 and RRII 422. The polyclonal seedlings planted along the tea plantation as shade trees measured better girth (25.6 cm). However the traditional shade tree, Silver oak (37 cm) planted in the same period were found superior to *Hevea*.

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

With a major objective to evaluate the physiological and biochemical basis of adaptations of ortets selected from extreme climatic zones (both cold and drought prone) in India, twenty three clones (16 ortets and 7 clones) were planted in RBD pattern with

three replications during the planting season of 2012 in three different regional stations namely, RES Nagrakatta, RRS Dapchari and CES, Chethackal.

The ortets namely, RRSA 98, GH-9, DAP-1, GH-1 and RRSA 315 showed better photosynthetic performances at CES, Chethakkal during stress free period while NGK-47, DAP-34, DAP-36 and NGK-1 showed lower photosynthetic rate among the ortets tested. Effective quantum yield of PS II found to be better in ortets, RRSA 315, GH-9, GH-1, GH-3 and RRST 24 whereas it was in the lower side for DAP-34, DAP-36 and NGK-47. Degree of leaf yellowing and senescence was enumerated during summer at CES, Chethackal. Leaf yellowing under drought stress was comparatively low in ortet GH 3 and in the check clone RRII 430 whereas severe in RRSA 98, RRST 59 and NGK 1. While analyzing the *in vitro* drought tolerance the ortets NGK 47, GH 3 and RRSA 315 showed more tolerance than the other ortets. Enumeration of number of leaves retained under drought condition showed that clones RRII 422, RRII 430, RRII 417, GH 3 and RRSA 98 with maximum number of leaves in plant whereas, ortets NGK 1, RRST 39, GH 1 retained lowest number of leaves.

Among the ortets, GH 1 and GH 3 showed better growth at RES, Nagrakata and DAP 36 had the lowest girth. RRII 414 recorded the maximum girth and RRII 105 was the least among the check clones. During severe winter period, ortets GH-9, DAP-35, GH-1, DAP-1, and RRSA 29 showed better photosynthetic rates while GH-3, NGK-69, NGK-1, RRSA-585 and NGK-47 had lower photosynthetic rates. The effective quantum yield (ΦPSII) was better for ortets namely., GH-9, GH-1, DAP-1, and RRST 34 and GH-3, NGK-69, NGK-1, RRST-37. Maximum quantum yield of PS II in detached leaves was the highest in ortets GH-9, RRST-37, DAP-1, DAP-34 and GH-1 while NGK-1, RRSA-585, GH-3 and DAP-35 recorded the lowest.

In Dapchari, GH 3, RRSA 585 and RRSA 98 recorded better growth. DAP 36 had the lowest girth. Among the check clones RRII 414 showed the better growth.

1.11. Drought Survey

During 2012, drought survey was done in southern Kerala – Kottarakkara, Punalur and Nedumangad regions in one year old rubber plantations (sample size 100). In general, the management practices adopted by the planters in this region are poor compared to other regions surveyed earlier. The percentage of planters resorting to irrigating the young plants in this area is nearly 3%. More than 90 per cent of the planters do not follow management practices like mulching, shading and cover-cropping, which leads to casualties (3%) of young plants during summer in this region (Table Phy. 4). Intercropping in the initial year is seen more in this part of Kerala compared to other regions surveyed in the previous years. More than 70 per cent of the plots surveyed showed leaf yellowing with a lower intensity of leaf tip dryness and leaf shedding.

Table Phy. 4. **Percentage of planters adopting the recommended management practices during first year of planting in South Kerala**

	Mulching (%)	Shading (%)	Weeding (%)	Cover crop (%)	Casualty (%)
Kottarakkara	3	-	27	1	2
Punalur	6	1	19	1	3
Nedumangad	0	-	44	1	3

1.12. Physiology and proteome studies of cold stress in *Hevea brasiliensis*

In the controlled environmental conditions maximum photochemical efficiency of PSII (Fv/Fm) and effective quantum yield of PSII (ΦPSII) were stable in SCATC 88/13 followed by Haiken 1 under low temperature conditions. The rate of lipid peroxidation was severe in RRII 105 and RRIC 100 and two stress proteins were found in the chloroplast protein profile (Fig. Phy.3a) of Haiken 1 and SCATC 88/13 that may probably be related to cold tolerance in these clones.

More than sixty differentially expressed proteins were found when proteome profiles of control and cold stressed plants of susceptible (RRII 105) and tolerant (RRIM 600, SCATC 88/13 and Haiken 1) clones were developed by two-dimensional electrophoresis and compared (Fig. Phy.3b). The expression pattern of many proteins was found common among the tolerant clones than the susceptible clone.

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

Pre-dawn leaf water potential data showed that RRII 430 and RRIM 600 were better in maintaining leaf water potential than Tjir 1, RRII 414 and RRII 208. Chlorophyll retention (CCI) was better in RRII 430 followed by Tjir 1 and RRIM 600 than RRII 414 and RRII 208. Leaf samples were collected in respective days and proteome studies are in progress.

2. Physiology and yield

2.1 Intercropping with tree crops in rubber

Rubber trees were intercropped with tree species *viz.* mahogany and pathimugom in 2001 and trees were tapped from 2010 onwards. Growth and yield of rubber trees being monitored and found that the girth of trees was not significantly affected by

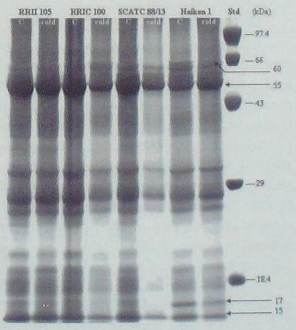


Fig.Phy.3a **Chloroplast protein profile of young plants of *Hevea* grown under control (C) and cold temperature for 72 hours (cold)**

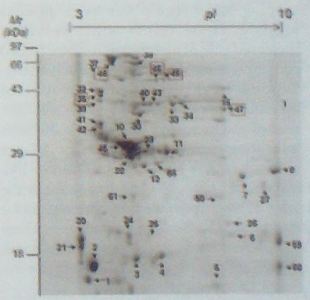


Fig.Phy.3b **Proteome profile of cold exposed RRII 105. Boxes indicate newly synthesized proteins after cold exposure**

intercropping with tree crops. Annual girth of rubber trees alone, rubber with 3 rows of Mahogany + 1 row Pathimugom, 1 row of Mahogany + 1 row of Pathimugom measured 60.9cm, 61.9cm and 60.8cm, respectively. Similarly the yield of rubber trees did not show any significant variation among the treatments. Rubber trees without any intercrop yielded 58.5 g/tree/tap, whereas that of 3 rows of mahogany as well as 1 row of Mahogany trees per tap yield was recorded as 55.5 g/tree, 59.2 g/tree, respectively.

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

This project was concluded during the reporting period. A completion report was submitted. The possible mechanisms of tapping mediated biomass loss that was not accounted either by removal of latex or standing biomass known as 'k factor' was explored. In tapped trees, non-phosphorylative alternative respiration was found significantly increased in and around tapping panel region due to continuous tapping. Those clones which recorded increased rate of alternative oxidase (AOX) activity tended to lose more shoot biomass. Tapped trees had higher amount of accumulated carbohydrates in the soft bark tissues and increased ATP level in the latex indicating increased sink activity. A large amount of ATP and other resources like sugars and proteins were lost through the latex. All these possible factors accounted for the missing biomass in tapped trees. From the present study it was found that clones PB 235 and PB 260 are the L-T clones for the South Karnataka region.

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

With an objective of selecting Latex-Timber clones in 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 annual girth increment and annual block yield during 2011-12 and 2012-13 were recorded. Among the clones PB 235 was shown to be the best yielding clone closely followed by RRII 105. The annual shoot biomass increment was also the highest in PB 235 possessing desirable character as a L-T clone.

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

Latex ATP content was measured in different clones with different yield potentials (high, medium and low) and the regression analysis of latex yield with same day latex [ATP] showed a direct relationship during the peak yielding months (September, October and November). This result suggested that energy metabolism has a role in controlling rubber biosynthesis.

2.5. Clonal variation in latex regeneration mechanism in *Hevea brasiliensis*

Latex samples were collected from seven clones with different yield potentials and protein biosynthetic capacity was measured *in vitro* using labeled aminoacids (Leucine, Arginine, Lysine and Phenylalanine-marked with ^{14}C) during peak yielding and stress seasons. Incorporation of radioactively labeled amino acids into protein in latex showed differences between clones. Higher rate of incorporation was noticed in clones PB 260, RRIM 600 and RRII 105 compared to low and medium yielding clones. Reduction in protein biosynthetic capacity was observed in clones RRII 105, PB 217 and PB 260 during stress period. However, no marked reduction was observed in clone RRIM 600 and low yielding clones (RRII 33 and RRII 38).

2.6. Studies on rubber biosynthesis in *Hevea* clones

Many enzymes are involved in rubber biosynthesis, from acetyl CoA genesis to IPP polymerization. A study aims at magnitude of expression of genes corresponding to major enzymes involved in rubber biosynthesis in high /low yielding *Hevea* clones was continued. Primers were designed and synthesized for the genes such as hmgr1, cis-prenyl transferase 1, idp isomerase, Patatin like inhibitor, Rubber biosynthesis stimulator protein, REF and endogenous control gene (GAPDH). Based on the primer efficiency (slope value), primers were selected for expression analysis.

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

The plasmid of (pRSETA/hmgr1) construct was transformed in to expression specific BL21(DE3)pLysS) cells. The bacterial culture was induced with 1mM IPTG at 37° C for 4 hours. The pRSETA in BL21(DE3) pLysS was used as the negative control. The protein was collected from each sample and quantified. The protein was subjected to SDS polyacrylamide gel electrophoresis (PAGE) analysis and visualized using Coomassie blue stain. An increase in the intensity of band in the profile corresponding to HMGR1 with a size of 64.6 kDa (including His Tag protein) was observed. The maximum intensity of band represented hmgr1 was observed after 3.5 hour induction. The plasmid from the clone (pRSETA/hmgr1) was isolated and confirmed by restriction digestion analysis.

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

Expression analysis of ETRs by Quantitative PCR:

Hevea trees (RRII 105) of uniform girth and yield under S2d3 6d/7 tapping system were selected and latex samples were collected before stimulation and different time intervals (third, fifth and seventh day) after stimulating the trees with ethephon. Total RNA was prepared and relative quantification of *ETR1* and *ETR2* was carried out at different tapping days after stimulation and compared with unstimulated control trees. Significant up regulation of *ETR1* (about 1.5 and 1.6 fold on the third and 7th day, respectively) with a considerable dip on the 5th day (to the levels of unstimulated trees) was noticed. However, *ETR2* level went up only on the third day (to 1.4 fold) followed by significant reduction to a lower level of about 0.6 fold. The preliminary data on its expression indicated that both *ETR1* and *ETR2* were up-regulated immediately after stimulation followed by a significant reduction in its levels in the subsequent tapping days.

3. Tapping panel dryness

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

The experiment was continued at CES, Chethackal in clone RRII 105 under $^1/_2$ Sd36d/7 tapping system with an objective to reduce the ethylene mediated stress responses in the tapping panel by applying the ethephon away from the tapping area

without compromising latex yield. Rubber yield of individual trees belongs to all the groups were recorded. Stress components like Peroxidase (PX), Hydrogen peroxide (H_2O_2), Malondialdehyde (MDA), Proline, β-cyanolalanine synthase (β-CAS) and Cyanide (CN) were analysed in the soft bark tissues at definite intervals (one month after stimulation). Biochemical parameters such as sucrose, thiols and ATP were analysed in the fresh latex collected during every month. Significantly high ATP and very low sucrose was observed in trees applied with 5% ethephon above and below the tapping panel compared to normal stimulation. Sustainable rubber yield and comparatively less magnitude of stress indicators were observed in treatment with ethephon application away from tapping panel.

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

About 100 trees were selected and regular TPD scoring was done. Bark samples were collected from TPD (10%, dry and wet portion of 40-60 % bark and dry and wet portion of barks from 80-90 % TPD affected) and normal trees. The relative expression level of about 30 genes under different levels of TPD was quantified by using quantitative PCR (qPCR). cDNA obtained from the above samples were used as template for the qPCR analysis. The results indicated that there is significant up-regulation of peroxidase in the dry portion of bark in trees with 40-60 % stage of TPD. While one NAC tf (DRT 5b) was found to be significantly up-regulated in both wet and dry regions of bark with 40-60 % TPD, another NAC tf (TPD 24) was found up-regulated only in the wet region of tapping cut in trees with 10 % TPD.

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

To study the effect of soil pH on the incidence of TPD and abiotic stress in rubber plants a lime application experiment was continued. Lime application was skipped for the year 2012-13 to study the soil buffering capacity. Soil samples (top and bottom soil) in all the blocks were collected and the pH was analyzed. The pH of the soil was maintained in the same level during this year without lime application for the period 2012-13. There were no much variations in growth and yield between treatments. TPD scoring is being continued on monthly basis.

4. Secondary metabolites

4.1. Quantification and identification of inositols in *Hevea*

Other than annual renewal of patent on L-quebrachitol production, further steps were initiated toward licensing the technology. In order to commercialize the method developed further contacts have been made with government organizations, Biotechnology Consortium of India Limited (BCIL) and some leading R& D companies.

4.2. Water relations of latex with reference to the contents of inositols and sugars

Biochemical components estimated from the latex were compiled and analysed in terms of effect of solutes in water relations of latex. The data showed that sugar levels of latex is significantly correlated ($r = 0.67$) with osmoticum of latex. Relationship of latex osmolarity and cationic concentration was worked out. Latex osmolarity is significantly corrletaed ($r = 0.57$) with composition of all cations and not with volume.

RUBBER TECHNOLOGY DIVISION

In the current year, the activities of the Division were focused mainly on evolving improved techniques in processing of latex, vulcanisation of latex, latex stage incorporation of fillers, reinforcement of NR using polymeric filler and silica, scorch control of peroxide vulcanisation, rubber nanocomposites based on modified clays and rubber recycling.

1. Primary processing

1.1. Low protein natural rubber (LPNR) processed through gamma ray irradiation technology

An attempt was made to produce low protein natural rubber (LPNR) by exposure of fresh NR latex to gamma radiation followed by creaming. The creamed latex was then diluted, coagulated, dried and processed in the conventional way. By this process the nitrogen content could be reduced to less than 0.2 per cent . There was an increase in gel content after gamma ray irradiation. (Table Chem.1) The LPNR showed good cure characteristics along with higher level of vulcanization and higher cure time in comparison with ISNR grade, for the ACSI mixes. For the carbon black filled mixes the level of vulcanization and cure time were comparable with ISNR grade. The low protein rubber also showed very good mechanical and dynamic properties (Table Chem.2 and Chem. 3). The improvement in mechanical and dynamic properties is attributed to the partial removal of proteins and formation of gel during exposure of fresh NR latex to low doses of gamma radiation.

Table Chem. 2. **Formulation of mixes used for evaluation of LPNR**

Ingredients	Parts
Natural rubber	100
ZnO	5
Stearic acid	2
HAF black (N220)	40
Antioxidant (HSL) *	1
Naphthenic oil	7
CBS **	0.6
Sulphur	2.5

*2,2,4-trimethyl – 1,2 – dihydroquinoline
**N-Cyclohexyl-2-benzothiazole sulfenamide

Table Chem. 3. **Technological properties of low protein rubber**

Parameters	ISNR 5	LPNR
Modulus 300%, MPa	5.82	5.63
Tensile strength, MPa	26.8	27.8
Elongation at break, %	910	890
Tear Strength, N/mm	85.2	89.8
Abrasion loss, mm^3	106	100
Heat build up, ΔT, °C	11	10
Compression set , %	38.9	32.3

2. Latex technology

2.1. Radiation vulcanised latex (RVNRL)

Standardised a method to improve quality of RVNRL films using centrifuged

Table Chem. 1. **Raw rubber properties of creamed latex before and after gamma ray irradiation**

Parameter	Un-irradiated latex		Pre-irradiated latex	
	creaming	After creaming	Before creaming	After creaming
Nitrogen content, %	0.46	0.49	0.24	
Acetone extractable, %	4.25	3.42	4.19	
Gel content, %	2	-	36	

latex. It was observed that centrifuged latex prepared from fresh NR latex exposed to low doses of gamma radiation had higher green strength and lower levels of nitrogen. These factors favoured radiation vulcanisation and films had very good mechanical properties.

3. Rubber Technology

3. 1. Reinforcement

3.1.1. NR/ Polymeric filler system

Different cure systems such as conventional vulcanisation (CV), semi efficient vulcanisation (semi-EV) and efficient vulcanisation (EV) were tried in NR/ PF composites. The vulcanizates with semi-EV system gave better technological properties than the other two systems (Table Chem.4).

Table Chem. 4. **Technological properties of NR/PF with different cure systems**

Parameters	CV	Semi-EV	EV
Modulus, 100 %, MPa	4.60	5.74	4.00
Modulus, 300 %, MPa	10.62	12.24	9.82
Tensile strength, MPa	28.16	26.65	21.33
Elongation at break, %	620	555	530
Tear strength , N/mm	68	78	52
Din abrasion loss, mm^3	109	102	116
Heat build-up, ΔT, ° C	11	11	13

The compounds having varying concentration of polymeric filler were subjected to strain sweep using Rubber Process Analyser (RPA). The un-vulcanised compounds as well as the vulcanizates were subjected to this analysis. For a comparison 50 phr HAF black (N330) filled NR compound was also included. The compounds with polymeric filler showed a lower storage modulus (G′) at lower strain than the HAF filled compound both in un-vulcanised and vulcanized ones. (Figures Chem.1 & 2). This indicated better polymer filler interaction for the NR/PF composites.

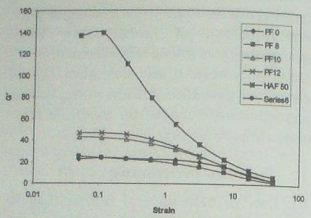


Fig. Chem. 1. **G′ vs Strain of un-vulcanized sample**

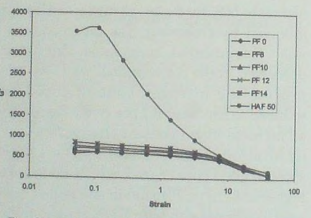


Fig. Chem. 2. **G′ vs Strain of vulcanized sample**

3.1.2. Latex filler masterbatches

Latex filler master batches were prepared using ISAF black (N220) filler and silica. Carbon black/silica master batch containing 50 phr filler showed comparable mechanical properties along with a lower heat build up. A comparison with conventionally compounded black filled NR containing all carbon black (control), as shown

Table Chem. 5. **Mechanical properties of master batch in comparison with dry rubber mix**

Parameters	Master batch *	Control**
Modulus 300 %, MPa	12.1	9.1
Tensile strength, MPa	28.9	27
Elongation at break, %	550	585
Heat build-up ΔT, °C	16	19
DIN Abrasion loss, mm^3	135	127
Tear strength, N/mm	106	108

* contains 40 phr ISAF black and 10 phr silica filler
** contains 45 phr ISAF black and 5 phr naphthenic oil as plasticiser
(All compounds contain ZnO-5 phr, Stearic acid-1phr, CBS-0.9 phr.and Sulphur 2.5 phr as other compounding ingredients)

3.1.3. Silica reinforcement of ENR

Studies on reinforcement of rubber by silica filler showed that reinforcement properties such as modulus and abrasion resistance improved with incorporation of suitable grade of epoxidised natural rubber but this was accompanied by a reduction in tear strength. Attempt was made to improve the tear strength by replacing the naphthenic oil used as plasticizer for filler incorporation either partially or fully by rubber seed oil. The study revealed that compared to naphthenic oil, rubber seed oil containing mixes exhibited higher tear strength and lower abrasion loss. In continuation to the study a comparison of silica reinforced natural rubber containing naphthenic oil as process oil with that of rubber seed oil, oleic acid, and epoxidised polybutadiene with two levels of epoxy content was carried out. As seen from Table Chem.6 it was found that the non conventional process oil containing composites exhibited better technological properties than composites prepared using naphthenic oil.

3.2 . Recycled rubber

A new radical capping agent has been identified for devulcanisation in a mechano-chemical process. Retention of properties after devulcanisation using this process depended on the type of cross linking: the highest efficiency is for efficient vulcanisation system.

3.3. Nanocomposites

33.1. Natural rubber layered clay nanocomposites

Natural rubber nanocomposites were prepared by melt intercalation process using

Table Chem. 6. **Technological properties of 50 phr silica filled NR mixes containing different plasticizers**

Plasticizer used	Tear strength (N/mm)	Hardness Shore A	Abrasion loss (mm3)	Compression set (%)	Heat build-up, ΔT, (°C)
Naphthenic oil	84.9	64	109.7	27.3	6
Rubber Seed Oil	101.9	63	99.0	30.2	7
Oleic Acid	91.1	66	99.8	25.8	8
Epoxidised polybutadiene 1	104.5	64	107.9	29.8	9
Epoxidised polybutadiene 2	92.1	65	102.9	31.4	8

Table Chem. 7. **Technological properties of nanocomposites.**

Parameters	A	B	C	D	E	F
Modulus 300 %, MPa	2.81	3.10	3.35	3.39	3.47	3.78
Tensile strength, MPa	27.45	31.95	32.23	30.88	30.63	29.83
Elongation at break, %	645	680	680	680	695	670
Tear strength, N/mm	36.5	37.4	40.0	38.9	39.3	36.9
Hardness, Shore A	44	48	50	52	54	56
Heat build-up, ΔT, °C	2	4	5	8	9	11
Din abrasion loss, mm^3	235	177	169	164	157	159
Compression set, %	29	32	34	36	39	40

Haake rheocord.. Octadecylamine modified montmorillonite clay (MMT- ODA) was used as a filler in this study. From the data given in Table Chem. 7 is inferred that the technological properties improved with filler loading up to 4 phr and with further loading there was no appreciable improvement in the overall mechanical properties.

3.4 Peroxide vulcanisation

The combination of DCP/TEMPO/N,N'-m-dimaleimide cure system imparted scorch safety in peroxide vulcanisation and the problem was poor mould release after vulcanisation. A new system consisting of another peroxide, TEMPO and a co-agent was investigated The cure system consisting of di(t-butyl peroxy isopropyl) benzene/ TEMPO/ trimethyl propane trimethacrylate was attempted. The new system had good mould release property, better scorch safety and better mechanical properties like tensile strength, tear strength and modulus than a conventional DCP cured EPDM rubber mix. (Table Chem.8).

Table Chem. 8. **Technological properties of EPDM rubber based vulcanizates**

Parameter	EPDM-DCP	EPDM - new vulcanisation system*
Modulus,200%, MPa	10.21	6.56
Modulus,300%, MPa	-	12.31
Tensile strength, MPa	16.34	17.32
Elongation at break, %	269.00	383.00
Tear strength , N/mm	67.00	64.00
Compression set, 100°C, 72 h, %	8.30	14.10

*di(t-butyl peroxy isopropyl) benzene/ TEMPO/ trimethyl propane trimethacrylate

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, Development of Spheri block is being attempted.

4.2 Development of rubber mounts for submarines

Developed the formulation for a silicone rubber based mount for use in submarines at Mazagon Dock Ltd, Mumbai, and also made trial mounts.

5. Collaborative work with rubber industry

Based on the findings made in the Institute, initiated collaborative research programmes with different rubber industries in the following areas for technology transfer.

1. Reinforcement of natural rubber using polymeric fillers
2. Filler masterbatch from fresh natural rubber latex
3. Deproteinised natural rubber
4. A novel method for easy coagulation of skim latex and recovery of high quality skim rubber.

6. Development / advisory work

6.1. Rubber tapping knife was developed.

6.2. Tested and report given for the damage of 7 nos of tyres referred from various Consumer Disputes Redressal Forum

6.3. Tested and report given for 35 no of polythene samples for the RP Department and tested 3 no of logo material for its suitability as sticker in rubber bales for marketing division.

6.4. Developed a microcellular rubber ball simulating that of cricket ball further to a demand from an entrepreneur.

TECHNICAL CONSULTANCY DIVISION

The major services of technical consultancy are R&D activities on industrially important rubber based projects and testing/certification of rubber products as per relevant standards. The division caters to the needs of new entrepreneurs as well as existing rubber goods manufacturers. The services offered by the Technical Consultancy Division include (i) raw material/rubber products testing as per national and international standards (ii) preparation of project profiles and technical bulletins to entrepreneurs (iii) solutions for trouble shooting/cost reduction of factory processes (iii) demonstration/practical training for quality improvement/product development (IV) advisory/analytical support to customs for import of reclaimed rubber and (iv) conducting awareness meetings/lectures to entrepreneurs.

1. Testing support to industries

For the testing of raw materials and rubber compounds/products, consistent support was offered especially to Small and Medium level Entrepreneurs (SME). The details of the products came for testing during the reporting period are given below.

Dry rubber products

Different types of dry rubber products tested in the division include, pre-cured/ conventional tread, bonding gum, black vulcanizing cement, tyre flaps, inner tubes, rubber channels/ tubes, floor mats, Hawaii soles, sponge rubber, O-ring, bushes, engine mounts etc.

Latex rubber products

The main latex products came for testing include examination and surgical gloves, latex adhesive, latex thread, balloons, condoms, protein analysis of latex products and evaluation of chemicals/ latex based paints.

2. Polymer identification and reverse engineering

A total of 246 samples were received for polymer identification based on FTIR and elemental analysis. 255 samples were received for reverse engineering. Total number of samples tested and the revenue collected during the period are given below (Table TC. 1).

Table TC. 1 Total number of sample listed and the revenue collected

No. of clients	692
No. of products tested	1767
No. of parameters analyzed	8576
Total revenue collected	RS.10,81,079

3. Product Development

44 no. of different types of products were developed in the current period.

Standard rubber sample sheet

Degree of abrasiveness of the DIN abrader' abrasive paper in compliance with ISO 4649 was determined using a standard rubber sample sheet. This standard sheet was developed from a specially mixed rubber compound and supplied to RUBCO on payment basis.

Oil seals for Naval Aircraft yard, Kochi

In connection with the Indigenization of spares of all western origin air-crafts, 15 rubber seals were successfully reverse engineered for Navy, Kochi and the results were delivered.

Development of rubber based munitions for Tear Smoke Unit, BSF

Tear Smoke Unit (TSU) an autonomous body working under the Ministry of Home Affairs, Government of India has approached us for the development of rubber bullets and other less lethal ammunitions (Fig. TC. 1) used for riot control and law and order operations. Component analysis and testing of the products are under progress.

Fig. TC. 1 Rubber bullets and other less lethal ammunitions

New process development for small scale units

Division has recently introduced the cold vulcanization system which has been implemented successfully in the rubber band manufacturing sector. Presently the cold vulcanization process is being standardized for latex foam and the trials in this respect are progressing well.

4. Evaluation of chemicals/rubber compounds

In the reporting period, four grades of low PCA oils were analysed and the reports were transferred to Indian Oil Corporation. Similarly three grades of carbon blacks, two grades of process additives, two grades of peptizers and three grades of SBR were evaluated and the detailed reports were communicated to the clients.

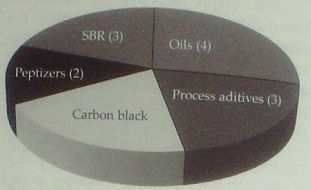


Fig. TC. 2. Evaluated chemicals/rubber compounds.

5. Project profiles/Technical bulletins

As per the request of the entrepreneurs, 38 project profiles and 14 technical bulletins were issued on payment basis.

6. Advisory services

Matters relating to various aspects like selection of raw material, dosage of ingredients, processing conditions, recent regulation etc. were always a subject of concern for small and medium scale product manufacturers. 1991 queries on technical matters were addressed during the reporting period.

7. Entrepreneur's promotion meeting/publications

In order to popularize the services of the Technical Consultancy Division, a video programme on the various activities of the Division has been displayed in the following events.

1. International Rubber Conference IRC-2012, Kovalam, 28-30 October 2012 Kovalam, Thiruvananthapuram.
2. 25th Kerala Science Congress, Thiruvananthapuram, 29-31January 2013.

3. 7th International Rubber Exhibition, Conference and Tyre show on 22-24th Jan, 2013, Mumbai.
4. Index-2013, Mammen Mappillai Hall, Kottayam, 25-31 Jan 2013
5. Kudumbasree Meet, organized by the New Mans College, Thodupuzha

8. RRII-AIRIA regional meetings

Concerned officials delivered talks on the manufacturing of rubber products in the meetings which are listed below.

1. Latex compounding and products manufacture, National Rubber Conference on new challenges and opportunities for rubber industry, organized by All India Rubber Industries Association, Habitat Centre, New Delhi, 16 -17 March 2012.
2. Latex Products and Recent Developments in India, Radisson Blu Hotel, Chennai, 22-23 June 2012.
3. Rubber Nanocomposites, National Rubber Conference, Lalit Hotel, Mumbai, 2-3 November 2012.

9. NABL Accreditation

In connection with the NABL implementation of the Division, training was given to the officers by an external expert and following documents were prepared.

1. Management and technical requirement procedures
2. Procedure for the testing parameters
3. All the old instruments were replaced with new ones
4. Completed the calibration of all equipment and analytical devices.

10. Infrastructure Facilities

The infrastructure and sophisticated instrumental facilities introduced in the Division include universal testing machine (5 kN and 100 N), particle size analyser (micro range), UV spectrometer, GCMS, FTIR, Rheo meter (MDR), Mooney viscometer, Goodrich flexometer, Shore A hardness tester, digital hardness testing machine, micro hardness tester, TGA 4000, Dispergrader, Din abrader, rubber process analyser, Ross flex tester, Haake rheocord, Intermix, Mixing mill and Hydraulic presses.

11. New projects

A project report has been submitted to the Ministry under the ASIDE scheme for setting up a Tyre Testing Station at RRII also submitted a proposal for the continuous foaming technology. In order to avoid the delay in the testing and certification of products, an online consultancy service was proposed.

12. Research Projects

(a) New machines for sheet processing

With increasing labour cost, a new automated sheet processing machine suitable for smallholders has been developed by coupling the dual machines in a single frame. The operator can select the operation with a gear. It has got 50% savings in foot-print and also a proportionate reduction in processing shed area. The new machine is user friendly and cost effective (Fig. TC. 3).

Fig. TC. 3 New Sheet Processing Machine

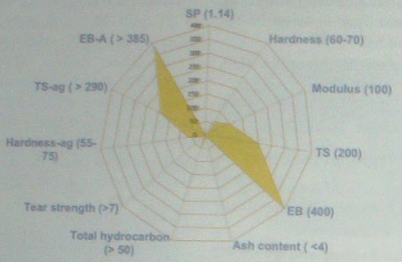


Fig. T.C. 4. FCCD establishing the relationship with different ingredient and quality parameter

(b) Development of cost effective tread formulation having different abrasion resistance indices

A cost effective formulation which meets the specifications of a tread as per ASRTU is a concern to most of the tread manufacturers. Hence, a project was under taken to produce tread formulation which can meet all the specification requirements. A Face Centered Central Composite Design (FCCD) was proposed for establishing the relationship with different ingredients and quality parameters (Fig. TC. 4).

(c) Rubber compounds with Improved flex crack resistance

Mixes with three types of carbon black fillers, *viz.* HAF, FEF and GPF at varying loading were used in nitrile rubber for optimizing flex crack resistance (Fig. TC. 5). Vulcanizates which can give good flex crack resistance and low heat build-up were formulated. These materials are ideal for products requiring oil resistance and flex-crack resistance (*e.g.* axial boot of automobiles).

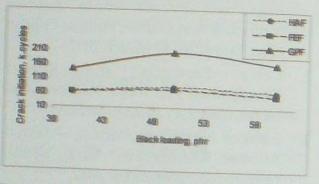


Fig. TC. 5 Flex crack resistance against different loadings of different carbon blacks.

(d) Cell structure and performance of expanded rubber

Expanded rubber sheets have got wide applications as they can be produced with varying hardness. The technological properties of expanded rubber are highly depended on its cell structure. Blowing agent DNPT at different loading was used to correlate the expansion and technological properties of Hawaii soles. The technological properties of these mixes as per IS10702 have been studied. As the loading of blowing agent DNPT was increased beyond 6 phr, the technological properties were found to deviate from the specification limit.

(e) NR based EMI shielding composites

Compared to conventional metal-based EMI shielding materials, electrically conducting polymer composites have gained popularity because of their light weight and other properties/processing advantages. NR based shielding composites were developed and characterized for their shielding effectiveness.

Specially formulated natural rubber composites showed an EMI shielding effectiveness of 29 dB at frequency of 5GHz (Fig. TC. 6). The shielding effectiveness can be further increased by suitably formulating the compound.

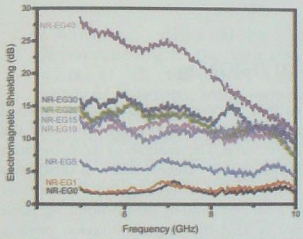


Fig. TC. 6 Effect of frequency on electromagnate shielding of NR based shielding composite

(f) Characterization and restriction of bloom

Bloom refers to the initial formation of star-shaped clusters when solid compounding materials re-crystallize at the vulcanizate surface. A typical formulation with conventional ingredients was prepared, and its blooming effect was assessed. Photographs at regular intervals were taken for visual grading of bloom. DCP and ZnO are found to bloom in NR vulcanizate. ZnO in combination with stearic acid exhibit lesser bloom. This might be due to the formation of zinc stearate which minimizes the presence of insoluble and unreacted ZnO. Also, the zinc complexes formed rarely migrate to the surface as they are bound within the material.

(g) Variation of particle size / extractable protein in different processing / product development techniques used in natural rubber latices

The objective of the study is to find out the effect of different latex processing and manufacturing techniques on extractable protein content. The protein content of the latex films prepared from different concentration methods was estimated and a relationship was established which will be useful for the manufacturing process.

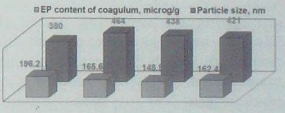


Fig. TC. 7 shows the range extractable protein contents and particle sizes of different types of natural rubber latics.

(h) Peroxide vulcanization of natural rubber

The peroxide vulcanization of rubber has got lot of advantages compared to sulphur. The effect of different process additives on peroxide vulcanization has been investigated in the study. A Face Centered Central Composite Design (FCCD) was used to establish the relationship between vulcanizate properties and compounding ingredients.

(i) Stabilisation of nano ZnO dispersion and latex compounding

Stabilization of nano ZnO dispersions were studied in detail. Unlike other synthesis methods, mechano-chemical process gave high yield and lower particle size 35 nm. For the stabilization of ZnO nano particles various capping agents including both ionic and non-ionic types were used. DLS technique was used to monitor the particle size of ZnO nano particles. Figure below shows the DLS results of ZnO nanoparticles in SLS sonicated after 4 minute. Zeta potential analysis indicates that a potential of -43.2 mV is imparting good stability to the ZnO dispersion.

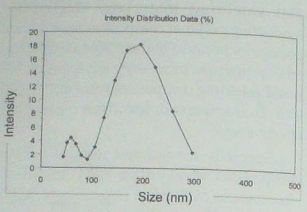


Fig. TC.8 DLS results of ZnO nano particles in SLS sonicated dispersions

ECONOMICS DIVISION

During the reporting period the Division undertake 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) inter-crops and by-products; and (v) inter-divisional collaborative projects. Five projects handled at RRII HQ and one at RRS Agartala were completed and reported during the period. A Summary of the findings and policy suggestions evolved from the studies conducted at RRII HQ is presented below.

1. Heterogeneity in tariff policy and differential levels of protection under AIFTA: The case of rubber and rubber products

This study attempted to provide a conceptual basis for disaggregate level analysis of the implications of AIFTA on rubber and rubber products based on a critical assessment of the key provisions of the Agreement. The analysis of the provisions revealed that categorization of tariff lines under the six different groups is the most crucial component predominating tariff policy and the implementation period. Destination-wise shares of tariff lines and average final tariff are given in Table Eco. 1. India's strategy of pursuing an inverted tariff structure on rubber and rubber products under the AIFTA might have been guided by the composition of the total value of exports to and imports from ASEAN. However, a negative balance of trade in rubber and rubber products with ASEAN and a higher annual average growth rate of imports of finished rubber products underline the need for a closer monitoring of the trends in external trade with the trading block at the disaggregate level.

Table Eco. 1. **Destination-wise shares of tariff lines and average final tariffs (%)**

Country	Raw material			Intermediate products			Finished products		
	Tariff elimination	Tariff reduction	Exclusion list	Tariff elimination	Tariff reduction	Exclusion list	Tariff elimination	Tariff reduction	Exclusion list
India	65.6(0.0)	6.3(5.0)	28.1(31.11)	11.5(0)	77.0(5.0)	11.5(10.0)	58.6(0)	41.4(5.0)	0
Malaysia	86.7(0.0)	13.3(4.3)	0	2.4(0)	97.6(6.9)	0	21.4(0)	64.7(7.6)	13.9(22.7)
Vietnam	100.0(0.0)	0	0	100.0(0)	0	0	45(0)	0	55(23.2)
Thailand	97.9(0.0)	2.1(5.0)	0	75.0(0)	16.7(5.0)	8.3(20.0)	76.5(0)	18.5(5.0)	5(10.0)
Indonesia	77.1(0.0)	22.9(2.4)	0	41.7(0)	58.3(4.5)	0	16.8(0)	83.2(4.7)	0
Myanmar	100.0(0.0)	0	0	50.0(0)	8.3(1.0)	41.7(5.0)	44.3(0)	29.5(3.8)	26.2(5.1)
Philippines	47.9(0.0)	0	52.1(3.0)	83.3(0)	16.7(5.0)	0	66.4(0)	33.6(4.8)	0
Cambodia	75.0(0.0)	25.0(5.0)	0	100.0(0)	0	0	63.8(0)	32.8(5.0)	3.4(13.0)
Brunei Darussalam	100.0(0.0)	0	0	100.0(0)	0	0	32.2(0)	0	67.8(13.4)
Lao PDR	100.0(0.0)	0	0	83.3(0)	16.7(5.0)	0	99.2(0)	0.8(5.0)	0

Figures in parentheses are the average final tariffs at the end of the implementation period
Source: GOI, 2010

2. Changing dimensions of intercropping in the immature phase of NR cultivation: A case study of pineapple intercropping in central Kerala

Intercropping in the immature phase of rubber plantations had been the outcome of a major policy decision implemented in 1957 with the core objective of achieving self sufficiency in NR production. However, the priorities and strategies of intercropping have undergone important changes during the past five decades due to a number of factors including changes in the socio-economic determinants, crops grown, objectives and R&D efforts. The study revealed growing popularity of contract farming in pineapple intercropping in NR with three different contractual arrangements in Table Eco. 2. The results of the analysis highlighted the growing divergence between the recommended and

Table Eco. 2. **Contractual arrangements in pineapple intercropping**

Operations	Type		
	1	2	3
Land preparation	Contractor	Contractor	Contractor
Pineapple intercropping	Contractor	Contractor	Contractor
Procurement of rubber planting materials	Contractor	Grower	Grower
Maintenance of rubber plantation (first three years)	Contractor	Contractor	Grower
Type of compensation to growers	All expenses related to planting and maintenance for 3 years	All expenses as in Type 1 except the cost of planting materials	Rent paid Rs. 10000/- to Rs. 25,000/- acre
Share of growers (%)	58.9	5.4	35.7

adopted agro-management practices in intercropping of pineapple under contract farming and the potential challenges to the agronomic sustainability of NR cultivation.

3. An economic analysis of the socio-economic dimensions of participatory experimental trials on low frequency tapping (LFT)

The study was a socio- economic evaluation of the demonstration plots having S/2 d3 tapping system with stimulation. The demonstration plots were established in the rubber smallholdings in different locations to popularize low frequency tapping (LFT) system by participatory monitoring and evaluation. The analysis showed that the average size of demonstration plots (0.9 ha) is higher than the average size of rubber smallholdings in Kerala (0.5 ha) indicating that holding size is one of the factors prompting the adoption of LFT. The dependence on hired labour for tapping is higher among the sample holdings. In the case of holdings dependent on hired labour the (size of holding) number of trees emerges as the key factor facilitating the adoption of LFT. The resistance from tappers towards LFT was observed only in the smaller holdings mainly due to: (i) loss of tapping days; and (ii) increase in work load due to higher yield from unit area. The growers overcome the resistance by (i) resorting to self-tapping; (ii) by assuring employment to tappers in other grower's holdings; or (iii) by offering incentives for extra production. Availability of family labour is a key factor influencing the adoption of LFT in smaller size groups with less than 1 ha area under rubber. Despite the positive signals emerging from the scheme the scale neutrality of LFT remains suspect in the unique regional context of Kerala with smaller size of the holdings and higher dependence on hired labour. The study highlighted the need for appropriate institutional arrangements to overcome the in-built deficiencies of size and rigidities of the labour market for the effective implementation of LFT from a long-term perspective.

4. Uncertain prices and segmentation of market as a survival strategy: The case of latex processing industry in India

Historically, the price of Preserved Field Latex (PFL) in India was linked with

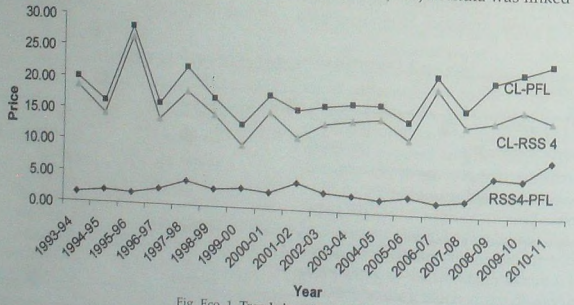


Fig. Eco. 1. Trends in price difference

the price of RSS 4. Since the middle of 2000s price of PFL is delinked from RSS 4 price. The study analysed the factors behind the delinking of PFL price from RSS 4 and the trends in price movements of PFL, RSS 4 and Centrifuged latex (CL) and the inter-linkages. Fig. Eco. 1 shows the trends in price differences. The preliminary observations of the study highlighted the crucial role of the growing narrowness of the domestic market due to the significant growth in the import of latex based value added products rather than the import of CL for the segmentation of the latex market. Hence, a two pronged strategy of rehabilitating the latex based manufacturing segment so as to ensure commensurate rate of growth in latex consumption and rejuvenating the latex processing industry to reinforce the focus on exports is suggested.

5. Adoption of intercrops in the traditional rubber growing regions in India: Emerging trends in the smallholder sector

The study confined to the smallholdings which availed planting subsidy (2004-10) showed that among the various intercrops, banana was the most popular intercrop in the traditional region spread over five agro-climatic zones. However, the results revealed notable differences in the extent of adoption of intercrops and its size-class-wise preferences. The highest level of adoption of intercrops was observed in Kanyakumari region (72.9 %) followed by Central Kerala (72.2 %) and South Kerala (68.2 %) during the seven year period under study (2004-10). Adoption of intercrops was found to be the lowest in North Kerala (36.20%). The analysis revealed that pineapple replaced banana as the choicest intercrop in Central Kerala. The size of holding was found to be a key determinant in the selection of intercrops. A positive relationship was observed between the size of holding and adoption of commercial crops such as banana and pineapple whereas subsistence crops such as tapioca, amorphophallus and colocasia are preferred in the smallest size-class.

LATEX HARVEST TECHNOLOGY DIVISION

The division continued research and advisory activities on various aspects of crop harvesting. The collaborative programme on popularising Low Frequency d3, tapping among small holdings initiated under all the Regional Offices of the Rubber Board during 2009-10 was concluded in the month of March, 2013. Controlled Upward Tapping (CUT) demonstration trials initiated during 2008-09 in various regional stations continued successfully and reported atleast 50 percent yield increase from old trees. The bio-degradable polythene under evaluation served for both the monsoons (total service period of 9-10 months).

1. Low Frequency Tapping

The division continued various experiments, onfarm trials and advisory trials on Low Frequency Tapping under different

Table LHT. 1. **Dry Rubber Yield (kg 400 trees^{-1}) and other attributes under various frequencies of tapping**

Tapping system	Tapping days		Yield	DRC%	Girth incrmt.	TPD
	Actual	Expected			(cm year^{-1})	%
S/2 (RG) d2 6d/7	149	150	2188	32.6	1.9	4.8
S/2 (RG) d3 6d/7	103	104	1663	36.2	2.5	6.1
S/2 (RG) d3 7d/7 ET 2.5% Pa 2/y*	119	121	2220	34.3	1.5	4.2
S/2 (RG) d3 6d/7 ET2.5% Pa 3/y*	104	104	1976	36.3	2.4	5.3
S/2 (RG) d4 7d/7 ET 2.5% Pa 4/y*	91	91	1937	35.8	1.3	2.1
S/2 (RG) d4 6d/7 ET2.5% Pa 6/y*	78	78	2109	37.2	2.0	2.3
S/2 (RG) d6 7d/7 ET 2.5% Pa 10/y*	60	60	2085	36.7	1.5	7.1
S/2 (RG) d7 6d/7 ET 2.5% Pa12/y(m)	52	52	2017	39.0	2.7	2.7
CD (0.05)	-	-	265	-	-	-

agro-climatic conditions. The comprehensive study initiated during 2010-11 at Koney Estate of Harrisson Malayalam to understand the performance of clone RRII 105 under different tapping systems continued successfully. The study also covered tolerance of the lactiferous system under different tapping frequencies and stimulation based on biochemical indicators. Yield under different frequencies were comparable except for S/2 d3 without yield stimulation. Annual average DRC ranged from 32.6 under d2 to 39 percent under d7 and there was no definite trend with respect to Tapping Panel Dryness and annual girth increment (Table LHT.1). There was no sign of degradation of the laticiferous system after stimulation in d/6 and d/7, as indicated by high thiol, without accumulation of stress indicators like proline and phenol. Among the low frequency tapping systems, d4 showed balanced and activated metabolism with sufficient sucrose, high energy availability [ATP], latex regeneration capacity and protection of the laticiferous system.

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

The programme initiated in 2009-10 (phase I) and extended in 2010-11, proceeded smoothly and concluded in March, 2013 as per the original schedule. In general, an average yield of more than 5kg/tree was realized by the participants and not even a single grower reported any increase in TPD due to LFT and stimulation. Nearly one third of the growers are utilising family labour for tapping and 30 per cent follow d3 frequency with Sunday rest, whereas 70 per cent follow d3 frequency without Sunday rest leading to higher number of tapping days per year. Accordingly stimulation rounds per year was modified (reduced from 3 to 2). General information from this programme is that for the success of LFT, regular and correct tapping, effective and timely rainguarding, panel washing using fungicides to prevent diseases and proper yield stimulation under the scheduled levels etc. are essential. Delayed and second collection of latex during few taps after stimulation can reduce field coagulum (Scrap). Instead of skipping tapping due to rain in the months of June-July, delayed tapping (even at 9.00am) ensures regularity in tapping. Shifting from high frequency tapping to LFT must be done only in the low yielding months of February-April.

The experiment on Low Frequency Tapping (LFT) with different levels of yield

stimulation in clone RRII 105 laid out in the Experimental Farm Unit of Rubber Research institute of India located at Kottayam, Kerala (9°32'N; 76°36'E) was concluded.

Cumulative dry rubber yield over fifteen years under d3, d4 and d6 frequencies of tapping with yield stimulation is comparable to unstimulated alternate daily tapping (Fig. LHT.1). Cumulative dry rubber yield obtained with d3 (3 stim/y), d4 (5 stim/y) and d6 (12 sim/y) systems were 104%, 99% and 92% of d2 frequency of tapping.

Cumulative yield under d3, d4 and d6 frequencies of tapping resulted in 51%, 59% and 69% yield increase in BO-1 panel and 13%, 30% and 58% yield increased in BO-2 panel over d2 system of tapping (Fig. LHT.2).

Mean annual yield under third daily tapping, fourth daily tapping and weekly tapping frequencies were comparable to that of alternate daily tapping (Table LHT.2). Mean annual tapping days during the study period under d2, d3, d4 and d6 frequencies of tapping were 144, 98, and 74 and 51 days, respectively. Thus, by adopting d3, d4 and d6 frequencies of tapping, requirement of tapper can be reduced by 32%, 49% and 65% respectively, compared to alternate daily tapping.

There are several other benefits under LFT such as the low incidence of tapping panel dryness, extended period of tapping on the same panel and increase in tree girth. Present study clearly showed that tapping under d3, d4 and d6 along with appropriate stimulation can result in comparable production to that of alternate daily tapping. Sustainable yield can be achieved under LFT

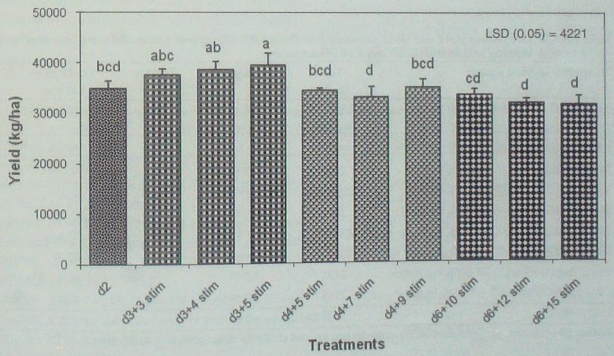


Fig. LHT.1. **Cumulative yield (kg ha^{-1}) of clone RRII 105 under different frequencies of tapping (cumulative of fifteen years).**
Values followed by same letters are not critically different from each other. Vertical bar represent SE; n=15.

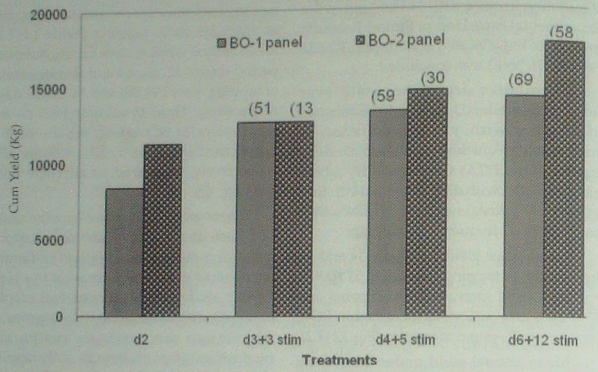


Fig. LHT. 2. **Cumulative yield (kg ha^{-1}) of clone RRII 105 under different frequencies of tapping in BO-1 and BO-2 panel. Values in parenthesis indicate percent increase over d2 frequency of tapping**

Table LHT. 2. **Dry rubber yield and other parameters of clone RRII 105 tapped under different frequencies of tapping and stimulation (Mean of fifteen years)**

Treatment/Tapping system	Yield (kg/ha)	Yieldkg/tree	Tapping days	Yieldkg/tap/ 400 trees
T1- S/2 (RG) d2 6d/7 (Control)	2331 ^{bcd}	7.1 ^{a}	144	19.8 ^{e}
T2 - S/2 (RG) d3 6d/7. ET 2.5% Pa3/y*	2505 ^{abc}	6.4 ^{bc}	98	26.3 ^{d}
T3 - S/2 (RG) d3 6d/7. ET 2.5% Pa4/y*	2575 ^{ab}	6.7 ^{ab}	98	27.3 ^{d}
T4 - S/2 (RG) d3 6d/7. ET 2.5% Pa5 /y*	2633 ^{a}	7.2 ^{a}	98	29.5 ^{cd}
T5 - S/2 (RG) d4 6d/7. ET 2.5% Pa 5/y*	2299 ^{bcd}	6.0 ^{cd}	74	32.2 ^{bc}
T6 - S/2 (RG) d4 6d/7. ET 2.5% Pa 7/y*	2212 ^{d}	6.0 ^{cd}	74	32.4 ^{bc}
T7 - S/2(RG) d4 6d/7. ET 2.5% Pa 9/y*	2336 ^{bcd}	6.2 ^{bc}	74	33.6 ^{b}
T8 - S/2(RG) d6 6d/7. ET 2.5% Pa10/y*	2236 ^{cd}	5.5 ^{bc}	51	42.8 ^{a}
T9 - S/2 (RG) d6 6d/7. ET 2.5% Pa12/y(m)	2134 ^{d}	5.3 ^{c}	51	41.4 ^{a}
T10 - S/2(RG) d6 6d/7. ET 2.5% Pa 15/y*	2115 ^{d}	5.2 ^{c}	51	40.2 ^{a}
LSD (P=0.05)	281	0.6	-	3.3

Within the column, values followed by same letters are not critically different from each other.

with yield stimulation and the grower can benefit from reduction in cost of production and increased economic life of rubber trees.

The demonstration plot at Central Experiment Station (CES) under weekly tapping with monthly application of 2.5%

ethephon continued to give promising yield during 2012-13. It was 2790 kg/400 trees with mean per tap yield of 60.6 kg and average DRC of 39.2%. Incidence of tapping panel dryness was 6.3%. Mean yield of eleven years of tapping was 2669kg/400 trees.

The exploratory trial at CES under d10 frequency of tapping with once in 20 day's stimulation continued to give promising yield during 2012-13. Yield of 3477 kg/400 trees was obtained in the second year of BI-1 panel. TPD percentage was very low (3.4 %). Overall mean yield of eleven years of tapping was 2472 kg/400 trees.

2. Controlled Upward Tapping

During eighth year, yield under S/4U d3, S/4U d4, S/3 U/d6 with periodic panel change and basal panel tapping under d6 frequency was comparable (Table LHT.3). Annual panel change under d6 frequency of basal tapping also was comparable to periodic panel change of d3, d4 and d6 frequencies of tapping.

The collaborative all India project on Controlled Upward Tapping (CUT) clearly indicated usefulness of CUT to considerably enhance production and productivity of NR from old and senile trees. By large scale implementation of CUT in India, the productivity can be increased at least by 200 kg per ha.

3. Other experiments

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

The randomized block design experiment with eight treatments and six replications laid out in the year 1997-98 at the Experimental Farm, Unit, RIT , Pambady, Kottayam (Dist.) in clone RRII 105 was consolidated and concluded. Data on yield, related parameters and incidence of Tapping Panel Dryness were monitored till 2011-12. Fifteen years data were compiled, processed, statistically analysed

Table LHT.3. **Performances of low frequency tapping with different harvesting practices in clone RRII 105 at EFU, RIT, Pampady.**

Tapping system	Yield*(kg 400 trees^{-1})	Tappingdays
S/4U d3 ET5%La 6/y ;S/2 (RG) d3 ET2.5% Pa 2/y	2543 a	100
S/4U d4 ET5%La 9/y(3w); S/2 (RG) d4 ET2.5% Pa 4/y	2571 a	75
S/2 (RG) d4 ET2.5% La Pa 6/y	2446 ab	75
S/4U d4 ET5% La 18/y (3w) with annual panel change	2494 a	75
S/4U d4 ET5% La 18/y stim. with bi-annual panel change	2371 abc	75
S/4U d6 + ET5% La 12/y (2w) ;S/2 (RG) d6 ET2.5% Pa 6/y	1984 bcd	52
S/2 d6 ET2.5.% Pa 12/y (m)	2181 abcd	52
S/4U d6 ET5% La 24/y (2w) with annual panel change	1880 d	52
S/4U d6 ET5% La 24/y (2w) with bi-annual panel change	1970 bcd	52
S/3U d6 ET5% La 9/y (3w) ; S/2 (RG) d6 ET2.5% Pa 6/y	2202 abcd	52
S/2 (RG) d6 ET2.5.% La Pa 12/y (m)	2272 abcd	52
S/2 (RG) d6 ET2.5% La Pa 12/y (m)	2442 ab	52
S/2 (RG) d6 ET2.5% Pa 12/y (m)	1894 cd	52
USD (0.05)	491	

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

Table LHT. 4. **Dry rubber yield and Tapping Panel Dryness status in clone RRII 105 under d2 and d3 frequencies of tapping with and without rainguarding and stimulation**

Treatments	Dry rubber yield** (kg 400 trees^{-1})	Dry rubber yield** (g t^{-1}t^{-1})	TPD trees (2011-12)
S/2 (RG) d2 6d/7	2773 a	51.6 d	6
S/2 d2 6d/7	2306 bcd	64.2 c	5
S/2 d2 6d/7 ET. 2.5% Pa 3/Y	2458 bc	69.2 c	7
S/2 d2 6d/7 ET. 2.5% Pa 5/Y	2196 cd	63.6 c	3
S/2 (RG) d3 6d/7 ET. 2.5% Pa 3/Y*	2328 bc	59.7 cd	3
S/2 d3 6d/7 ET. 2.5% Pa 3/Y	2047 d	85 b	2
S/2 d3 6d/7 ET. 2.5% Pa 5/Y	2223 cd	92.8 ab	4
S/2 d3 6d/7 ET. 2.5% Pa 7/Y	2513 ab	101.2 a	1
LSD (0.05)	277.1	9.5 -	

* ET 2.5 % Pa from 2001 ** Mean of fifteen years

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

and consolidated. Overall picture of the data indicated that, crop loss in non rain guarded trees can be only partially compensated by stimulation under d2 frequency of tapping. However under d3 frequency of tapping crop loss in non rain guarded trees can be compensated by 5 or 7 rounds of stimulations per year. Irrespective of the system of tapping non rainguarded trees showed higher per tap yield (g/t/t) and was highest under d3 frequency of tapping. Tapping Panel dryness was lower under d3 frequency of tapping (Table LHT 4).

Evaluation of mini and reduced spiral tapping

In the experiment at EFU, RIT, S/4 d3 continued to give good yield in the 14th year and the fourteen year average yield also confirms the advantage of this system (Table LHT 5). Two farmers had initiated S/4 d3 with yield stimulation at 45 days interval (excluding February, March & April) and the trees were opened at 45cm girth. In the location near Piravom, yield was 3.41 and 4.01 kg/tree/year respectively during first and second year of tapping. In the second location at Ottappalam, the yield was 3.81kg/

Table LHT 5. **Evaluation of mini and reduced spiral taping in comparison to S/2 d3**

Treatments	Dry rubber (kg 400 trees^{-1})		
	2012-13	Mean Yield*	Panel
S/2 (RG) d3 ET ET 2.5% Pa3/y	1521	2023 (100)	BI - 1(4)
Mc 5 (RG) d3 ET 2.5% Pa24/y	1532	1930 (95)	BO - 4 (2)
Mc 5 (RG) d3 ET 5% Pa12/y	1832	2033 (100.5)	BO - 4 (2)
S/R (RG) 10 d3 ET 2.5% Pa24/y	1884	2499 (124)	BO - 4 (2)
S/R (RG) 10 d3 ET 5% Pa12/y	1694	2283 (113)	BO - 4 (2)
S/4 (RG) d3 ET 2.5% Pa12/y	1679	2476 (122)	BO - 4 (2)
S/3 (RG) d3 ET 2.5% Pa12/y	1647	2491 (123)	BI - 1 (1)

*Mean of 14 years. Figures in parenthesis is % of Control, NS

Table LHT. 6. **Commercial yield of S/4 tapping in comparison to S/2**

System	Kg ha^{-1}	Kg tree^{-1}	No. of trees ha^{-1}
S/2 (RG) RG) d3 6d/7 ET 2.5% Pa 3/y*	561	3.9	144
S/4 (RG) d3 6d/7 ET 2.5% Pa 6/y*	700	2.6	267
S/4 (RG) d3 6d/7 ET 2.5% Pa 9/y*	720	2.6	280

tree/year in the first year of opening. The Grower has also reported that the time taken for tapping 500 trees with S/4 cut was only 150 minutes.

The results of commercial evaluation of reduced spiral tapping at B C Cheruvally estate in comparison to S/2 d3 is promising (Table LHT 6).

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

An RBD experiment on vertical tapping was initiated with five treatments and five replications. Dry rubber yield data from all tapping days were collected, compiled, processed and statistically analysed. Dry rubber yield of 34 g t-1t-1 could be obtained with vertical tapping cut of 10 cm with 24 rounds of yield stimulation with ethephon. Vertical tapping cut of 22 cm with 6 rounds of stimulation, dry rubber yield of only 28 g t-1t-1 could be obtained which was observed to be at par with 8 rounds of stimulation. However, dry rubber yield (g t-1t-1) from vertically tapped trees were significantly lower compared to S/2 d3 6d/7 (Table LHT 7). Further modification in length of cut, stimulation rounds, etc. may yield better results.

Table LHT 7. **Dry rubber yield (g^{-1} t^{-1}t^{-1}) under conventional (half spiral) and non conventional (vertical tapping) tapping systems**

Sl. No.	Treatments	Dry rubber yield (g^{-1} t^{-1}t^{-1})
1	S/2 (RG) d3 6d/7 ET. 2.5 % Pa 3/Y	77.2 a
2	Vert. (22 cm) (RG) d3 6d/7 ET. 2.5 % Pa 6/Y	27.8 b
3	Vert. (22 cm) (RG) d3 6d/7 ET. 2.5 % Pa 8/Y	26.0 b
4	Vert. (10 cm) (RG) d3 6d/7 ET. 2.5 % Pa 12/Y	26.9 b
5	Vert. (10 cm) (RG) d3 6d/7 ET. 2.5 % Pa 24/Y	33.8 b
LSD (0.05)		15.6

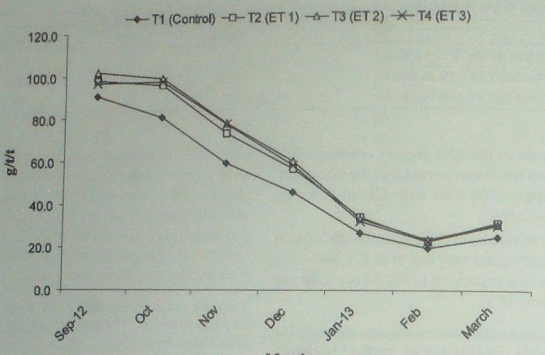
Values followed by same letter/s are not critically different from each other

Testing and evaluation of products : Evaluation of "Mortex" as an yield stimulant in rubber

The randomized block design experiment at Harrisons Malayalam Limited, Kaliyar Estate, Thodupuzha, Idukki (Dist.) in clone RRII 105 to evaluate "Mortex" in comparison with ethephon was consolidated and concluded. Higher yield could be observed in trees stimulated with ethephon and "Mortex". Response of rubber trees to ethephon and "Mortex" application were comparable (Tables LHT 8) indicating that by applying 'Mortex", grower do not get additional benefit, rather than incurring extra expenditure as the cost of "Mortex" is approximately 6 times higher than ethephon.

Table LHT 8. **Overall response clone RRII 105 to ethephon and mortex application under d3 system of tapping (Mean of 3 years): Kaliyar estate, Thodupuzha**

Treatment	Kg/ tree	g^{-1} t^{-1}t^{-1}	TPD Trees (11-12)
S/2 (RG) d36d/7	7.6	76.5	2/64
S/2 (RG) d3 6d/7 ET 2.5 Pa 3/Y	7.8	81.6	3/64
S/2 (RG) d3 6d/7 MTX 2.5 Pa 3/Y	8.1	81.8	5/64
S/2 (RG) d3 6d/7 MTX 2.5 Pa 6/Y	7.8	78.7	7/64
LSD (0.05)	NS	NS	-



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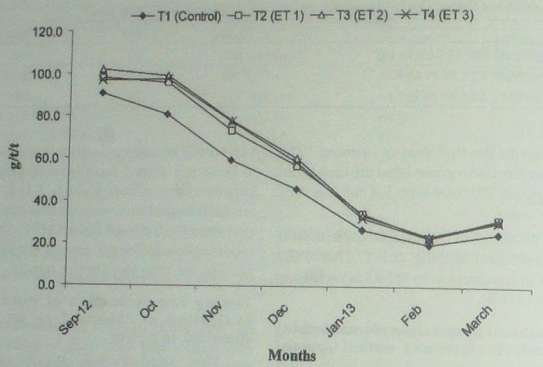

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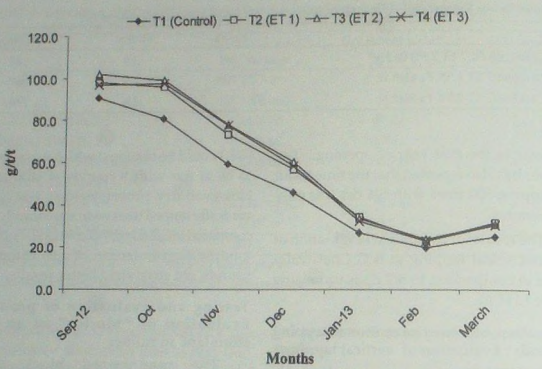

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I. Development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping

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

1.1. Transcriptome derived microsatellites

Transcriptome sequencing of *Corynespora* challenged and control leaf samples of RRII 105 and GT1 was performed using the Illumina HiSeq 2000 platform. RNA-seq data set was analysed for large-scale microsatellite/SSR mining. Microsatellite sequences bearing simple dinucleotide repeat motifs (> 10 repeats) and trinucleotide repeat motifs (>bearing 7 repeats) were identified in control transcripts of RRII 105 (C1) and also in GT1 (C2). Simple sequence repeats comprising of 347 dinucleotide and 154 trinucleotide repeats unique in RRII 105 were identified. Similarly, 347 dinucleotide and 183 trinucleotide repeats were found unique in GT1. A common set of 286 dinucleotide and 283 trinucleotide repeats existing in both RRII 105 and GT1 were also identified. Altogether 980 dinucleotde and 620 trinucleotide repeats were identified in control transcripts.

1.1.1. Genetic characterization of popular clones and wild accessions

Fluorescent labeled primer pairs were used in multiplexing for multicolor SSR genotyping of rubber clones. Multicolored allelic profiles generated could be used to discriminate rubber clones.

Genotyping of wild accessions of *Hevea brasiliensis* along with five other wild *Hevea* species *viz.*, *H. benthamiana*, *H. spruceana*, *H. pauciflora*, *H. camargoana* and *H. nitida* was continued with genomic and genic (EST-derived) microsatellite markers to assess the extent of genetic diversity.

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

1.2.1. Identification of SNPs in transcription factors (TFs) from disease transcriptome

Transcriptional control of the expression of stress-responsive genes, regulated by transcription factors (TFs), is a crucial part of the plant response to a range of abiotic and biotic stresses. Therefore, an effort was made to identify TFs controlling disease resistance transcripts from *Corynespora* challenged RRII 105 and GT1 transcriptomes. Genes belonging to the WRKY family, Myb, GATA, ethylene responsive and some of the putative TFs were found up-regulated in treated plants. Twenty up regulated TFs with 5′ or 3′ UTRs were identified and primers were designed for PCR amplification of those regions from four *Hevea* clones GT1, RRII 105, RRIM 600 and RRII 430 for SNP detection. Finally 13 transcription factor genes, mostly at their 5′UTRs were successfully amplified and sequenced. Sequencing of PCR products from each of the rubber clones under study resulted in a total aligned sequence data of 5075 bases for each genotype. Respective reference sequences derived from transcriptome sequencing of either RRII 105 (T1) or GT1 (T2) treated with *Corynespora cassiicola* were also aligned. Altogether 38 SNPs including 4 indels were identified from the analysed regions.

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

HMG-CoA reductase (HMGR) *gene*

The entire *HMGR* gene was cloned and sequenced to reconfirm the SNPs identified

earlier by PCR product sequencing. Haplotype analysis was carried out using DnaSP and Haploview software. In total, 14 SNPs including two indels were identified and confirmed from the combined PCR product as well as cloned fragment sequence data. RRII 105, RRII 118 and RRIC 52 showed complete homozygosity whereas RRIM 600 and GT1 appeared to be highly heterozygous. RRII 105 and RRII 118 shared the same alleles while RRIC 52 alleles were different. Haplotype analysis by DnaSP confirmed the presence of five haplotypes. Using Haploview software it was confirmed that the identified haplotypes formed a single haplo-block.

HMG-CoA synthase (*HMGS*) gene

A highly variable 800 bp intron-exon junction of *HMGS* gene having five unique SNP combinations for all the five genotypes was re-amplified and cloned. Allelic status of SNPs from this region was confirmed by sequencing of both PCR products and cloned fragments. Seven haplotypes were identified from this short region indicating that the site was highly prone to recombination events. The above combinations of alleles could clearly differentiate all the five clones. The identified haplotypes are; GCGAT, ACTGC, GCGAG, ACTGG, ACGAG, GTGGT and ATGAT.

Geranylgeranyl diphosphate synthase (*GGDPS*) gene

The entire genomic region of *GGDPS* gene was amplified and sequenced from five clones for SNP detection. Interestingly the gene appeared to be intron less. A total of 15 SNPs were identified from the 1.3 kb region of which two were from the 5'UTR region and the rest from coding region. Fourteen SNPs out of 15 in RRII 105 were in homozygous state whereas the other clones had mostly heterozygous SNPs. Seven haplotypes were identified in the five rubber clones. A non-synonymous SNP resulting in a substitution of aspartic acid to glycine (acidic to neutral) was identified in the clone RRIC-52 at 1097 nt position, which may be responsible for variation in structural and functional properties of the encoded protein. All the other SNPs were either silent or resulted in synonymous mutations.

Farnesyl diphosphate synthase (*FDPS*) gene

Allelic variants of *FDP* synthase gene (due to a large indel in first intron, FDP2 fragment) were detected through gel-based analysis. Different alleles from selected popular clones, wild accessions and other *Hevea* species were amplified separately, cloned and sequenced. Phylogenetic analysis using these allelic sequences was carried out to understand their relationship. Popular clones, except RRII-118 and RRII-5, were clustered together. Interestingly, morphologically much different *H. pauciflora* was also found in the same group with 100% sequence homology. RRII118 was grouped along with Acre (A19) as well as Rondonia accessions (R10). The lowest of all the identified allele was noticed in three Rondonia accessions with homozygosity only in R6. Surprisingly, this rare allele, which had a major deletion of 337 bp showed maximum homology to a transposable element. Three *Hevea* species *viz. H. benthamiana, H. spruceana* and *H. nitida* were clustered together although SNPs existed in the respective allelic fragment.

Rubber elongation factor (*REF*) gene

Complete *REF* genomic region of around 1.7 kb was amplified and sequenced from five genotypes (RRII 105, RRII 118, RRIM 600, RRIC 52 and GT1) using 3 sets of overlapping primers. A total of 32 SNPs were identified with an SNP frequency of 1 SNP every 53 bases, which is the highest among

all the rubber biosynthesis genes analyzed so far. RRII 105 and RRII 118 were found completely homozygous for all these SNPs, whereas RRIM 600, RRIC-52 and GT1 were highly heterozygous. Interestingly, the allelic status of RRIM 600 and RRIC-52 were found to be similar at all the loci.

1.2.2.1. SNP genotyping using locked nucleic acid (LNA) primers

Allele-specific LNA primers were designed and used for the differentiation of 'C' and 'T' alleles at nucleotide position 1918 of the FDPS gene. The technique was optimized and successfully used for the genotyping of forty popular clones. Another LNA primer based on A/T alleles at nucleotide position 924 in *HMGR* gene was also optimized used to genotype these clones.

1.3. Construction of a consensus genetic linkage map for understanding genetic architecture of quantitative trait loci controlling disease resistance, latex yield and timber quality in rubber (*Hevea brasiliensis*)

A concerted effort was taken to generate a framework linkage map using a segregating progeny population derived from a diverse interspecific cross between RRII 105 (*H. brasiliensis*) X F4542 (*H. benthamiana*). Genetic characterization of the progeny population of 93 individuals was initiated using 60 selected SSR markers.

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 (3284 bp) from the clone GT1 showing tolerance to *Corynespora cassicola* was characterized for the first time. Conceptual translation of the coding sequences had characteristic NB-ARC domain and leucin-rich repeat (LLR) motif.

Sequence tagged site (STS) marker derived from resistance *(R)* gene

Two sequence variants of *R* gene were identified in GT1 clone. A 75 bp indel was noticed in the coding region of these two sequence variants. Based on this indel, an STS marker (R-STS) was generated and employed in genotyping of 40 rubber clones. Existence of two alleles for R-STS was noticed among the cultivated clones. RRII 105 was found to be homozygous for the upper allele, whereas GT1 was heterozygous owing both the alleles.

2.1.1.1. Resistance protein encoded by *R* gene

Protein structure prediction was performed with conceptual translation (amino acid sequence) of the cloned *R* gene through homology modeling. Domain analysis resulted highest matches with apoptotic protease activating factor. Besides above, characteristic domains viz. coiled coil, leucine-rich repeat, toll-like receptor2 existed in *R* gene.

2.1.1.2. Functional characterization of resistance (*R*) gene

The involvement of *R* gene in disease tolerance was assessed through real-time quantification of gene expression after challenging tolerant (GT1) and susceptible (RRII 105) clones of rubber with *Corynespora cassiicola*. Differential expression of the R gene was noticed between these two clones even in unchallenged condition (control) and expression level was significantly higher in

tolerant clone GT1. However, at 6 hours following infection, the expression levels reduced considerably in both the clones. At 12 hours, expression level of *R* gene was found to reduce drastically (5 folds compared to the expression level at 0 hour) in case of RRII 105, whereas expression shot up in GT1 reaching initial level of expression at 0 hour. However, at 24 hours expression level increased in RRII 105 by 30% but decreased in GT1 by 50% of the expression level noticed at 12 hours following infection in respective clones. From this observation it is evident that increased level of *R* gene expression in GT1 (8 folds more than in RRII 105) at 12 hours when pathogen establishes infection on host, may be one of the reason for tolerance to *Corynespora cassiicola* observed in GT1.

2.1.2. Role of caffeic acid O-methyltransferase (*HCOMT2*) gene in disease tolerance

Increase in lignification is often observed in response to pathogen attack. Since lignin is a non-degradable mechanical barrier for most microorganisms, it increases the tolerance of the host by blocking pathogen invasion. The involvement of *HCOMT2* in disease tolerance was assessed through real time quantification of gene expression after challenging tolerant (GT1) and susceptible (RRII 105) clones of rubber with *Corynespora cassiicola*. Differential expression *HCOMT2* was noticed between these two clones, even in unchallenged condition and expression level was more in RRII 105 over the tolerant clone GT 1. However, at 4 hours following infection, the expression levels shot up in both the cases. At 12 hours, expression level was found to reduce significantly (12 fold compared to the expression level at 4 hours) only in case of RRII 105. Even though an increasing trend of gene expression level was noticed in RRII 105 at 24 hours of infection, it couldn't reach up to the control level. Whereas in GT 1, expression shot up significantly after 12 hours and eventually at 24 hours it had increased by 62 fold compared to the control indicating that this form of *COMT* might participate in the hypersensitive reaction in GT1 in response to *Corynespora cassiicola* infection.

2.1.3. Genes involved in host tolerance to Corynespora leaf disease

2.1.3.1. Transcriptome analysis

Transcriptome sequencing of *Corynespora cassiicola* challenged leaf samples along with control was performed for identification and functional analysis of disease responsive genes. About 132 million paired end reads were generated through Illumina HiSeq 2000 platform. Genes/transcripts showing differential expression between *Corynespora cassiicola* challenged and control leaf samples were listed out to place them in respective metabolic pathways for understanding the functional relevance in disease tolerance.

2.1.3.2. Cloning of *GRAS* transcription factor gene

Differentially expressed GRAS transcription factor having significant role in disease tolerance was identified. Full-length cDNA (2328 bp) cloning was performed based on the sequence information obtained using RACE for 5′ and 3′ of the DDRT-PCR product. Coding sequence comprising of 2088 bp encoding 695 amino acids showed maximum homology with GRAS of *Ricinus communis*. GRAS (GAI, RGA, SCR) gene families share a variable amino-terminus and a highly conserved carboxyl-terminus that contains five recognizable motifs.

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

Expression profiling of stress related gene(s) in rubber clones from Northeast India

Expression analysis of aquaporin (*PIP1*) and myo-Inositol phosphophate synthase (*MIPS*) genes in leaf samples of RRII 105, RRIM 600, SCATC 88/13 and Haiken grown in NE (cold stressed) was performed through Real time PCR. Down regulation of *PIP1* was noticed in all cold samples and very low level of expression was noticed in RRII 105 among all the clones grown under cold. Lower expression of *MIPS* was noticed in all cold samples compared to RRII 105 control. However, both *PIP1* and *MIP* expression was relatively high in Haiken among the cold samples. Another stress responsive gene, metallothionein *MT-3a* was upregulated in RRII 105 exposed to cold.

2.3. Methylation dynamics of *Hevea brasilinesis* genome

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

2.3.1. Real time PCR studies for establishing correlation between DNA methylation and gene expression

QPCR experiments were carried out for establishing correlation between the gene expression patterns of *HMGR* and *REF* and their respective promoter methylation during pre and post cold stress on *Hevea* clones maintained in growth chamber. Initial results from RRII 105 plants indicated that the expression of both the genes was coming down after inducing cold stress. These results were viewed in the context of methylation patterns detected by bisulfite sequencing in the CCAAT box of *HMGR* gene and the cytosine preceding the TATA box of *REF* gene after inducing cold stress in RRII 105.

2.3.2. Identification of genes responsible for de-nova DNA methylation and de-methylation in rubber

RACE was performed for both 5′ and 3′ to get full-length sequence information of the partial cDNA fragments of DRM transferase (Methylase, 759 bp) and DNA N-glycosylase (De-methylase, 986 bp) amplified from rubber. RACE products of 5′ and 3′ were found to be ~1.3 kb and 500 bp respectively for DRM transferase gene, and ~1 kb and 700 bp respectively for DNA N-glycosylase gene. Cloning of these products is in progress.

2.3.3. Methylation-sensitive (MS) AFLP for identification of somaclonal variants in *Hevea*

Identification of epigenetic variation in tissue culture derived plants gives a better explanation for the phenotypic variation exhibited by them despite their similar genetic makeup. An attempt was made to identify whether epigenetic variation exist in polyembryony derived rubber plants generated by tissue culture using MS- AFLP technique. Analysis using different primer combinations was carried out. All the combinations analyzed showed clear variation in the banding pattern between the *Msp*I and *Hpa*II digests. The presence of more number of bands in the *Msp*I digest set compared to *Hpa*II set indicated presence of methylated regions in *Hevea* genome. But variation in AFLP profiles couldn't be detected among the four polyembryony-derived plants digested with *Hpa*II for all the primer combinations analyzed. This indicated the absence of methylation variation among these four plants. Based on the methylation AFLP studies it is assumed that methylation variation, which is a major factor responsible for somaclonal variation, is meager or nil in polyembryony-

derived plants and they are epigenetically true to type.

3. Cloning and characterization of agronomically important genes

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

Caffeic acid O-methyltransferase (*COMT*)

Plant O-methyltransferases (OMTs) play important roles in secondary metabolism through phenyl propanoid pathway. Two *COMT* genes from rubber, designated as *HCOMT1* and *HCOMT2* were identified and full-length cloning of *HCOMT2* cDNA was reported last year. Further an effort was made to characterize other form (*HCOMT1*) of the gene. Through RAGE technique we could generate sequence information of the difficult 5′ genomic region of *HCOMT1* along with ~ 800 bp promoter region. Coding region of *HCOMT1* was 1086 bp showing maximum homology with *Ricinus communis*. To determine the genomic organization of *HCOMT2* gene, genomic sequences of *HCOMT2* (1779 bp) from both the rubber clones RRII105 and GT1 were characterized, which consisted of 4 exons and 3 introns. Sequence variability couldn't be noticed between RRII105 and GT1.

3.2. Cloning and characterization of stress responsive genes

Cloning of *NAC* transcription factor gene

Full length *NAC* transcription factor gene involved in stress tolerance was cloned. Rapid amplification of genomic DNA ends (RAGE) technique was adopted. *EcoRV* library yielded a 1387 bp fragment, which was found to be the 5′ end of NAC transcription factor through sequence homology. Full-length sequence of *Hevea* NAC transcription factor (*HbNAC*) was deduced. Sequence length was found to be 1753 bp encoding 282 amino acids.

CENTRAL EXPERIMENT STATION, CHETHACKAL

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

The Station meets the needs of the scientists of various disciplines of Crop Improvement, Crop Management, Crop Protection, Crop Physiology and Latex Harvest Technology. The station has under A and B Divisions 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-Rex), 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 216183 kg (PFL), and 88230 kg. (scrap) and 17105 kg. (coagulum). A total of 298 tapping days was possible in the year and 45 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 5434.

Expression profiling of stress related gene(s) in rubber clones from Northeast India

Expression analysis of aquaporin (*PIP1*) and myo-Inositol phosphophate synthase (*MIPS*) genes in leaf samples of RRII 105, RRIM 600, SCATC 88/13 and Haiken grown in NE (cold stressed) was performed through Real time PCR. Down regulation of *PIP1* was noticed in all cold samples and very low level of expression was noticed in RRII 105 among all the clones grown under cold. Lower expression of *MIPS* was noticed in all cold samples compared to RRII 105 control. However, both *PIP1* and *MIP* expression was relatively high in Haiken among the cold samples. Another stress responsive gene, metallothionein *MT-3a* was upregulated in RRII 105 exposed to cold.

2.3. Methylation dynamics of *Hevea brasilinesis* genome

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

2.3.1. Real time PCR studies for establishing correlation between DNA methylation and gene expression

QPCR experiments were carried out for establishing correlation between the gene expression patterns of *HMGR* and *REF* and their respective promoter methylation during pre and post cold stress on *Hevea* clones maintained in growth chamber. Initial results from RRII 105 plants indicated that the expression of both the genes was coming down after inducing cold stress. These results were viewed in the context of methylation patterns detected by bisulfite sequencing in the CCAAT box of *HMGR* gene and the cytosine preceding the TATA box of *REF* gene after inducing cold stress in RRII 105.

2.3.2. Identification of genes responsible for de-nova DNA methylation and de-methylation in rubber

RACE was performed for both 5′ and 3′ to get full-length sequence information of the partial cDNA fragments of DRM transferase (Methylase, 759 bp) and DNA N-glycosylase (De-methylase, 986 bp) amplified from rubber. RACE products of 5′ and 3′ were found to be ~1.3 kb and 500 bp respectively for DRM transferase gene, and ~1 kb and 700 bp respectively for DNA N-glycosylase gene. Cloning of these products is in progress.

2.3.3. Methylation-sensitive (MS) AFLP for identification of somaclonal variants in *Hevea*

Identification of epigenetic variation in tissue culture derived plants gives a better explanation for the phenotypic variation exhibited by them despite their similar genetic makeup. An attempt was made to identify whether epigenetic variation exist in polyembryony derived rubber plants generated by tissue culture using MS- AFLP technique. Analysis using different primer combinations was carried out. All the combinations analyzed showed clear variation in the banding pattern between the *Msp*I and *Hpa*II digests. The presence of more number of bands in the *Msp*I digest set compared to *Hpa*II set indicated presence of methylated regions in *Hevea* genome. But variation in AFLP profiles couldn't be detected among the four polyembryony-derived plants digested with *Hpa*II for all the primer combinations analyzed. This indicated the absence of methylation variation among these four plants. Based on the methylation AFLP studies it is assumed that methylation variation, which is a major factor responsible for somaclonal variation, is meager or nil in polyembryony-

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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.

1.2.1. Evaluation of potential primary clones in Clonal Nursery

A nursery trial of eight potential primary clones of *Hevea* (*viz.* GH 1, GH 3, GH 4, GH 5, GH 6, GH 8, GH 9 and GH 10) obtained from Sarutari Research Farm under RRS, Guwahati was started in 2008 along with two check clones (RRIM 600 and RRII 429). Girth was high in GH 9 followed by GH 8 and GH 6 with minimum in GH 10 after five years of planting in clonal nursery. Average juvenile yield (g/t/ten tappings) was significantly higher in GH 9 followed by GH 8 and GH 1compared to RRIM 600 with the minimum in GH 10 after five years of planting.

1.2.2. Large scale trial for evaluation of potential primary clones

The trial was started in 2010 with ten potential primary clones of Sarutari Research Farm (viz.GH 1, GH 2, GH 3, GH 4, GH 5, GH 6, GH 7, GH 8, GH 9 and GH 10) along with two check clones (RRIM 600 and RRII 429) in RBD with three replications (plot size: 16 plants/clone) at RRTC, Hahara in Assam for evaluation of growth and yield performance in primary clones. Planting of polybag plants of different primary clones at vacancy points in field was completed.

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

Four RRII 400 series clones of *Hevea* (*viz.* RRII 417, RRII 422, RRII 429 and RRII 430) along with two check clones (RRIM 600 and SCATC 88/13) were planted in blocks (Plot size: 80 plants/clone) in Umsiang (2009) and in Byrnihat, Bhakuagoog and Sonapuat in four different growers' field for evaluation.

Girth of clones at 3rd year was recorded from Umsiang. Girth of all the clones were similar after three years of planting (Table Ghy. 1). Incidence of die-back symptoms due to cold injury was found in RRII 422 in the on-farm trial at Byrnihat(26%) after two years of planting.

Table Ghy.1. **Mean girth of RRII 400 series clones at Umsiang**

Clones	Mean girth at 3rd year of planting(cm)
RRII 417	26.1
RRII 422	23.2
RRII 429	25.5
RRII 430	25.8
RRIM 600	25.3
SCATC 88/13	24.1
CD (P = 0.05)	NS

2. Crop management

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

The experiment on Integrated Nutrient Management system for young rubber (Clone RRIM 600) with cover crop initiated in 2008 at RRTC, Hahara, RRS, Guwahati was continued. There were seven treatments comprising combinations of different doses of inorganic fertilizers with and without

biofertilizers, a biofertilizer alone treatment and a no fertilizer control. The biofertilizers applied were *Azotobactor, Pseudomonas, Phosphobacteria* and *AMF*. Growth of the plants under the treatments T_2, T_4, T_6 and T_7 was significantly superior to control (Table Ghy. 2).

Table Ghy. 2. **Girth of rubber plants during different years of growth**

Treatments		Girth at 4th year of planting (cm)
T_1	Control	23.59
T_2	Standard practice	26.66
T_3	25% N & P + BF *	24.68
T_4	50% N&P + BF *	27.03
T_5	75% N&P +BF *	25.08
T_6	Standard practice +BF	27.21
T_7	BF alone	26.62
SE		0.68
CD(P = 0.05)		2.11

* Full dose of K

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

The experiment initiated to study the impact of different biological bunds at RRTC, Hahara, RRS, Guwahati was continued. There were seven treatments, *viz.* vetiver, lemon grass, palmarosa, guinea grass, rice bean, cover crop and natural cover. The quantity of eroded soil collected in trench was recorded and 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

The onfarm intercropping trial at Morugdara, Kamrup district in Assam initiated in 2010 continued (Clone RRIM 600 with a spacing 22′ x 11′). The yield and benefit cost rates of the different intercrops are shown in Table Ghy. 3.

Table Ghy. 3. **Yield and cost benefit ratio of different intercrops within in rubber**

Crops	Yield (kg ha^{-1})	BCR
Mustard	96	2.05
French Bean	75	1.10
Radish	600	1.90
Pumpkin	750	1.96
Upland Paddy var.Luit	220	1.50
Jhoom Paddy	200	1.20
Matimah (*V. mungo*)	70	1.50
Maize	250	2.20
Sesamum	50	2.17

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

The experiment initiated during 2011 to study the comparative effect of zero tillage and normal planting techniques of RRIM 600 was continued.

The treatments involved combinations of three types of planting materials viz.

Table Ghy. 4. **Survival percentage and girth of rubber plants in different system of planting**

Treatment	Survival percentage (%)		Girth 2012 (cm)
	2011	2012	
Polybag plants with normal planting	87	70	4.62
Polybag plants under zero tillage	88	76	3.97
Budded stump plants with normal planting	79	72	3.82
Budded stump plants under zero tillage	76	70	4.02
Root trainer plants with normal planting	70	67	3.87
Root trainer plants under zero tillage	80	70	3.92
SE			0.22
CD (P = 0.05)			NS

budded stumps, polybag plants and root trainer plants and two methods of planting *viz.* planting in pits of standard size (75 cm^3) and reduced size (In holes just to accommodate the plants). The highest survival percentage was recorded for polybag plants under zero tillage. Girth of the plants was not by different treatments (Table Ghy. 4).

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

Root trainers *viz;* earthen and bamboo of required quantity has been made for starting of the experiment at Sarutari farm under RRS, Guwahati. Same experiment was also simultaneously initiated at HBSS, Parliar, Kanyakumari.

3. CROP PROTECTION

3.1. Survey on pests and diseases of rubber

Survey on pests and diseases of rubber was carried out in 52 pockets covering 26 locations in Assam, Meghalaya, Tripura and northern part of West Bengal. The range of severity of powdery mildew disease (PDI) was 20 to 50% on lower branches of the affected trees in most of the locations. Incidence of brown root disease was noticed on four/five year old rubber plants in most of the locations with maximum at Gamaichora Monuar Tilla in Tripura. Incidence of collar root and bark necrosis were found on four to five year old rubber plants in some locations in Assam. Minor incidence of Colletotrichum leaf spot was noticed in nurseries on semi-matured leaves in some locations. Infestation of scale insect was found in few locations in Assam, Meghalaya and Northern part of West Bengal during the study period.

3.2. Isolation and identification of fungal pathogens of rubber

Fungal pathogens from diseased samples of rubber collected during the survey was isolated and identified. Two species of *Colletotrichum i.e. Colletotrichum acutatum* and *Colletotrichum gloeosporioides* were isolated and infection of *Colletotrichum acutatum* was found to be more than 60% compared to *Colletotrichum gloeosporioide* causing *Colletotrichum* leaf spot disease of rubber.

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

The three short-listed wild accessions of *Hevea* germplasm was evaluated in field condition at Sarutari Research Farm by dusting with spores of *Oidium* and found a high level of tolerance in AC 587 to powdery mildew disease as compared to other two accessions. Further assessment of AC 587 & RO 1737 was carried out in polybag plants and high level of tolerance of the clone AC 587 to powdery mildew disease was confirmed.

3.4. Management of purple root disease of *Hevea* in immature plantation

A trial was conducted in six-year-old rubber plants of RRIM 600 and RRII 105 at RRTC, Hahara in Assam with five treatments and three replications. Observations in the treated and untreated plot was carried out and the root system of all affected plant was found healthy. Fructification of the pathogen *Helicobasidium compactum* was not developed at the plant base of treated plants as observed in the untreated plots. Three collateral hosts of the pathogen causing purple root disease were observed in rubber plantation at RRTC, Hahara. Microbial population in soil was

maximum in healthy plant followed by untreated control and propiconazole treated plants and the minimum was in-hexaconazole plot indicating that the fungicide propiconazole is more efficient in the management of purple root disease as well as in the maintenance of soil health. Maximum girth was observed for healthy plants.

3.5. Microbial activity, disintegration of leaf litter and nutrient release under mature rubber plantation and natural forest cover

Litter bag method was adopted to study the rate of decomposition of four types of forest litters (*viz*. rubber, sal, teak, bamboo) under two ecosystems. Decomposition rate was rapid under natural forest cover than rubber plantation. The total microbial population was more in decomposed litters under forest covers. The rate of decomposition of bamboo litter was faster than the rubber and it was followed by teak and sal.

3.6. Dynamics of microbial status of rubber, tea, forest and barren soils in Assam and N. Bengal region

Studies on seasonal variation of microbial populations in rubber plantation, forest, tea and barren soil revealed a decrease in populations from pre- winter to winter season. The population of bacterial and fungal population was found to be high in the forest soils and it was followed by rubber, tea and barren soils. Amongst the most dominant fungal species, *Trichoderma, Aspergillus, Penicillium, Fusarium* and filamentous yeasts were common on the entire ecosystem. The soils of all the four different systems are acidic in nature.

3.7. Exploitation of naturally occurring antagonistic agents against diseases of rubber

Microorganisms from the rhizosphere soils of rubber were isolated and tested their antagonistic activity against rubber pathogens like *Colletotrichum gloeosporioides; Corynespora cassiicola, Phellinus noxius* and *Helicobasidium compactum* by dual culture technique. Six fungal and five bacterial isolates showing antagonism against various pathogens were selected.

4. CROP PHYSIOLOGY & LATEX HARVEST TECHNOLOGY

4.1. Effect of application of Stimulant away from tapping cut

Effect of stimulation on different areas of bark away from tapping cut was studied. Six different treatments were imposed along with unstimulated trees as control. The experiment was carried out in clone RRIM 600 under S/2 d3 system of tapping in blocks. Application of 5% ethephon on bud union showed significantly higher yield than that of the unstimulated trees during 2nd year.

4.2. Shallow tapping – an option to stress alleviation in *Hevea* plantation during winterseason in NE region.

Tapping rest during winter is mandatory for NE India to avoid low winter temperature stress which caters to yield loss of around 40-45 tapping days every year. It would be worth experimenting whether shallow tapping (tapping half of the bark consumed by normal tapping) during rest period can compensate the yield loss. The dry rubber yield and TPD in RRIM 600 clone at Sarutari Research Farm in Assam was recorded with different tapping systems. There was no significant difference between the yield in different tapping system. The

plants showing above 75% TPD was more in shallow and continuous tapping with 148 tapping days each in comparison with the normal S/2 d3 tapping where the plants did not show severe TPD with 105 tapping days each. The continuous tapping system without rest showed better yield in this region.

5.1. Controlled upward tapping (CUT)

The experiment on CUT in clone RRIM 600 was concluded. Among the treatments periodic panel change system of S/4 U d2 ET 5% La (m), S/2 d2 ET 2.5% Pa 2/y recorded highest yield. Under CUT 142-181% increase in yield over normal tapping was observed.

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The station continued its research on evaluation of clones, intercropping, nutritional requirement, remote sensing study and latex harvest technology. The other aspects of investigations were marketing of rubber and advisory services to rubber growers.

1. Crop improvement

1.1. Development of clones

In recombination breeding, evaluation of 1860 hybrid progenies is progressing in three seedling nursery trials. 25 hybrids have been selected for further evaluation based on test tap yield in the seedling nursery evaluation. In the clonal nursery evaluation of selected hybrids (2008), mean test tap yield of 99/5/9 (10.4 g $t^{-1}t^{-1}$) and 98/46 (11.6 g $t^{-1}t^{-1}$) over two years was on par with RRIM 600 (16.4 g $t^{-1}t^{-1}$). In the clonal nursery evaluation of selected hybrids (2009), 98/38 (10.9 g $t^{-1}t^{-1}$) had better test tap yield than RRIM 600 (9.1 g $t^{-1}t^{-1}$).

In a SST of poly cross progeny evaluation, among the eleven selected ortets 114 (52.2 g $t^{-1}t^{-1}$) gave significantly higher yield than RRIM 600 (41.6 g $t^{-1}t^{-1}$). Among the 13 ortets from four regional stations in the North Eastern region, RRSG 248 recorded highest mean test tap yield (23.7 g $t^{-1}t^{-1}$) followed by RRST 37 (19.6 g $t^{-1}t^{-1}$) while check clone RRIM 600 recorded 22.6 g $t^{-1}t^{-1}$. Twelve ortets from traditional area along with RRII 403 and RRII 407 and check clones, are being evaluated in clonal nursery (2009). Clone RRII 407 exhibited significantly higher test tap yield (12 g $t^{-1}t^{-1}$) than RRIM 600 (9.1 g $t^{-1}t^{-1}$). Among the ortets, P 132 recorded highest test tap yield (9.1 g $t^{-1}t^{-1}$).

1.2. Evaluation of clones

In the large scale trial (LST) planted in 1995 with ten clones, PB 311 (49.57 g $t^{-1}t^{-1}$) had the highest mean yield over eight years followed by RRII 105 (49.25 g $t^{-1}t^{-1}$). In the LST (1996) consisting of thirteen clones, RRII 429 (49.9 g $t^{-1}t^{-1}$) and RRII 422 (50.5 g $t^{-1}t^{-1}$) showed significantly superior yield (over ten years) compared to RRIM 600 (42.6 g $t^{-1}t^{-1}$) (Table Agar. 1).

Potential clones are being evaluated in four on-farm trials. In Killamura block plantation (1997), mean yield over seven years of tapping was highest for PB 235 (1023 kg $ha^{-1}yr^{-1}$). At TFDPC plantation, Bagafa, South Tripura (2000), RRIM 600 (1195 kg $ha^{-1}yr^{-1}$) gave the highest mean yield over four years of tapping. In the on farm trial at TRPC plantation, Pathalia (2005), RRII 429 recorded the highest girth (40.6 cm) in 2012. At Hirapur Block plantation (2009), casualty due to cold stress was observed in RRII 422.

Table Agar. 1. **Yield performance of thirteen clones at Agartala**

Clone	Girth (cm) Feb '13	Mean yield (g $t^{-1}t^{-1}$) over 10 years
RRIM 600	70.2	42.6
RRII 429	74.6	49.9*
RRII203	73.1	34.2
PB 217	71.7	32.2
RRII 51	67.3	29.5
RRII 414	65.7	34.9
RRII 430	64.0	43.2
RRIC 100	72.5	39.8
RRII 422	67.9	50.5*
RRII 105	67.6	40.0
RRII 417	70.3	44.9
RRII 176	78.6	34.3
PB 235	69.9	38.8
CD (P = 0.05)		4.4

Four clonal nursery evaluations involving 57 pipeline clones from traditional area, 11 popular clones and 19 potential clones are in progress. In the clonal nursery evaluation (2009), PB 255 recorded significantly higher girth and test tap yield (11.6 g $t^{-1}t^{-1}$) than RRIM 600 (6.6 g $t^{-1}t^{-1}$). Clones RRIM 712, and PB 314 also had significantly higher test tap yield than RRIM 600. Among the pipeline clones from the traditional region P 072 and P 026 had test tap yield on par with RRIM 600 (clonal nursery, 2009). Field trial involving 49 clones for standardising DUS testing norms is also in progress. In the study on the influence of root stock on survival of susceptible clone RRII 422 under low temperature condition, RRII 422 budded on selected tolerant root stock had better survival compared to susceptible root stock.

2. Crop management

The experiment to study the influence of organic manure and inorganic fertilizers on growth of young rubber trees continued. As in the previous years, the treatment with FYM (20 kg) and 50% inorganic fertilizer was superior recording 48.3 cm girth. The girth of plants receiving inorganic fertilizers alone was 44.7 cm and that of control was 35.8 cm. Integrating FYM with 50% of recommended dose of inorganic fertilizers achieved higher tappability also. Improvement in soil physical and chemical properties was also observed due to the application of FYM along with inorganic fertilizes.

The field experiment, to study the influence of bio-fertilizers (*Azotobactor*, *Phosphobacteria*, *Pseudomonas* and VAM) with and without graded doses of inorganic fertilizer on growth of young rubber was continued. At the end of the fourth year of plantation, it was observed that a combination of 50% N, 50% P and 100% K along with biofertilizer, significantly increased the girth of the plants.

On evaluation of a cropping system model, *Colocasia* recorded highest BCR (3.08), followed by cowpea (1.99), maize (1.86), rice (1.82) and amorphophalus (1.72) after five years of intercropping. Among the short term crops, Banana recorded higher BCR (2.79) over pineapple (1.78) both in model I and II. In the tea-rubber intercropping trial, mean green tea leaf yield was 224 kg ha^{-1} as intercrop and 1920 kg ha^{-1} as monocrop. Rubber yield showed that clone RRIM 600 (2016 kg ha^{-1}) and PB 235 (1914 kg ha^{-1}) were significantly superior in comparison to other clones like GT 1 (1542 kg ha^{-1}) and RRII 105 (1198 kg ha^{-1}). In the experiment on assessment of growth of *Hevea* under zero tillage condition, the results indicated that pits of larger dimensions have no advantage on growth of plants. In another trial on specific package of practices for immature *Hevea*, the moisture percentage in all the three layers of soil (0-15 cm, 15-30 cm and

30-60 cm) was higher in vertically mulched pits than in conventional mulching and control.

3.1 Latex Harvest Technology

Shallow tapping experiment was initiated in clone RRIM 600 with an objective to alleviate the cold stress during winter period by shallow tapping (October to March). Higher yield was observed in normal tapping (S/2 d2 6d7) compared to shallow tapping. In the different systems of tapping experiment, the clone PB 235 continued to give the highest yield under S/2 d3 system of tapping compared to S/2 d4 and S/2 d6 system of tapping. In another experiment, yield in S/2 d3 system tapping was at par with S/2 d2 system in clone RRIM 600 and higher than the S/2 d4 system of tapping.

3.2 Remote Sensing

Prediction of future distribution of Natural Rubber (NR) was attempted using the Maximum Entropy (MAXENT) species distribution model in South and Northeast India. The preliminary results of model simulation on present distribution of NR were validated with the satellite imagery based on the present rubber distribution map of the state. The distribution of NR was predicted for south and northeast India for the year 2020 and 2050 using the bioclimatic variables of SRES-A1B scenario generated by the Intergovernmental Panel for Climate Change (IPCC). The model simulation has revealed that the suitable region at present for NR in northeast is 23095 km^2 (8.8% of total area) which is mainly concentrated in the western part of Brahmaputra valley of Tripura, Goalpara district of Assam and part of Meghalaya state. The model predicted suitable regions for NR cultivation in northeast region in 2020 as 31940 km^2 (12.2%), expanding the NR cultivation upwards in Assam valley and in the Northern part of Tripura and Mizoram. The prediction for 2050 indicates that there is no significant difference in suitable regions in North East India between 2020 (31940 km^2) and that of 2050 (31535 km^2).

In Western Ghats region the present distribution of NR is spread over 30963 km^2. Part of Northern region in Western ghats will become suitable for NR by 2020. However, there is no significant temporal difference in the total area under NR in the Western Ghats region. The most suitable area of 4310 km^2 at present may decrease to 2496 Km^2 in 2020. Present distribution of NR predicted by MAXENT model indicates that southern part of the Western Ghats i.e., Kerala and southern part of Tamil Nadu will be dominated by NR.

4. Economics

The study on primary processing and marketing of Natural Rubber in Tripura is completed. The database consisted of samples drawn from public sector undertakings such as Tripura Forest Development & Plantation Corporation Limited (TFDPC) and Tripura Rehabilitation Plantation Corporation Limited (TRPC), smallholders organized under Block Planting Units (BPU) - Rubber Producers' Societies (RPS) network, individual growers in the unorganized sector and market intermediaries. The relevant information was gathered from 357 growers and 47 market intermediaries. The type/grade-wise quantity of NR sold by TRPC indicated the dominance of sheet rubber accounting for around 88% of the total quantity sold during the year 2011-12. Sheet rubber (38.6%) and preserved field latex (PFL) (31.1%) were the

major forms of rubber sold by TFDPC. More than 93% of the growers in the unorganized sector were selling their produce as unsmoked sheets are sold to village traders/ subagents of dealers. The average deduction while selling unsmoked sheets to the subagents is Rs 36.8 kg^{-1} from the consumer's price. About 92 % of BPUs and 60% of RPSs are marketing their produce as PFL and the marketing channel for PFL has been dominated by Manimalayar Rubbers Ltd. for 100% of BPUs and 90% of RPSs. The Manimalayar Rubbers sells the PFL so procured to the local consumer the DS group. Normally, the RPS/BPU process sheet rubber only during monsoon and peak seasons (since the local purchaser the DS group, could not buy the entire quantity at this time). The farm gate price realized by the beneficiaries/ members for one kg of dry rubber is Rs. 35.2 less than the Cenex price prevailing in Kottayam market.

5. Advisory work

Discriminatory fertilizer recommendation based on soil was offered to 255 rubber growers of this region. A total of 1674 latex samples were analysed for DRC and other latex parameters. A total of 4795 m of bud-wood (of high yielding clones) were supplied to the 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

In 2011 populations, growth data of 338 poly-cross seedlings were recorded

1.2 Clonal Nursery Evaluation

A clonal nursery was set up with three selections from Tura, four each from Agartala and Guwahati with RRIM 600 as the check clone. The experiment is in RBD design with 12 clones and three replications and the plot size is 6. After two years, RRIM 600 showed significantly higher plant height (3.64 m), where as Agartala selection RRSA 121 showed the highest plant girth (13.6 cm) at 30 cm from the bud union.

1.3 Evaluation of Clones (LST)

Establishment of LST with 18 clones is under progress.

1.4 On-Farm Evaluation of Select Clones

Three on-farm trials have been set up in the North & 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 North Garo Hills and 400 plants of four clones viz. RRII 417, RRII 422, RRII 429 and RRIM 600 were planted in the West Garo Hills of Meghalaya. 100 plants were used per clone. Growth data have been recorded from all the on-farm trials. In North Garo Hills, highest girth was recorded in PB 235 (23.04 cm) followed by RRII 429 (22.95 cm), and lowest was in RRII 422 (16.50 cm). Highest girth increment was recorded in RRII 429 (8.20 cm) followed by

Table Tura. 1. **Growth parameters of different clones in the on-farm trial at Mendipathar, North Garo hills of Meghalaya**

Clones	Girth (cm)			Girth Increment (cm) (January to October 2012)		
	Rabha	Momin	Mean	Rabha	Momin	Mean
PB 235	21.31	24.77	23.04	6.68	8.29	7.49
RRII 417	16.85	25.87	21.36	5.71	8.48	7.10
RRII 429	18.01	27.89	22.95	7.06	9.34	8.20
RRII 422	12.63	20.38	16.50	4.67	6.78	5.73
RRII 203	17.21	25.22	21.22	6.50	7.39	6.95
RRIM 600	18.68	24.03	21.35	5.70	6.67	6.19
LSD (P<0.05)	5.12	1.25				
CV (%)	24.3	18.0				

PB 235 (7.49 cm) and lowest was in RRIM 600 (6.19 cm) (Table Tura. 1).

In West Garo Hills highest plant height (4.44 m), mean girth (12.65cm) and number of whorls (9.56) were recorded in RRII 417 followed by RRIM 600 while RRII 429 had the least of all.

1.5 Half-Sib progeny evaluation

In 2008 and 2009 selections, on the basis of the test-tap yield of the progenies, top 20% of the population were selected for further evaluation in clonal nursery trials.

1.6 Evaluation of poly-cross progenies from four stations of NE region

Poly-clonal seeds were collected from four locations in the NE region and planted in RBD with 5 replications (360 progenies). Nagarakata polyclonal progenies showed the maximum plant girth (12.2cm) and test tap yield (5.19 g t^{-1} 10 tap^{-1}) followed by Agartala (11.33cm girth and 4.57 g t^{-1} 10 tap^{-1}) and least in Tura polyclonal progenies (10.77 cm plant girth and 3.29 g t^{-1} 10 tap^{-1}) selections. Around 15-20% were selected on the basis of test tap yield and cut back in the field for further study.

1.7. Fundamental studies on the nature of wintering and flowering in *Hevea* clones in Garo Hills of Meghalaya

10 clones in the 1985 clonal trials are included in this study. Clonal variations in wintering pattern and flowering was observed.

2. Crop physiology & Latex Harvest Technology

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 results indicated that annual average total volume of latex was 158.5 ml/t/t, yield was 48.7 g/t/t and DRC % was 33.1 %. Complete defoliation occurred in last week of January and refoliation in last week of February while flowering was noted 2nd week of March and during defoliation and refoliation period DRC ranged was from 26.6 – 28.3 %. Lowest soil moisture recorded in the month of February/March.

In the controlled upward tapping system (CUT), treatment wise monthly yield was recorded and result showed that highest yield was recorded in T2 followed by T1, T3

and lowest was in T4 (Table Tura. 2). Highest TPD was observed in T1 (8.92 %) while lowest was in T4 (6.89 %). Under CUT 33-60 % increased yield was observed over normal tapping of basal panel.

Table Tura. 2. **Annual yield (g $t^{-1}t^{-1}$) under different treatments of ethephon application in Controlled Upward Tapping system. (2012-13)**

Treatment	Yield	TPD
(g t^{-1} t^{-1})		(%)
T1. S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (3w)	90.6	8.9
T2. S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (m)	93.3	7.5
T3. S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (m)	83.8	7.2
T4. S/2 d2 6d/7 ET 2.5% Pa (2/y), S/4U d2 6d/7 ET 5%. La (6w)	72.1	6.9
CD (P = 0.05)	5.31	NS

Shallow tapping – an option to stress alleviation in *Hevea* plantations during winter season in NE region, monthly yield (g $t^{-1}t^{-1}$) and DRC (%) were recorded and presented in Table Tura. 5. Results indicated maximum yield in normal tapping system (46.0 g $t^{-1}t^{-1}$) followed by Normal continuous tapping (42.1 g $t^{-1}t^{-1}$) and LFT/normal tapping (39.3 g $t^{-1}t^{-1}$) and lowest was in shallow/Normal tapping system (37.3 g $t^{-1}t^{-1}$). Low DRC was recorded in normal continuous tapping system (33.1%) while higher in shallow/Normal tapping tapping system (34.0%,). Normal continuous tapping system showed higher TPD (8.9%) followed by the shallow/ normal tapping system (7.2%), LFT/normal tapping (6.5%) and minimum was in normal tapping system (5.6 %).

The experiment on location specific stimulant application on ethylene induced stress responses in the tapping panel of the *Hevea* trees was continued. RRIM 600 clone was selected for the study with 6 treatments. Results showed that maximum annual average yield (56.2 g $t^{-1}t^{-1}$) was recorded under T3 (Bark application of 5% ethephon at 150 cm above from bud union and near the bud union) and lowest was in (T6) control (28.43 g $t^{-1}t^{-1}$), while higher DRC (33.98 %) was recorded in control (T6) and lowest was in T3 (32.9 %).

3. Crop Management

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

The experiment was continued. The highest girth (93.60 cm), girth increment (3.12 cm), yield (58.8 g $t^{-1}t^{-1}$), DRC (35.73 %) and latex volume (168.3 ml $t^{-1}t^{-1}$) were recorded under the treatment combination of $N_{60}P_{30}K_{45}$ kg ha^{-1} and the minimum was recorded in $N_0P_0K_0$. Application of NPK fertilizers, significantly increased the OC content (0.79 to 1.45 %), available phosphorus (7.0 to 12.5 mg kg^{-1}) and potassium (81.0 to 119.0 mg kg^{-1}) in treatment ($N_{60}P_{30}K_{45}$).

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

Soil samples were collected during each month at a depth of 0-15 cm, 15-30 cm and 30-60 cm for soil moisture study. The trend was same as that in 2011-12. Annual mean of soil moisture were between field capacity and permanent wilting point *viz.* 22.48, 23.23 and 24.05% at the depth of 0-15, 15-30 and 30-60 cm, respectively.

3.3. Analytical/ Advisory work for fertilizer recommendation

42 soil samples were collected from the rubber growing areas and analyzed. Results indicated that in the surface soil (0-30 cm) OC content was in the medium range (0.92-1.32 %), available phosphorus was in the low range (0.8 -4.1 mg kg^{-1}) and available potassium was in the medium range (67-85 mg kg^{-1}). The soil is acidic in nature with pH ranging from 4.6- 5.4 and fertilizer recommendation were given to the growers.

REGIONAL EXPERIMENT STATION, NAGRAKATA, WEST BENGAL

1. Crop Improvement

1.1. Evaluation of clone

Experiments on multidisciplinary clone evaluation was initiated in 1990, 1991 and 1993 in the non-traditional area of sub-Himalayan West Bengal in order to identify promising clones suitable for the area. In terms of girth, RRIM 612, RRII 118, SCATC 93/114, RRIM 703 and Haiken were found better than RRII 105 in trial I and II. In terms of girth while SCATC 93/114 performed significantly better than RRIM 600 in trial III, none were superior in trial II.

The mean yield (Table Nag. 1) of clones RRIM 703, SCATC 93/114, SCATC 88/13, RRII 300, PB 311,GT 1, PB 235, RRII 208, RRII 118, PB 5/51 RRII 203, PR 107,RRIM 605, PB 86, HK 1 and RRIM 612 were superior to the check clone RRII 105 in trial I and II. In trial III such as PB 235, PB 310, RRII 208 and HK 1 were found superior to check clone RRIM 600, trial IV clones HK 1, PB 280, PR 261, RRII 308 and RRII 208 clones showed superior yield compared to RRIM 600.

1.2. Evaluation of germplasm

Evaluation of germplasm in hot-spot areas like Nagrakata, West Bengal was initiated in 1998 with 21 germplasms lines. Among the wild accessions, maximum girth was found in RO 3172 followed by RO 2890, RO 5348, RO 2635 and RO 5557. Girth of fourteen clones was superior to the check clone RRII 105. In terms of yield, RO 5363 was comparable to clone RRII 105. In general,

Table Nag. 1. **Pattern of yield ($g\ t^{-1}\ t^{-1}$) 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 ($g\ t^{-1}\ t^{-1}$)
GL1	22.04	HK1	42.45	HK1	59.51*
GT1	44.74	PB235	59.38**	PB235	49.10
HK1	33.60	PB260	36.93	PB280	53.78**
PB235	42.21	PB310	43.75	PR261	50.76
PB311	46.07*	PB86	34.65	RRIC104	34.84
PB5/51	38.97	PR107	31.48	RRII105	49.73
PB86	34.11	RRIC102	36.60	RRII208	50.21
PR107	36.82	RRII208	43.33	RRII300	47.85
RRII118	41.10	RRIM600	39.38	RRII308	50.41
RRII203	38.73	RRIM612	30.26	RRIM600	47.71
RRII208	41.21	SCATC93/114	33.15	SCATC93/114	34.16
RRII300	48.19**	Collective mean	39.21	Collective mean	48.19
RRIM605	34.89				
RRIM612	32.23				
RRIM703	49.24**				
SCATC88/13	48.80**				
SCATC93/114	48.82**				
RRII105	29.80				
Collective mean	39.53				
CD (Pd"0.05)	12.49	CD (Pd"0.05)	7.61	CD (Pd"0.05)	11.19

**Significant at 1% level; * Significant at 5% level.

the performance of Rondonia accessions were better compared to the Acre and MatoGrosso accessions.

1.3. Performance of polyclonal seedlings

Mean girth of the Polycross seeds, (collected from seed garden of Kanyakumari, Tamilnadu) at twenty second year was 69.00 cm and the average block yield was 35.78 g t^{-1} t^{-1} where 36% plants showed above average yield. Selected ortets were maintained at the nursery for further evaluation.

1.4. Multi trait screening of half sib progenies for cold tolerance and yield attributes

Seeds were collected from open pollinated fruits from seven clones and seedlings were raised in polybag. Significant difference in seed weight was observed, RRII 429 showed highest seed weight among the clone (Table Nag. 2). Shoot die back and cold injury was seen in all the clones in winter season.

Table Nag. 2. **Seed weight**

Mother Clone	Average Wt. (gm.)	Germination (%)
RRII 208	219.13 *	93
RRII 417	192.87	90
RRII 422	236.33 **	84
RRII 429	336.53 **	95
SCATC 88/13	206.53 *	72
RO 5363	196.47	83
RRIM 600	195.60	97
CD at 5 %	07.45	

1.5. On farm evaluation of promising clones

In an experiment to study the performance of promising clones, mean of shoot length and number of leaves was highest in RRIM 600 (Check Clone) followed by SCATC 88/13. However, highest survival rate was recorded in SCATC 88/13 followed by RRII 422 (Table Nag. 3).

Table Nag. 3. **On farm evaluation**

Clone	Shoot length (cm)	No. of leaves	Survival rate in polybags %
RRII 208	29.57	10.96	65.75
RRII 417	25.13	10.65	56.25
RRII 422	22.00	8.52	78.52
RRII 429	27.07	12.08	67.60
SCATC 88/13	30.64	12.27	91.46
RRIM 605	23.18	11.78	58.66
RRIM 600	34.01	12.53	67.60

1.6. Intraclonal Variability Studies

In the experiment initiated in 2011 to study the tree to tree variability in yield and yield related component (Volume, DRC and yield) in three clones, highest variation in volume was observed in RRII 300, while DRC % in RRII 105. However, RRIM 600 showed minimum variation in terms of yield and yield related components.

2. Crop Management

2.1. Nutritional trial

The experiment on NPK in three factorial design was continued. The girth data during this year did not show any regular pattern in different treatments. Also no significant difference in yield noted among the treatments.

2.2. Inter-planting trial

The inter-planting trial on rubber in tea was continued in the Dooars area of West Bengal. Green tea leaf yield in inter-planted plots, Rubber + Tea, 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. Rubber yield in pure plot was better than the inter-planted plots due to more plant stand.

3. Crop Physiology

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

Comparative study on the performance of the seedling plants raised from polycross local seeds with that from the traditional environment, the girth at 50 cm height of all the plants was similar in this year. Superiority of Kanyakumari seedling over the growth of seedlings of other three places, which may be due to early plant establishment, was still maintained during the reporting period. The first year juvenile yield of the seedling plants showed that the yield efficiency of all the plants was similar although the yield efficiency of Tura and Kanyakumari was more than the other two.

3.2 Physiological Evaluation of Rubber Clones in Abandoned Tea Growing Areas of Dooars Belt of North Bengal

Plants of all clones were established in the field. Girth was also similar in all the clones. However, the girth of RRII 422 was significantly higher in high pH soil than that of the control plot. Girth in high pH soil was found to be better than that of the normal pH soil. The chlorophyll content index (CCI) was high in RRII 429 while cultivated under high pH soil Table Nag. 4).

Effective quantum in yield (ϕPSII) was almost similar in both soil types. The maximum potential quantum yield of PSII (Fv/Fm) no variation between clones. In normal soil, it was higher than the high pH soil. Therefore, there was not much difference in the performance of the clones in normal and high pH soil at this stage.

3.3. Evaluation of Ortets for abiotic stress tolerance in different agro-climatic regions

Budgrafted plants of ortet selections from Nagrakata, Tura, Agartala, Guwahati (cold) and Daphchari (drought) were field planted in August 2012 under the agroclimate of sub-Himalayan West Bengal. The girth data of GH 1 and GH 3 was significantly higher than the check clone RRIM 600 after 8 months of field planting; however, girth increment over months showed no significant difference during the reporting period (Table Nag. 5). During the winter season some plants dried/died due to low temperature. As a result of winter shock casualties occurred in Ortet trials at RES, Nagrakata.

The Chlorophyll content index of NGK 69 and RRII 429 was significantly higher than RRIM 600 during pre-winter period. The maximum potential quantum yield of PSII (Fv/Fm) in 23 genotypes (under dark) showed that the reduction in Fv/Fm from

Table Nag. 4. **Growth pattern of different clones growing in high and normal pH soil**

Clones	Girth of plants after 12 months of planting (cm)		% Improvement over control	Chlorophyll content Index (December'12)	
	High pH soil	Normal oil		High pH soil	Normal soil
RRII 208	24.66	18.94	+ 5.72	46.39	43.99
RRII 417	19.90	18.50	+ 1.40	42.21	54.34
RRII 422	19.02	10.18	+ 8.84	36.14	40.01
RRII 429	21.80	20.20	+ 1.60	46.80	60.16
RRIM 605	18.75	14.03	+ 4.72	40.41	47.76
RRIM 600	19.43	16.58	+ 2.86	54.19	54.52
CD (Pd"0.05)	6.30	6.07	7.48	10.14	12.23
Population average	20.59	16.41	+ 4.19	44.36	50.13

Table Nag. 5. **Pattern of growth and effect of cold stress in ortets / clones**

Ortet/ Clone	Girth (cm)	GI (cm/month)	No of casualties out of 21 plants
DAP 1	4.52	0.13	0
DAP 34	4.60	0.14	3
DAP 35	4.49	0.28	2
DAP 36	3.60	0.37	2
GH 1	6.15*	0.02	0
GH 3	6.37**	0.04	0
GH 9	5.10	0.27	0
NGK 1	5.02	0.24	4
NGK 47	5.14	0.39	1
NGK 69	5.79	0.15	1
RRII 105	4.82	0.39	1
RRII 414	5.82	0.22	0
RRII 417	5.52	0.31	4
RRII 422	5.59	0.32	8
RRII 429	5.16	0.33	1
RRII 430	5.34	0.40	2
RRIM 600	5.62	0.33	0
RRSA 315	5.01	0.39	3
RRSA 585	5.20	0.39	0
RRSA 98	5.77	0.41	12
RRST 24	4.93	0.51	2
RRST 37	4.86	0.62	0
RRST 39	3.88	0.72	2
CD (≤0.05)	0.42	NS	

post-monsoon period to pre-winter period was maximum in RRII 429 and lowest in DAP 1 and RRST 39. Similarly, effective quantum yield (ϕPSII) in 23 genotypes (under light) maximum reduction in clone RRIM 600 and minimum was in DAP 1.

3.4. Effect of stimulant application away from tapping panel

In order to understand the effect of stimulant applied away from tapping cut, six different treatments were imposed along with unstimulated trees as control. The experiment was undertaken in BO2 (virgin) panel (5th year) of RRIM 600 in S2d3 system of tapping in blocks of forty plants each. The 2nd year yield data on kg/tree/yr and kg/ha showed that yield in C and F were significantly higher than that of the unstimulated (E) trees. The data on % of plants showing above 75% TPD ranged from 2.5 to 7.5% only (Table Nag. 6).

3.6. Water mining of rubber trees in West Bengal: Sap flow System

To measure the quantum of water mining through transpiration by rubber tree, sap flow measurement system was installed. The measurement was made on 12 year old trees of two clones, RRII 429 and RRII 417. The mean water consumption was 20.0 litre $tree^{-1}day^{-1}$. Maximum rate of sap flow per day was observed during November followed by December and the minimum in the month of February coinciding with complete defoliation of the canopy.

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

System ofstimulation application	Yield (Kg/tree/ year)	Yield (Kg/ha–400 plants)	% of plants showing above 75% TP
A. Bark application of 5% Ethephon above 125cm from the bud union	3.67	1470	0
B. Bark application of 5% Ethephon on the bud union	4.15	1660	0
C. Bark application of 5% Ethephon at both A and B positions	4.36	1746	2.5
D. Bark application of base material (diluents oil) at both A and B positions	3.76	1506	0
E. Unstimulated trees.	3.84	1538	0
F. Panel application of 2.5% Ethephon (standard practice).	4.50	1800	7.5
CD (≤0.05)	0.43	172	

)plants) above 75% TP

REGIONAL REESEARCH STATION DAPCHARI, MAHARASHTRA

The mandates of this station are to develop suitable clones and location specific agro technology for prevailing 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, studies on identification of reliable juvenile and mature characteristics for clones to file a DUS norms), environmental physiology (irrigation requirement and irrigation methods, drought studies, physiological evaluation of selected ortets from various agroclimates of India), Latex Harvest Technology and suitable crop management practices (to mitigate the drought and soil moisture conservation) are being carried out.

1. Environmental Physiology

1.1. Drip and Basin method of irrigation

For the identification of suitable drought and high temperature tolerant clones, three experiments namely, (i) physiological adaptation of cold and drought tolerant ortets selected from varying agroclimatic regions of India, (ii) irrigation scheduling / response of methods of irrigation and (iii) cost benefit analysis of irrigated and rainfed grown rubber plants was being conducted in this station. Accounting the water mining rates of mature rubber plantation of clone RRIM 600 is also started recently.

The irrigation experiment started during 1987 with ETc based irrigation scheduling in two methods of irrigation (basin and drip) was continued. A significantly higher girth was registered in basin irrigation at 1.0 ETc (79.67 cm) level but which is on par with other two levels of same irrigation method, 0.25 and 0.50 ETc (75.13 cm, 74.92 cm), respectively.Trees under different levels of basin irrigation (1.0, 0.25, 0.50 ETc) showed higher girth (79.67, 74.92 cm) as compared to drip system and control (65.03 cm) .

The results indicated higher dry rubber yield (44.50 g $t^{-1}t^{-1}$) with basin irrigation scheduling at 0.50 ETc level as compared to control (27.0 g $t^{-1}t^{-1}$). Reduced irrigation (0.25 ETc) in basin and drip recorded rubber yield of 42.4 and 34.4, 37.1 g $t^{-1}t^{-1}$ respectively.

1.2. Cost evaluation trial

The cost evaluation trial started during 1987 to account the expenses incurred towards various inputs, farm practices and irrigation was also continued. The results indicated better growth and summer as well annual yield of trees under reduced level of irrigation ($1/5^{th}$ ETc). In the deep soil area it was 21.39 g $t^{-1}t^{-1}$ compared to the higher level of irrigation 1.0 ETc, (16.90 g $t^{-1}t^{-1}$).

1.3. Evaluation of environmental stress tolerance and physiological adaptations of cold and drought tolerant ortet selections from varying agroclimatic regions of India

In order to evaluate the physiological and biochemical basis of adaptation potential and to find out various mechanisms involved in cold and drought tolerant traits in related ortets from extreme climatic zones (both cold and drought) by interchanging the clones to different agro-climatic regions and to study the G x E interaction for growth and yield

under varying agro-climates. A significant difference in girth, height and no. of leaf whorls were recorded in young field planting. The lowest girth was recorded in Dap 36 (2.2 cm) which is less than RRII 105 (2.5cm). while the highest girth was recorded in RRII 422 and RRII 430 (4.6 cm) followed by RRII 417, RRII 429 (4.2 cm) and ortet RRSA 98 (3.9 cm). The lowest height was registered in Dap 36 (71.1 cm) while RRII 414 the highest height of 159.6 followed by RRII 430 (146.7 cm) and RRSA 98 (144.3 cm). Lowest no. of leaf whorl was recorded in Dap 34 (1.3). The clone RRII 414 and RRSA 98 registered the highest number of whorls (2.8) followed by NGK 69 (2.7), Dap. 1 and GH 1 (2.5). No foliar drying was seen in Dap.1. RRII 105 (3.8%) recorded the highest drying followed by NGK 1, GH 3 (3.1%), RRII 422 (2.9 %), NGK 699 (2.8 %). Among the ortets RRSA stands first rank for all growth parameters while the lowest rank registered by RRST (Table Dap. 1).

Table Dap. 1. **Growth parameters and visual scoring of % drying in various ortets selected from various location of India**

Clones	Girth (cm)	Height (cm)	No. of whorl	% Drying
RRII 105	2.5	70.8	1.4	3.8
RRIM 600	2.7	100.1	2.1	0.5
RRII 414	4.4	159.6	2.8	2.7
RRII 417	4.2	128.7	1.7	0.2
RRII 422	4.6	135.3	2.4	2.9
RRII 429	4.2	133.9	1.9	1.2
RRII 430	4.6	146.7	2.0	2.1
DAP 1	3.3	116.5	2.5	0
DAP 34	2.9	86.0	1.3	0.8
DAP 35	3.5	105.0	1.9	4.0
DAP 36	2.2	71.1	1.5	1.5
RRSA 98	3.9	144.3	2.8	4.0
RRSA 315	3.7	127.2	2.1	1.4
RRSA 585	3.4	119.0	2.0	1.0
NGK 1	2.7	141.0	1.8	3.1
NGK 47	3.1	122.9	2.2	2.5
NGK 69	3.2	128.1	2.7	2.8
GH 1	3.2	127.2	2.5	1.2
GH 3	3.6	125.8	2.4	3.1
GH 9	2.9	111.1	1.6	0
RRST 24	2.4	82.8	1.5	0
RRST 39	2.8	96.5	1.6	2.5
RRST 37	2.5	101.5	1.9	2.2
Dap	2.98	95.65	1.8	1.58
RRSA	3.67	130.97	2.3	2.13
NGK	3.0	130.67	2.23	2.8
GH	3.23	121.37	2.17	1.43
RRST	2.57	93.6	1.67	1.57
SE.d	0.35	13.91	0.37	2.03
CD (P = 0.05)	0.71	28.03	0.76	NS

2. Latex Harvest technology

The study on controlled upward tapping initiated during 2009 with objective to identify best CUT practice suitable to this region yield for clone RRII 105 was continued.

The treatment S/3 U d3 6d/7 ET 5% La with application of ethephon once in three weeks recorded higher yield of 42.24 g t^{-1} t^{-1} (1132.20 kg ha^{-1}) than the other treatments. Lower yield was recorded under S/4U d3 system with stimulation once in a month (30.30 g t^{-1} t^{-1} 812.20 kg ha^{-1}). Results revealed that S/3U d3 tapping system with application of ethephon once in three weeks proved promising. In general, CUT recorded high yield in all the treatments than the normal basal panel tapping.

3. Crop Improvement

Development of drought tolerant clones, screening of wild *Hevea* accessions for drought tolerance and future evaluations from modern clones, ortets selection, clones from half sib progeny of prepotent clones along with newly released 400 series, study on unique characteristics of each clones to

file DUS norms were continued to be the thrust areas. A total of 10 experiments are being conducted to screen wild Hevea accessions and to evaluate the growth and yield performance of clones, pipe line clones, wild *Hevea* accessions, progeny evaluation from half sib clones and polycross seedlings, selections from half sib clones from prepotent clones and selected ortets for further evaluation and experiment on identification of reliable juvenile and mature characteristics for clone identification (50 divergent clones) in *Hevea*: standardization of distinctiveness, uniformity and stability (DUS) testing norms for evolving specific guidelines for varietals registration.

3.1. Ortet selection

The large scale evaluation of 14 ortets of Dapchari along with 4 control clones (RRII 105, RRII 208, RRII 430 and RRIM 600) was started during 2008 to evaluate the growth and yield performance. Among the ortets, significantly higher girth was found in OS 37 (19.11 cm) than clone RRII 105 (14.74 cm) while OS 42 (13.23 cm) had lowest girth. Highest first branching height was recorded in OS 111 (329.3 cm) while it was 315.7 cm in clone RRII 208 recorded highest girth of 17.62 cm. OS 216 registered lowest per cent of yellowing (3.89 %) while OS 136 registered (13.84%). Among the check clones RRII 430 registered 9.05% of yellowing followed by RRII 105 (9.04 %) while RRII 208 (4.39 %) registered 4.4%.

3.2. Germplasm screening

A Screening for wild Hevea accession for drought under Dapchari condition was laid out in July 2003 using one hundred thirty wild Hevea accessions along with three selected clones viz., RRII 105, RRIM 600, Tjir 1 as a check control in Augmented block design for screening against drought tolerance. The observation on pre and post drought growth and RWC % were recorded. The Accessions showed a wide variability for all characters studied. In general, Mato Grosso accessions were superior for all the growth characters studied than those from Randonia and Acre provenances. Among the control clones RRIM 600 and RRII 208 was superior to RRII 105. 25 potential drought tolerant accessions were identified based on 3-4 years field performance for further detailed studies are in progress.

3.3. Further evaluation of selected wild Hevea accessions

A total of three experiments comprising of 25, 47 and 11 selections are being evaluated and is at initial stage.

A field evaluation of selected Hevea clones for drought tolerance at RRS, Dapchari was laid out in July 2007 using 25 selected drought tolerant clones of wild Hevea accession for drought under Dapchari condition along with five HP clones and RRII 105, RRIM 600, TJIR 1, RRII 430, RRII 208 as a check clone in Rectangular Lattice Design. The observation on growth was recorded. The Accessions showed a wide variability for all characters studied.

A clonal nursery experiment with clones selected from half sib progeny of prepotent clone was initiated in 2010 with objective to evaluate the clones in a clonal nursery and advance the potential ones showing drought tolerance along with rubber yield to LST and PCE to reduce the breeding cycle trial is in initial stage. A one small scale field evaluation trial of selected wild Accessions (11 no) for drought tolerance further field evaluation of selected wild accessions are planted in 2010 with objective to evaluate juvenile and mature performance under drought condition is in initial stage.

3.4. Clonal nursery evaluation

Evaluation of half sib progeny of 15 clones in nursery was started in 2011 for the early selection based on dependable juvenile traits under rainfed condition.

Observation on growth parameters were recorded in the plot of clonal nursery evaluation of Pipe line clones (50) for drought tolerance.

In connection with the experiment on identification of reliable juvenile and mature characteristics for clone identification in *Hevea* to develop DUS testing norms for evolving specific guidelines for varietal registration in rubber (laid out in 2011) with 50 divergent clones, morphological data on individual plants in the juvenile stage covering 40 traits were recorded from all three locations. Data was also recorded for a set of traits from mature plants of the popular RRII clones (RRII 5, RRII 105, RRII 118, RRII 203, RRII 208 and RRII 400 series).Data was recorded on wintering pattern in a set of 25 clones at RRII for the third year, and the data are being analysed. Quantitative data on girth and height have also been recorded.

4. Crop Management

The experiment initiated in 2008 to study the effect of vertical mulching and Kaoline spray on moisture conservation, growth and yield of rubber continued. There was no significant difference among the treatments in terms of girth and plant height.

REGIONAL RESEARCH STATION, DHENKANAL, ORISSA

The Regional Research Station in Dhenkanal district represents dry sub humid climate. The station continued its research activities on Crop Improvement and management, with the particular objective of identifying clones suited to prevailing drought prone conditions.

Crop improvement

Crop Improvement includes evaluation of clones and of polyclonal population. There are five clone evaluation trials. The trials were laid out to evolve most suitable clones under the dry sub humid climate.

Clone Evaluation

In the 1987 experiment, both GT 1 (79.3 cm) and RRIM 600 (75.5 cm) exhibited significantly higher mean girth over RRII 105 (71.6 cm) while maximum mean yield was found in RRII 105 (43.8 g t^{-1} t^{-1}). Minimum was found in GT 1 (38.45 g t^{-1} t^{-1}).

In the 1990 clone trial, SCATC 93/114 (88.5 cm) followed by RRII 208 (86.5 cm) showed superior girth. SCATC 88/13 exhibited highest yield (66.7 g t^{-1} t^{-1}) while RRIM 600 yielded 50.8 g t^{-1} t^{-1}. SCATC 93/114 was the lowest yielder (31.5 g t^{-1} t^{-1}).

In another experiment (1991), the performance of *Hevea* clones were compared with polyclonal seedlings. Polyclonal seedlings exhibited better growth and adaptability as compared to other clones. Clones such as RRII 208 (72.7 g t^{-1} t^{-1}) and PR 255 (84.3 g t^{-1} t^{-1}) recorded higher mean yield. In this trial also, RRII 208 exhibited higher growth, yield and adaptability in this region. PR 261 and polyclonal seedlings showed comparatively lower yield (Table OR.1).

Table OR 1. **Performance of different clones**

Clone	Girth (cm) Mar'13	Mean yield ($g\ t^{-1}\ t^{-1}$)
RRII 5	82.7	58.8
RRII 105	80.6	67.7
RRII 208	87.9	72.7
RRII 300	85.8	62.4
RRIC 102	84.2	74.9
RRIM 600	78.0	63.8
GT1	92.4	61.4
PR 255	86.4	70.6
PR 261	80.0	52.0
Polyclonal	104.3	45.4
SE(m)	3.03	8.46
CD(P=0.05)	9.09	N/A

In the 1999-00 clone trial, highest mean girth was recorded in RRII 300 (60.9 cm) and IRCA 111 (55.4 cm). The lowest girth was recorded in IRCA 109 (45.1 cm). Maximum mean yield was observed in IRCA 109 (45.0 $g\ t^{-1}\ t^{-1}$) followed by RRII 105 (43.5 $g\ t^{-1}\ t^{-1}$) and RRII 352 (40.7 $g\ t^{-1}\ t^{-1}$) while lowest mean yield was found in RRII 51 (30.2 $g\ t^{-1}\ t^{-1}$).

Polyclonal Trial

In a trial (1989), conducted to evaluate growth and yield performance and adaptability of polyclonal seedlings in Orissa conditions, maximum annual mean yield was recorded in O2 (94.6 $g\ t^{-1}\ t^{-1}$) followed by O5 (89.8 $g\ t^{-1}\ t^{-1}$) . Ten elite polyclonal trees were selected and are under field evaluation.

Ortets Evaluation

Among the ten ortets planted along with few modern clones planted for further evaluation (2008), OR 4 (28.6 cm), OR 8 (28.3 cm) and OR 7 (27.2 cm) exhibited superior girth while clone RRII 208 attained 24.0 cm girth.

Latex Harvest Technology

Controlled upward tapping trial

Preliminary observations indicated that clone RRIM 600 under treatment T3 (S/3 U d2 ET 5% (m), S/2 d2 ET 2.5% (2 y) showed maximum mean annual yield of 86.0 $g\ t^{-1}\ t^{-1}$ and the yield trend was same as that of previous year.

REGIONAL RESEARCH STATION, PADIYOOR, KANNUR

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

1. Crop Management

1.2. Water requirement studies

Observations 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. Significant difference in girth increment was observed among the different treatments. Summer yield differences were not significant (Table Pad. 1).

Table Pad.1. **Effect of irrigation on growth and yield**

Treatment (IW/CPE)	Girth (cm)		Girth increment	Summer yield
	12^{th} yr	13^{th} yr	13^{th} yr (cm)	$g\ t^{-1}t^{-1}$
1.2	62.6	62.8	0.2	26.5
0.9	60.2	61.6	0.3	26.4
0.6	65.2	65.9	0.5	31.2
0.3	60.9	61.6	0.7	29.5
Control	59.9	61.4	1.5	29.9
CD (P = 0.05)	NS	NS	0.6	NS

Table Pad. 2. **Effect of applied fertilizer on growth (11th year) of rubber**

Treatment	Girth (cm)		
	RRII 105	RRII 429	RRII 414
30:30:20	52.9	48.3	54.7
60:30:20	47.2	48.9	55.5
90:60:40	45.2	48.1	55.4
120:60:40	54.1	49.3	53.9
Mean CD (P = 0.05)	5.7		

1.3. Response to applied fertilizers in high yielding clones

The experiment was initiated in June 2002 using budded stumps as the planting material. The treatments comprised of 3 clones (RRII 105, RRII 414 and 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. The experiment was laid out in RBD with 3 replications. The different levels of applied fertilizer did not significantly influence growth of any of the clones studied though a significant clonal difference in growth was evident (Table Pad.2).

1.3. Water consumption in rubber nurseries

Polyhouse raised root trainer plants utilized a total of 75.3 L per plant through irrigation for a total duration of 312 days from seeding to two-whorl stage while the quantity utilized for a polyhouse grown polybag plant for the same stage of growth was 45 L per plant for 290 days. Polybag plants raised in the open conditions utilized 46.6 L of water through irrigation for a total growing period of 289 days from seeding to two-whorl stage.

2. Crop Improvement

2.1. Large scale evaluation of clones

The trial planted in 1996 with 11 clones indicated the significant superiority in girth and girth increment of IRCA 130 followed by PB 330. IRCA 130 also exhibited significantly high annual and summer yield followed by PB 255. IRCA 130 followed by PB 255 were significantly superior for branching height and bole volume also (Table Pad. 3).

Table Pad. 3. **Growth performance of modern *Hevea* clones**

Clones	Girth (cm)	Annual yield ($g\ t^{-1}t^{-1}$)	Summer yield ($g\ t^{-1}t^{-1}$)
PB 255	63.5	58.7	38.7
PB 314	63.9	45.5	27.3
PB 330	69.8	40.4	22.7
PB 28/59	63.3	48.4	30.2
RRIM 703	56.9	35.4	20.3
IRCA 18	68.1	39.8	19.1
IRCA 109	60.9	36.2	24.9
IRCA 111	61.5	36.1	23.7
IRCA 130	69.3	67.7	49.8
IRCA 230	64.3	40.8	22.9
RRII 105	63.3	48.4	29.2
CD	4.7	12.4	10.2

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

The trial planted in 1996 under high altitude conditions (974 m MSL) with 10 selections and 5 clones indicated the superiority of the clones RRII 203 and RRIC 100. The selections P 270, P 213 and Iritty were also superior to that of RRII 105 with respect to girth and yield. Mean annual yield of RRII 203 was found to be on par with that of PB 86, RRIC 100, P 270 and P 213 and significantly superior to RRII 105 (Table Pad. 4).

Table Pad. 4. **Growth and yield in high altitude area**

Clones	Girth (cm)	Summer yield ($g\ t^{-1}t^{-1}$)	Annual yield ($g\ t^{-1}t^{-1}$)
RRII 105	48.5	21.9	29.7
RRII 203	67.7	44.9	54.3
RRIC 100	65.5	43.2	48.1
RRIC 102	51.8	14.8	20.7
PB 86	56.6	28.4	39.4
P1	63.7	27.1	31.9
P 2	52.3	19.5	24.4
P90	54.4	20.9	27.7
P121	51.4	18.9	22.9
P155	47.9	20.6	24.9
P213	62.9	32.8	42.3
P270	65.6	38.6	46.8
P280	58.9	21.4	25.4
P296	59.4	23.4	26.6
Iritty	63.2	31.5	43.3
CD (P = 0.05)	12.0	15.5	22.8

HEVEA BREEDING SUB STATION, KADABA, KARNATAKA

The major thrust areas of research in the station are to evaluate clones under different biotic and abiotic stress factors and to identify clones suitable for commercial cultivation. Brief account of the progress of research projects during the financial year, April 2012 to March 2013 are detailed below:

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

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

In the trial 1988A, a total of 15 ortet clones and three control clones are under evaluation. Mean yield over ten years of tapping indicated T2 to be the highest yielder with 70.0 g $t^{-1}t^{-1}$ closely followed by the ortets namely O 17 (65.0 g $t^{-1}t^{-1}$), O 15 (60.2 g $t^{-1}t^{-1}$). Yield of control clones GT 1, RRII 105 and RRIM 600 was 61.9, 43.5 and 42.5 g $t^{-1}t^{-1}$ respectively. Regarding the growth, ortet O 47 recorded highest girth (124.8 cm) followed by T 2 (113.7 cm) and O 17 (110.2 cm). Though O 47 (121.3 cm) exhibited highest girth, its yield was only 50.1 g $t^{-1}t^{-1}$.

Sixteen ortet clones and three control clones *viz.* RRII 105, RRIM 600 and GT 1 were under evaluation in the trial 1988 B. Details on growth and yield performance of the clones are given in Table Net. 1. After ten years of tapping high yielding clones were T1 (78.2 g $t^{-1}t^{-1}$) followed by GT 1 (70.8 g $t^{-1}t^{-1}$).

Table Net. 1. **Performance in growth and yield in the small scale ortet trial (1988 BC) at HBSS, Kadaba**

1988 B Clone	Girth (cm) at age 25 years (Dec.2012)	Mean yield overten years of tapping (g t-1t-1)	1988 C Clone	Girth (cm) at age 25 years (Dec., 2012)	Mean yield over ten years of tapping (g t-1t-1)
T 1	114.2	78.2	GT 1	93.5	82.8
GT 1	92.3	70.8	O 55	124.6	79.3
O 53	89.5	50.8	C 140	99.9	78.0
O 40	85.6	47.7	O 49	80.6	69.5
RRII 105	72.7	47.0	RRII 105	83.0	65.0
C 150	80.6	43.6	O 11	102.7	63.7
O 38	87.4	43.4	O 26	106.9	62.2
O 54	85.2	43.0	O 56	92.1	59.5
O 16	73.9	42.0	O 30	84.4	56.5
RRIM 600	76.2	40.3	O 39 A	90.0	49.6
PO	78.6	37.6	C 10/9	91.4	46.7
O 46	85.2	34.1	C 6	62.6	45.4
O 14	74.0	31.7	O 51	82.7	39.5
O 37	80.7	30.0	C 32	82.5	35.9
C 151	70.2	28.4	RRIM 600	73.3	31.3
O 23	66.1	28.1			
O 22	72.3	24.4			
O 57	67.2	20.6			
C 9	65.5	17.8			
CV (%)	11.2	28.3	CV (%)	15.7	20.1
CD (P<0.05)	14.8	18.7	CD (P<0.05)	23.6	19.4

The third trial (1988 C) consists of 14 ortet clones and three control clones namely RRII 105, RRIM 600 and GT 1 (Table Net.1). After ten years of tapping, GT 1 recorded maximum yield (82.8 g $t^{-1}t^{-1}$) followed by ortet O 55 (79.3 g $t^{-1}t^{-1}$), C 140 (78.0 g $t^{-1}t^{-1}$) and RRII 105 (65.0 g $t^{-1}t^{-1}$).

2. Large scale clone trial 1989

In this trial 14 clones under evaluation. After ten years of tapping, maximum yield was found in clone RRII 203 (66.0 g $t^{-1}t^{-1}$) followed by KRS 25 (55.5 g $t^{-1}t^{-1}$). Check clones RRII 105 and RRRIM 600 yielded 46.0 and 34.4 g $t^{-1}t^{-1}$ respectively (Table Net. 2).

Table Net. 2. **Performance in growth of clones over 24 years and yield over ten years of tapping (2002-2012) in the large scale clone trial (1989) at HBSS, Kadaba**

Clone	Girth (cm) atage 24 years (Dec 2012)	Mean yield overten years of tapping (g $t^{-1}t^{-1}$)
RRII 203	97.5	66.0
KRS 25	90.7	55.5
PB 255	80.5	54.6
KRS 128	83.1	48.8
KRS 163	81.8	47.9
RRII 308	86.5	46.7
RRII 105	75.9	46.0
SCATC 88/13	68.2	40.2
PR 255	67.8	38.6
HAIKEN 1	63.2	36.7
RRIM 600	76.6	34.4
PR 261	73.0	32.4
RRII 300	80.5	30.9
SCATC 93/114	71.0	16.1
CV (%)	5.5	20.0
CD (P<0.05)	7.3	14.3

3. Large scale clone trial 1990 A

In this experiment 1990, 15 clones are under evaluation which have completed seven years of tapping. Maximum yield was recorded for clone PB 235 (65.3 g $t^{-1}t^{-1}$) closely followed by PB 260 (65.2 g $t^{-1}t^{-1}$) and HP 372 (63.4 g $t^{-1}t^{-1}$). Tjir 1 was the lowest yielder (23.5 g $t^{-1}t^{-1}$).

4. Estimation of genetic parameters 1990B

This trial was started with an aim to evaluate 12 parent clones and their progenies for estimating genetic parameters. Details on

Table Net. 3. **Growth and yield performance of clones and their progeny in the trial on estimation of genetic parameters (1990B) at HBSS, Kadaba over nine years of tapping (2003-2012)**

Clone	Girth (cm) atage 23 years (Dec., 2012)	Mean yield over nine years of tapping (g $t^{-1}t^{-1}$)
Parents		
PB 235	98.9	78.2
RRII 203	93.6	57.4
RRII 105	75.6	49.3
GT 1	82.9	45.2
PB 5/51	76.7	40.8
PB 213	74.3	38.5
PB 242	77.9	38.3
PB 252	85.5	38.2
RRIM 600	63.8	33.7
IAN 45/873	61.4	30.4
PB 86	64.5	28.1
Tjir 1	73.2	27.2
CV (%)	4.9	8.6
CD (P<0.05)	6.3	6.1
Progeny		
RRII 203-HS3	110.1	46.6
GT 1-HS2	111.0	45.4
PB 235-HS10	119.2	43.9
PB 242-HS7	111.1	38.6
PB 5/51-HS11	106.3	37.4
RRIM 600-HS1	96.3	36.3
RRII 105-HS8	101.7	33.2
IAN 45/873-H	105.5	33.0
PB 252-HS4	101.8	32.7
PB 86-HS5	93.8	30.9
PB 213-HS12	117.9	30.0
Tjir 1-HS9	99.0	26.2=
CV (%)	7.4	16.7
CD (P = 0.05)	13.2	10.2

growth and yield performance of the clones are provided in Table Net. 3.

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. Growth and yield performance continued same pattern of previous years.

6. Large scale clone trial 2000

The trial has 8 clones under evaluation and has completed 12 years of growth and four years of tapping. Maximum yield was noted for clone RRII 414 (72.7 g $t^{-1}t^{-1}$) followed by RRII 430 (65.3 g $t^{-1}t^{-1}$) and RRII 422 (64.2 g $t^{-1}t^{-1}$). Parent clones RRII 105 and RRIC 100 respectively yielded 42.2 and 42.7 g $t^{-1}t^{-1}$ respectively.

HEVEA BREEDING SUBSTATION, THADIKARANKONAM, KANYAKUMARI

The research activities pursued at the station during 2012-13 could be categorized under two projects, namely crop improvement and improvement of plant propagation in *Hevea*. The former project consists four sub-projects viz. clone evaluation, hybridization and clonal selection, new generation polyclonal seed garden and evaluation of new pipeline clones under the mega project Participated Clone Evaluation.

1. Crop improvement

1.1. Clone evaluation

Field performance of 30 high yielding indigenous and exotic clones is being evaluated under nine large scale clone evaluation experiments. In the large-scale clone evaluation experiment (1994), IRCA 130, RRIM 703 and PB 314 gave crop above 130 g $t^{-1}t^{-1}$ (Table Par. 1). However, the pooled data for 12 years of tapping revealed PB 255 (88.15 g $t^{-1}t^{-1}$) as the best yielder exhibiting significant yield difference from the control clone RRII 105 (64.51 g $t^{-1}t^{-1}$). Incidentally high TPD was noticed in IRCA 130, RRIM 703 and PB 314. Out of the five IRCA clones introduced recently from Cote d'Ivoire IRCA 109 (79.86 g $t^{-1}t^{-1}$) presented the maximum yield, followed by IRCA 111 (77.81 g $t^{-1}t^{-1}$) and IRCA 130 (77.47 g $t^{-1}t^{-1}$) up to the 12th year of tapping.

Table Par. 1. **The mean girth and mean yield and the pooled data of 12 years of tapping**

Clone	Mean girth (cm)	Mean yield (g $t^{-1}t^{-1}$) 2012-13	Pooled (12 yrs)	TPD over 12 years
RRII 105	80.86	98.98	64.51	13.33
PB 314	90.94	130.47	80.26	51.96
IRCA 130	85.40	139.22	77.47	39.27
PB 28/59	83.70	65.08	60.13	15.76
IRCA 109	90.14	94.10	79.86	15.38
PB 330	93.62	82.96	63.18	30.17
IRCA 18	87.51	82.82	65.79	20.20
RRIM 703	100.2	137.72	70.82	34.47
IRCA 111	101.6	81.19	77.81	19.47
PB 255	96.14	119.06	88.15	20.55
IRCA 230	96.49	115.66	67.30	7.43
G. mean	91.54	104.29	72.3	33.81
CD ($P = 0.05$)	12.25	26.63	22.07	–

In the 1994 block evaluation, out of the 13 modern clones, RRII 105 (68.96 g t^{-1} t^{-1}) was ranked first with respect to 12 years of tapping, followed by PB 28/59 (63.75 g t^{-1} t^{-1}). In this trial the bark damage due to sun scorch during the initial years of planting exhibited a highly significant positive correlation with the TPD incidence recorded towards the end of tapping in BO-2 (Table Par. 2). However, it requires further studies to establish any positive correlation between sun scorch damage in the initial years and TPD incidence at a mature stage. The yield performance of PB 311 (59.85 g t^{-1} t^{-1}) worth close monitoring as it presented reasonably good yield with the minimum occurrence of TPD (17.98%).

Table Par. 2. **Percentage of sun-scorch damage and TPD incidences after 11 years of tapping**

Clone	Sun-scorch damage at 2nd year of planting	TPD incidence after 11 years of tapping
RRII 5	23.82	25.01
RRII 50	21.16	21.76
RRII 105	28.91	40.97
RRII 176	23.76	29.61
RRIC 102	21.07	20.64
PB 217	9.91*	16.71*
PB 235	53.08*	41.74*
PB 260	32.51	28.83
PB 311	16.77	17.58
PB 28/59	27.04	26.58
PR 255	41.09	29.41
PR 261	14.11	21.44
Mean	26.1	26.69
SE	3.54	2.85

A large scale clone trial at New Ambadi Estate was initiated during 1996 as part of a multi-location trial to analyze the G x E Interaction of selected *Hevea* clones at five diverse agro-climatic conditions. The yield performance of 12 modern *Hevea* clones, including five hybrid clones belonging to the RRII 400 series and their parents are being monitored. In this trial, RRII 203 consistently performed better than RRII 105 till 10th year of tapping, but in the 11th year of tapping, RRII 105 (58.4 g t^{-1} t^{-1}) and RRII 203 (58.07 g t^{-1} t^{-1}) performed equally well. Among the hybrid clones belonging to the RRII 400 series RRII 422 (50.29 g t^{-1} t^{-1}) and RRII 430 (50.01 g t^{-1} t^{-1}) performed better in terms of yield while RRII 414 (44.55 g t^{-1} t^{-1}) was not up to the mark.

In the observational trial initiated at Vaikundam Estate (2000), RRII 422 (61.05 g t^{-1} t^{-1}) was found to be the best yielder and RRII 414 (51.78 g t^{-1} t^{-1}) the least up to the 8th year of tapping. RRII 429 and RRII 414 contracted high TPD of 35.71 and 28.57% respectively. In order to study on the performance of hybrid clones, five large scale clone evaluation experiments representing five diverse agro-climatic conditions in Kanyakumari District were initiated.

1.2. Hybridization and clonal selection

Two breeding orchards consisting 26 clones (1987) and 25 clones (1988) respectively were well maintained and hand pollinations were attempted at different parental combinations. Out of a total of 62 potential high yielders isolated, 10 were included in a large scale clone evaluation experiment initiated during 2008 at RRII, Kottayam and another set of 14 pipeline clones were planted in a small scale trial at Vellambimalai during 2011.

1.3. New generation polyclonal seed garden

A polyclonal seed garden consisting nine modern clones as parents was established in an area of 9 ha of land at New Ambadi Estate, Maniankuzhy during 2000. The seed garden was well maintained and the cultural operations were closely

monitored. The mother plants raised out of poly-cross seeds collected during 2012 were test tapped and the promising high yielders were kept cut back for further evaluation. The seedling trees of poly-cross origin transplanted at Nagamalai (Isfield Estate) are being closely monitored for high yield and promising secondary characters.

1.4. Participatory clone evaluation experiment

Juvenile growth of 11 pipeline clones and three check clones recorded during 2013 at Tharuvaiyar (2008) and Vithura (2008) are presented in Table Par. 3. The clone with code No. 10 exhibited maximum growth both at Tharuvaiyar (33.08 cm) as well as at Vithura (38.53 cm). Two OFTs were initiated at Bethany Estate during 2010 (Phase II). The OFT initiated at Bethany Estate (Phase III) during 2012 were well maintained and all the vacancies were filled during the planting season in 2013.

2. Root trainer planting technique

In the field trial initiated at Churulacode (2002) root trainer plants continued to exhibit better yield (56.6 g t^{-1} t^{-1}) than polybag plants (54.7 g t^{-1} t^{-1}) up to the 7th year of tapping. Two demonstration plots of root trainer plants (200 each of root trainer and polybag plants) were established in the South Zone Vellayani and Mukampalai and a third demonstration plot in the Central Zone (Pazhamthottam). Large scale production of root trainer plants was initiated at Central Nursery, Karikkattoor and four Regional Nurseries under Rubber Board from 2013 onwards. Training was imparted on root trainer planting technique to various groups of farmers.

Table Par. 3. **Mean girth of OFTs at Tharuvaiyar and Vithura (2008)**

Clone	Girth (cm)	Girth of RRII 105 for the analysis of covariance	Girth (cm)	Girth of RRII 105 for the analysis of covariance
	Thadikarankonam		Vithura	
P10	33.08	30.48	38.53	35.80
P15	22.97	23.80	37.12	32.96
P 76	26.79	31.30	33.64	36.80
P21	31.06	31.89	33.71	39.75
P88	26.48	29.41	31.42	36.94
P84	23.06	23.17	33.34	33.64
P60	21.95	28.07	33.41	35.15
P61	27.95	22.89	38.08	36.90
P67	25.10	26.05	35.87	33.79
P68	26.46	28.27	34.67	35.89
P74	26.79	26.50	35.25	38.16
RRII 105	32.54	28.75	32.43	37.00
RRII 414	31.47	26.24	32.87	39.34
RRII 430	28.49	24.11	33.62	36.11
Mean	27.44	27.21	34.57	36.3
SE	1.89	1.73	1.46	1.42

LIBRARY AND DOCUMENTATION CENTRE

During the year, 66 books were added to the stock of the library. The library subscribed 44 foreign and 72 Indian journals. About 42 other journals were also received as gift/exchange. Provided online access of journals, literature searches, press clipping, reprographic services, etc.

Natural Rubber Research: A Bibliography 2005-2010 was brought out during the period. *New Additions List 2012, List of Current Periodicals 2012* and two issues of *Documentation List* were compiled and distributed. Databases were updated by adding the details of 10 books and 372 journal articles. Arranged the distribution of 455 numbers of Press Clippings and 489 numbers of other SDI bulletins.

Organized the distribution of 653 issues of the journal *Natural Rubber Research* and 14 numbers of RRII Annual Report. Arranged the sale and distribution of 555 numbers of RRII publications. Photocopies of about 16948 numbers of information materials were provided during this period.

AGROMETEOROLOGY

1. RRII HQ

1.1. Climate resource characterization of the rubber growing areas

Box plot and whisker plot analysis was carried out in parameters such as Tmax, Tmin, sunshine and rainfall in Kottayam to study the distribution and spread of the dataset and frequency of occurrence of the events. Percentiles of Tmax, Tmin ,rainfall and sunshine for Kottayam on weekly ,monthly and seasonal basis has been worked out since they serve as indicators to characterize various regions.

1.2. Why NE India is hostile for Abnormal leaf fall disease in Natural Rubber?

Differences in thermal and moisture regime in an ALF disease prevalent region (Kottayam, South India) and a free region (Agartala, Northeast India)) have been examined based on the indices defined by the WMO Expert Team on Climate Change Detection, Monitoring and Indices. Temperature thresholds at 90th, 50th and 10th percentile and the number of days exceeding these thresholds for maximum temperatures and below the threshold for minimum temperatures were worked out and compared between the regions .

Observed temperature profiles of Tmax & Tmin were well within the range for Phytophthora incidence at Kottayam whereas in Agartala thermal regime falls beyond the favorable range for the disease incidence.

The precipitation regime of the selected regions were also compared using the indices defined by WMO.

Out of 120 days in the season there were 90 wet days (RFe"1mm), around 60 heavy precipitation days (RFe"10 mm) and 25 very heavy precipitation days (RFe"30mm) in Kottayam where as it was 60, 30 and 10 days in Agartala. Maximum number of consecutive wet days was 20-30 days in Kottayam as against 5-10 days in Agartala It

was observed that conditions congenial for disease to occur prevail in Agartala too, but what is shielding Agartala from this disease might be the overriding influence of the temperature regime over the precipitation regime.

2. Studies on climate change

Change point analysis and cusum analysis for temperature and precipitation time series of Kottayam were carried out for the period (1973-2011) and Kerala for the period 1871-2011. Climate change impact analyses were also carried out for All India and Sub divisional temperature and precipitation time series for the period 1901-2007 and 1813-2007.

Change point analysis for Tmax at Kottayam showed that changes have occurred twice during 1979 and 1998 at confidence levels of 91 and 100 % respectively. Maximum temperature (Tmax) changed from 31.0 to 31.6 ^{0} C and from 31.5 to 32.1^{0} C during 1979 and 1998 respectively. Change point analysis of the seasonal rainfall detected two changes in the time series of summer rainfall of Kottayam during 1969 and 1979 at confidence levels of 97 and 92 % with confidence levels of 3 and 2. Rainfall shifted from 386mm to 637 mm and from 637 to 484 mm during 1969 and 1979 respectively. No significant change was detected in the time series of Southwest monsoon, Northeast monsoon, winter and annual rainfall.

Change point analysis for Northeast monsoon for the State exhibited four changes in the precipitation time series during 1877, 1975, 1961 and 1992 with a confidence interval of 100, 94, 96 and 97 per cent at confidence levels of 6,5,3 and 2 respectively . The time series of Southwest monsoon rainfall did not exhibit any change over the years.

3. Monsoon variability

Precipitation time series of the state of Kerala during the monsoon for the period 1871-2011 have been employed for the study. Rainfall anomalies were worked out for all the months, seasons and annual rainfall. Anomalies were subjected to running means of wavelength 11 years . Linear changes in the precipitation time series have been estimated by the least square regression analysis. Regression analysis was employed to find out the interdependence of June and July rainfall between themselves and also on SW monsoon, NE monsoon and total monsoon.

A decline in SW monsoon and a corresponding increase in NE monsoon have been observed during the last decade of the 20th century. The decline in SW monsoon was compensated during NE monsoon which was well depicted in the time series of total monsoon. When the performances of individual months were considered a declining trend was noticed for June and July rainfall towards the end of the century. Rainfall during August did not exhibit any trend and maintained consistency throughout the study period. An insignificant positive trend was observed for September, October and November rainfall. It can be concluded that there exists a major shift in the rainfall pattern towards the end of the century, as seasonal rainfall during SW monsoon showed a downward trend and NE monsoon showed an upward trend. This could be better termed as climate variability.

Regression analysis showed that SW and total monsoon was influenced by June and July rainfall significantly (0.01 level). It was observed that June and July rainfall did not influence or show any dependence between themselves and also on the rainfall of other monsoon months.

2. RRS, AGARTALA

2.1. Resource characteristics of rubber growing areas

Resource characteristics of water budgeting were studied to understand the water deficit intensity and direction for a specific place. Weekly and monthly basis water balance parameters have been worked out in the rubber growing regions of NE India. Potential evapotranspiration estimated by the modified Penman method was utilized in the standard T-M model and the water deficit, water surplus, storage and runoff for the respective periods were studied for the stations of Agartala, Guwahati, Tura, Nagrakata and Dhenkanal with data ranging from 15 to 29 years.

Annual march of evapotranspiration (PET) showed a double peak for Dhenkanal of 6.2 during May and 4.4 mm day^{-1} during October against the normal peak in Apruil in other stations. Lowest PET values were noticed during December and January with the lowest curve obtained for Guwahati. Water deficit conditions as shown in Fig. 1a-e exists from December to March in all stations except Dhenkanal where deficit periods extend from December to May. Monthly runoff values were highest during July, with Nagrakata having more than 800 mm. High water deficit were also noted for Dhenkanal and Agartala (164 and 162mm respectively).

3. Climate change studies in the rubber growing tracts of the NE region

Monthly, seasonal and annual trends were computed for the various data sets for five regional stations of RRII in the NE region *viz.* Agratala (Tripura), Guwahati (Assam), Tura (Meghalaya), Nagrakata (N.Bengal) and Dhenkanal (Orissa) to understand the nature of future change in climate

The observed surface meteorological parameters considered were maximum, minimum and average temperature, relative humidity, wind speed, bright sunshine hours, evaporation, rainfall and eainy days for respective periods. In most stations significant increasing trends were observed for the minimum temperature which contributes the similar trend in the average temperature. No significant change was noted in the maximum temperature. Striking feature in Agartala was the significant decrease in evaporation and bright sunshine hours, with an R^2 of 0.76 and 0.51 respectively. There was no change in the amount of rainfall and rainy days in any of the stations. September month in Agartala showed a significant increasing trend in maximum temperature while minimum temperature increased during the monsoon months which have contributed to the annual increase compared to any other months. Characteristic trends in slopes were noted for the trend analysis. The morning relative humidity showed decreasing trend over the years for the post-monsoon and winter periods. A significant reduction in sunshine hours was noted by 1 hour in ten years for the December and January months in Agartala.

4. Forewarning of pests and diseases in rubber

The identification of critical weather parameters for the development of diseases and its intensity is important to take appropriate prophylactic measures in the hot spot areas of rubber growing tracts. In view of this, rubber plantations in three different locations in the NE region *viz.* Agartala (Tripura), Guwahati (Assam) and Nagrakata

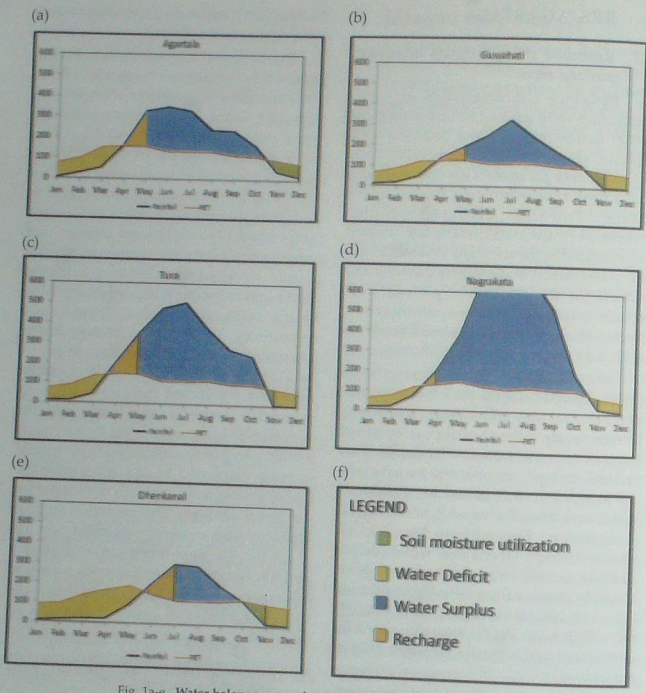

Fig. 1a-e. **Water balance curves for the five regional stations of RRII**

(W.Bengal) have been selected for the experimental trial. Daily based Diseased leaf collections on daily basis were made before the onset of disease and with the advent of daily weather changes. Evaluation of sample size of Leaf Collection Baskets (LCB) was carried out in RRS, Agartala to find out the optimum number of LCBs required in a hectare so as to capture the true representation of diseased leaf fall in rubber. The experiment was carried out in the mature clones of RRIM 600 and RRII 105.

Power Analysis with two years of leaf collection data was used to find out the probable number of baskets required for the analysis. This analysis gives the minimum sample size required in an ANOVA within a particular significant level. Assuming that uniform leaf fall is experienced during the wintering period, the sample size of leaves collected in leaf baskets were evaluated with 25 baskets in a hectare of land. It was found that the leaves under RRIM 600 gave better representation of the mean number of leaves per leaf basket. Power Analysis for ANOVA, revealed that the minimum sample size of leaf baskets needed was five replications with ten baskets/replication amounting to 50 numbers (95% significance) to represent the leaf fall in a rubber plantation in Agartala.

5. Agrometeorological Database Management

Several meteorology parameters are being collected from five research stations and one block planting unit in the NE region and daily basis and the data were posted after tabulation on to standard forms adopted in the format of India Meteorological Department (IMD).

Weekly meteorological data on surface meteorological parameters including rainfall were obtained from IMD were used for the database management.

ANNUAL EXPENDITURE

Expenditure at a glance (2012-13)

Head of Account	Expenditure (₹ in lakhs)
Non-Plan*	
Non-Plan RRII	669.77
Projects (CES)	549.08
Total	**1,218.85**
Plan	
Research Scheme	2,449.28
NERDS Research Component	402.55
Total	**2,851.83**
Grand Total	**4,070.68**

*Non-plan expense includes non-plan projects (CES)

SCIENTIFIC ADVISORY COMMITTEE RECOMMENDATIONS (2012-2013)

1. To increase the planting density of the clone RRII 105 to 550 plants/ ha. from the previous recommendation of 500 plants/ha.
2. **Biodiversity in Rubber plantations:**
   - Shade tolerant perennial crops with canopy underneath rubber *viz.* coffee, vanilla, *Glyricidea* standards , *Garcinea* and cocoa can be cultivated along with rubber without adverse effect on growth and yield of rubber. One row of these crops can be grown between two rows of rubber. Number of plants that can be cultivated as intercrop in one hectare of rubber plantation are : Coffee – 445 Nos. ; *Garcinea* – 445 Nos. ; Cocoa- 445 Nos.; vanilla- 650 Nos. Providing shade is beneficial for establishment of these crops. All other cultivation practices including manuring should be followed as per the recommendation for the respective crops. Yield of these crops will decrease after canopy closure.
   - In mature rubber plantations, permitting more bio-diversity through crop with canopy underneath rubber will improve soil moisture status during dry season and sustain soil nutrient status.
   - *Alpinia calcarata* can be a medicinal plants suitable for cultivation in mature rubber.
   - Short duration vegetables can be cultivated during the wintering period in mature rubber plantations on level lands for family consumption.
3. Level beds with a layer of about 5 cm thickness of sand or coir pith (leached by exposing to sun and rains for 6 months) as a medium for germination of rubber seeds.
4. Four Brush cutter models (i) Oleomac Model: 744T (ii) Mitsubishi Model: KK-BC2-JP45 (iii) Sun max Model: SMH-270 –GX 35 (iv) Sun max Model: SM-6400 Premium for mechanized weeding in rubber plantations.
5. MAK rubber spray oil (BD) manufactured by M/s. Bharat Petroleum Corporation Ltd. as a carrier for copper oxy chloride for spraying against leaf diseases in rubber plantations.
6. **Interim Planting recommendations**: **Planting materials suitable for Karnataka and South Konkan :**

   GT1, RRII 203, PB 260, PB 217* , RRII 414, RRII 430, RRII 422, RRII 429, PB 280, PB 313, PB 314, PB 235, RRII 5, RRII 300, PB 311* and RRII 105*. Polyclonal seedlings are advisable for planting in areas where proper management is not possible.

   (RRII 105 is highly susceptible, RRII 203 and PB 217 are moderately susceptible to Corynespora leaf disease, and hence recommended control measures are to be adopted. PB 311 is wind susceptible (hence avoid planting in wind prone areas).

Planting materials suitable for North Konkan:

RRIM 600, RRII 208, RRII 105, RRII 6, RRII 5, PB 260, PR 255, RRIC 100 perform well in the region. The drought tolerant , stable clone RRII 430 could be planted in the region. Life saving irrigation to be given to all clonal plantings in the first three years of planting. If cultivation under rainfed / unirrigated condition is intended, polyclonal seedlings are the best choice.

7. Wood Pecker chain saw model WP-5200 supplied by M/S. Remys Agro quipments Pvt. Ltd, Kaloor, Kochi for rubber tree felling and cutting operations .
8. Mist blower STIHL-SR 450 supplied by M/s Andreas STIHL Pvt. Ltd.,Pune for low volume spraying and sulphur dusting in rubber plantations.
9. HTP sprayer-WP-22K supplied by M/s Remys Agro Equipments Pvt. Ltd., Kochi for high volume spraying in rubber plantations.
10. Rainguard compound supplied by M/s. STP Ltd. Palarivattom, Cochin for rainguarding rubber trees.
11. Tapping knife ("Jorwin (Manimooli)) supplied by Sri. T.P.George, Thadathil (H), Manimooli , Nilambur for its use as Tapping knife in rubber plantations.
12. Revised usage of formic acid for coagulation of RSS in the North East. Usage during November to middle of March as 3ml diluted to 300 ml, and for other months as 1.5 ml diluted to 300ml (for Next day of sheeting). Similarly for same day sheeting, use 4 ml diluted to 400 ml (during November to middle of March) and 2ml diluted to 400 ml for other months.
13. Recommended the following clones for planting in North East India

Category I	RRIM 600
Category II	RRII 105, GT1, PB 235, RRII 203, RRII 208, RRII 429, RRII 417 and RRII 430.
Category III	RRII 5, RRII 422, RRII 118, PB 260, PB 310, PB 311, RRIM 703, SCATC 88/13, SCATC 93/114, Haiken1 and polyclonal seedlings. RRII 422 has been downgraded from Category II to Category III due to high cold susceptibility during the early period of establishment.

LIST OF MAJOR EQUIPMENTS AT RRII

Air permeability tester
Atomic Absorption Spectrophotometer
Autoclave (Cylindrical and Horizontal)
Ball mill
Bio – Cabinet
Bio safety cabinet
Biomedical Freezers (-30°C)
BOD Incubator
Brook field viscometer
Carbon Nitrogen Analyser
Centrifuges (High speed refrigerated)
Chemical fume hoods
Chlorophyll Content Meter
Chlorophyll index meter
Climatic chamber
Coir foam testing equipment (indentation hardness, flexing, compression set A & B testers)
Compression set apparatus (25% strain)
De- mattia Flexometers
Deep freezer (-20° & -80°)
Deep freezers
Differential scanning calorimeter
Din Abrasion machines
Disper grader
DMA 50
DNA electrophoresis unit
DNA isolation machine
Eddy covariance system
ELISA reader
Environmentally controlled shakers
Flame photometer
Flash chromatograph &accessories
Fluorescence Monitoring System
Fluorescence spectrophotometer
Freeze Driers
FTIR spectrometers
Gas chromatograph–mass spectrometer
Gel blotting apparatus
Nanodrop Spectrophotometers
Oxygen electrode
Ozone chamber
P700 chlorophyll fluorescence measurement system
PAM-2000 portable flurometer
Particle size analysers
PCR machines
Phase contrast Microscope & accessories
Phosphor Image Analyser
Phosphor imager
Plant growth chamber
Plate reader
Polarizing Microscope & accessories
Projection microscope with accessories
Protein separating systems - 2D
Real time PCR machines
Recirculating Cooler
Refrigerated and Heating Circulator
Refrigerated high speed micro centrifuge
Refrigerated shaker
Refrigerated Table Top Centrifuge
Remote Sensing and Geographical Information System (RS & GIS)
Research microscope and image analyser
Ross flexing Machine
Rotary Evaporator
Rubber Process Analyser
Sap flow meter
Sequencing gel electrophoresis unit
Sequi-Gen GT system (sequencing system)
Soil Respiration Analyser
Sonicator
Specific gravity balance
Spectro radiometer
Spectrophotometer – nanodrop

Gel documentation & image analyzer
Gel documentation systems
Gel Dryer & Pump
Gel electrophoresis apparatus - 2D
Geldoc systems
Genetic Analyzer 3500 XL
Goodrich Flexometers
GPC
Hardeness tester (shore A,D,M,0)
High speed microcentrifuge
High speed Table Top Centrifuges
High Voltage Power Pack
High-Speed Centrifuge
Histo Embedder
HPLC system
Hybridization oven – Incubator shaker
Hydraulic press (14" × 14",)
Image processing and Analysis System
Incubator & Accessories
Incubator shaker with cooling
Infrared thermometer
Inverted MicroscopeIRGA- Portable photosynthesis system
Isoelectric focusing unit
Laminar Air Flow Hoods
Latex foam testing equipment (indentation hardness,flexing, compression set A&B testers)
Leaf area meter
Linear PAR Ceptometers
Liquid scintillation system
Measuring mixer (80 cc)
Micro centrifuge with cooling
Micro pH meter
Microtome – Base sledge
Microtome – Rotary with knife sharpener
Mini IEF electrophoresis unit
Mooney viscometer
Moving die rheometer
Muffle furnaces
Spectrophotometers
Speed vac concentrator system
Stereo Microscope
Submerged Electrophoresis System
Temperature controlled incubator shaker
Temperature Recorder
Thermo gravimetric analyzer
Thermocouple psychrometer
Tissue processor
Two-roll mixing mill (6" × 13")
Ultracentrifuges
Universal testing machines (50N, 100N, 5 kN)
UV Spectrophotometer
Vertical electrophoresis unit
Walk in environmental Growth Chamber
Walk in Fume Hood
Water potential system
Water purification system
Wet sieving apparatus
Zeta potential analyzer

NORTH EAST

Atomic Absorption Spectrophotometer
Chlorophyll content meter.
Flame photometer
Fluorescence Monitoring System
Leaf Area Meter
Luminometer
Nitrogen Analyzer
PAR/LAI Ceptometer
Portable photosynthesis system
Spectronic 20D Spectrophotometer
Stereo microscope
UV-spectrophotometers
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K.K. Jayasooryan	Scientist A
P.M. Sebastian	Farm Officer
Rajen Mathew	Farm Officer
K.K. Benny	Farm Officer

Latex Harvest Technology Division

K.U. Thomas, M.Sc., Ph.D.	Joint Director
R. Rajagopal, M.Sc., M.Phil., Ph.D., Dip. Stat.	Senior Scientist 3
K. Karunaichamy, M.Sc., Ph.D.	Scientist C
P. Babuji	Farm Officer (upto 30.11.2012)
T.V. Somaraj	Assistant Farm Officer

Rubber Technology Division

K.T. Thomas, M.Sc., LPRI, M.Tech., Ph.D.	Joint Director (upto 30.10.2012)
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.N. Madhusoodanan, M.Sc.	Scientist C 7
Joy Joseph, M.Sc.	Assistant Rubber Processing Technologist (S3)
Manoj Kurian Jacob, M.Sc.	Scientist A
Valsa George, M.Sc.	Assistant Scientific Officer
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.	Junior Technical Officer

Technical Consultancy Division

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K.S. Mohandas, M.Sc., M.Tech.	Scientist B
Jinu Jacob George, M.Tech., Ph.D.	Rubber technologist
Shera Mathew, M.Sc., M.Tech., Ph.D.	Assistant Rubber Technologist
M.A. Fancy, M.Tech.	Junior Rubber Technologist (HG)
K.K. Sasidharan, M.Tech.	Assistant Rubber Technologist
Reethamma Joseph MSc., Ph.D.	Assistant Rubber Technologist
Mathew Joseph, B.Tech.	Assistant Rubber Technologist
Sheela Joseph, M.Sc., B.Ed., M. Tech.	Junior Scientific Officer
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	Radiological 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
S. Veeraputhran, M.A., M. Phil.	Scientist S3
T. Siju, M.Sc. (Ag.)	Scientist B
Joby Joseph, M.A.	Scientist B
K. Rajesh , M.A. Ph.D.	Scientist A

Project Monitoring

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) (upto 30.04.2012)
A.S. Ajitha, M.A., M.L.I.Sc.	Senior Librarian
P.U. Lalithambika, B.Com.	Section Officer
V.R. Sujatha, B.Sc., M.L.I.Sc.	Librarian
N. Latha, M.Sc., M.L.I.Sc.	Assistant Librarian (HG)

Statistics and Computer

B. Biju, M.Sc., M.C.A.	Assistant Director (Systems)
P. Aneesh, M.Sc., P.G.D.C.A.	Statistical Inspector
Suma George, P.G.D.C.A., M.C.A., B.Ed.	Computer Assistant

Instrumentation

Thomas Baby, M.Sc., M.Phil., Ph.D.	Deputy Director
M.R. Anilkumar, Dip. in Inst. Tech.	Instrumentation Officer
K.R. Suni	Assistant Instrumentation Officer

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

T.V. Thomas	Assistant Farm Manager
A.K. Peeusmon	Farm Officer

Security Wing

PV. Suresh Babu	Assistant Security Officer

Central Experiment Station, Chethackal, Kerala

P. Mallinath Priyadarshan	Deputy Director
N.K. Suresh	Medical Officer
K.R. Suresh Kumar	Assistant Director (Finance)
K.U. Saramma	Section Officer
T.R. Divakaran	Farm Manager (upto 31.07.2012)
T.G. Sasi	Farm officer
T.V. Somaraj	Farm officer
K.K. Kunjachan	Farm officer

Regional Research Station, Padiyoor, Kerala

Radha Lakshmanan, M.Sc. (Ag.), Ph.D.	Senior Scientist
V.J. George	Farm Manager (upto 30.11.2012)

Regional Research Station, Guwahati, Assam

D. Chaudhuri, M.Sc.(Ag.), Ph.D.	Project Co-ordinator (Scientist D)
Gitali Das, M.Sc., Ph.D.	Deputy Director
Gopal Chandra Mondal, M.Sc., Ph.D.	Plant Pathologist
Hiranyakumar Deka, M.Sc., Ph.D.	Scientist C
Mrinal Choudhury, M.Sc. (Ag.)	Scientist B
N. Ashith Raj, M.Sc. (Ag.)	Scientist A

Regional Research Station, Agartala, Tripura

Sushil Kumar Dey, M.Sc., Ph.D.	Deputy Director
Shammi Raj, M.Sc., Ph.D.	Agrometeorologist (Scientist C)
Debasis Mandal, M.Sc.	Scientist S3
P. Deepthi Antony, M.Sc. (Ag.)	Scientist B
Debabrata Ray, M.Sc. (Ag.)	Scientist B.
Bhaskar Datta, M.Sc. (Ag.)	Scientist A
Gaurav Sharma	Scientist A
Jiban Chakraborty, B.Com.	Assistant Director (Finance)
Tapan Kumar Pal, M.Sc.	Assistant Scientific Officer
Haradhan Bhowmik, B.A.	Assistant Farm Manager
C.L. Benny	Farm Officer

Regional Research Station, Tura, Meghalaya

A.P. Thapliyal, M.Sc., Ph.D.	Deputy Director (upto 20.05.2012)
R.P. Singh, M.Sc. (Ag.), Ph.D.	Scientist S3
Debabrata Pandey	Scientist B
Umesh Chandra	Scientist A

Regional Experiment Station, Nagrakatta, West Bengal

Shyamal Kheroar	Scientist A
Sandeep Kumar, M.Sc., Ph.D.	Scientist A
Thampy Andrews	Farm Officer

Regional Research Station, Dapchari, Maharashtra	
Meena Singh, M.Sc. (Ag. Bot.), Ph.D.	Plant Physiologist
S. Ravichandran	Scientist A
Kishore Shivaji Parkar	Section Officer
P.R. Mohanan	Farm Officer
Dilip Ganapat Shende	Junior Scientific Officer
Regional Research Station, Dhenkanal, Orissa	
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Hevea Breeding Sub station, Nettana, Karnataka	
T.R. Chandrashekar, M.Sc., M.Tech. Ph.D.	Senior Scientist
M.J. Manju, M.Sc. (Ag.)	Scientist S3
Hevea Breeding Sub station, Paraliar, Tamil Nadu	
T.A. Soman, M.Sc., M.Phil., Ph.D.	Scientist C
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Thomas Eappen, M.Sc., B.Ed.	Scientist S3
Regional Soil Testing Laboratory, Muvattupuzha, Kerala	
Regional Soil Testing Laboratory, Kozhikode, Kerala	
Joyce Cyriac, M.Sc.	Scientist S3
P.K. Madhusooddhanan, B.Sc.	Scientific Officer
K. Jayasree, M.Sc.	Junior Scientific Officer
Regional Soil Testing Laboratory, Nedumangad, Kerala	
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C. Viswambharan, B.Sc.	Assistant Scientific Officer
Regional Soil Testing Laboratory, Taliparamba, Kerala	
Suresh Babu Ayyappanchalil, M.Sc.	Junior Scientific Officer

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REGIONAL SOIL TESTING LABORATORIES IN KERALA

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Front Cover Photo
Rubber Research Institute of India Main gate
(Background with field 3 replanted during 2014)

Photo by
Dr. Vinoth Thomas, Scientist C, Botany

Back Page Photos
1. Inaugural function of International Rubber Conference, Kovalam, Kerala, 28-31 October, 2012.
2. IRRDB Annual Meetings, Kovalam, Kerala, 1-2 November, 2012.
3. View of participants, IRRDB Annual Meetings 2012.

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Continued from inside front cover

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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