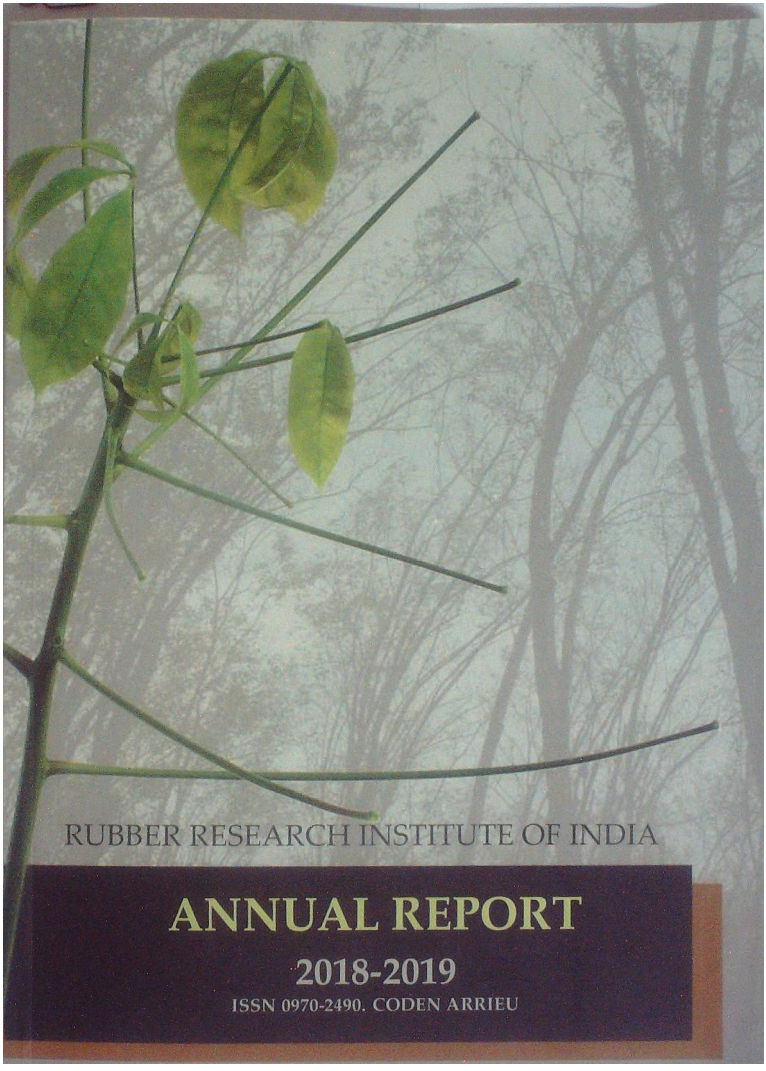

RUBBER RESEARCH INSTITUTE OF INDIA

ANNUAL REPORT

2018-2019

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Rubber Research Institute of India

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

Location

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

Organization

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

Continued on inside back cover

ANNUAL REPORT
2018-2019

RUBBER RESEARCH INSTITUTE OF INDIA
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THE RUBBER BOARD

The Indian Rubber Board was constituted under the Rubber (Production and Marketing) Act, 1947, which came intoforce 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 intoforce 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.

Chairman
Dr. Shanmuga Sundaram IAS
(w.e.f.24.11.2017)

Mr. D. Anandan IAS (w.e.f.01.07.2018)

Rubber Research Institute of India
Dr. James Jacob
Director

Crop Improvement
Dr. Kavitha K. Mydin
Joint Director

Botany
Deputy Director (vacant)

Biotechnology
Joint Director (vacant)

Principal Scientist (Officer-in-charge)
Dr. R.G. Kala

Genome Analysis Laboratory
Dr. Thakurdas Saha (up to 31.10.18)
Principal Scientist

Germplasm
Joint Director (vacant)

Dr. Jayashree Madhavan
Principal Scientist (Officer-in-charge)

Crop Management
Joint Director (vacant)

Agronomy and Soils
Dr. M.D. Jessy
Joint Director

Fertiliser Advisory Group
Dr. Mercykutty Joseph
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Crop Physiology

Climate Change and Ecosystem Studies
Joint Director (vacant)

Plant Physiology
Dr. K. Annamalainathan
Joint Director

Crop Protection
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Joint Director (upto 12.09.18)

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Joint Director (vacant)

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Principal Scientist (Officer-in-charge)

Economics Research
Toms Joseph
Joint Director

Latex Harvest Technology
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Rubber Technology
Joint Director (vacant)

Rubber Technology
Deputy Director (vacant)

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Scientist (Officer-in-charge)

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

Hevea Breeding Substation, Kadaba
Dr. P. Deepthy Antony
Scientist (Officer-in-charge)

Hevea Breeding Substation, Thadikarankonam
Dr. M. Suryakumar
Scientist (Officer-in-charge)

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Deputy Secretary (upto 29.06.18)

Finance & Accounts
CA Zachariah Kurian
Joint Director

Instrumentation
Deputy Director (vacant)

M.R.Anilkumar
Instrumentation Officer (Officer-in-charge)

Library & Documentation Centre
N. Latha
Documentation Officer

Statistics & Computer
B. Biju
Assistant Director (Systems)

DIRECTOR'S REVIEW

The year 2018-19 saw price of natural rubber and its domestic production somewhat stabilizing; however a substantial extent of mature rubber holdings remained untapped citing unremunerative price and low productivity of rubber. Rubber-based multi-cropping systems assume particular relevance during low price situations. Results from several field trials conducted by the Agronomy/Soils group of RRII continued to prove the economic and ecological benefits of intercropping even as growth and productivity of rubber trees were not adversely affected. Microclimates inside mature rubber plantations where native plants are left unweeded are more congenial for producing more latex. Similar results have been reported from other countries as well. The traditional concept of monoculture of natural rubber needs a serious review at the global level. This is very important from a small grower's sustainability perspective. As the mean holding size continues to decrease, returns from rubber monoculture becomes smaller and when rubber price is low the situation becomes untenable. Intercrops offer a source of additional income. Cocoa, coffee, nutmeg and several other species, particularly shade-loving crops can be profitably cultivated in mature rubber holdings. We have shown for many years that immature rubber holdings can support a variety of seasonal, annual and perennial crops which will provide additional income and provide nutrition to the grower household. Whether planting design of rubber can be drastically altered to release more land and light to enable long term intercropping needs further consideration. Results emerging from the trial on different planting systems initiated at Cheruvally estate in 2007 offer useful insights in this regard.

There are several simple, easy to adopt and low cost farm innovations made by the Agronomy/Soils group that have not reached to a large section of the grower community. For example, latex coated newspapers and gunny bags or biodegradable plastics used as mulch give good control of weeds while conserving soil moisture, similarly multi-cropping in mature rubber holdings. But these are not being widely practiced by growers. There are many more recommendations such as low frequency tapping; region-specific clones *etc.* that are not seen widely adopted by growers, despite confirmed research data. It is important to examine the reasons for poor adoption of

these recommendations that can increase productivity, reduce cost and thus improve profitability of rubber cultivation.

A pioneering study conducted by RRII in Kottayam and Idukki districts showed that quality of surface water was the best in rubber dominated landscapes than crops such as cardamom or tea. However, irrespective of the land use type, bacterial contamination was widespread which is a matter of serious concern from the stand point of community health. It is understood that bacterial contamination of water bodies of Kerala is a major issue, apart from other concerns such as high levels of pesticide residues, nitrates *etc.*

RRII continued to make significant advances in use of geospatial techniques in the natural rubber plantation sector. Digital mapping and estimating acreage under rubber cultivation was completed in the entire country using satellite-based remote sensing data. Follow-up studies based on RubSIS recommendations of fertilizer application were initiated. Climate studies continued to be a priority area of research during the reporting year. Out of the 68 districts in NE region, 42 districts were found reasonably suitable for rubber cultivation based on long time climate date. Earlier studies had shown that as climate continues to warm, more areas may become climatically suitable for rubber cultivation in the NE region and climate stress may seriously affect growth and productivity in the other rubber growing regions of the country, including the traditional areas. Net primary productivity of rubber growing regions was estimated from satellite-based data which was the lowest in Palakkad district compared to other rubber growing districts of Kerala. This is likely due to severe drought and hot conditions prevailing in Palghat region during summer months. Based on life cycle analysis (30 years) one ha of rubber plantation could sequester as much as 777 MT CO_2.

The Breeding group continued to make significant studies during the reporting year. The large number of clone trials at various locations which are under different stages of growth and the 300 odd advanced clones in the pipeline offer good prospects for identifying high yielders as per the "smart clone vision" of RRII. In addition to high yield, extent of increase in yield in response to ethephon application has become an important trait in clone development programmes. This is to ensure that new high yielding clones are amenable to low frequency tapping for which stimulant application is a pre-requisite.

The different breeding strategies adopted by RRII has witnessed a paradigm shift from biparental mating and ortet selection to polycross breeding and half-sib selection over the years. Producing a large number of seedlings through careful selection from half-sibs will markedly improve the chances of identifying a smart clone. Promising wild accessions collected from Amazon and advanced clones brought from almost all rubber growing counties in the world are already included in these breeding strategies. But the large size of the seedling population will pose an insurmountable difficulty in screening them for useful traits unless a fast and efficient protocol is developed. Marker assisted selection offers a clear solution. While the molecular biology/genome team at RRII continued to develop QTL and other markers associated with high yield, disease tolerance, climate-resistance *etc.* the genetic engineering group of RRII continued to produce GM rubber plants incorporating several genes of agronomic importance. Significant advances were made in the areas

of poly ploidy, poly embryony and production of gynogenic haploids through ovule culture and embryosac culture. Another interesting development was production of Corynespora tolerant rubber plants from cells "recruited" after *in vitro* habituation with Corynespora toxin. Progress made by the Molecular Biology/Biotechnology team offers great prospects for future genetic improvement research for high yield, disease tolerance and climate-resilience underpinning the importance of multi-disciplinary research.

The international clones recently imported from 10 different countries through the IRRDB exhibited wide genetic diversity (30-70%) offering great opportunity for exploiting it for crop improvement research. The Cirad-Michelin Selections (SALB tolerant clones developed in the Michelin plantations in Bahia, Brazil received at RRII from Cambodia and Ghana after long quarantine) showed maximum diversity even as their largely Brazilian parentage had little genetic sharing with Asiatic clones which clustered separately. Metagenomics studies initiated in rubber soils open several avenues of exciting scientific concepts and unknown territories and possibly with potential practical applications.

Pathology research revolved around studies on yield loss and chemical control measures. With a large repository of excellent genetic resources available with RRII, Pathologists and Breeders should dream of developing disease tolerant clones that will require minimal spraying of chemical pesticides which is both economically and ecologically desirable. Here again marker assisted selection protocols can help. Whole genome sequence data of *Corynespora* isolates should be exploited for further advancing the science and understanding the mechanism of fungus-*Hevea* interactions.

Crop Physiology group continued to generate more evidences from *in vitro* studies to indicate intrinsic tolerance of RRII 430 to high temperature stress and tolerance of SCATC 88/13, RRII 208 and RRIM 600 to low temperature stress. Ethephon-induced activation of sucrose transporter and glutamine synthetase genes (as well as several other genes associated with rubber biosynthesis) is an interesting result. Activities of these genes could act as proxy indicators of yield potential as well as response to stimulation. Latex Harvest team showed more results to prove the technical feasibility of d/7 tapping. Further reducing the tapping frequency (d/10 *etc.*) also appears to be possible.

Rubber Technology group, including the Consultancy team made significant contributions that benefit the processing and products developing sectors. A novel method was developed to make good quality sheet rubber from ammonia-preserved latex with suitable pre-treatment. Also novel processes were developed to make good quality low protein solid rubber from latex and maximum leaching of proteins from surgical gloves. Studies on latex carbon-black master batch, silica reinforcement of natural rubber, devulcanization of used rubber, finger printing of latex, shelf-life of gloves *etc.* gave promising results. A project on evaluating any possible cytotoxicity of crumb rubber powder made from end of life tyre was initiated. More than 40 entrepreneurs received technologies for making various rubber products such as adhesives, automobile components, fire resistant mats, rubber soles, dipped and foamed gird, treads, tube valves, rubber moulds *etc.*

Economics research team successfully prepared a road map for attaining self-reliance in natural rubber production by 2030.

However, given the poor rate of re/new planting, it is more likely that self-reliance in natural rubber production may not be achieved and this can hurt the Indian rubber products manufacturing industry badly in the coming years. Our studies have shown that by adopting scientific agronomic practices and cost saving recommendations, latex harvesting from existing mature holdings is still profitable even as the price of rubber is low. There is growing apprehension among rubber growers that rubber plantation sector is becoming sunset sector and they are gradually beginning to lose interest in rubber cultivation. This is an immediate and prolonged challenge that needs to be addressed with top priority.

Technical advises to growers on selection of clones, soil fertility management, disesase management and stimulation and tapping were continued. During the year technical know how for 42 rubber products were transferred to the entrepreneurs. Advisory services were provided to 416 clients from the industry sector. More than 852 test reports were issued based on testing of 833 samples for more than 3402 parameters. In addition to this, consultancy services through 2385 letterss/e-mails were given. Provided advisory on trouble shooting of 13 factory processes. During the reporting year the Quality Control Division of RRII issued NOC for the import of 5.79 lakh tonnes of natural rubber in 5247 consignments of which major share was block rubber followed by sheet rubber and small portions of crepe rubber and latex.

During the reporting year also no new recruitments were made and vacancies in the scientist cadre continued to increase. Three issues of Rubber Science were published with 27 research articles during the reporting year. A total of 32 research articles, 42 conference papers 19 popular articles, one book chapter and one PhD thesis were published and two scientific recommendations were approved.

Dr. James Jacob

AGRONOMY AND SOILS DIVISION

Developing sustainable farm practices, reducing cost of cultivation, generating additional income and improving biodiversity continued to be the major thrust areas of research of the Division. Various experiments on nutrient management, soil and water conservation, intercropping and ground cover management were in progress. Experiments to develop good agricultural practices to reduce gestation period and enhance yield of rubber were continued. Follow up studies of RubSIS on liming and fertilizer skipping was initiated at different locations. Soil sample collection for the project on fertility mapping of rubber growing regions of North-East India was in progress.

1. Nutrient Management

The trial initiated to evolve a fertilizer recommendation for root trainer rubber plants was in progress. A fertigation experiment was also initiated to reduce water consumption of root trainer plants.

The field experiment started during 2001 at Central Experiment Station (CES), Chethackal to study the effect of long term application of inorganic and organic manures on the growth and yield of rubber and on the physico- chemical properties of soil was continued without applying fertilizer and FYM to understand the residual effect of the treatments. Superiority of the treatment 25 per cent fertilizer + 75 per cent FYM in enhancing the growth of rubber was maintained. Significantly higher yield was also recorded in this treatment compared to all other treatments.

The field trials initiated during 2011 to study the effect of secondary and micronutrients on growth of rubber and soil properties at three locations, *viz.* Cheruvally, Palappally and Thamarassery estates were continued to the mature phase. The field trial initiated to evaluate 'Geogreen' organic manure on growth of young rubber at SFCK, Chithalvetty estate was concluded. Integration of Geogreen with fertilizer improved soil fertility status, but the positive effect was not reflected on growth of plants.

Continued the field experiment on target application of water soluble fertilizers into the root zone of young rubber plants. Girth of plants after one year was not significantly different between the standard practice and water soluble fertilizer treatments.

The study on rhizosphere chemistry under varying soil pH was in progress. Cation exchange capacity and exchangeable K were significantly higher in the rhizosphere soil compared to bulk soil.

2. Soil and water conservation

The experiment on evaluation of various biological/vegetative hedges for soil and water conservation initiated during 2009 in Mundakayam estate was continued. Significant difference was not observed between treatments either on growth of rubber or quantity of soil eroded.

3. Intercropping and cropping systems

Experiment initiated to develop a multispecies rubber based cropping system for Tamil Nadu region was in progress. The growth of rubber was significantly higher in intercropped area than control. The leaf yield of *Dracaena fragrans cv. Massangeana* was 35-40 leaves per plant per year. The experiment to study the effect of retaining pineapple after the normal intercropping period on performance of rubber and soil properties was in progress. Growth and yield of rubber was not influenced by retaining pineapple after the normal intercropping period.

The experiments on intercropping perennial crops with rubber initiated at CES, Chethackal in 2001 were continued and the growth and yield of rubber continued to be not influenced by cultivating coffee or nutmeg as intercrops. Yield of coffee was 50 per cent of that of monoculture. Average yield of nutmeg was 115 fruits $tree^{-1}$, which was comparable with that of monoculture. Experiment on inter planting of rubber with timber trees *viz.* teak, wild jack and mahogany initiated in 2001 at CES, Chethackal was continued. Growth of rubber was not significantly influenced by row spacing and type of timber intercrops. No significant difference in yield of rubber was observed between spacing, type of intercrops and interactions.

Initiated a field experiment at CES, Chethackal to find out the feasibility of establishing various crops *viz.* coffee (Selection 13), pepper, turmeric, amorphophallus, colocasia, chilli and *Heliconia* as intercrops in mature rubber plantation under tapping and the effect of these intercrops on growth and yield of rubber and soil physico-chemical properties. Planting of coffee was undertaken.

4. Ground cover management

An observational trial to find out the feasibility of establishing *C. caeruleum* under partial shade (after the intercrop pineapple) initiated during 2015 at Kaliyar estate was continued. *C. caeruleum* established well and survived in the field in summer also. The dry matter production after three years was about 3,720 kg ha^{-1}.

The pot culture study and field experiment with different flora *viz.* grasses, soft weeds and leguminous cover crops to study the differential effect of flora on soil nutrient dynamics were in progress.

The field experiment on the effect of legume covers and natural flora on the growth of rubber, soil physico-chemical and biological properties, biomass and nutrient turnover laid out during 2012 at CES, Chethackal was continued. Growth of rubber under both cover crops was comparable and the highest growth was recorded in plots where Pueraria was established. Rubber + $1m^2$ weeding also recorded a comparable girth to that of *Mucuna* plots.

The study on weed spread of *Mikania micrantha* Kunth from rubber plantations showed that the intensity of *Mikania* was severe within plantations with least human disturbance and where ample sunlight was available. The frequency of occurrence of this noxious weed reduced to moderate levels when moving away from the epicenter area outwards. The study showed that rubber plantations infested with *Mikania* were a good source for the weed spread to nearby localities.

5. Planting techniques

The field experiment initiated in 2010 to study the impact of mechanized land preparation on soil erosion and growth of

plants was continued. Significantly higher rate of soil erosion observed up to six years after planting in plots where inter-rows were tilled for cultivating intercrops was not observed during 2018-19 also.

The experiment to evaluate different planting systems in 2007 at Cheruvally estate continued. Canopy growth continued to be asymmetrical and girth of plants in the control (square system) was comparable with that of twin system of planting. All other planting system were inferior to control with respect to girth.

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

The field experiment initiated at CES, Chethackal during 2008 to evolve an agronomic package to reduce the immaturity period of rubber continued. The treatments with combinations of two types of planting material and two management options were in progress. Growth of direct-seeded green budded plants under integrated management were significantly superior to all others. The experiment to evaluate the field performance of one- whorl, two-whorl and three-whorl polybag and root trainer rubber plants initiated at CES, Chethackal during 2008 was continued. Three-whorl polybag plants were significantly superior to all other types of planting material in terms of growth. Yield was being recorded from the experimental area.

7. Rubber growing soils

The experiment to estimate root respiration components of soil respiration in different rubber based systems was concluded. In banana-rubber system the contribution of root respiration towards soil respiration was more than rubber-pineapple and mature rubber systems. The total soil respiration was also higher in rubber-banana system followed by rubber-pineapple and mature rubber systems.

8. Stress management

The experiment evaluating efficacy of latex coated news paper as mulch was continued. Results showed that low cost mulch materials such as latex coated newspaper and gunny were effective in preventing weed growth and conserving soil moisture for a period of six to eight months. Cost of mulch material for 1 m^2 area was Rs.20/- for newspaper and Rs.55/- for gunny (both coated with 400 ml latex each), and Rs.25/- for biodegradable plastic, which was durable for three years.

9. Environmental aspects of rubber cultivation

The study on assessment of water quality initiated during 2015 in watersheds dominated by rubber, tea and cardamom in Kottayam and Idukki districts of Kerala was concluded. The study showed that most of the quality parameters for drinking water were within the acceptable limits in all three land use systems. Compared to rubber system, significantly higher contents of nitrate, Ca, Mg, and K were observed in water samples collected from tea and cardamom dominated watersheds. Significantly higher dissolved oxygen content in surface water of rubber watershed during all the four seasons indicated better health of water resources in rubber dominated landscapes. Pesticide residues were below the level of quantification in samples from all three land use systems. The study showed that bacterial contamination was the major water quality problem in the study area, rendering the water unsuitable

Table Ag. 1. **Total dissolved solids (TDS) in groundwater and surface water (mg L^{-1})**

Groundwater						
Season	Rubber(n=27)	Tea(n=20)	Cardamom(n=22)	RxT	RxC	TxC
Post-mon. 2015	26.0(9-60)	67.3(16-169)	164.5(30-377)	**	**	**
Pre-mon. 2016	47.8(8-149)	87.4(7-215)	171.0(18-400)	**	**	*
Post-mon. 2016	54.6(5-250)	113.9(16-459)	210.0(63-489)	**	**	**
Pre-mon. 2017	50.7(9-180)	117.4(10-466)	212.8(17-589)	*	**	*
Surface water						
	Rubber(n=10)	Tea(n=9)	Cardamom(n=12)	RxT	RxC	TxC
Post-mon. 2015	25.9(13-48)	72.4(31-174)	93.1(44-179)	**	**	*
Pre-mon. 2016	34.4(15-68)	64.0(18-95)	102.4 (50-162)	**	**	**
Post-mon. 2016	22.7(7-48)	75.8(36-113)	83.6(28-219)	**	**	NS
Pre-mon. 2017	57.5(12-89)	104.9(53-168)	106.6(76-184)	**	**	NS

Figures in parentheses are the range of values BIS acceptable limit -500 mg L^{-1}

Table Ag. 2. **Dissolved oxygen content in surface water from three cropping systems (mg L^{-1})**

Season	Rubber(n=10)	Tea(n=9)	Cardamom(n=12)	RxT	RxC	TxC
Post-mon. 2015	8.1(7.1-8.6)	5.4(4.4-7.3)	6.8(6.3-7.0)	**	**	**
Pre-mon. 2016	7.0(6.1-7.5)	5.5(3.4-6.7)	6.0(3.1-8.8)	**	NS	NS
Post-mon. 2016	7.4(6.8-7.8)	5.7(4.3-6.1)	6.0(5.6-6.6)	**	**	NS
Pre-mon. 2017	7.1(6.5-7.9)	5.4(4.5-6.4)	6.0(4.3-7.0)	**	**	NS

Figures in parentheses are the range of values WHO specified limit 6.0 mg L^{-1}

for drinking purpose in all land use systems studied.

10. Level of adoption of agro-management practices

The primary data collected through the questionnaire on the extent of adoption of various agro management practices in rubber plantations of Kerala, Tamil Nadu, Karnataka, Goa and Maharashtra were compiled. The results showed that banana was the widely adopted intercrop in all states especially in Tamil Nadu (94%) and Goa (100%). In Maharashtra around 50 per cent of the farmers adopted pineapple intercropping where as in Kerala, Karnataka and Tamil Nadu, the percentage of adoption was 11, nine and three, respectively. More than 86 per cent of the rubber growers in South India adopted manual method of weed control in immature and mature rubber plantations. Only two per cent of the growers applied herbicides in rubber plantations for weed control. Construction of terraces for conserving soil and water was the widely accepted conservation practice (85%) in all states and combination of conservation practices for controlling soil erosion was followed only in Kerala.

11. Soil fertility mapping and soil health monitoring of traditional rubber growing regions of Kerala, Tamil Nadu and Karnataka

In continuation to the development of the on-line fertilizer recommendation system for rubber (RubSIS), follow-up studies on various aspects were initiated at

different locations. Two block trials each were initiated at Malankara Estate, Thodupuzha and Thevervelil Estate, Perunadu to study the effect of liming on soil properties and performance of rubber during immature and mature phases. Field experiemnts were initiated at four locations in Kerala and at one location in Karnataka to study the effect of skipping of fertilizers on yield and growth of rubber and on soil properties. Another field trial was initiated at TR&T, estate Mundakayam to find out whether application of Mg along with lime will improve performance of rubber.

FERTILIZER ADVISORY GROUP

The major objective of the group is to provide soil and leaf analysis based fertilizer recommendation to both large estates and small and medium growers. The services were provided through the central soil and leaf testing laboratory at RRII headquarters and seven regional laboratories. Advices on fertilizer use were provided during the visit of the growers to the laboratory or as clarifications on telephonic enquiries or email queries. Of late, the major activity of the regional laboratories was estimation of dry rubber content of latex samples.

- 73816 latex samples were tested for dry rubber content.
- Offered site-specific fertilizer recommendation to individual fields from large estates on the basis of analysis of 380 leaf samples and 712 soil samples.
- Eleven leaf samples and 254 soil samples from smallholdings were analyzed and offered site-specific fertilizer recommendations.
- Advices to smallholders were provided through telephone and visit of the farmers to RRII.

Table FAG. 1. **Details on soil, leaf and latex analyses and the revenue**

Sample details	Number	Revenue (Rs.)
Soil	966	1,35,405.00
Leaf	391	1,94,346.00
DRC of latex samples	73816	43,55,144.00
Total Revenue		46,84,895.00

CLIMATE CHANGE AND ECOSYSTEM STUDIES DIVISION

The major areas of research in the Division are analysis of climate change process and its impact on rubber cultivation in traditional and non-traditional rubber growing regions of India. Developing information system on rubber cultivation using RS-GIS platform to identify existing areas under rubber cultivation and suitable new area where rubber cultivation can be extended. Meteorological data management system in rubber growing regions is regularly updated. Ecosystem level studies in rubber plantations like species diversity, microclimate parameters, ground water table, effect of land use change etc. were also undertaken.

During this reporting period first phase of mapping and estimation of rubber acreage in the whole of India was completed. A total of sixteen rubber growing states have been mapped using remote sensing satellite data. Agro-climatic suitability analysis of NE was completed using 'area under curve' method and climate tolerance limits (CTL) parameters. Out of the 68 districts in the NE region, a total of 42 districts in 5 states (Assam-18, Manipur-9, Meghalaya-4, Mizoram-7 and Tripura-4) were identified as normally suitable and 54 and 62 districts in the 10 and 20 per cent variability limits, respectively. The impacts of El nino indicators on the seasonal rainfall and temperature were analyzed in rubber growing regions. Studies on vegetation dynamics and understory vegetation on

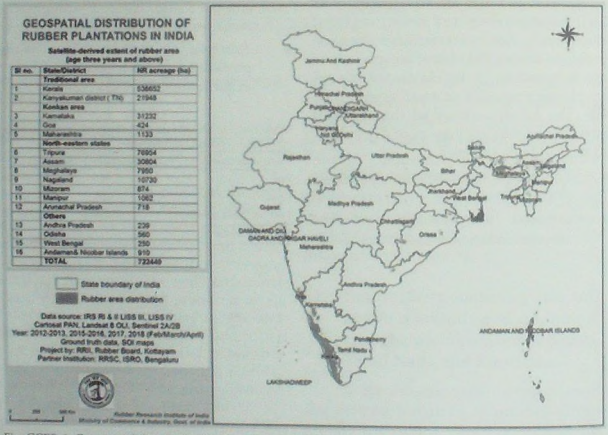

Fig. CCES. 1. Geo-spatial distribution and acreage of rubber plantations in India

microclimate in rubber plantations are progressing.

1. Developing rubber based information system using remote sensing and GIS.

1.1. Current status of mapping of rubber plantations in India

First phase of mapping and estimation of rubber acreage in the whole of India was completed during 2018-2019. Sixteen rubber growing states have been mapped using remote sensing satellite data. Total extent of rubber plantations (age 3 years and above) estimated in the country as per remote sensing data was 7,22,440 ha (Fig. CCES. 1).

2. Identification of potential areas for rubber cultivation in North-East India using satellite data, long term climatic data and soil fertility status

Updating and mapping of acreage estimation of rubber plantations in Tripura

Sl no	Districts (and year)	NR area (ha)
1	South Tripura (2018)	20453
2	Sepahijala (2018)	19792
3	West Tripura (2015)	8859
4	Gomati (2015)	9556
5	Khowai (2015)	5346
6	Dhalai (2015)	5996
7	Unakoti (2015)	4338
8	North Tripura (2015)	2634
	TOTAL	76,954

Source: Landsat 8 OLI (30m) March-April 2018 and 2015 Ground truth points

Fig. CCES. 2. Updated map of distribution of rubber plantations in Tripura

was completed during the reporting year. Mapping of rubber plantations in Arunchal Pradesh was completed. Further acreage estimation of rubber plantation in Assam using latest satellite data was initiated and about seven districts were mapped during 2018-2019.

2.1. Tripura

Mapping of rubber plantations in Tripura was updated. Using satellite data of the year 2015 the estimated rubber area in the state was 76,954 ha (Fig. CCES. 2). Area under NR was the highest in South Tripura and Sepahijala districts and the least was in North Tripura district. Present estimate showed nearly 7.3 per cent of total geographical area in Tripura is under rubber cultivation.

During the period 2010-2015, a total of about 28,583 ha of rubber area has increased in Tripura. Extent of expansion was the highest in South Tripura, Dhalai and Sepahijala districts whereas area under NR expansion was the least in North Tripura district. Other districts such as West Tripura, Gomati, Khowai and Unakoti have considerable extent of expansion of rubber area from 2010 to 2015. Present study showed that approximately 5,000 ha of NR area has increased every year in Tripura during 2010-15.

2.2. Arunachal Pradesh

Mapping and estimation of NR acreage in Arunachal Pradesh was completed during the period 2018-2019. In this state, NR plantations were distributed in East Kameng, East Siang, Namsai and Changlang districts (Table CCES. 1). These districts are bordering with Assam state. Satellite-derived extent of NR plantations in Arunachal Pradesh (age 3 years and above) as of the year 2016 was 718 ha (Table CCES. 1). Interpretations of satellite data showed that substantial extents of young NR holdings (age 3 years or less) are present in the state.

Table CCES. 1. **Satellite-derived area under rubber (age 3 years and above) in Arunachal Pradesh as of 2016**

District	NR area (ha)
East Kameng	180
East Siang	440
Namsai	78
Changlang	20
Total	718

3. Net primary productivity (NPP) of rubber plantations

MODIS based net primary productivity (NPP) of rubber plantations in different agro-climatic regions were studied. Satellite based annual NPP (2014) was downloaded, processed and analysed for rubber plantations in Kerala, Kanyakumari district in Tamil Nadu, Karnataka, Maharashtra, Goa and Tripura. Differences in NPP were observed in the different in agro-climatic conditions. Generally annual NPP of rubber (2014) was less in non-traditional areas compared to traditional region. In Kerala, high NPP was found in Southern and Northern districts whereas Palakkad district showed low NPP. About 65 per cent of the total rubber growing regions in Kerala showed high NPP. Rubber growing regions in Karnataka showed low NPP (18%) especially in Chickmagaluru and Shivamoga districts.

4. Identification of agro-climatically suitable areas for natural rubber cultivation in non-traditional regions of India

Agro-climatic suitability analysis for the cultivation of NR in the North-East states

was completed. Mean monthly climatic parameters of maximum temperature, minimum temperature, amount of rainfall, number of rainy days and potential evapotranspiration (P_{ET}) was obtained for a period of 102 years (1901-2002) from the India Water Portal website (www.indiawaterportal.org) for the NER comprising of a total of 68 districts. Original datasets were utilised to extract the mean monthly values of rainfall, maximum, minimum and average temperatures, vapour pressure and potential evapotranspiration from 1901 to 2002.

Monthly Aridity Index (A_I) defined as the ratio of annual rainfall and potential evapotranspiration (UNESCO, 1979) was considered as a primary variable for assessing the suitability of the rubber tree crop in an area. Higher values of $A_I \geq 1$ indicate less aridity and better climate suitability for crop growth whereas warm arid regions are characteristic of low rainfall and high P_{ET} rates and thus very low A_I values.

The Area-Under-Curve (AUC) is denoted by the total area under a curve in graph units. The agro-climatic conditions prevailing in Kottayam and Kanyakumari districts in the traditional NR growing region are generally considered as the most optimum. Considering these two districts as the benchmark, the extent of deviation in the climatic parameter (A_I) of the NER districts from Kottayam and Kanyakumari were determined. The percentage variations in AUC for each NER district from the respective chosen standard curves were calculated separately from the monthly A_I values mentioned above. Selection of a district was made if the AUC provides a 100 per cent match with that of either Kottayam or Kanyakumari district, which is considered here as the normal category. In order to find out how the agroclimates vary from that of the traditionally suitable regions, the percentage AUC values falling within the 10 and 20 per cent categories were considered in the study.

The "crop criteria" for growth and yield tolerance were considered in terms of the Climate Tolerance Limits (CTL) defined as the extreme tolerance limits of important climatic parameters. Suitability of districts was considered based on the respective variability levels in either the AUC or the CTL analysis.

In terms of suitability based on the AUC analysis it was found that the normal, 10 and 20 per cent variability limits showed 24, 34 and 49 districts, respectively, while the CTL based analysis showed a corresponding 28, 34 and 50 districts, as suitable for NR cultivation, respectively. Out of the 68 districts in the NER, a total of 42 districts over 5 states (Assam-18, Manipur-9, Meghalaya-4, Mizoram-7 and Tripura-4) were identified as normally suitable and 55 and 62 districts in the 10 and 20 per cent variability limits, respectively. No districts in the states of Arunachal Pradesh and Sikkim were chosen in the normal AUC and CTL analyses. Unlike the normal AUC, no districts in the state of Manipur qualified under the normal CTL analysis.

5. Climate resource characterisation of rubber growing areas

The distribution and intensity of rainfall during the two catastrophic flood events of 1924 and 2018 in Kerala was studied. Rainfall data of Kerala state during heavy flooding period from 8th to 16th August 2018 from 60 rain gauge stations was documented and plotted the rainfall in the outline map of Kerala using GIS platform. It was observed that of the two mentioned

years heavy flooding had occurred during 1924.

Rainfall during the 10-day period (8-17th August, 2018) departed more than three times from the extreme downpour from mean. Excess rainfall was not confined to the high ranges alone during 2018. Midland and coastal regions too recorded heavy downpour. Many rain gauge stations in midland recorded all-time high rainfall intensities of more than 20cm consistently for more than a week. It can be interpreted that flood was caused by multi-day extreme rainfall and also partly due to high reservoir storage.

Details of cyclonic disturbances that contributed to the flood of August 2018 were collected. It was observed that the depression formed in northwest Bay of Bengal on 7-8th and 15-17th August had catastrophic effects. More interpretation is in progress in terms of track, intensity and position of the disturbance which caused unusually heavy showers.

6. El nino/La nina episodes and regional climate in rubber growing regions of India

El nino events from 1951-52 to 2015-16 were characterized in terms of initiation, duration, maturity and intensity. It was observed that the SW monsoon rainfall is not affected by El nino characteristics like duration of the episode/event or maximum ONI (ONI when the event/episode is matured) or formation, maturity or decay of the event.

SW monsoon rainfall of the Kerala State and of RRII, Kottayam was found decreasing with increasing Oceanic Nino Index (ONI). However, both the associations were non-significant. It was observed that SW monsoon rainfall of a small spatial scale was not influenced by ONI.

SW monsoon rainfall of large spatial extent was influenced significantly ($p<.01$) by the ONI of June to September. When area-averaged seasonal rainfall series of seven homogeneous zones and the whole India, (developed using highly quality-controlled data from well spread network of 316 rain gauge stations) was regressed with ONI (Jun-Sep), SW monsoon rainfall was observed to be influenced inversely and significantly ($p<.01$) by the ONI of June to September. NE monsoon rainfall and monsoon total (SW + NE monsoon) rainfall did not have any association with respective ONI.

All India annual T_{max}, SW monsoon T_{max} and NE monsoon T_{max} were influenced significantly by the ONI of the respective seasons. ONI influences all the three temperature series significantly ($p<.01$) and positively. On a small spatial scale such as RRII, influence of ONI on temperature time series was not significant.

7. Vegetation dynamics in rubber plantation ecosystems

The experimental plots in four ha area was initiated at CES, Chethakkal. The plots are (1) normal rubber cultivation, (2) unweeded rubber cultivation, (3) controlled weeded cultivation. Another one ha area (4) was left unweeded without rubber plantations. Initial vegetation survey was conducted in the study area using quadrate method. Dominance of invasive exotic species such as *Clidemia hirta, Centrosema pubescence, Lantana camera, Alternanthera braziliana etc.* noticed in all the three experimental plots with rubber, while in the fourth one (without rubber) species diversity was high. It was noticed that high growth rate of invasive alien species has

affected the establishment and growth of rubber saplings.

8. Impact of understory vegetation on microclimate and yield components of natural rubber

Measurement of microclimatic parameters in weeded and unweeded rubber plantations were carried out in plantations at Paika (Kottayam district) and RRS Padiyoor (Kannur district). Temperature inside the un-weeded plantation was lower than the weeded plantation by an average of 0.5°C at Padiyoor and 0.2°C at Paika, Kottayam. Relative Humidity inside the un-weeded plantation was higher than weeded plantation by an average of 2.6 per cent at Padiyoor and 2.4 per cent at Paika. This indicates that rubber plantations with good understory vegetation such as intercrops and other species can maintain the microclimate better than weeded plot.

BOTANY DIVISION

Classical breeding procedures including biparental hybridization, polycross breeding and ortet selection produced good results during the reporting year. Long term performances of hybrids and selections from polycross breeding were utilized for a comparative study of the two major breeding procedures in rubber namely biparental hybridization and polycross breeding. Ortets selected from small holdings and large estates were also evaluated in small scale and large scale trials. Response to ethephon stimulation in the RRII 400 series clones as well as clones in the pipeline was evaluated. Ninety superior pipeline clones were evaluated in central large scale trials and satellite on-farm trials spread over 44 locations in traditional and non-traditional regions for selecting the best clones though participatory clone evaluation with growers. Screening of a second batch of clones in the pipeline for abiotic stress tolerance in non-traditional regions was initiated. Twenty six clones recently imported under a multilateral clone exchange program with five IRRDB member countries were multiplied for further large scale evaluation of their suitability to the agro climatic situation in India. A compilation of results from 10 multi-locational evaluations of PB clones introduced from Malaysia in 1985 indicated scope for release of three of these clones in a region specific manner in the traditional rubber growing area. Long term performances of IRCA clones introduced from Cote d'Ivoire in 1991 also indicated scope for up gradation of two of these clones to Category two of the planting recommendations for the traditional region.

1. Evolving high yielding clones for the traditional area

1.1. Hybridization and clonal selection

Hybrids evolved from earlier Hand pollinations (HPs) were evaluated over the long term in 15 small scale trials while hybridization programs were continued during the reporting year.

1.1.1. Small Scale Trials (SST)

Selections of hybrid clones from three SSTs (1995 A, B and C) were evaluated for their response to stimulation. In the 1995 A trial comprising of 34 experimental clones, all the selected high yielders showed good response to stimulation with an increase in yield ranging from 55.9 to 100.5 per cent (Table Bot. 1). Four clones (89/7, 89/27, 89/145 and 89/309) showed more than 100 g t^{-1} t^{-1} on stimulation. Of these, response to stimulation was comparatively moderate in two top yielding clones (89/27 and 89/309) which showed more than 60 g t^{-1} t^{-1} in unstimulated conditions.

Table Bot 1. **Response to stimulation in selected high yielders (mean of 2 years)**

Clone	Pre-stimulation yield (g t^{-1} t^{-1})	Yield on stimulation (g t^{-1} t^{-1})	% increase
89/7	54.4	100.7	84
89/27	63.6	102.7	65
89/64	23.8	45.6	89
89/79	49.5	83.6	72
89/95	51.5	82.2	56
89/124	50.7	87.9	83
89/145	54.0	107.4	101
89/243	40.1	79.7	97
89/308	55.9	99.4	78
89/309	60.8	100.1	71
89/349	50.8	89.0	77
RRII 105	39.2	75.2	93

The comparative study on response to stimulation among selections was continued in the 1995 B trial which is comprised of 26 WxA hybrids and their parents. Selections showed comparable or superior response to stimulation with reference to check clone, RRII 105 as well as other parental clones *viz.* RO 142, RO 87 and RRIM 600 (Fig. Bot.1).

In SST 1995 C, 17 W x A hybrid clones that resulted from six cross-combinations under the 1990 HP program were evaluated for growth and yield performance. The trees in the trial are in the 15th year of tapping and currently under ethephon stimulation. Among the selected clones, clone 90/109 (63.0 g t^{-1} t^{-1}) recorded the highest yield followed by 90/55 (60.8 g t^{-1} t^{-1}) and 90/102 (60.3 g t^{-1} t^{-1}). Meanwhile, the highest girth was recorded in 90/55 (100.5 cm), followed

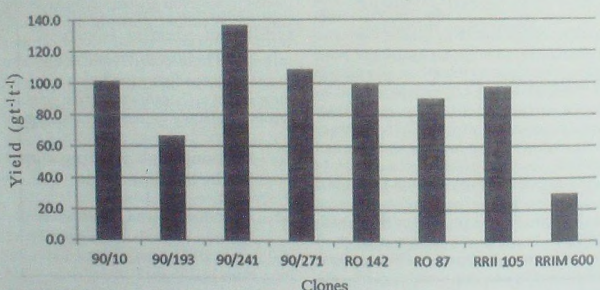

Fig. Bot. 1. Variability in response to Ethephon stimulation among WxA hybrid selections

by 90/109 (93.2 cm), 90/129 (89.4 cm), 90/277 (88.3 cm).

In the 1998 A SST, 16 hybrid clones evolved from 1993 HP are under evaluation. Response to stimulation in 11 high yielding clones selected from the 16 clones under evaluation was undertaken by applying three rounds of stimulation as per recommended practice. All the high yielders showed very good response to stimulation with an increase in yield ranging from 35 to 73 per cent (Table Bot. 2). Clones 93/5, 93/263, 93/37, 93/45 which were selected for further evaluation showed good response to stimulation ranging from 49 to 73 per cent substantiating its potential as promising clones.

Table Bot. 2. **Response to stimulation in selected high yielders (mean of 2 years)**

Clone	Mean yield ($g\ t^{-1}\ t^{-1}$) (8^{th}-12^{th} year)	Pre-stimulation yield ($g\ t^{-1}\ t^{-1}$)	Yield on stimulation ($g\ t^{-1}\ t^{-1}$)	% increase
93/39	53.9	38.4	59.4	54
93/5	47.4	46.3	67.0	49
93/2	54.9	45.8	67.6	50
93/17	58.0	49.6	74.1	56
93/248	54.2	60.3	79.4	35
93/48	67.3	54.5	82.4	52
93/263	47.8	56.3	86.4	62
93/45	51.8	58.8	91.1	56
93/250	52.4	63.3	92.5	48
93/37	58.3	57.2	94.8	73
93/172	62.2	77.4	105.9	43
RRII 105	53.6	42.5	67.6	67

Trees in 1998 B SST are currently in the 14^{th} year of tapping. Response to stimulation in one high yielding clone selected from the five clones under evaluation showed very good response to stimulation with 53 per cent increase in yield.

In another SST (1999) comprised of progenies from 1994 hybridization programme, there are five superior selections *viz.* 94/87 (RRII 105 x RRIM 703), 94/92 (RRII 105 x Mil 3/2), 94/296 (RRII 105 x RRII 118) and 94/567 (RRIM 600 x RRII 203), identified based on superiority in growth and yield. A study was initiated for assessing the yield response of above selections under Ethephon stimulation.

In SST 2001 A, top yielding clones *viz.* 95/413, 95/425, 95/121, 95/304 and 95/410 maintained their superiority in terms of growth and yield performance with dry rubber yield ranging from 50 to 65 $g\ t^{-1}\ t^{-1}$ as compared to check clone RRII 105 (45-55 $g\ t^{-1}\ t^{-1}$, mean of three trials). Among the 36 clones under field testing in 2003 trial, two selections *viz.* 96/417 and 96/459 maintained high yield performance during 14^{th} year of evaluation. Among 21 hybrid progenies under clonal nursery evaluation (2008), six selections (02/688, 02/638, 02/690, 02/844, 02/514 and 02/335) registered superior growth and yield performance when compared to check clone RRII 414. The above selections have been identified for evaluation under next phase of Farmer Participatory Clone Evaluations.

The clonal nursery of hybrid clones (Wickham x Amazonian wild germplasm) derived from the progenies of 1997 hybridization programme was maintained with identity of the accessions at Central Experimental Station (CES), Chethackal. The population is a repository of WxA hybrids serving as source bush nursery and reference collection of the original WxA hybrids which includes many selections under large scale evaluation through Participatory Clone Evaluation Programme (Table Bot. 3). The accessions were assessed for *Oidium* powdery mildew incidence under unsprayed condition in the field. There was no variation for the disease among the accessions as all the accessions

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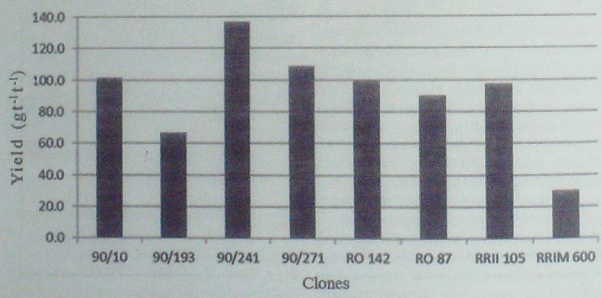

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showed powdery mildew infection albeit varying intensity.

Table Bot. 3. **Pedigree of some of the Wickham x wild germplasm hybrids in the clonal nursery**

Sl. No.	Clone	Parentage
1.	97/4	RRII 105 x MT 1014
2.	97/10	RRII 105 x MT 1027
3.	97/213	RRII 105 x MT 1005
4.	97/238	RRII 105 x MT 1021
5.	97/13	RRIM 600 x MT 999
6.	97/47	RRIM 600 x RO 380
7.	97/62	RRIM 600 x MT 1021
8.	97/98	RRIM 600 x MT 1005
9.	97/255	RRIM 600 x MT 1005
10.	97/125	RRIM 600 x MT 1014
11.	97/166	RRIM 600 x AC 495
12.	97/196	RRIM 600 x MT 1027

1.1.2. New generation hybrids

With an aim to develop high-yielding clones through transgressive introgression, hybridizations were carried out during 2011-14 using high-yielding Wickham clones (RRII 105, RRII 414, RRII 429 and RRII 430) as female parents and superior Wickham / Amazonian hybrids (95/10, 95/34 and 95/274) as male parents. Presently, 353 hybrid seedlings from 14703 hand pollinations and more than a thousand half-sibs of the above parents are being evaluated in seedling nurseries. Based on the test tap yield recorded consecutively for two years, 205 half sibs and 63 hybrids were selected and multiplied along with control clones. The plants are being maintained in root trainer nursery at CN, Karikkattoor for field planting during 2019.

Hybridization programmes involving different bi-parental cross combinations were continued in 2019 in the Breeding Orchard at RRII Farm, Kottayam. Hybridizations were done involving 27 cross combinations of selected parents and a total of 7106 hand pollinations were performed in 2019. From the earlier hybridizations, a total of 410 seedlings which included 150 hybrids and 260 half-sibs, were planted in seedling nursery in 2018 for further evaluation.

1.2. Ortet selection

Clonal nursery evaluation of ortets selected from the South Andamans, evaluation of ortets selected from non-traditional regions and large scale evaluation of ortet clones selected from large estates and small holdings were continued.

1.2.1. Clonal Nursery Evaluation

From the clonal nursery evaluation of ortets and hybrids 2007, five clones *viz.* RRSG 9, RRSG 4, Par 18, Par 10 and P 270 with promising test tap yield and girth, were found superior to RRII 105 and were on par with the modern check clones RRII 414 and RRII 430 (Fig. Bot. 2). These plants were selected for the final phase of evaluation under Participatory Clone Trial (PCE, Phase 6).

In clonal nursery trial (2012) of Andaman ortets at CES, Chethackal, ortets selected from the oldest surviving seedling populations (source GG1 and GG2 seedlings of PBIG, Malaysia) located at south Andamans, were assessed for test-tap yield in the 5th year of planting. Yield of the ortets ranged from 64 g t^{-1} $15t^{-1}$ (And-Or 103/8) to 156 g t^{-1} $15t^{-1}$ (And-Or 88/4) when compared to check clone RRII 105 (133 g t^{-1} $15t^{-1}$) and RRII 430 (203 g t^{-1} $15t^{-1}$). Four ortets performed better than RRII 105.

2. Evaluation of clones

Nine large scale and three on-farm evaluations were in progress during the year.

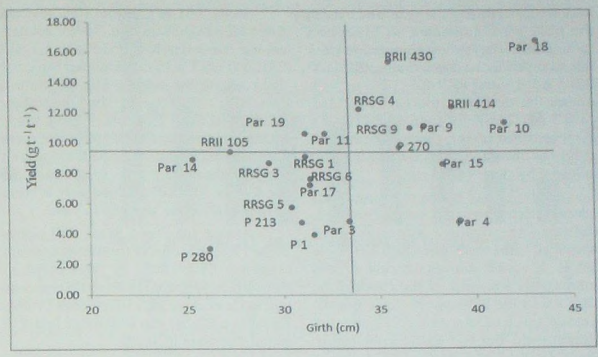

Fig. Bot. 2. Selection of clones for high yield and girth

2.1. Large scale evaluation (LST)

In the two LSTs of RRII 400 series clones at CES (LST I and LST II), significant clonal variation for yield under stimulation was observed. When evaluated for response over the 16th and 17th years of tapping, clones RRII 417 and RRII 429 in Trial 1 and RRII 430 and RRII 410 in Trial 2 were superior to RRII 105 with respect to yield under stimulation in the renewed panels. RRII 105 gave a yield of 66 g t^{-1} t^{-1} in both the trials. Clones RRII 414, RRII 402, RRII 403, RRII 52, RRII 422 and PB 330 maintained high yields above 66 g t^{-1} t^{-1} in the two years and were on par with the check. Among the high yielding clones of the RRII 400 series, RRII 429 gave the highest response of 28.7 per cent improvement in yield on stimulation, followed by RRII 417 which responded with 15.9 per cent improvement in yield. The two high yielding clones RRII 430 and PB 330 did not respond well to stimulation in terms of per cent yield improvement. RRII 105 showed around five per cent response in terms of yield improvement when stimulated. Clone PB 330 (0.38 m^3 $tree^{-1}$) exhibited maximum bole volume. Among the RRII 400 series, clones RRII 417, RRII 414, RRII 407, RRII 402 and RRII 410 with 0.16 m^3 $tree^{-1}$ showed promising timber yield.

In the 1994 LST, 12 clones were under evaluation. The trees in the trial were subjected to ethephon stimulation during the year. Stimulated yield and girth of the clones were recorded. The highest girth was recorded in hybrid 86/120 (101.6 cm) followed by ortet clone O 65 (94.3 cm), RRII 51 (81.1 cm), RRIM 712 (80.5 cm) and 55/180 (80.4 cm). The highest stimulation yield was recorded in RRIM 728 (163.0 g t^{-1} t^{-1}) followed by RRIM 712 (133.0 g t^{-1} t^{-1}), 86/120 (119.0 g t^{-1} t^{-1}) and RRIM 722 (118.0 g t^{-1} t^{-1}).

In LST 1999A, nine clones were under evaluation. Girth and yield of the clones

were recorded during the year. The trees in the trial were subjected to Ethephon stimulation. The highest stimulation yield was recorded in the check clones RRIM 600 (131.5 g t^{-1} t^{-1}) and RRII 105 (130.6 g t^{-1} t^{-1}). Among the test clones, ortet clones 12 (116.8 g t^{-1} t^{-1}), 46 (114.6 g t^{-1} t^{-1}) and ortet 4 (114.2 g t^{-1} t^{-1}) recorded higher yields. The highest girth was recorded in clone 12 (75.3 cm) followed by clone 4 (70.5 cm).

In the LST of nine promising ortets selected from Cheruvally estate, five ortets showed significantly superior girth compared to the check clone RRII 105 (Table Bot. 4). Six ortets showed dry rubber yield comparable with that of the check clone RRII 105 in the BO-1 panel (Table Bot. 4). Among the trial clones Cyo 72 showed highest yield, but was found to be highly susceptible to ALF disease. Six clones showed comparable yield with check clone in the first year of BO-2 panel with Cyo 41 as the top yielder. Cyo 41 showed significantly superior girth and very low incidence of ALF disease. Selections *viz.* Cyo 41 (P 126), Cyo 35 (P 133) and Cyo 48 (P 129) are also being evaluated in the PCE trials.

In the LST at CES, of ortets selected from Mundakkayam and Koney estates, among the ortets KO9 (53.8 g t^{-1} t^{-1}) and RRII 430 (49.7 g t^{-1} t^{-1}) exhibited superior yield among the ortets while the control clone RRII 105 (67.9 g t^{-1} t^{-1}) had the highest yield in the trial. In terms of girth, ortets MO28 (64.2 cm) and MO45 (63.4 cm) were found superior while clone RRII 430 was on par with them (62.1 cm). In contrast, the check clone RRII 105 attained only 56.9 cm girth though it was superior in yield.

Among the Prang Bazar (PB) clones in the 1989 LST, PB 235 showed maximum response (Fig. Bot. 3) to Ethephon stimulation compared to other PB clones as well as check clone RRII 105. Clones PB 260, PB 217 and PB 280 also responded better to stimulation than RRII 105.

2.2. On-farm evaluation

To evaluate the performance of pipeline clones in the South Karnataka region, an on-farm trial was laid out at KFDC, Karnataka with 14 high-yielding pipeline clones and seven control clones. The same pipeline clones are also under

Table Bot. 4. **Girth (cm), yield (g t^{-1} t^{-1}) and ALF incidence in ortet clones**

Clone	Girth (cm)	ALF incidence	Yield (g t^{-1} t^{-1}) (Mean in BO-1)	Yield (g t^{-1} t^{-1}) (First year in BO-2)
Cyo 72	59.2cd	Very high	47.6a	39.5b
Cyo 30	64.6b	Low	46.0a	46.7ab
Cyo 43	60.7bcd	Medium	45.1ab	50.9a
RRII 105	57.3de	Medium	40.9ab	51.1a
Cyo 41	74.3a	Very low	40.5ab	51.4a
Cyo 35	65.0b	High	40.0abc	44.0ab
Cyo 18	63.4bc	Medium	36.9bcd	50.7a
Cyo 48	77.0a	Low	31.2cd	42.9ab
Cyo 68	52.8e	Medium	28.2d	28.2c
Cyo 31	61.3bcd	Medium	14.5e	26.1c
CV			14.18	14.5

Values followed by same letters are not significantly different based on DMRT RRII 105 is the check clone

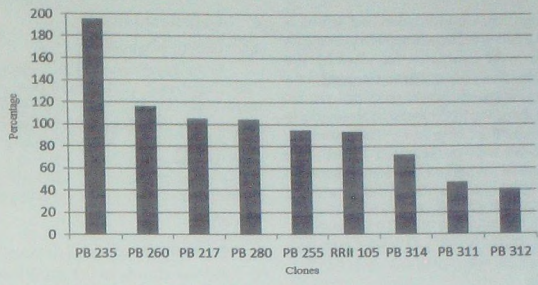

Fig. Bot. 3. Clonal response (%) to Ethephon stimulation

evaluation in PCE Phases I (2008), II (2010) and III (2012), in various other locations. The clones in the above on-farm trial were evaluated for growth performance in the fourth year of planting. Six pipeline clones showed better growth compared to RRII 105. Pipeline clone P 102 showed the highest girth.

An on-farm trial with 12 clones *viz.* RRII 105, RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, PB 330, P 021, P 026, P 064, P 070 and P 081 planted in Chithalvetty estate, Punalur in 2016, was maintained.

2.3. Investigations on genotype x environment interactions

In continuation of the physiological evaluation of pipeline clones, second set of 37 pipeline clones were bud-grafted and raised in nurseries at CES, Chethackal, RRS, Agartala and Dapchari for field planting in 2019. Physiological parameters such as leaf membrane permeability for drought and cold stressed conditions, stomatal density, chlorophyll index were studied in a selected set of one-year-old plants of pipeline clones. In terms of membrane permeability under drought stress, clones P 200, P 180, P 173 were found tolerant while clones P 196, P 112, P 202, P 096, P 065 and P048 were found susceptible. Under cold stress, clones P 196, P 112, P 173 were found highly tolerant while P 174, P 181, P 071, P 080, P 200 and P 073 were found susceptible. In terms of chlorophyll index, clones, P 180, P 173, P 048, P 181 and P 201 were found tolerant. In terms of stomatal density, clones P 096, P 073, P 180, P 080, P 200, P 071 and P 047 were found tolerant along with check clone RRIM 600.

3. Polycross progeny evaluation

In the observational trial of polyclonal seedlings (Nettana seed garden origin) and check clone RRII 105, polyclonal seedlings showed superior girth and yield. Eighty five polyclonal seedlings performed better than clone RRII 105. While 18 seedlings had more than 100 g t^{-1} t^{-1}, 56 progenies had more than 50 g t^{-1} t^{-1} showing better possibility for selection.

In order to identify pre-potency in PB clones to develop high yielding clones

through half-sib evaluation and selection, 337 half-sib seedlings from high yielding PB clones were evaluated for yield by test tapping. The yield under test tapping in the 3rd year showed that 40 seedlings yielded more than 20 g 10 t^{-1} of which five seedlings had more than 50 g 10 t^{-1}. The promising half-sibs will be selected for further evaluation. When the correlation between girth and test tap yield was analysed, correlations of 0.6 and 0.3 were observed in population and top yielding 40 seedlings, respectively. Yield and girth within the selected seedlings were not correlated.

Forty one selections from the polycross progeny population at Kottayam were bud grafted. 1868 buddings were done in all, and the budding success was 86 per cent. A root-trainer nursery of 1622 plants was raised at Central Nursery (CN), Karikkattoor and the nursery is being maintained. All the selections from Nagrakata and Dapchari were cutback for bud wood generation.

4. Genetic studies

4.1. Genetic parameters

In the genetic analysis experiment at CES, Chethackal, parents and progenies were assessed for growth and yield parameters. Among full-sib families, RRII 105 x RRII 118 achieved maximum yield with more than 65 g t^{-1} t^{-1} in the 16th year of tapping (Fig. Bot. 4). Clone RRII 33 maintained its lowest yield trend of less than 20 g t^{-1} t^{-1}.

5. Participatory evaluation of rubber clones in the pipeline

5.1. Source Bush Nurseries of pipeline clones

At the CES,Chethackal, 282 pipeline clones were maintained in 17 source bush nurseries.

5.2. PCE trials under mature phase

A total of six Central Large Scale Trials at CES,Chethackal and RRII Farms and 39 satellite On Farm Trials are in progress

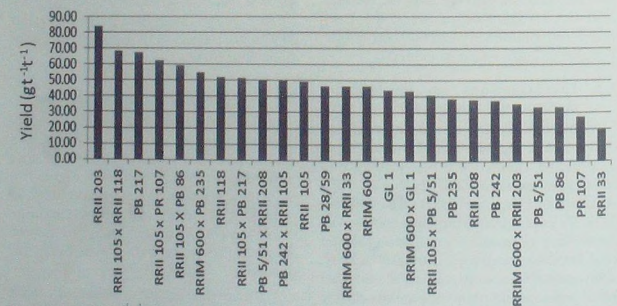

Fig. Bot. 4. Yield (g t^{-1} t^{-1}) of parental clones and their full-sibs

under five phases of Farmer Participatory Clone Evaluation.

5.2.1. Phase 1 (2008)

In the Central large Scale Trials, there was significant clonal variation for yield in the second year of tapping. All the three check clones were comparable in yield in LST 1, while RRII 105 was inferior to RRII 414 in LST 2. Clone RRII 414 was the highest yielding check clone and showed superior summer yield in both the trials. Among the pipeline clones, P 021, P 067 and P 074 in LST 1 and P 044, P 066 and P 026 in LST 2 were the highest yielders with >60 g t^{-1} t^{-1} but were statistically comparable with RRII 414 which gave yields of 59.96 and 66.95 g t^{-1} t^{-1} in LST 1 and 2, respectively.

A pooled analysis of clonal performances in the first year of tapping across the LSTs and several OFTs was done. The mean yield of the first batch of 11 pipeline clones and three check clones in the first year of tapping was 36.54 g t^{-1} t^{-1} across seven locations. The clonal performance was best in Calicut Estate followed by Thuravaiyar Estate, Kanyakumari and Mooply Estate, Thrissur. The check clone RRII 430 was the highest yielder in Adirappally, followed by P 067 and P 015. In Calicut, clone P 088 was the highest yielder followed by RRII 430 and P 015. In Mooply Estate, pipeline clone P 068 along with RRII 105 registered the highest yields followed by P 074. In Manikkal Estate, Mundakkayam, clone P 067 was the highest yielder followed by P 060 and RRII 430. In Vithura, clone P 015 was the highest yielder followed by RRII 430 and P 067. In the Central Large Scale trial at CES (LST1) clone P 015 was the highest yielder followed by RRII 105 and RRII 430. In Kanyakumari two of the pipeline clones *viz*. P 010 and P 061 out yielded the highest yielding check clone RRII 430.

The performance of the second batch of 11 pipeline clones and three checks in the first year of tapping was compared in six locations. The mean yield in the first year was 37.16 g t^{-1} t^{-1} across locations and clones. The mean yield was highest in the Punalur region, in Shaliacary estate followed by Be Be Estate. In the first month of tapping in Kanjirappally, P 026 was the highest yielder followed by P 072. In Shaliacary estate, RRII 430 was the highest yielder followed by pipeline clones P 066 and P 026. In Kootickal estate, pipeline clone P 066 was the highest yielder followed by RRII 430 and P 026. In Perinthalmanna, P 026 was the highest yielder followed by P 015. In Be Be estate, RRII 430 was the highest yielder followed by P 026 and P 066. In the Central LST 2, RRII 414 was the highest yielder followed by P 015 and P 087. RRII 430 was the highest yielder in two out of the six locations.

When averaged across the seven locations where the Batch 1 clones were planted, RRII 430 recorded the highest yield in the first year of tapping closely followed by pipeline clones P 067 (half sib progeny of parent clone PB 215) and P 015 (parentage RRII 203 x PB 5/51). In terms of mean yield in the first year across the six locations where the Batch 2 clones were tested, the pipeline clones P 026 (PB 242 x RRII 105) was the best performer followed by P 066 (half sib progeny of parent clone PB 28/83). These two clones out yielded the check clones RRII 414 and RRII 430. Clone P 015 also performed well in terms of initial yield in these locations.

Under the Phase 1 trials, based on initial yield in farmer participatory trials in various locations, two hybrid clones evolved through bi-parental mating *viz*. P 015 and

P 026 along with two clones evolved by polycross breeding *viz.* P 066 and P 067 exhibited high yield, comparable to the high yielding check clones RRII 430 and RRII 414.

5.3. PCE trials under immature Phases 2 to 5

5.3.1 Phase 2 (2010)

Girth, tappability and secondary observations like disease incidence were recorded from the Central LST and eight OFTs. In the LST, there was significant clonal variation among the 14 pipeline clones and three checks. Check clones RRII 414 and RRII 430 were the most vigorous. Pipeline clones P 044, P 080 and P 070 were comparable with the check clones in terms of girth.

Among the OFTs, Shaliacary estate was the most favourable location for growth of the clones, followed by Calicut estate. Mean girth across locations was the best in clones RRII 430 and RRII 414. Among the pipeline clones P 044, followed by O 054, P 070 and P 064 registered high mean girth of more than 50 cm in the seventh year.

In terms of tappability, Bethany and Vaniampara estates were the best locations registering 73 per cent tappable trees, followed by Shaliacary estate with 71.1 per cent tappable trees while the mean tappability across locations 6½ years after planting was 60 per cent. Among the clones, the check clones RRII 414 and RRII 430 registered the highest tappability of 88 and 87 per cent, respectively.

Clones under OFT in Pudukkad Estate were under tapping. Among the pipeline clones, P 44 and P 64 maintained their superior growth and yield trend.

5.3.2. Phase 3 (2012)

In the 2012 LST at CES, Chethackal, among the pipeline clones, P 110 (32 cm) showed good growth performance after RRII 430 and RRII 417 (34 cm). P 101, P 102 and P 104 performed better in terms of girth than RRII 105 (28 cm). In the 2012 OFT at Chemoni Estate, Thrissur, pipeline clone P 110 with 44 cm mean girth maintained its superior growth trend only matched by check clone RRII 417 with the same girth, six years after planting. Three more pipeline clones (P 142, P 104 and P 101) showed better growth than the popular check RRII 105 (38 cm). In Phase 3 Kumbazha estate (Pathanamthitta), clones P 110 and P 104 showed high vigour (50-51 cm) comparable to RRII 430 (52cm) and RRII 417 (49 cm) in the 7th year of planting. Leaf retention under ALF was high in P 114 and P 142. In the trial at Cheruvally estate, among pipeline clones, maximum girth was recorded in P 110 (38 cm), followed by P 101 (37 cm) P 104 (36 cm) and P 142 (34 cm), and minimum girth was recorded in P 158 (26 cm). At Calicut Estate, Kozhikode, two pipeline clones showed better growth than RRII 105 in the sixth year after planting. P 110 showed better girth than RRII 430. Most of the experimental clones were better than RRII 105 in terms of disease tolerance. At KFDC, Nettana, growth parameters and disease incidences were recorded. Based on three years' girth data, clone P 126 (34.9 cm) was found superior which was followed by RRII 417 (29.8 cm), P 129 (26.5 cm), PB 330 (25.8 cm) and RRII 430 (24.8 cm) while RRII 105 exhibited 23.7 cm. P 126 and RRII 417 continued to be superior throughout the three year period.

5.3.3. Phase 4 (2014)

Twenty three pipeline clones along with four check clones are being evaluated in Central Large Scale Trial (CLST) at Kottayam and two on-farm trials at Chemoni Estate (Thrissur Dt., Kerala) and

Kailiyadu Estate (Palakkad Dt., Kerala). The pipelines include four Wickham x Wickham hybrids, 13 Wickham x Amazonian hybrids, four selections from polycross progenies and two ortet selections. The above pipeline clones were evaluated for their initial growth and field tolerance to abnormal leaf fall (ALF) caused by *Phytophthora*, fourth year after planting. There was significant difference in girth among the experimental clones in terms of growth and adaptive traits. Among the pipeline clones, ten clones performed above population mean with P 129 (CyO 48; Cheruvally Ortet) and PB 330 (PB 5/51 x PB 32/36) showing maximum girth (32 cm) better than RRII 105 and comparable with check clone RRII 430. Other check clones RRII 414 and RRII 417 had high girth. Among WxA hybrids, P 177 (RRIM 600 x RO 380) and P 171 (RRIM 600 x MT 1021) showed better girth (31 cm).

In the OFT at Chemoni, P 129 (Cheruvally ortet) and P 174 (WxA hybrid) were found to perform better after check clones RRII 414, RRII 417 and RRII 105. Interestingly, the same clone performed well in the Large Scale Trial at Kottayam. Among WxA hybrids, P 174 (RRIM 600 x MT 1014) showed better performance when compared to RRII 430. At Shoranur also, P 129 performed well. Among WxA hybrids, P 171 (RRIM 600 x MT 1021) was found better. The initial performance of pipeline clones at three different locations (two OFTs and one LST) indicated emergence of location preferences among the clones. Regarding ALF incidence, there was significant difference in leaf retention among the pipeline clones in both the LST and OFTs. While there was indication for environmental influence on disease incidence among the pipeline clones, the disease tolerance trait of wild germplasm accession RO 142 and other WxA hybrids observed during small scale evaluation was further established in the LST and OFTs.

In Bethany estate, Kanyakumari, RRII 414 (49.5cm) had highest girth in the 5th year of planting P 126, P 171 and P 172 were found superior in terms of girth (37cm). At Thiruampady Estate, Kozhikode, four pipeline clones showed better growth compared to RRII 105 and RRII 414. Among the pipeline clones, Cheruvally ortets P 126 and P 133 along with a hybrid P 168 and a half sib P 071 showed maximum growth. Most of the experimental clones showed good level of disease tolerance. At the Manikal estate, girth attained by the clones during the year is shown in the descending order with the highest girth in P 129 followed by P 133, P 71, P 120, P 168, P 174, P 126, P 171, P 180, P173, P 48, P 89, P 73, P 177, P 172, P 93 and P 121. Among the check clones, the highest girth was recorded in RRII 414.

5.3.4. Phase 5 (2016)

Under PCE Phase V, vacancy filling was carried out and the trials was maintained for further evaluation. In the OFT at Cherupittakavu Estate, Punalur, early growth performance of pipeline clones and check clones was assessed in the third year of planting. At State farming corporation, Punalur, growth of three year old plants was good except for the plants supplied against fire damage affected plots.

5.3.5. Phase 6 (2019)

Multiplication of planting materials for Phase 6 of PCE trial was initiated. The pipeline clones selected for the new phase included half sibs, ortets and hybrids selected from clonal nurseries. Check clones for each region was selected based on Region Specific Clone Advisory (2017), with

RRII 430 as the common check. Five locations were selected including one LST in CES, Chethackal and four OFTs at Kanyakumari, Punalur, Padiyoor and Nettana.

6. Breeding for other specific objectives

Breeding programmes for abiotic as well as biotic stresses were emphasised in the year under report.

6.1. Breeding for drought tolerance

In SST 1999 A, analysis of average yield over nine years showed maximum yield in 94/50 (70 g t^{-1} t^{-1}) followed by 94/23 and 94/44 (69 g t^{-1} t^{-1}) and 94/101 (64 g t^{-1} t^{-1}). The clones were assessed for response to stimulation. Maximum yield after stimulation was recorded in 94/50 (108 g t^{-1} t^{-1}) followed by 94/44 (108 g t^{-1} t^{-1}).

In an attempt to develop drought tolerant clones for the non-traditional area, the drought tolerance capacity of the selected progenies, developed through hybridization between high yielding clone (RRII 105; female parent) and a drought tolerant clone (PB 280; male parent) and the reciprocal crossing (PB 280 x RRII 105), were evaluated in a clonal nursery trial in the drought prone area (RRS, Dapchari). Forty experimental clones along with nine control clones were planted in the trial. Nineteen clones showed superior girth than the most vigorous check clone RRII 208. Three hybrid clones (96/114, 96/69 and 96/66) exhibited test tap yield consistently superior to the top most yielding check clone RRIM 600. Clone 96/69 which showed superior yield had the highest girth as well.

Growth and yield performance of selections was monitored for 13 years of tapping among 15 hybrids and check clones in 1998 A SST at CES. Selections, which are also under evaluation in Phase 5 of PCE *viz.* 93/214 (69 g t^{-1} t^{-1}) and 93/216 (57 g t^{-1} t^{-1}), maintained superior yield trend compared to RRII 105 (45 g t^{-1} t^{-1}). In order to develop clones tolerant to drought, 17 pipeline clones were laid out in a large scale trial at Dapchari during the year along with 3 check clones in Randomized Block Design with three replications and 16 trees per plot.

6.2. Breeding for disease tolerance

Under disease resistance breeding programme, the superiority of top selections was further ascertained through second round of test tapping carried out four years after planting in the nursery. Top hybrid selection from RRII 414 x Fx 516 had very high yield of 343 g t^{-1} $15t^{-1}$ and top selection from half-sibs of Fx 516 had 302 g t^{-1} $15t^{-1}$. Interspecific hybridization was carried out among high yielding RRII clones of *Hevea brasiliensis* (RRII 430, RRII 414, RRII 105) and other *Hevea* spp. *viz. H. spruceana* and *H. camargoana* as well as RO 380 (a putative genetic variant of *H. spruceana*) in order to fortify the high yielding RRII clones with disease tolerance. After establishment in nursery evaluation trails, the putative hybrids from the above interspecific hybridization programme were evaluated for nursery test-tap yield. The test-tap yield of hybrids ranged from 1 g t^{-1} $15t^{-1}$ to 54 g t^{-1} $15t^{-1}$. Four full-sibs had more than 50 g t^{-1} $15t^{-1}$ while seven progenies had more than 40 g t^{-1} $15t^{-1}$. Preliminary observation on disease incidences of the progenies indicated putative tolerance of many of the progenies against shoot rot and other leaf sport diseases as indicated by absence of apparent disease symptoms under field conditions.

7. Anatomical investigations

7.1. Wound callus as explant for tissue culture

During the hardening process, root trainer plants develop callus tissue at the root apex through air pruning. The callus has immense potential for root architecture modification. Structural organisation and metabolic status of this starved tissue may have ample scope to be used as explant in tissue culture experiments. Callus developed on rooted cuttings of selected clones were used as explant in tissue regeneration. The tissue culture process is underway.

7.2. Petiolar anatomy for identification of *Hevea* clones

A set of petiole anatomical traits *viz.*, shape of the vascular bundles in the abaxial and adaxial side, inter-vascular continuity, medullary bundles, intrusion of cortical cells into the pith, shape of pith *etc.* were used for identification of six *Hevea* clones *viz.* RRII 414, RRII 417, RRII 422, RRII 430, RRII 105 and RRIC 100. Among these, shape of vascular bundles, features of inter-vascular continuity and intrusion of cortical cells, proportion of vasculature and pith area in the stele are observed to be reasonably stable anatomical traits for clone identification. RRII 414 shows a close affinity to the female parent RRII 105, and RRII 430 shows more resemblance to its male parent RRIC 100. The other two clones *viz.* RRII 417 and RRII 422 are intermediate with respect to these traits. Structural traits showed more resemblance between RRII 414 and RRII 422. Among the six clones studied, RRII 430 showed both morphological and anatomical identity with RRIC 100. It is obvious that compared to morphological traits, anatomical characteristics represent the environmental adaptation of clones and hence, are more stable and reliable adjuncts for identification of modern *Hevea* clones.

8. Studies on propagation

8.1. Drought tolerant rootstocks

A study aimed at developing drought tolerant rootstocks for the non-traditional area by evaluating the drought tolerance capacity of the seedlings from non-traditional areas compared to seedlings from traditional areas was carried out in a nursery trial at RRS, Dapchari. Seeds were collected from three drought prone non-traditional rubber growing areas namely Maharashtra (RRS, Dapchari), Orissa (RRS, Dhenkanal) and Karnataka (HBSS, Nettana) and from traditional areas namely Kerala (CES, Chethackal) and Tamil Nadu (HBSS, Paraliar). Assorted seeds as well as seeds from polyclonal seed gardens and drought tolerant clone RRIM 600 and drought susceptible clone RRII 105 were collected from each of above locations. Seeds were also collected from drought tolerant clone RRII 203 located at Orissa. The seedlings were maintained under rain-fed condition at Dapchari. Bud-grafting of those seedlings which survived drought condition at Dapchari was carried out using drought susceptible clone RRII 105 as scion. Bud-grafting of the unselected seedlings at Central Nursery, Karikkattoor was also done using RRII 105 as scion. Field planting and maintenance of both droughts survived and control plants budded with RRII 105 was carried out for evaluation of the drought survived stock as against the unselected stock.

8.2. Modified root trainer plants (MRTP) for disease control through crown budding

Modified root trainer plants were raised in the nursery as per the standardised methodology. Crown budding on thirty MRTP was carried out in the nursery. Ten MRTP with crown bud success was transplanted in the field.

9. International Clone Exchange

Replenishment of domesticated germplasm with clones from other rubber growing countries via bilateral and multilateral exchanges was successfully achieved. A total of 44 high yielding and disease resistant clones were imported from 10 countries. Import arrangements are being done for two Nigerian rubber clones.

9.1. Bilateral clone exchange

In the SBN at CES, no major disease incidences or plant quarantine pathogens were observed in clones imported from Vietnam (eight clones) and China (five clones).

9.2. Multilateral Clone Exchange

Ten CMS SALB-resistant clones imported from Ghana and Cambodia were monitored for major diseases including plant quarantine pathogens. Three clones (USM1, Philippines; RRISL 203 and RRISL 219, Sri Lanka) were also imported. The above clones were bud-grafted and polybag nursery was established at CES. Clones imported from other countries including those from Vietnam (8), China (5), Cambodia (5), Thailand (5), Indonesia (3) and Sri Lankan (3) were also maintained in the SBNs at CES. In the SBNs of the imported clones from Thailand, Indonesia and Sri Lanka, disease incidence during the year was recorded. *Colletotrichum* and *Oidium* leaf diseases were recorded in varying intensities, shoot rot was also noted. Sri Lankan clones RRISL 211 and RRISL 219 recorded zero level to very low level incidence of diseases.

Twenty six clones introduced from China, Vietnam, Cambodia, Indonesia and Thailand was multiplied for laying out large scale evaluations in 2019.

9.3. International *Hevea* clone museum

The international *Hevea* clone museum, consisting of high-yielding as well as SALB-resistant clones imported from various countries, was maintained with identity at RRII Main Campus at Kottayam.

10. Arboreta of *Hevea* clones and forest species

The arboretum of *Hevea* consisting of 55 clones as demonstration-cum-research plot was maintained at RRII Main Campus, Kottayam. The arboretum of 63 assorted forest plant species along with *Hevea*, planted in 2014, was maintained at RRII Main Campus.

11. Multilocational evaluation of Prang Besar Clones

A report on the long term performance of four PB clones *viz.* PB 312, PB 314, PB 280 and PB 255 in LSTs and OFTs across the traditional rubber growing region was tabled for approval to release the best among the PB clones in terms of growth and yield. Data from the following field evaluations in Kerala, Tamil Nadu and Karnataka were collated:

- Large Scale Evaluation - 1989, RRII Farm, Kottayam, Kerala
- Large Scale Evaluation - 1989, Central Experiment Station, Chethackal, Kerala
- Large Scale Evaluation - 1994, Keeripari, Kanyakumari, Tamil Nadu
- Large Scale Evaluation - 1989, Hevea Breeding Sub-Station, Nettana, Karnataka
- Large Scale Evaluation - 1996, Regional Research Station, Padiyoor, Kannur, Kerala
- Small Scale Evaluation - 1991, Hevea Breeding Sub-Station, Nettana, Karnataka
- On-Farm Evaluation - 1988, Koney Estate, HML Ltd., Konni, Kerala

Table Bot. 5. **Global mean girth and yields in station trials and on farm trials**

Clone	Girth at opening (cm)			Rubber yield (kg ha^{-1} year^{-1})		
	Station Trials	On Farm Trials	Mean	Station Trials	On Farm Trials	Mean
PB 255	54.2	53.4	53.8	2579	2029	2304
PB 280	55.0	47.3	51.1	3123	2162	2643
PB 312	55.7	45.7	50.7	2812	1763	2288
PB 314	54.5	49.7	52.1	2782	2047	2415
RRII 105	54.2	48.3	51.2	2180	1917	2049

- On-Farm Evaluation - 1988, Malankara Estate, Thodupuzha, Kerala
- On-Farm Evaluation - 1992, Shaliakary Estate, Punalur, Kerala
- On-Farm Evaluation - 1994, Mount Horeb Estate, Sasthamkotta, Kerala

The growth and yield of clones PB 280, PB 255 and PB 314 were superior to that of RRII 105 over long term in various locations in the traditional region (Table Bot.6). These clones are highly susceptible to abnormal leaf fall and powdery mildew but have shown tolerance to Corynespora, pink disease and tapping panel dryness. Clones PB 280 and PB 255 are promising in timber yield too.

12. Multilocational evaluation of IRCA clones

Long term performance of five IRCA clones *viz.* IRCA 18, IRCA 109, IRCA 111, IRCA 130 and IRCA 230 introduced from Cote de Ivoire in 1991 were evaluated and a report was submitted for upgradation of the best two clones. The following were the field trials from which data was collated:

- Large Scale Evaluation, 1992, Central Experiment Station, Chethackal, Kerala
- Large Scale Evaluation, 1994, Kanyakumari, Tamil Nadu
- Large Scale Evaluation, 1996, Regional Research Station, Padiyoor, Kerala
- On Farm Evaluation, 2010, HML Malankara Estate, Thodupuzha, Kerala
- On Farm Evaluation, 2010, HML Mooply Estate, Thrissur, Kerala
- On Farm Evaluation, 2010, BC Cheruvally Estate, Erumely, Kerala
- On Farm Evaluation, 2010, Calicut Estate, Kozhikode, Kerala

Table Bot. 6. **Girth and long term yield across three station trials in the traditional region**

Clone	Girth at opening (cm)	Mean yield over 8-10 years (g t^{-1} t^{-1})
IRCA 18	51.2	48.2
IRCA 109	49.5	51.5
IRCA 111	53.4	57.0
IRCA 130	52.2	72.9
IRCA 230	52.5	48.6
RRII 105	48.7	59.0

Clones IRCA 130 and IRCA 111 were the best in terms of growth and yield in the traditional region. Initial observations on growth in OFTs also show these clones to be on par with RRII 105.

13. New Project

A project on Alternative sources of natural rubber: Studies on the feasibility of cultivation of guayule rubber (*Parthenium argentatum*) as an alternative source of NR in arid/semi-arid areas and marginal lands of India' has been initiated. Seeds of 18 varieties of Guayule from USA are awaited.

GERMPLASM DIVISION

The genetic resources of *Hevea* being conserved at RRII belong to three gene pools - the domesticated gene pool with clones derived from the original Wickham collection of 1876, the wild germplasm belonging to the 1981 IRRDB collection, and the collection of other *Hevea* species. Apart from the conservation of these resources, the management of the wild germplasm collection – its agronomic evaluation, screening for diseases, drought and cold stress resistance, timber latex traits and utilization in crop improvement, is the focus of the Division.

1. Introduction, conservation and documentation

1.1. Domesticated genepool (Wickham collection) from secondary centers

183 Wickham clones are being conserved in a budwood nursery (the clone museum) at RRII, Kottayam, and three arboreta (Germplasm Gardens) at CES, Chethackal. The clones in the bud wood nursery are being assessed for their tolerance levels to the primary leaf diseases. The arboreta serve the primary purpose of conservation and scientific data collection as and when necessary, and are a source of clonal flowers for breeding when required.

Five IRCA clones introduced from the Cote d' Ivoire in 1992 are being conserved and evaluated in Germplasm Garden 92. Among the five clones, IRCA 130 and IRCA 111 recorded the highest girth (86.3 and 85.4 cm respectively) as compared to the control clone RRII 105 (71.7 cm). IRCA 130 was superior to all other clones for yield (69.4 g t^{-1} t^{-1}). In the Germplasm Garden 94 comprising 20 Wickham clones, RRIM 609 was the highest yielder (100.9 g t^{-1} t^{-1}), followed by RRIC 100 (71.5 g t^{-1} t^{-1}) and RRII 23 (64.9 g t^{-1} t^{-1}), compared to 54.7 g t^{-1} t^{-1} for the popular clone RRII 105. RRII 23 also continued to be the most vigorous clone (111.2cm). Four other clones had girth more than 100 cm, compared to the RRII 105 (80.7cm). RRII 23 was used as a parent in this year's WxA hybridization programmes.

1.2. 1981 IRRDB wild germplasm

This gene pool, originally introduced during 1984-1990, is being conserved in field gene banks in the form of budwood nurseries and arboreta.

1.2.1 Conservation nurseries

3576 accessions have been re-established in compact new conservation-cum-source bush nurseries (SBNs) from 2003 to 2008, while the older nurseries continue to serve as a source of flowers for hybridization programmes. This year, six wild accessions (MT 43, MT 185, AC 2004, MT 2217, RO 2871 and MT 4788) were used in a WxA hybridization programme with elite Wickham clones. Ten wild accessions with a high number of latex vessel rows were identified from 701 wild accessions in SBN 2005: these will be included in the breeding pool for yield improvement. The large number of potentially useful accessions identified during the characterization and preliminary evaluation in the juvenile stage for yield, yield contributing traits like latex vessels, disease and drought tolerance traits, are being put into Further Evaluation Trials. Simultaneously, these are also being established in separate budwood nurseries (the Germplasm Working Collection) in order to ensure better care and accessibility. So far, 119 potential wild accessions have

been established in the GWC, to which potentially useful accessions will be added as and when identified.

1.2.2. Heveatum

A separate *Hevea* arboretum (or Heveatum), comprising all the available genetic resources, is being established in phases at Teksragre farm, Tura, Meghalaya, with the primary intention of ensuring an insurance collection, as well as facilitating free cross pollination and genetic mixing between the different gene pools. So far, a total of 466 wild and Wickham accessions have been established here, and 128 multiplied for the next phase. Another arboretum comprising 120 accessions established earlier, is being maintained at Central Experiment Station, Chethackal.

1.3. Other *Hevea* species

This gene pool is being conserved as an arboretum established in 2006 at CES. It conserves six accessions of five other species available at RRII *(H. benthamiana, H. spruceana, H. nitida, H. camargoana* and two accessions of *H. pauciflora)*, along with five natural putative interspecific hybrids, two *H. brasiliensis* clones, and FX 516 (an interspecific cross between *H. brasiliensis* and *H. benthamiana*).

2. Characterization and preliminary evaluation

The last three trials in the Preliminary Evaluation Trial format were planted at RRS, Padiyoor in 2000 (A&B) and 2002. Nine relatively high yielding selections from here AC 3131, AC 552, RO 2136, RO 1313, AC 567, AC 1964, RO 341, MT 4351 and RO 210, and vigorous accessions for timber traits MT 4219, AC 4140, MT 387, AC 647 and RO 2883 are being conserved as male parents for future WxA hybridization programmes.

3. Further evaluation and selection

Detailed evaluation of selections from preliminary evaluations are carried out in clonal nurseries (CNs) for accessions with 50-80 per cent of the test tap yield of the controls, while those with more than 80 per cent yield are evaluated in field trials (FETs) at normal spacing.

3.1. Clonal nursery evaluation

Of the three wild accessions (AC 2199, MT 1056, AC 2027) with good growth and test tap yield higher than RRII 105 identified from a clonal nursery planted in 2010 at Central Experiment Station, accession AC 2027 was multiplied and raised in a polybag nursery at HBSS, Nettana for planting the next FET in 2019.

3.2. Further evaluation trials

All accessions with more than 80 per cent of the control yield on preliminary evaluation, as well as those with potential secondary traits, were subjected to detailed evaluation in FETs in statistically laid out trials at normal spacing. There are currently six FETs comprising 117 accessions.

The FET 2003 comprising 22 wild accessions and three control clones was evaluated for growth and yield traits. RO 2629 recorded the highest girth followed by MT 2233 and AC 626 compared to the check clone RRII 105, while the accession RO 2629, AC 4149 and AC 716 recorded the highest yield.

Among the 22 wild accessions in FET 2005, MT 43, MT 4788, AC 2004 and MT 1077 had the highest yields in the 4th year of tapping (24.1 – 16.4 g t^{-1} t^{-1}), compared to the check clones RRII 105, PB 260 and RRIM 600 (22.5, 17.5 and 15.3 g t^{-1} t^{-1} respectively). ALF was high this year. The second round of visual assessment of ALF

showed an almost similar trend as last year, though with greater severity. The three high yielders MT 43, AC 2004 and MT 1077 also had the least incidence, while MT 4788 had the maximum disease incidence among the 22 accessions.

Among the immature trials, in FET 2008, RO 2846 (69.6 cm), AC 176 (67.5 cm) and MT 200 (66.2 cm), recorded the highest girth in the eleventh year of growth as compared to the check clone RRII 105 (57.3 cm). Among the 13 accessions evaluated in FET 2010 at CES, Chethackal, there were six accessions with girth higher than clone RRII105 and two accessions with girth on par with clones RRII 430 and RRII 414. In another FET 2013 at CES, a set of 22 selected wild accessions along with three control clones are in the fifth year of growth. The wild accession AC 167 (33.4 cm) followed by AC 5280 (29.8 cm) and RO 2784 (27.3 cm) recorded the highest girth, compared to the check clone RRII 105 (24.1 cm).

3.3. On-farm trials

Selections from FETs are subjected to multi location evaluation in On-Farm Trials for confirmation of yield potential. The first OFT was established in 2010 at five locations *viz*. B.C. Cheruvally estate in Erumely, Malankara estate in Thodupuzha, Mooply estate in Trissur, Calicut estate in Kozhikode and Bethany estate in Kanyakumari for evaluating the performance of the three selected IRCA clones (IRCA 130, IRCA 111, IRCA 109) and one wild accession (AC 166). The trees were opened for tapping in Mooply and Malankara estates.

At Mooply estate, Trissur, among the four test clones, wild accession AC 166 recorded the highest girth (55.1 cm) followed by IRCA 130 (48.8 cm). Among the check clones, RRII 430 (59.3 cm) was superior to RRII 414 and RRII 105. Among the test clones, mature yield was highest in IRCA 130 (58.6 g t^{-1} t^{-1}), while the yield of wild accession AC 166 was on par with RRII 105 (38.9 and 35.7 g t^{-1} t^{-1}, respectively) in the first year of tapping. Among the check clones, RRII 430 recorded highest yield of 72 g t^{-1} t^{-1}. Tappability per cent was highest for IRCA 111 (78.6).

At Malankara estate, clone IRCA 130 recorded the highest girth (50.6 cm) followed by IRCA 111. Among the check clones, RRII 414 (62.9 cm) was superior to RRII 430 and RRII 105. IRCA 130 recorded highest tapability per cent (87.2). Among the test clones, highest mature yield was recorded by IRCA 130 (25.9 g t^{-1} t^{-1}) and the performance of wild accession AC 166 was poor in this estate. Among the check clones, RRII 430 recorded the highest yield (51.1 g t^{-1} t^{-1}).

In both BC Cheruvally estate (Erumely) and Calicut estate (Calicut), AC 166 and IRCA 130 recorded the highest girth as compared to the check clone RRII 105.

At Bethany estate, Ullimala, maximum growth was recorded for clones IRCA 109 (50.7 cm) and IRCA 111 (48.6 cm). The mean girth of the check clones was 51.5 cm, 53.0 cm and 51.5 cm, respectively for RRII 105, RRII 414 and RRII 430. The tappability for the trial plots ranged from 90 (IRCA 130) to 97.9 per cent (IRCA 109). This trial was damaged in the cyclone Ockhi, and had to be wound up.

4. Screening for stress tolerance

4.1. Screening for biotic stress tolerance

A set of 41 short listed wild *Hevea* accessions along with two control clones are under evaluation for confirmation of field tolerance to *Corynespora* disease in a 'hotspot' trial at Ulickal nursery, Iritty, Kanoor.

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

In the clonal nursery of 40 potential half-sibs of nine clones at RRS, Dapchari, 20 half-sib progenies from eight pre-potent clones were selected on the basis of test tap yield and growth under drought stress. These selections were multiplied at RRS, Dapchari and a polybag nursery was raised for a bud wood nursery of these selections, which will be advanced to a large scale trial at RRS, Dapchari for developing drought tolerant clones. In the clonal nursery at RRS, Padiyoor with 31 potential half-sibs of eight clones, six half-sibs from three pre-potent clones were selected on the basis of test tap yield and growth under drought stress. These selections will be advanced to LST.

In the further field evaluation of selected *Hevea* clones at RRS, Dapchari in collaboration with Botany Division, the growth during the summer and peak periods of growth in the 34 selected *Hevea* clones planted in 2007 comprising 23 wild accessions, five HP clones and six 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 showed girth superior to drought tolerant clone RRIM 600. Highest girth was recorded by accession MT 4856 (53.7 cm) followed by MT 1619 (52.4 cm). Out of 5 hybrid clones evaluated, hybrid 93/105 recorded highest girth. Among the check clones, RRII 430 recorded the highest girth of 54.3 cm. Among the wild accessions, highest mature yield was recorded by MT 4788 (22.6 g $t^{-1} t^{-1}$) followed by MT 4856 (14.4 g $t^{-1} t^{-1}$). Among the hybrid clones, 93/105 recorded highest mature yield of 25.2 g $t^{-1} t^{-1}$ and the check clone RRII 430 recorded highest yield among all (50.1 g $t^{-1} t^{-1}$) proving its drought tolerance. Around 200 OP seeds were collected from this field trial and raised a seedling nursery. The growth and yield performance of these seedlings will be studied for selecting potential recombinants of wild and Wickham clones.

4.2.2. Cold tolerance

Sixty four wild *Hevea* accessions are under evaluation for cold stress in two trials at Regional Experiment Station, Nagrakata, West Bengal. Highest girth was recorded inRO 2902, MT 923 and MT 5105 as compared to the check clones SCATC 93/114 and RRIM 600 in Trial 1, while accession RO 2727, MT 915 and RO 3197 recorded the highest girth compared to that of the controls Haiken 1 and RRIM 600 in Trial 2. The accession AC 4653, AC 3514 and MT 915 recorded the highest yield among all the wild genotypes.

5. Screening for timber characteristics

5.1. Field screening

Twenty five genotypes were evaluated for annual girth to identify potential timber clones in evaluation trial at RRS, Padiyoor. The accession MT 941, MT 1032 and AC 650 recorded the highest girth as compared to the control clone RRII 118, which indicated its timber potential.

6. Utilisation of *Hevea* germplasm

6.1. Hand pollination programmes

Six promising hybrids from the 2009 hand pollination programme at CES, Chethackal, involving three wild accessions and six cultivated Wickham clones, were under multiplication for further evaluation. At RRS, Padiyoor, 29 seedling progenies derived from two cross combinations in

2009, along with 25 OP seedlings of RRII 105, were evaluated in a seedling nursery. Ten hybrid progenies from two parental combinations and seven OP seedling progenies were selected and multiplied and a polybag nursery was raised at HBSS, Nettana for conducting a Clonal Nursery evaluation at HBSS, Nettana. A bud wood nursery of these selections is maintained at RRII campus.

From the 110 progenies of the 2009 and 10 interspecific HPs, six were selected for further evaluation and mutiplied. At RRII, from the 104 hybrids of the 2013 WxA HP involving two cross combinations and one interspecific combination (*H. brasiliensis* x *H. benthamiana*), 11 were selected on the basis of two rounds of test tapping and multiplied for further evaluation. In HP 2014, juvenile evaluation in the 104 progenies of the three surviving WxA combinations and one interspecific combination, 17 accessions were multiplied for further evaluation.

In the 2016 HP, of the 100 WxA hybrids planted in the seedling nursery, of the 75 surviving hybrids from 3 WxA cross combinations, progenies of the cross with the *Oidium* tolerant parent RO 2871 showed the highest vigour and survival percentage. However, the highest incidence of ALF was also observed in this combination (66%), while the least in the combination RRII 105 x RO 4599 (50%).

In the 2019 HP, 1026 cross of 6 wild and 2 Wickham parents were carried out, with initial fruit set of 59 (5.7%).

6.2. Open pollinated progeny evaluation

Growth of the surviving 282 OP seedlings collected from the further evaluation trials FETs 2003 and 2005, which comprised preliminary selections from the wild germplasm, interspersed with high yielding Wickham control clones was monitored. 137 seedlings have reached the girth of more than 7cm, and will be tapped next year.

6.3. Phenotyping of mapping population for QTL identification

QTLs were identified for three major diseases from this population (results reported in section under Pathology Division).

7. Other studies

7.2. Studies on Alternative Sources of Natural Rubber yielding plants

Ceara rubber (*Manihot glaziovii*) - Seven germplasm accessions from Palakkad Region and four plants multiplied through stem cutting collected from Vaikom region are being conserved at RRII.

BIOTECHNOLOGY DIVISION

Crop improvement through biotechnological interventions is the prime objective of research in the Biotechnology Division. Genetic transformation in rubber was fine tuned for developing *Hevea* transgenic plants integrated with genes of agronomic importance. Experiments were done for incorporation of genes with desirable traits for imparting enhanced biotic and abiotic stress tolerance, improving latex yield and growth. Perfecting the system for development of antibiotic marker free transgenic plants was also attempted. Research programmes aimed to complement conventional breeding were also carried out. Other than these, development and perfection of protocols for the propagation of elite *Hevea* clones from different explants, development of ploidy variants, *in vitro* approaches for disease tolerance and cloning and characterization of genes are also being envisaged.

1. Development of transgenic plants

1.1. Genetic transformation for stress tolerance

1.1.1 Multiple gene integration by repeated transformation

Repeated transformation was performed by infecting *hmgr*1 transgenic cell lines with *Agrobacterium* strain EHA 101 harboring *MnSOD* gene construct. New putatively transgenic cells emerging from the infected tissues were proliferated in the antibiotic medium. Frequency of transformation was 15 per cent. GUS assay was performed in the transgenic cell lines. GUS positive transgenic cell lines were sub cultured in the embryo induction medium for inducing somatic embryos. Somatic embryogenesis was achieved in two transgenic cell lines. PCR analysis was performed with DNA extracted from the two cell lines using *hmgr*1 and *SOD* primers to monitor the presence of the transgene. Positive amplification of *hmgr*1 and *HbSOD* transgene was observed in the two transgenic cell lines.

1.2. Genetic modification with *HMGR*1 gene for improved latex yield

1.2.1. Gene integration studies

Gene integration was studied in the acclimatized *hmgr*1 positive transgenic plants, of zygotic origin, by Southern hybridization. Genomic DNA was extracted from the *hmgr*1 transgenic plants and digested with two restriction enzymes namely *Hind*111 and *Xba*1; the digested products were separated in an agarose gel and hybridized with digoxigenin labeled *hmgr*1 probe. Gene integration was confirmed in the PCR positive plants and the copy number of the transgene was different among the transgenic plants. The latex yield was recorded in the selected transgenic plants where better yield was observed compared to control. Expression studies in these plants showed higher transcript level of the *hmgr* 1 transgene. The data were compiled and published.

1.2.2. Development of HMGR1 transgenic plants from clonal explants

Genomic DNA was extracted from the transgenic cell lines obtained from the infected callus of clone RRII 105. PCR was carried out using promoter specific primers to determine the presence of the transgene in the cell lines. Positive amplification was observed in the transgenic cell lines.

*HMGR*1 integrated embryos and plantlets were developed *in vitro* from the clonal explants of *Hevea*. The plantlets were transferred to small polybags for hardening.

1.3. Genetic transformation with *Osmotin* gene

1.3.1 Molecular reconfirmation of gene integration and the identification of integration pattern

Molecular characterization of already developed transgenic plants were carried out. DNA was isolated from all the transgenics and their respective control plants. PCR was done with gene specific as well as npt II primers and all the plants were tested PCR positive. DNA was digested with *Hind* III and *Xba* enzymes and Southern hybridization was done with dig labeling system. Positive signals were obtained in all the *transgenic* plants tested.

1.3.2. Evaluation for cold tolerance

Transgenic and control callus were subjected to cold treatment from 1-25 days at three different temperatures (4°C to -20°C and -30°C) and growth was assessed after subculture. It was observed that transgenic calli showed better growth and survival after cold treatment compared to control. The selected lines were multiplied and kept for embryo induction.

1.3.3. Experiments on plant hardening

Since hardening is a major hurdle in plant tissue culture, experiments were carried out for improving hardening. It was observed that selection of plants is an important criteria for hardening. Plants were transferred for acclimatization at different stages of development such as plants prior to leaf emergence, plants with medium matured leaves and plants with matured leaves. Germinated embryos just after the shoot emergence and before the leaf emergence was found to perform better compared to others. The nutrient absorption of tissue culture plants was found to be impaired after two to three weeks of hardening. Foliar spray of nutrient solution was found to be effective for improving the hardening process to some extent. Plants were transferred to polybags filled with vermicompost and soil. Around 50 somatic plants and ten transgenic plants were transferred for hardening. Out of which ten somatic plants were hardened and established in the field. Out of the 10 transgenic plants regenerated, thre plants could overcome the initial hardening phase and survived until three months. However they failed to develop further. Hardening experiments are being continued.

1.4. Development of transgenics integrated with *IPT* gene for TPD tolerance

Transformation experiments were carried out with embryogenic callus induced in leaf explants of clone RRII 105, as the target tissue, during the reporting period. Several transgenic lines incorporated with ipt gene could be obtained. From among them, ten transgenic callus lines tested to be GUS positive were proliferated. The proliferated callus was sub cultured in embryo induction medium fortified with stress inducing compounds as well as phytohormones. Somatic embryo induction and transgenic plant regeneration was also attempted from the proliferated transgenic callus of clone RRII 105 obtained from earlier experiments. Embryo induction with good frequency was obtained from seven lines. Transgenic embryos germinated *in vitro* and full plant regeneration obtained. Hardening of the plants is being attempted.

1.5. Development of biotic/abiotic stress tolerant plants with HSP31 gene

Genetic transformation experiments were carried out with the HSP31 gene construct to incorporate HSP31 gene into *Hevea* for imparting abiotic/biotic stress tolerance. *Agrobacterium* mediated genetic transformation was carried out using proliferated embryogenic calli of *Hevea* clones RRII 105 and RRII 414 using the gene construct in pBIN vector in *Agrobacterium* strain LBA4404. Very high transformation efficiency (above 50%) was obtained. Since the construct does not have a qualitative marker all the proliferating transgenic lines obtained were maintained by regular subculture and somatic embryo induction was attempted. Somatic embryogenesis from the transgenic callus of clone RRII 105 and RRII 414 could be obtained from several transgenic lines. The embryos induced in clone RRII 105 could germinate and full plant regeneration obtained *in vitro*. Attempts for hardening of the plants are being carried out.

1.6. Development of antibiotic marker-free transgenics

New transformation experiments were performed for validating the *PNS* gene construct in *Hevea*, using zygotic embryo derived callus as the explant. Results are awaited.

A project proposal entitled "Development of marker free transgenics for enhancing stress tolerance in *Hevea brasiliensis*" was submitted to DST for external funding.

1.7. Gene identification for development of climate resilient transgenic plants

Preliminary attempts were made to identify genes imparting tolerance to both abiotic/biotic stresses in *Hevea*. Genes which were over expressed under drought, cold and disease conditions were selected from the transcriptome data. It was observed that the transcripts of small heat shock proteins (sHSPS), namely 15.7, 17.5 and 18.2 and translationally controlled tumor proteins (*TCTP*) genes were activated under abiotic/biotic stress conditions. Primers were designed for the amplification of the respective genes from *Hevea brasiliensis*.

Genomic DNA of clone RRIM 600 was used for the PCR amplification of the sHSPS (15.7, 17.5 and 18.2) and TCTP genes. The PCR product was gel purified, cloned into pGEM T vector and sequenced. Plasmids were isolated from the recombinant colonies and sent for sequencing. The sequence data was compared and after editing the vector sequences it was observed that sHSP 15.7 with 761 bp had one intron and two exons, the other two forms namely Hsp 17.5 with a length of 474 bp and Hsp 18.2 of length 423 bp were intron less. It was observed that the *TCTP* gene of length 835 bp had three introns and four exons.

A project proposal entitled "Development of *Hevea* transgenic plants integrated with the translationally controlled tumour protein (TCTP) towards improved vegetative growth and tolerance to tapping panel dryness syndrome" was submitted to SERB, DST for external funding.

2. Propagation of elite *Hevea* clones

2.1. Somatic embryogenesis and plant regeneration from immature anther

Plantlets derived from immature anther of *Hevea* clone RRII 414 were hardened and acclimatized. These plants

were field planted along with control plants. Experiments on induction of callus and embryogenesis were repeated during the current year. After surface sterilization, immature anther was inoculated on callus induction medium. Callus induced was got proliferated on the same medium and the cultures were kept for embryogenic callus induction.

2.1.1. *Sequence analysis of HbSERK gene in Hevea*

Somatic Embryogenesis Receptor-Like Kinase (*SERK*) is the only one gene that is involved in the transition of somatic cells to an embryogenic state, which is capable of differentiating into an embryo at later stage of development. The *SERK* gene identified in *Hevea* was 408 bp in length. Comparative analysis of *SERK* cDNA sequences with genomic sequence showed the presence of 2 exons separated by one 204 bp intron. *In silico* translation of *HbSERK* gene based on the predicted ORF yielded a 136 amino acid protein. Comparison with *SERK* sequence in NCBI data bank revealed its similarity with *Arabidopsis thaliana, Medicago truncatula, Cocos nuciferla* and *Araucaria angustofolia*. *HbSERK* showed 78 per cent similarity to that of *AtSERK1* nucleotide sequence level and 99% at amino acid level to *AtSERK1*. This high similarity between *HbSERK* and *AtSERK1* at amino acid level indicates that these two genes are orthologs. Multiple sequence analysis of *HbSERK* proteins displayed the presence of Ser/Thr protein kinase in subdomain VI. In the kinase domain of *HbSERK*, the 29 amino acid residue activation loop (A-loop) is present in subdomains VII and VIII which is 100 per cent identity to *AtSERK1*. Similarly, as in *AtSERK1*, *HbSERK* A-loop also contains 4 threonine residues and one tyrosine residue. The unrooted phylogenetic tree constructed using *HbSERK* and other reported *SERK* genes indicated that *HbSERK* was tightly clustered with *AtSERK1* and *MtSERK1*.

2.2. Somatic embryogenesis from leaf explants

Cultures were initiated with leaf explants of clones RRII 105 and RRII 414 and plant regeneration attempted. Embryogenic callus initiation and somatic embryogenesis could be obtained in presence of stress inducing compounds such as poly ethylene glycol and phytagel in the embryo induction medium. The embryogenic callus was proliferated and sub cultured in fresh medium every 50 days for further embryo induction. The proliferated callus was also used as target tissue in genetic transformation experiments. Several plants were regenerated *in vitro* and experiments carried out for hardening. Plants could survive the initial phase of hardening in soilrite with controlled humidity and watering once in three days with 1/10th Hoagland solution.

2.3. Self-rooted clones by *ex vitro* adventitious rooting

Shoots from bud grafted plants maintained in polybags in the glass house were experimented for *ex vitro* rooting. The mature shoots after hormone pulse were transferred to soilrite and rooted *ex vitro* under humidity control. Clonal plants rejuvenated through somatic embryogenesis were established as stock plants in nursery. Bud grafted plants were generated from green buds collected from these stock plants. Shoots from these plants are also being tried for *ex vitro* rooting. Suitable conditions and potting medium necessary for rooting has to be optimized after necessary experimentation.

3. Development of homozygous haploid plants

3.1. Development of gynogenic haploids through embryo sac culture

Mature female flowers were collected from different clones *viz*. RRII 105, RRII 414, RRII 422, RRII 429 and RRII 430. Embryo sacs were isolated and cultured over a few media combinations identified suitable for callus induction from previous studies. Callus induction could be obtained from the cultured embryo sacs in all the above clones, though the frequency of callus induction varied among the clones. These calli have been subcultured in callus proliferation medium. Haploid embryos obtained from previous cultures belonging to clones RRII 105 and RRII 414 were sub cultured for further development in maturation and germination media. A few of the embryos germinated and are in the plant regeneration phase.

3.2. Gynogenic haploids through ovule culture

Mature female flower buds of *Hevea brasiliensis* clone RRII 105 and RRII 422 were used for culture initiation. Unfertilized ovules were dissected and cultured in semi-solid medium supplemented with 2,4-D, BA and GA_3. Cultured ovules were subjected to temperature shock for four weeks. Embryo sacs were isolated from the enlarged ovules and cultured in the same media combination. Callus induction efficiency was 4 per cent and the frequency of proliferation was 2 per cent. The proliferated calli became embryogenic and proembryogenic masses appeared after two months of culture in the embryo induction medium. Somatic embryos induction was observed in the cultures raised from both the clones namely RRII 105 and RRII 422.

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

4.1. Induction of Polyembryony

4.1.1. Stock -scion interaction studies

Studies on stock scion interaction using uniform seedlings and their budgrafted counterparts were continued. An attempt was made to identify the impact of divergent root stock on the epigenome of scion in grafted rubber plants in collaboration with Genome Analysis division. Own rooted seedlings along with its five bud-grafted plants were used for a comparative analysis using genetic and epigenetic molecular markers. The plants were uniformly maintained and their DNA was subjected to MSAP analysis using the standard protocol. Polymorphic DNA methylation bands corresponding to CG as well as the plant-specific CHG types of methylation were observed. To understand the functional relevance of the four prominent loci showing variations in DNA methylation patterns, the corresponding MSAP bands from the bud-grafted plants were isolated and sequenced. Detailed sequence analysis revealed that the region of interest lies in the promoter region of a Leucine-rich Repeat Receptor Kinase (LRR-RK) gene. Cloning of selected polymorphic regions and bisulfite sequencing confirmed the presence of methylation in the promoter and coding region of important genes including an LRR receptor kinase gene. The entire LRR-RK gene from *Hevea* with promoter region was also extracted from *H. brasiliensis* clone RRII 105 whole-genome sequence data.

Altogether, the results from the study clearly indicate that heterografts in rubber are epigenetically divergent despite sharing the same scion material. The variation in DNA methylation patterns observed in the

bud-grafted *Hevea* plants can be specifically attributed to stock–scion interaction, because maximum uniformity among them was maintained in terms of growing environment (temperature, relative humidity, and light intensity), soil properties, nutrient, and water availability. The study assumes importance in *Hevea*, because accumulation and maintenance of epigenetic changes in functional genes and promoters during subsequent cycles of vegetative propagation may contribute towards intraclonal variability eventually leading to altered phenotypes. The study suggests that DNA methylation changes may accumulate in plants through successive vegetative propagation cycles (budding) and the cumulative effect may alter desirable phenotypic characters like yield which may affect the productivity of high yielding rubber clones in the future. Furthermore, the present study paves the way for better understanding of the stock–scion interaction process in rubber and it is anticipated that unravelling the mystery behind this complicated process may lead to a solution for the intraclonal variation among popular rubber clones. Data were compiled and a research article was published.

4.1.2. Development of uniform seedlings

A field planting with own rooted uniform plantlets and their budgrafted counterparts was established for further molecular studies. For scaling up of the existing pathway and development of more embryogenic calli lines, immature open pollinated fruits were inoculated in already reported media for developing embryogenic lines. Polyembryonic ovules were identified and cultured for proliferation. Yield recording was initiated in the polyembryony derived plants planted in field during 2009.

5. Development of polyploids

Embryogenic callus derived from clones RRII 105 and RRII 414 were treated with colchicine by culturing over medium supplemented with different levels of colchicine. However it was observed that the cultures did not respond positively and emergence of new callus from the colchicine treated callus did not occur. The experiments were repeated with colchicine purchased from two different suppliers. Results of these experiments are awaited.

6. *In-vitro* screening of rubber clones against *Corynespora* pathogen

6.1. *In vitro* tolerance of callus against cassiicolin toxin

Immature inflorescence of eight clones of *Hevea* belonging to tolerant and susceptible groups under study were used as the initial explants for callus induction. Fresh, proliferated calli were exposed to cassiicolin by transferring to proliferation medium additionally supplemented with different volumes of filter sterilized crude toxin (0.5, 1.0, 2.0 and 3.0 ml/100 ml medium). Calli inoculated on toxin free proliferation medium were used as control. The cultures were incubated under dark conditions at 25±2°C. After three weeks in culture, the effect of toxin was assessed through visual evaluation of various parameters such as percentage of necrosis, colour and morphological changes of the callus.

It has been observed that in the toxin enriched medium, percent survival of calli from different clones under study showed marked difference. Moreover the response was well in accordance with the findings of the detached leaf technique. Overall growth and survival of intact callus could be

observed only in the tolerant group. Clones GT1 and FX 516 were found to be the most tolerant, being unaffected even at the highest level of toxin tried (3.0 ml). Clones RRII 414 and RRII 430 showed tolerance up to 2.0 ml of toxin. Regarding the susceptible clones, even the lowest level of toxin (0.5ml) was lethal to PB 217 and PB 260 while in the case of RRII 105 and RRII 203, browning and necrosis of the calli was observed with 1ml toxin onwards.

6.2. *In vitro* screening for *Corynespora* tolerance using cassiicolin

RRII 105, the most popular and widely cultivated clone, which at the same time susceptible to CLFD, was selected for this experiment. Embryogenic calli were cultured over medium fortified with different levels of cassiicolin toxin. After six weeks in culture, the toxin insensitive calli were selected, proliferated in toxin free medium, and again exposed to cassiicolin toxin for two months. Tolerant calli obtained after two cycles of such selection were transferred to toxin free media for further development including embryo induction and maturation. Upon transferring the mature embryos to germination and plant regeneration media, a few of the embryos regenerated into plantlets. An initial survival rate of 20 per cent was obtained when the regenerated plantlets were subjected to hardening. Those surviving plants are in the next stage of acclimatisation in big poly bags.

GENOME ANALYSIS LABORATORY

On-going research projects in the Genome Analysis Laboratory are grouped under four major areas of research *viz.* (1) development, optimization and validation of molecular tools for the assessment of genetic diversity and evolutionary relationships in rubber and genetic linkage mapping (2) development of genetic markers for biotic and abiotic stress tolerance and understanding the stress adaptation processes through transcriptome analysis (3) cloning and characterization of agronomically important genes and (4) rubber genome sequencing and *de-novo* assembly. Besides the above research programs a collaborative project has been initiated with CSIR-NEERI on conversion of tropical forests to rubber plantations in Kerala and its impact on the soil environment and different eco-restoration strategies.

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

1.1. Genetic assessment of international rubber clones received through clone exchange program of IRRDB

RRII could successfully establish best 40 clones from almost all rubber growing countries: Indonesia, Cambodia, Thailand, Philippines, Vietnam, Sri Lanka, Myanmar, Ghana and Cote D'Ivoire through clone exchange program. It is necessary to do the genetic assessment of these clones along

with the high yielding clones existing in RRII, especially the 400 series clones and RRII 105 using molecular markers. This will help in understanding the extent of genetic diversity existing among these clones compared to RRII clones and also to develop clone specific SSR profiles for their identity.

So far 23 SSR markers were used in the characterization of 34 international clones along with 5 popular clones. In total 107 alleles were generated. Genetic relationship established among these clones revealed that the clones were highly diverged (genetic diversity ranged from 30% to 70%). Among these 34 clones seven clones belong to Cirad-Michelin Selection (CMS) received from Cambodia (5 clones), Ghana (2 clones) showed maximum diversity (>60%). Twelve clones could be identified based on unique fingerprints generated by SSR markers. Two clones, USM1 from Philippines and IRCA 331 from Cote d'Ivoire were found to be genetically identical, although they were from two different countries. Pedigree of the clones was reflected in clustering patterns. For example, two of the Vietnam clones RRIV1 and RRIV5 were clustered together due to their common parentage (RRIC 110 x RRIC 117). Cluster analysis clearly separated the clones of Indian origin. SNP genotyping of these clones was also performed by HRM analysis using an SNP from MVK gene (MVK2628A/G). All the clones were clearly grouped in to three genotypes (AA, GG or AG) along with reference clones.

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

1.2.1. *Cis-prenyltransferase (CPT) gene isoforms associated with rubber biosynthesis*

Protein sequences of *CPT* gene from *Hevea brasiliensis* and orthologs of *CPT* from other plants species and representative organisms from major domains of life were subjected to phylogenetic analysis to understand the evolution, diversity as well as relationship of *CPTs* prevailing among living organisms. Simultaneously, DNA based phylogenetic analysis of all the *Hevea CPTs* were performed to classify them based on their sequence structure similarity.

Phylogenetic analysis of *CPT* sequences revealed that *Hevea CPTs* formed a separate cluster from the other plant species indicating their uniqueness. DNA based phylogenetic analysis indicated the presence of three different forms of *CPTs*. Error in the existing nomenclature of *CPTs* as cis-1, cis-2, cis-6 *etc.* was also identified based on sequence homology studies. The unique nature of *CPT* gene in *Hevea* and the differences between various *CPT* gene isoforms was estimated. Extensive sequence analysis with reference to *CPT* genes from whole genome, transcriptome and nucleotide databases revealed that variants/isoforms of this gene are existing in *Hevea*, which may have a functional role in the synthesis of extra-long chain backbone of natural rubber. The rest of the sequences designated as *CPT* isoforms were mostly dehydrodolichyl diphosphate synthases associated with the synthesis of shorter chain isoprenoids.

1.2.2. *Rubber elongation factor (REF) gene*

Phylogenetic tree was constructed using the amino acid sequences of REF and/or SRPP from relate plant species to estimate the unique nature of REF in *Hevea*. The uniqueness of *Hevea* REF class of proteins was proved by the phylognetic analysis. The analysis also revealed that REF share approximately 50 per cent homology with another latex related protein called small rubber particle protein

(SRPP). During the analysis, only one *Hevea* protein (AC096096) named as SRPP was clustered along with the proteins designated as REF in other plant species. The other *Hevea* REF sequences including the five sequences from the present study formed a single separate cluster demonstrating their uniqueness.

1.2.3. *Identification of novel polymorphic SNPs for segregation analysis in new interspecific progeny population*

A mapping population derived from an interspecific cross between *H. brasiliensis* clone RRII 105 (high yielder with low disease resistance potential) and *H. benthamiana* F4542 (low yielder with high level of disease resistance) was used for the construction of high density genetic linkage maps of *H. brasiliensis* and *H. benthamiana*. PCR product sequencing of the entire genomic region of *HMGR, GGDPS* and *REF* from *H. benthamiana* was done to identify novel polymorphic SNPs in this mapping population. Sequence analysis revealed the presence of 21 SNPs. Interestingly all the 21 SNPs were homozygous in the clone RRII 105 whereas only seven out of 21 were homozygous in *H. benthamiana*. Eighteen out of 21 were novel SNPs whereas three SNPs (HMGR924A/T, HMGR1666C/T and HMGR1693G/T) identified were reported earlier from other clones.

Sequence analysis of *GGDPS* gene revealed the presence of 14 SNPs. Out of the 14 SNPs, four were novel SNPs which were not reported earlier. Fourteen polymorphic SNPs were also identified in the *REF* gene out of which 8 were novel SNPs which were not reported earlier. Interestingly all the fourteen SNPs in *REF* gene were homozygous in RRII 105 whereas only four SNPs were homozygous in F4542.

1.2.4. *Comparative analysis of SNP number and frequency in the eight rubber biosynthesis genes*

Comparative analysis of the SNP number and frequency in the eight rubber biosynthesis genes was performed to understand the level of conservation of rubber biosynthesis genes in *Hevea*. The results revealed that the upstream genes like *HMGS, HMGR. MVK* and *PMVK* are highly conserved whereas the genes which are functionally more specific to *Hevea* like *REF* and *CPT* were the least conserved genes with very high SNP frequency rates. *REF* had the highest number of SNPs (32), highest SNP frequency (1/53) as well as highest number of non-synonymous SNPs (15). The rubber biosynthesis initiator molecule synthesizing genes like *FDPS* and *GGDPS* were moderately conserved. Interestingly FDPS also had the highest number of SNPs from non-coding regions.

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

2.1. NAC domain containing sequence analysis

NAC transcription factors are the largest families of plant transcriptional regulators involved in essential biological processes in development, stress responses and nutrient distribution in plants. NAC proteins consist of a conserved N-terminal region (NAC domain) with DNA-binding and oligomerization abilities, and a diverse C-terminal region which functions as a transcription regulatory domain.

Previously we reported successful cloning of *NAC* cDNA from both susceptible (RRII 105) and tolerant (RRIM 600) rubber

clones to abiotic stresses. Three dimensional structure of *Hevea* NAC was predicted through homology modeling. While comparing with *Arabidopsis* NAC protein, *Hevea* NAC showed the presence of five DNA binding residues instead of six. The AA motif 'YWKATGTD' present both in Arabidopsis and *Hevea* is the major DNA interaction region having specific role in recognition of DNA sequences. Four non-synonymous SNPs identified in the coding sequences of RRII 105 and RRIM 600 could not bring any change in these DNA binding motifs.

2.2. Construction of plant transformation cassette

The T-DNA binary vector pCAMBIA1301 ontaining 35S:NACtf:polyA cassette was constructed with the NACtf cDNA from both RRII 105 and RRIM 600 and mobilized into *Agrobacterium tumefaciens* strain LBA 4404 for plant transformation. Positive colonies of Agrobacterium with *NAC* constructs from both RRII 105 and RRIM 600 were identified through PCR amplification with *NAC* gene-specific primers. Two selected colonies were grown and used in transformation of tobacco leaf disc.

2.3. Characterisation of *NAC* promoter sequence

To develop strong inducible promoters to drive abiotic stress-inducible transgene expression in rubber, a promoter sequence along with partial coding sequence of *NAC-tf* gene (2820 bp) was amplified and cloned from genomic sequence of both the clones RRII 105 and RRIM 600 to identify *cis*- acting stress response elements controlling the expression of the downstream *NAC-tf* gene under abiotic stress. Transcription start site (TSS) at 165 bp upstream of ATG start codon was identified, which gave an idea of the exact size of the promoter sequence (2621 bp) cloned. Sequence analysis revealed a significant feature - a stretch of trinucleotide (ATT)-repeats known as short tandem repeats (STRs) at the upstream of the promoter sequence. Allelic variations based on repeat length polymorphisms were observed in both the clones. In RRIM 600 two alleles containing $(ATT)_{16}$ and $(ATT)_{15}$ repeats were identified. In RRII 105 also two alleles were identified based on repeat length variation *i.e.*, $(ATT)_{15}$ and $(ATT)_{14}$. Among these three alleles, one $(ATT)_{15}$ was found to be common and other alleles were different. Genetic variations in gene promoters play key roles in the determination of gene expression and phenotypes, and transactivation regulation of nearby genes due to polymorphic short tandem repeats (STRs) in promoter sequences has already been reported. Therefore, presumably allelic polymorphisms observed between RRIM 600 $[(ATT)_{16}]$ and RRII 105 $[(ATT)_{14}]$ in *NAC-tf* promoter sequence have a regulatory role on differential expression of *NAC-tf*. Although evidence to support STRs as functional elements continues to accumulate, little information is known about how they operate.

2.4. Methylation dynamics of *Hevea brasiliensis* genome

Identifying stress induced DNA methylation pattern in the promoter region of selected rubber biosynthesis genes (*HMGR*, *HMGS*, *FDPS* and *REF*) in cold susceptible (RRII 105, RRIC 100) and tolerant (RRIM 600) clones were continued.

A partial promoter region of *REF* gene was amplified from 24 DNA samples derived from RRII 105, RRIM 600 and RRIC 100 before imparting the stress, during second weeks under stress and after one month post stress. Cloning of bisulfite PCR

products of this gene from 16 plants was completed and plasmid DNA was isolated from multiple colonies for sequencing. Simultaneously, cloning of bisulfite converted *HMGS* gene promoter was completed from all the 24 plants. A total of 77 plasmid samples having bisulfite treated partial promoter region of *HMGR* gene were sequenced. Nine plasmids having partial promoter region of *HMGS* gene from the above three clones were also sequenced to identify methylation patterns associated with cold stress in the above clones. Sequence Analysis is in progress.

2.5. DNA methylation analysis of poly-embryony derived plants grafted on different rootstocks

MSAP experiments to assess the impact of grafting on poly-embryony derived bud-grafted plants was continued. Experiments were carried out in three sets of 10 year old own rooted plants and their two year old grafted plants in the field. Pre-amplification was carried out using selective primers and successful amplification was confirmed by running on gel. Six different primer combinations were selected for selective amplification. Initially two samples each from MspI digest set and HpaII digestive set was used for the reaction to check the efficiency of new primers. The experiment is in progress.

3. Cloning and characterization of agronomically important genes

3.1. Validating the role of CAld5H in model plant tobacco by the development of transgenics using the sense and antisense construct of *CAld5H*

3.1.1. Plant transformation and regeneration

Nicotiana tabacum was transformed by pCAMBIA1301-sense and antisense HbCAld5H1 constructs using the leaf-disk method. Untransformed tobacco plants served as controls. Hygromycin (50 mg L^{-1}) was used as a selective agent during *in vitro* regeneration. Differentiation of shoots was achieved on MS medium supplemented with 1 mg L^{-1} 6-benzylaminopurine (BA) and 0.1 mg L^{-1} napthaleneacetic acid (NAA). Rooting was obtained on MS basal medium devoid of growth regulators. Cephotaxime and Carbenicilin were used at 250 mg L^{-1} each during in vitro regeneration to remove excess bacterial growth. Transformed plants were grown in vitro for 6 weeks under a light-dark regime of 16 h (20–30 mE m-2 s-1, 27_C)/8 h (27_C) and then transferred to soil and grown to maturity in the green house.

3.1.2. Molecular characterization of transgenics

From the surviving explants, several GUS positive transformants were recovered. Ten independent transformants were regenerated with *HbCAld5H* sense construct while 25 independent transformants were regenerated with *HbCAld5H* antisense construct. Of these, three independent transformants (3-months-old) from each *HbCAld5H* sense and antisense constructs (CAS-A and CAS-B) were selected along with untransformed controls and analysed by PCR and Southern blotting. The *HbCAld5H* gene specific primers were used for PCR confirmation of transgenic tobacco plants with *HbCAld5H* sense and antisense transgenes. All positive transformants showed bands of 1542 bp for *HbCAld5H* sense, 842bp for CAS-A and 484bp for CAS-B transgenes. The same were not noticed in untransformed controls. The marker genes, GUS and hpt II (hygromycin) were also amplified from transgenic lines using gene specific primer for these genes.

3.1.3. Gene expression analysis and CAld5H activity in transgenic tobacco

Quantification of transgene expression and CAld5H enzyme assay were performed on 3-4 months old, three independent lines each of *CAld5H* sense, antisense (CAS-A and CAS-B) along with untransformed controls acclimatized in the green house. RT-PCR showed less transcript levels in antisense lines compared to sense lines in the leaf tissue. This indicates the up and down-regulation of *CAld5H* gene in transgenic lines. All the three lines each of CAS-A and CAS-B transgenic lines showed different amounts of reduction levels in *CAld5H* expression compared to control. The reduction level was more apparent in CAS-B (473bp) compared to CAS-A (842bp) suggesting that smaller antisense RNA may be more effective in gene silencing. On the other hand, a variation in degree of silencing was also evident in individual lines giving rise to different levels of *CAld5H* gene expression between transgenic lines generated using same construct.

3.1.4. Morphology of CAld5H sense and antisense transgenic tobacco lines

The primary transformants did not show much variation during in vitro development. However, after transfer to green house, the transgenic plants showed distinct variations in morphology. The CAS-B lines showed dwarfism compared to control, sense and CAS-A lines. The sense plants showed an increase in leaf size while it was reduced significantly in antisense plants compared to that of control plants. The leaves were stunted, wrinkled and curled in CAS-B lines and paler photosynthetic tissue was evident compared to control and sense plants. The sense plants showed robust leaf growth and development compared to antisense lines.

3.1.5. Anatomy and lignification pattern in the leaf tissue

The transverse sections from the leaf mid rib tissue of control and transgenic lines stained with toluidine blue revealed the lignified vascular tissue consists of protoxylem and metaxylem elements. Although sense plants showed more number of xylem elements in radial rows, their cell wall were thinner and often deformed compared to that of control and antisense lines. Weisner reaction revealed the relatively thicker, lignified secondary walls in the primary xylem elements of antisense plants (both CAS-A and CAS-B lines), however, the sense line showed thin cell walls with less lignin. Maule's reaction of these tissue showed the presence of guaiacyl units in the thicker secondary cell walls of proto and metaxylem elements of control and antisense lines. The thinner secondary walls of primary xylem elements in the sense plants also showed presence of guaiacyl lignin. These results suggest that the amount of guaiacyl lignin units are reduced by decreasing the secondary wall proportion of primary xylem elements in the vascular tissue of leaf tissue during overexpression of *CAld5H* gene. On the other hand, the increase in number of radial extent of xylem elements in the sense plants suggest the xylogenesis and vascular tissue differentiation could be enhanced with more number of cells with less secondary cell wall volume. The primary functions of leaf tissue (photosynthesis and transport of primary metabolites) are performed be chlorenchymatous and sieve cells while mechanical and conductive functions are of secondary importance. Therefore, the enhanced meristamatic activity and vascular tissue differentiation could be better for growth and development and leaf tissue in relation with their specific functional dynamics compared to stem and

root tissues. On the other hand, it is important to examine the effect of *CAld5H* overexpression on secondary growth in stem tissue where mechanical ad conductive functions are of priority.

4. Metagenomics and microbe identification in rubber ecosystems

Since rubber is a monoculture tree crop widely cultivated in large areas replacing the natural vegetations including forests, there are reports that the natural ecosystem as well as soil health is being depleted due to the lack of diversity in vegetation existing in rubber plantations. For providing additional income to the farmers and increasing biodiversity in rubber plantations, Rubber Board is promoting inter cropping in rubber plantations with diverse crops like, banana, pineapple, various vegetables, coffee, cocoa, *etc*. But so far no study has been undertaken to identify the impact of these cropping patterns on the microbial diversity thriving in the rubber soils and their contribution to the growth and health of rubber plants. Since the diversity and predominance of rhizosphere microbial population depend on a number of abiotic and biotic factors prevailing in that particular ecological niche, the metagenome analysis of soil samples from diverse locations/practices (intercrop, cover crop, native forests) will provide information on the beneficial micro-organisms prevailing in rubber soil. The information from such a study will also help us to identify the management practice which supports a healthy population of beneficial soil microorganisms. The study will also throw light on how the anthropogenic alteration of forests, through logging or replacement of native forest by plantation (which generally uses introduced species) affects the ecosystem structure and functioning of native forests of Kerala and how the mix of species affects ecosystem functioning in rubber plantations. Therefore a collaborative project on metagenomics of soil microbes from rubber growing areas with different cropping systems has been initiated in collaboration with CSIR-NEERI, Nagpur with the following major objectives 1) To investigate the spatial turnover of soil meiofaunal communities at the regional level, in response to environmental changes introduced by the practices of rubber monoculture, rubber with intercrops (cocoa), with natural flora and cover crop in comparison to native forests. 2) To confirm the positive impact of plantation management practices such as weeds crop and cover crop in the soil's physicochemical and biological properties leading to the restoration of biodiversity back into the landscape. 3) To study the soil microbial diversity prevailing under different management practices and to quantify the beneficial bacterial species under each system.

Soil samples were collected based on a nested design from 20 sites from a plot. In total 130 soil samples were collected from rubber grown under different management practices like, only rubber, rubber and weed, rubber and cover crop, rubber and inter crop (cocoa) and native forest. Total DNA was isolated from soil samples. To generate amplicons for sequencing and to check the quality of the isolated DNA for NGS, nematode specific primers (NemF and 18Sr2b) were used in a pre-amplification step followed by amplification with NF1 and 18Sr2B in a semi nested procedure. Amplicons of approximately 400 and 420bp was obtained from the two primer combinations showing that the DNA is of good quality. CSIR-NEERI have outsourced all 130 samples for getting sequenced using

both bacteria and archaea specific libraries at present.

5. Development of genetically modified rubber plants with agronomically desirable traits

5.1. Molecular characterisation of transgenic lines developed

5.1.1. Southern hybridisation of transgenic plants

Southern hybridization of transgenic plants integrated with *MnSOD, Osmotin* and *HMGR* genes was carried out. For Southern analysis of PCR positive transgenic plants, DNA samples were restricted with *XbaI* and *HindII* enzymes. In the case of *Osmotin* integrated transgenics, blotting of *Xbal* and *HindII* digest DNA samples from five lines revealed that all of them carry two copies of the *Osmotin* gene. Blotting was performed to validate the integration of *HMGR* gene in the 22 transgenic lines. For *HindIII* digest, three genotypes were present among 18 transgenic lines. In *XbaI* digest two genotypes were seen among 22 transgenic lines. Blotting of *XbaI* digest DNA samples from the two MnSOD transgenic lines developed indicate that both are of the same genotype

6. Genome sequencing and *de-novo* assembly of rubber (*Hevea brasiliensis*) genome

6.1. Transcriptome sequencing

6.1.1. Transcriptome annotation

Transcript sequences from various conditions and tissues were annotated against *Ricinus communis* protein data set using homology search. BLAST results were screened based on alignment length >= 50 and percentage identity >= 50 using in-house PERL script. The filtered BLAST result is given below in Table Gen.1.

6.1.2. Differential Gene Expression (DGE) Screening

CD-Hit clustering, transcript quantification and Differential Gene

Table Gen. 1. **BLAST result for transcriptome assemblies**

Sample		Total transcripts	Annotated (*Ricinus communis*)
COL_105_CO:	Control	75704	46267
COL_105_TP:	*Colletotrichum* challenged	59235	42167
COL260_CO:	Control	59888	41503
COL260_TP:	*Colletotrichum* challenged	57947	41704
PHY-600-CO:	Control (susceptible)	31860	18740
PHY-600-TP:	*Phytophthora* challenged	23816	14503
PHY-FX-CO:	Control (tolerant)	33985	26336
PHY-FX-TP:	*Phytophthora* challenged	26089	16801
RRI-BA-1:	Young bark	45446	38501
N:	Young bark	17216	12685
T:	Bark sample from TPD plant (20, 50 and 80% TPD samples pooled together)	10265	9120
YA:	Latex from high yielding germplasm accession	21600	17106
YB:	Latex from low yielding germplasm accession	23628	14323
YC:	Latex from low yielding clone	13712	12249

Table Gen. 2. **Differential gene expression result for YA, YB and YC samples**

Sample	Clustered transcripts	Up-regulated	Down-regulated
YA: Latex from high yielding germplasm accession vs YB: Latex from low yielding germplasm accession	38455	1297	1645
YA: Latex from high yielding germplasm accession vs YC: Latex from low yielding clone	26810	564	431

Expression (DGE) analysis were carried out for **YA**: Latex from high yielding germplasm accession, **YB**: Latex from low yielding germplasm accession and **YC**: Latex from low yielding clone samples. Differentially regulated transcripts were screened using in-house PERL scripts (Table Gen. 2).

6.2. Computational classification of cis-Prenyltransferases (CPTs)

Reclassification of cis-Prenyltransferases (CPTs) was carried out using computational approaches. A total of seven *CPT* gene family members and 1 CPT like sequence (*CPT1, CPT2, CPT3, CPT4, CPT5, CPT6, CPT7* and *CPTL*) were reported in the whole genome assembly of RRIM 600 (Lau *et al*., 2016). Extensive computational analysis on these sequences revealed highly conserved sequence pattern among *CPT1, CPT2* and *CPT3*, whereas *CPT4, CPT5, CPT6, CPT7* and *CPTL* were not showing conserved patterns with *CPT1, CPT2* and *CPT3*. Further the CPT2 and CPT3 sequences were found identical based on Untranslated Region (UTR) alignments. Thus, only two major CPTs (*CPT1* and *CPT2*/*CPT3*) were detected in RRIM 600 contradictory to what reported by Lau *et al*. (2016). To reconfirm the identity of *CPTs* in *Hevea* based on previous findings, a total of 29 *CPT* sequences were retrieved from NCBI's GenBank and subjected to computational analysis. Based on the results from BLAST analysis, multiple sequence alignments of protein, nucleotide and untranslated regions, open reading frame analysis, gene prediction analysis and sequence length variations, we identified three major CPTs in *Hevea brasiliensis* and designated them as *RubCPT1, RubCPT2* and *RubCPT3*.

7. Advisory work

Establishing the clonal identity of a rubber tree showing very good growth characteristics but very low latex yield from Karnataka-claimed to be RRII 400 series clone.

The clonal identity of the rubber tree was verified using molecular genetic markers like RAPD and SSRs. Marker profiling was done along with the parents of RRII 400 series clones (RRII 105 & RRIC 100), popular RRII 400 series clones (RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430) and GT1. Parents of 400 series clones were included to analyse the segregation pattern of alleles whereas GT1 clone was included as it is also widely cultivated in the rubber growing belt of Karnataka.

Comparative analysis of the allelic combination of the parents of 400 series clones, five standard 400 series clones and test sample confirmed that the plant being tested is a 400 series clone itself. Segregation pattern of the parental alleles in the five 400 series clones further confirmed that the suspected clone is RRII 414. The test plant showed perfect match with the allelic combination of RRII 414 alone for all the markers tested.

PLANT PATHOLOGY DIVISION

Plant Pathology Division is primarily concentrating on studies on economic and eco-friendly management of pests and diseases. Evaluation of new clones for disease tolerance, identification of genes involved in disease tolerance, identification of quantitative trait loci for disease tolerance, understanding biotic etiology of tapping panel dryness *etc.* were the focus area of research. In addition to research, the Division also takes up testing of spraying equipment, plant protection chemicals and analysis of water samples for bacterial contamination. Training on disease management, maintenance of spraying equipment and apiculture are the other activities undertaken by the Division.

Advisory work on disease management is also undertaken through field visits, telephonic advisory, WhatsApp and Online Rubber Clinic. About 1217 cases were attended through WhatsApp and 341 water samples were analysed during the reporting year.

1. Leaf diseases

1.1. Abnormal leaf fall disease

1.1.1. Disease survey

Abnormal leaf fall survey was carried out during 2018 disease season in Kerala and South Karnataka. During the survey, it was observed that about 70-80 per cent leaf fall was recorded in all the clones and regions. No difference was observed between the clones RRII 105, RRII 414 and RRII 430. All the clones were equally affected. In Kanyakumari region recorded only 20 per cent leaf fall. All other locations from Thiruvanathapuram to Kasargod recorded leaf fall ranging from 70 to 100 per cent.

The impact of abnormal leaf fall on growth and yield of four modern clones *viz.* RRII 414, RRII 422, RRII 429 and PB 260 gave varying results. The severity of ALF disease in general during 2018 was high. Among the clones, leaf fall of 40-50 per cent recorded in unprotected blocks of RRII 414, RRII 422, RRII 429 and PB 260. The girth of the trees in the sprayed blocks continued to be significantly higher in clones of RRII 429, RRII 414 and PB 260. The girth and girth increment (2018) between sprayed and unsprayed clones are presented in Table Path. 1.

Table Path. 1. **Comparison of growth under sprayed and unsprayed conditions**

Clone	Girth (2018) (cm)	Girth increment (cm)
RRII 414		
Sprayed	71.6 **	4.0**
Unsprayed	67.9	3.0
RRII 429		
Sprayed	71.4 **	5.1 **
Unsprayed	69.2	4.0
RRII 422		
Sprayed	68.3 NS	3.9 NS
Unsprayed	68.0	4.3
PB 260		
Sprayed	73.7 *	6.2 **
Unsprayed	71.8	5.1

The yield variation was noticed among the clones due to ALF. The ALF was more in the clone RRII 429 (25.3%). The clones PB 260, RRII 422 and RRII 414 registered yield drop 24.5, 13.7 and 9.3, respectively. The tapping panel dryness (TPD) was higher in PB 260 followed by RRII 414 and the lowest in RRII 422. The crown budded trees in CES Chethackal recorded higher yield than control PB 260.

1.2. Corynespora disease

Nursery evaluation of bio-agents (endophytic bacteria) and integrated control against Corynespora leaf fall disease on the clone RRII 105 was carried out at Ulickal nursery. The endophytic bacteria were applied in broth ($1x10^6$/mL) and encapsulated formulation with carbendazim (500 mg). The results showed that the integrated control (carbendazim 0.025% + Antagonistic endophytic bacteria in broth ($1x10^6$/mL)) and encapsulated form (Fig. Path.1) were effective and on par with recommended fungicide (Table Path. 3).

Table Path.2. **Effect of treatments on disease incidence**

Treatments	Disease incidence
T1 - Bio(broth) + Carbendazim (Alternate)	0.34
T2 - Bio (tablet) + Carbendazim (Together)	0.31
T3 - Control	1.55
T4 - Carbendazim	0.31
T5 - Bio (Talc) + Carbendazim (Together)	0.03
T6 - Bio (tablet) + Carbendazim (Alternate)	0.41
CD(P=0.05)	0.21

Evaluation of new fungicides, Pyroclostrobin metriam, Pencycuron, Isoprothiolne and integrated treatment were carried out at two hot spot locations at South Karnataka during 2018-19 disease season. The new molecule Pyroclostrobin metriam and integrated treatments are on par with recommended fungicide Carbendazim (Table Path. 3).

Table Path. 3. **Effect of fungicides on the control of Corynespora disease**

Treatments	Disease incidence
T1 - Bio (tablet) + Carbendazim (Together)	0.72
T2 - Pyraclostrobin metriam	0.79
T3 - Carbendazim	1.03
T4 - Pencycuron	2.17
T5 - Isoprothiolne	2.03
T6 - Control	4.21
CD (P=0.05)	0.40

1.2.1. Whole genome sequence of virulent Corynespora isolates

The strain HV where showed the presence of Cas 2 gene and high virulence in

Fig. Path.1. Corynespora disease severity between control plants and integrated treatment

the field (Sheradi-Karnataka) was selected for the subsequent experiments and genome sequencing. The genome of this Indian isolate of *C. cassiicola* was sequenced using Illumina Hiseq. A total number of 13654 genes were predicted by the repeat masked assembly using Gene Marker – ES Fungal V2. The predicted genes were annotated by BLAST searching of predicted mRNA (blast X) sequences against NCBI nr database; 12251 were identified in nr Blast X unique hits, the organism wise distribution 37 per cent belonged to the pleosporales. The specific functions of the predicted genes in the molecular, phylogenetic, and evolutionary basis were carried out using the database of Cluster's of Orthologous Groups of proteins (COGs), KEGG Orthology (KO) and Gene Ontology (GO). Among the 5142 COG functional category annotated genes 5.8 per cent of genes are involved in defense mechanisms. Effector Pv 2.0 was used to predict effector proteins in the annotated proteome. The algorithm is a machine learning classifier for fungal effector prediction. Effector P 2.0 achieves an accuracy of 89 per cent when compared to 82 per cent by its predecessor Effector P 1.0. A total of 1035 effector proteins were identified. Among them four were Chitinases, 25 were Glucanases and 20 were Pectinases.

1.3. Powdery Mildew disease

The efficiency of two bacterial bio-control agents along with recommended fungicide (wettable sulphur), a new fungicide trifloxystrobin + tebuconazole (Nativo) and integrated treatments were tested in poly bag plants. Treatments were imposed at eight days interval and disease intensity was assessed on 0-5 scale before each treatments. The integrated treatment of RH 34 with strifloxystrobin + tebuconazole (Nativo) was most effective. About 50-70 per cent control of the disease was obtained when compared with control.

1.4. Thread blight disease

A survey on the incidence and severity of thread blight disease was undertaken in Thodupuzha and Kothamanalam area. The incidence and severity at Thodupuzha and Kothamangalam area were 10-75 per cent and 10 per cent, respectively. Isolated the fungus and identified using ITS specific primers as *Pellicularia filamentosa*.

2. Tapping Panel Dryness (TPD)

The nested PCR primer combination and short primers synthesized from the existing **PLO** for phytoplasma detection yielded amplification in TPD/Partial TPD and certain healthy sample (Fig. Path. 2). All the sequence data from the amplified product gave homology to Phytoplsama like organism on optional phytoplasma blast analysis. The healthy plants that showed phytoplasma amplifications are under observation.

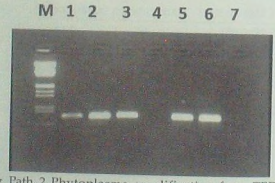

Fig. Path. 2. Phytoplasma amplification from TPD/ Healthy/Partial TPD plants with short primers M-marker, 1, 4, 7 (Healthy), 2, 3, 6 (TPD), 5 (Partial TPD)

3. QTL Marker Development for Disease Tolerance

3.1. Leaf diseases

3.1.1. Abnormal leaf fall disease

The major genes involved in the *Hevea-Phytophthora* interaction were studied and

a defence signalling network was predicted. The differential gene expression analysis identified upregulated genes in treated resistant clone FX 516. Gene enrichment analysis indicated that the pathogen triggers host DNA double-strand breaks and reduces plant defence responses. The biological interaction network created using genes highly expressed in challenged resistant clone revealed involvement of proteins like transcription initiation factor IIB-1 family protein, leucine-rich repeat transmembrane protein kinase, DNAJ heat shock N-terminal domain-containing family protein, SEC1-family transport family protein *etc.* which have been reported to play specific roles in biotic stress responses. Members of the complex gene family of WRKY transcription factors have been implicated in the regulation of transcriptional reprogramming associated with plant immune responses. In order to validate the transcriptomic data analysis 11 differentially expressed genes were selected for qRT-PCR analysis. The upregulation of these genes in resistant clone compared to susceptible clone in qRT-PCR analysis supported the transcriptomic data.

Wickham clones in the Clone Museum of RRII were assessed for their level of tolerance to *Phytophthora* using zoospores on leaf disc bioassay. One round of screening for *Phytophthora* tolerance is completed and the second round is being carried out with fresh isolate.

3. 1.2. Corynespora leaf disease

One hundred and sixty eight Wickham clones are maintained in the Clone Museum of RRII. These clones were assessed for their level of tolerance to *Corynespora* using toxin inoculation method through *in vitro* assay. Based on the resistance observed, clones were categorised into five groups namely highly resistant, resistant, moderately resistant, susceptible and highly susceptible. Two rounds of screening have been completed and those clones belonging to highly resistant and resistant categories have been shortlisted for confirmation through one more round of screening.

3.1.3. Powdery mildew disease

Wickham clones maintained in the Clone Museum of RRII were assessed for their level of tolerance to *Oidium*, which is an obligate parasite due to which its screening could be conducted only directly in the field. Clones were visually scored based on the severity of symptoms observed and percentage of disease severity index was calculated. Scoring for disease incidence was carried out thrice at an interval of 15 days during January and February, the season in which disease occurs in field following refoliation. One round of screening has been completed and will be reconfirmed during the next season.

3.1.4. Colletotrichum leaf disease

Wickham clones were assessed for their level of tolerance to *Colletotrichum* using toxin inoculation method. Two rounds of screening for *Colletotrichum* has been completed and clones belonging to highly resistant and resistant categories are shortlisted for confirmation through one more round of screening. Clones showing high levels of resistance and high level of susceptibility will be genotyped for identifying putative markers linked to resistance loci.

5. Construction of genetic linkage map, mapping of quantitative trait loci and development of high throughput markers for early detection of leaf diseases of rubber

The mapping population used in the study was derived from an interspecific cross between *Hevea brasiliensis* (clone RRII 105) as the maternal parent and *H. benthamiana* (clone F4542) as the paternal parent. An interspecific cross was chosen in order to ensure maximum genetic diversity between the parents to generate the maximum polymorphism in the progeny. Disease response of the parents and progeny to three major pathogens: *Phytophthora meadii, Corynespora cassiicola* and *Colletotrichum acutatum* were carried out in controlled laboratory condition. The *H. benthamiana* parent is resistant (in group R) and the *H. brasiliensis* parent is susceptible (in group S) to *Phytophthora*. Among the F_1 progeny that were assessed for resistance against *Phytophthora*, 5.9 per cent were found to be highly resistant and 10.6 per cent highly susceptible (Fig. Path.3). *Corynespora cassiicola* and *Colletotrichum acutatum* toxins were also tested on leaves of parents and mapping population. The *H. brasiliensis* parent was classed as highly susceptible to both pathogens (rated as 5) and the *H. benthamiana* parent was highly resistant to both pathogens (rated as 1). Of the F1 progeny tested with the *Corynespora cassiicola* toxin extract, 10.5 per cent were highly resistant and 21.1 per cent highly susceptible. Among the progeny tested with the *Colletotrichum acutatum* toxin, 9.2 per cent were highly resistant and 10.5 per cent highly susceptible.

The initial GBS linkage map was constructed using DArTseq. Consistent with the known haploid chromosome number of *Hevea* (n=18), each linkage map consisted of 18 linkage groups. Linkage maps were assigned and orientated based on the rubber draft genome assembly for *Hevea*. Using these maps QTL markers for

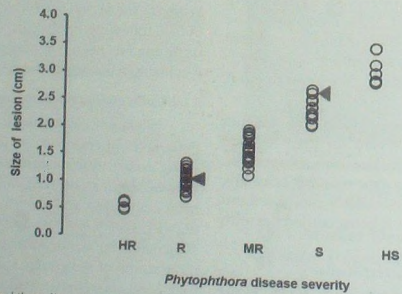

Fig.Path. 3. *Phytophthora* disease severity assessed with 85 progeny population. Five resistance categories are indicated as highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS). Disease severities of the resistant parent (*H. benthamiana*) and susceptible parent (*H. brasiliensis*) are indicated by green and red triangles, respectively

all these three diseases were identified using 'qtl' software in R package. Validation of QTL markers and fine mapping of the QTL regions for disease resistance was carried out using KASP marker technology. For *H. benthamiana*, QTLs for *Phytophthora* were detected in one region of LG 1 and two regions of LG 8, of the 85 F_1 progeny that were assessed for *Phytophthora* only 10 had the *H. benthamiana*-like genotypes in all QTL regions and of these 10, four had resistance level higher than *H. benthamiana* (Fig. Path.4). The genotypes at two markers in LG 1 and four markers in LG 8 were sufficient to distinguish highly resistant progeny for *Phytophthora* from the rest.

For *Corynespora* QTL detected in LG 1 of *H. benthamiana*, 12 had *H. benthamiana*-like genotypes three progeny had lower wilting rates in their leaves than the resistant parent *H. benthamiana* (Fig. Path. 5a). For *Colletotrichum* QTL detected in LG 18 of *H. benthamiana*, eight had *H. benthamiana*-like genotypes. Four progeny showed lower wilting rates in their leaves than the resistant parent *H. benthamiana* (Fig. Path. 5b). For the three diseases examined, two progenies (9_26 and 9_76) seemed to carry all three resistance genes and could be useful in pyramiding the resistance.

We identified eighteen progeny showing greater resistance to *Phytophthora* than that in the resistant parent *H. benthamiana* and also have detected some progenies with at least similar resistance to resistant parent for *Corynespora cassiicola* and *Colletotrichum acutatum*, which can be used in making future crosses.

Genetic analysis to identify genes or QTL markers that confer disease resistance was carried out. Closest and/or flanking DArTSeq GBS tags that were mapped to the QTL regions were BLASTN searched against the NCBI nucleotide database and significant hits with sequence identity higher than 90 per cent were selected. The

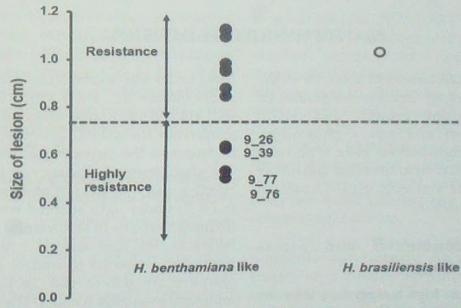

Fig.Path. 4. *Phytophthora* disease resistance of progeny based on KASP marker results. Size of lesion caused by the resistant parent (*H. benthamiana*) is marked with a spotted line in black. The progeny with resistant trait (*H. benthamiana* like) were separated from the others (*H. brasiliensis* like) using two markers in LG1 and four markers in LG8

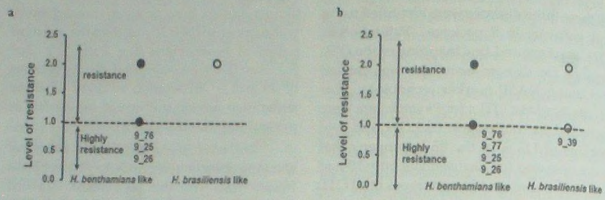

Fig.Path.5. *Corynespora* (a) and *Collectotrichum* (b) disease resistance of progeny based on KASP marker results. Level of resistance measured as rate of wilting in the leaf caused by the resistant parent (*H. benthamiana*) is marked with a spotted in black. The progeny with resistant to *Corynespora* and *Collectotrichum* (*H. benthamiana* like) were separated from the others (*H. brasiliensis* like)

CDS sequences were then annotated by BLASTX against the reference protein database and the putative functions of the proteins were identified using Pfam database. Pathogenesis-related proteins coding sequences (LRR, NB-ARC, Pkinase and Pkinase_Ser/Try type proteins) were identified in the candidate QTL regions. The production of PR proteins in the invasion of a pathogen attack is a primary mechanism that induces self-defence systems in plants. The candidate gene sequences identified in this study could be useful in designing primers for resequencing of these genes and useful in PR gene isolation.

PLANT PHYSIOLOGY DIVISION

The major areas of research in the Plant Physiology Division are studies on physiology of growth and yield, environment and stress physiology, secondary metabolites, gene expression analysis in relation to latex flow and rubber biosynthesis and ecological impact on rubber cultivation.

1. Environmental and Stress Physiology

1.1. Effect of high temperature stress on young plants of *Hevea*

A study was carried out to assess the photochemical efficiency of young plants of *Hevea* under high temperature stress. Four *Hevea* clones *viz.*, RRII 430, RRII 105, RRII 414 and Tjir 1 were subjected to high temperature stress (45 °C) *in vitro* and *in vivo* to determine the physiological responses. The photochemical efficiency in terms of effective quantum yield of photosystem II (x PS II) under light adapted conditions declined markedly in temperature stressed leaves at two, four and six hours after treatment compared to control ambient temperature where the decline was minimum even after 6 hours. The electron transport rates across PSI and PSII also declined in leaf discs exposed to high temperature indicating that temperature

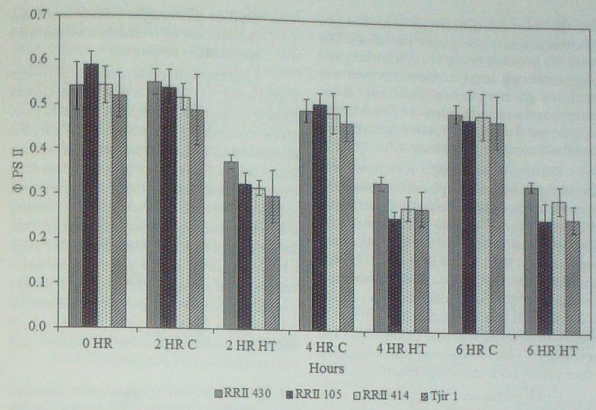

Fig. Phy.1. Effective quantum yield (φPS II) in leaf discs of different clones subjected to high temperature stress up to six hours *in vitro*. (C – control, HT – high temperature)

stress affect the photochemical efficiency of rubber clones. Maximum potential quantum yield (Fv/Fm) did not show much variation in the clones tested. Unlike Fv/Fm, x PS II reduced significantly after exposure to high temperature stress in all the clones, however, RRII 430 maintained better x PS II than other clones (Fig. Phy.1).

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

Three experiments were carried out using polybag plants raised at RRII experimental farm comprised of nine different clones having differential responses to drought and cold stresses. Leaf pigment composition and rate of decline varied in accordance with extension of stress. However, the reduction in pigments level was lesser in SCATC 88/13, RRIM 600 and RRII 208. Lipid peroxidation rate also showed almost the same trend. Accumulation of anthocyanin pigment in leaf was high in all the clones under low temperature stress. Chlorophyll stability index was better in clones, SCATC 88/13, RRII 208 and RRIM 600 than other clones studied when exposed to low temperature stress. Marked clonal variation was observed in cell viability rate under low temperature condition.

The proportion of xanthophyll pigments significantly altered in the treatment compared to control. It was noticed that increase in zeaxanthin and antheraxanthin was comparable in clone RRIM 600 and RRII 422 upon exposure to cold stress conditions. â-carotene and lutein

are the two predominant pigments of carotenoids, estimated in all the clones. The ratio of â-carotene to total xanthophyll pigments and total carotenoids was the same in all the clones. The level of á-carotene was extremely low. Under drought stress condition also same pattern of metabolic activity was observed.

During water deficit condition, leaf shedding and drying of plants was observed in some of the clones studied. Upon stress recovery from water deficit a fast development of new whirl was observed especially in RRII 430, RRIM 600, RRII 429 and RRII 208. Under low temperature and drought conditions, clones such as RRIM 600 and RRII 208 showed relatively stable metabolic activity whereas clones, RRII 430 and SCATC 88/13 were on par with each other under both the stress conditions.

Cold induced bud dormancy was noticed in all the clones and re-growth was noticed at a slow rate and thus varied with clones during recovery under tropical conditions. Re-growth after drought stress was comparatively faster than regrowth after cold stress. Analyses of samples collected after exposed to drought/cold stress in the controlled growth chamber and field for antioxidant system, adaptive mechanisms and responses are in progress.

1.3. Studies on drought effects on *Hevea* in relation to oxidative stress and antioxidant responses

Xanthophyll cycle activity showed significant variation among the clones during drought. Xanthophyll pigment pool size was higher in RRIM 600 and RRII 430 (two clones with fairly good drought tolerance) than other clones. The ratio of zeaxanthin to total carotene, total xanthophyll pigments and total chlorophyll was found high during drought condition. It was confirmed that RRIM 600 and RRII 430 have a higher ratio of xanthophyll cycle pigment to total carotene and total chlorophyll under drought condition. Analysis of biochemical parameters particularly xanthophyll pigments, oxidative stress responses and antioxidants are in progress.

1.4. Physiological adaptation of selected ortets under varying agro-climatic conditions in India

A multi-location trial with sixteen ortets and seven check clones was planted at three different locations in 2012, and CES, Chethackal being one of the locations. The young plants were allowed to grow in a closed planting design. During this reporting period the alternative plants were removed to allow normal spacing for the plant growth.

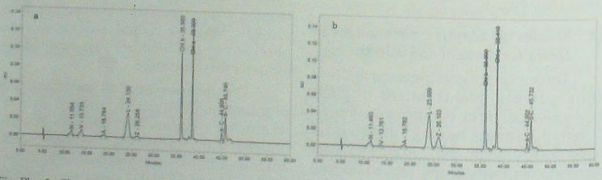

Fig. Phy. 2. Chromatographic profile of xanthophyll pigments in control (a) and drought condition (b) in leaves of young plants of *Hevea*

Plant girth at 50 cm height from the bud union and yield recording by test tapping was being done every Feb-March and September, since 2015. Mean girth of ortets during March 2019 ranged from 23.9cm for ortet NGK 69 to 41cm for RRSA 98 which was on par with that of check clone RRI I 430.

Mean yield of three years test tapping (ten tappings /season) was accounted from the yield data of peak yielding periods. The highest yield was recorded consistently in clone RRII 430 followed by clone RRII 417 among the elite clones whereas ortets RRSA 98 followed by RRSA 585 and DAP1 recorded higher yield and NGK 69 and DAP 34 recorded the lowest yield (Table Phy 1).

2. Production Physiology (Growth and Yield)

2.1. *Productivity enhancement of NR through HDP and growth regulation by application of PBZ*

Girth data was collected and analysed for three densities and two types of planting materials *viz*. polybag and root trainer plants. The data indicated a variation in girth between poly bag and root trainer plants in one treatment *viz*. PBZ@0.25g cm^{-1} and spacing 5m. Stem girth was lesser in root trainer plants in this treatment whereas all other treatments were on par with each other.

2.2. Intercropping with tree crops in rubber

The growth and yield of tree intercrops planted with rubber was monitored by measuring the annual trunk girth and cup lump yield taken fortnightly. The mean girth of rubber trees was 70.5 cm for control (rubber alone) and 70.5 cm and 70.3 cm for rubber with three rows of Mahogany and rubber with one row of Mahogany trees, respectively, along with one row of Pathimugam trees. The growth data indicated that tree intercrop did not affect the girth of rubber plants. The trees were continuously tapped for seven years under S/2 d3 system of tapping and from April 2017 onwards the tapping system was changed to low frequency tapping i.e. S/2 d7 and monthly stimulation was provided at 2.5 per cent ethephon since September 2017 onwards. On the contrary to growth of rubber plants, yield in pure stand of rubber and rubber intercropped with tree species exhibited significant

Table Phy. 1. **Girth and yield of ortets and check clones at CES, Chethackal**

Ortets/Clones	Girth (cm)	Yield (g)
	2019	Mean yield of three years ($10\ t^{-1}\ year^{-1}$)
RRII 105	29.8 ± 1.6	99.0 ± 8.8
RRIM 600	29.5 ± 1.1	88.4 ± 6.3
RRII 414	37.3 ± 1.9	130 ± 11.2
RRII 417	39.0 ± 1.7	206 ± 13.6
RRII 422	35.8 ± 1.3	145 ± 17.5
RRII 429	36.0 ± 1.4	107.6 ± 12.5
RRII 430	41.6 ± 1.0	211.0 ± 17.7
DAP 1	29.5 ± 1.3	118.2 ± 8.9
DAP 34	31.3 ± 1.2	47.0 ± 4.9
DAP 35	31.4 ± 1.3	104 ± 7.2
DAP 36	31.7 ± 0.8	96.7 ± 8.5
RRSA 98	41 ± 1.2	139.0 ± 6.5
RRSA 315	33.0 ± 1.4	83.5 ± 11.2
RRSA 585	32 ± 2.3	128 ± 9.8
NGK 1	30.5 ± 2.3	68.6 ± 5.4
NGK 47	29.4 ± 1.16	53.4 ± 4.5
NGK 69	23.9 ± 1.2	43.6 ± 5.1
GH 1	26.6 ± 1.0	66.4 ± 5.8
GH 3	27 ± 1.1	56.3 ± 3.9
GH 9	30.3 ± 1.0	83.0 ± 6.5
RRST 24	26.5 ± 0.9	60.7 ± 8.1
RRST 37	28.2 ± 1.0	73.2 ± 7.5
RRST 39	26.4 ± 1.2	69.3 ± 4.5

Table Phy 2. **Latex yield (mL t^{-1} t^{-1}) after three stimulations (May, Sep and Nov) in different clones under S/2 d3 tapping system**

Clones	Latex yield (May-Dec)		
	Control	Stimulated	% yield increase
RRII 105	235 ± 12.9	317 ± 26.0	34.89
PB 217	226 ± 13.8	301 ± 10.6	33.18
PB 260	165 ± 21.6	175 ± 11.8	6.0
RRIM 600	146 ± 4.2	152 ± 8.3	4.0
Tjir 1	66 ± 8.7	75 ± 2.6	13.6
RRII 33	42 ± 5.0	48 ± 4.2	13.4
RRII 38	75 ± 3.8	80 ± 2.2	6.66

variation with 98.6, 85.0 and 91.0 g t^{-1} t^{-1} for control, with three rows of Mahogany and one row of Mahogany trees, respectively. The result indicated that tree intercrops significantly influence the rubber yield but not the trunk girth. Among the intercrops, the Pathimugom stand was very poor with many trees drying and exhibited poor growth due to shading by the mature rubber trees, while Mahogany trees (59.3cm) were found growing better as intercrop in tree inter-crop trial at CES, Chethackal.

2.3. Effect of stimulation on latex regeneration mechanism in *Hevea brasiliensis*

Statistical analysis of three years data on yield and biochemical parameters related to latex regeneration was carried out to study the long term effect of stimulation in high, medium and low yielding clones. The data indicated that successive stimulations had significant effect on sucrose content and metabolism in clones RRII 105 and PB 217 under S/2 d3 system of tapping (Table Phy. 2, 3 &4). Metabolic activities of clone PB 260 was high with high rate of enzyme activities and low sucrose content but there was no significant yield increase after stimulation. TPD incidence was also high in clone PB 260.

Expression analysis of genes involved in latex regeneration (sucrose transporters and glutamine synthetase) showed up regulation after ethylene stimulation in clones, RRII 105 and PB 217 compared to other clones which is reflected in yield increase (Table Phy. 5)

Table Phy. 3. **Status of biochemical parameters related to latex production in control and stimulated trees of different clones under S/2 d3 system of tapping**

Clones	Sucrose (mM)		Pi (mM)		ATP (µM)	
	C*	S	C	S	C	S
RRII 105	13.56 ^{b}	5.82 ^{d}	23.36 ^{bc}	29.43 ^{a}	247.93 ^{b}	278.13 ^{a}
PB 217	20.16 ^{a}	11.01 ^{cc}	19.36 ^{de}	23.80 ^{b}	230.29 ^{c}	258.40 ^{b}
PB 260	3.80 ^{e}	2.72 ^{e}	21.37 ^{bcd}	20.59 ^{cd}	219.10 ^{cd}	216.40 ^{cd}
RRIM 600	11.75 ^{c}	6.10 ^{d}	17.58 ^{e}	21.3 $^{bcd\ bcd}$	193.13 ^{e}	225.93 ^{cd}
Tjir 1	14.12 ^{b}	10.48 ^{c}	13.67 ^{f}	20.76 ^{cd}	180.98 ^{e}	213.37 ^{d}
RRII 33	10.11 ^{c}	7.38 ^{e}	8.99 ^{g}	11.60 ^{fg}	123.13 ^{f}	128.03 ^{f}
RRII 38	15.03 ^{b}	10.04 $^{c\ c}$	12.04 ^{f}	11.06 ^{f}	132.33 ^{f}	139.70 ^{f}

*C-Control S-Stimulated

Values followed by common letters are not significantly different

Table Phy. 4. **Status of biochemical parameters related to oxidative stress inflicted to laticiferous system in control and stimulated trees of different clones under S/2 d3 system of tapping**

Clones	Thiol (mM)		Glutathione reductase (units)		SOD (units)		Proline (mM)	
	C	S	C	S	C	S	C	S
RRII 105	0.205	0.243	0.479 ^{c}	0.584 ^{a}	1.65 ^{bcd}	2.11 ^{a}	0.542	0.648
PB 217	0.267	0.304	0.405 ^{de}	0.508 ^{bc}	1.66 ^{bc}	1.72 ^{b}	0.492	0.555
PB 260	0.214	0.21	0.511 ^{b}	0.576 ^{a}	1.48 ^{de}	2.03 ^{a}	0.444	0.584
RRIM 600	0.177	0.166	0.422 ^{d}	0.519 ^{b}	1.27 ^{fg}	1.49 ^{cde}	0.584	0.763
Tjir 1	0.143	0.183	0.492 ^{bc}	0.566 ^{a}	1.40 ^{ef}	1.66 ^{bc}	0.645	0.772
RRII 33	0.109	0.123	0.382 ^{ef}	0.409 ^{de}	1.07 ^{h}	1.17 ^{gh}	0.64	0.854
RRII 38	0.175	0.192	0.375 ^{f}	0.387 ^{ef}	1.15 ^{gh}	1.25 ^{fgh}	1.006	1.218
	NS	NS					NS	NS

*C-Control S-Stimulated

Values followed by common letters are not significantly different

2.4. Molecular and biochemical basis of ethylene induced latex production in *Hevea brasiliensis*-ethylene receptors and signal transduction mechanism

Expression pattern of genes (ETR1, ETR2, EIN2, EIN3 and ERF) involved in ethylene signal transduction was studied in different clones (RRII 105, PB 217, RRIM 600, Tjir 1, RRII 33 and RRII 38) before and after stimulation. The data indicated that multiple ethylene receptors were up regulated after stimulation in high yielding clones compared to low yielders. Up-regulation of these genes evidently related to yield increase.

2.5. Location specific- stimulant application and ethylene induced stress response in the tapping panel of *Hevea* clones

Based on the results of the effects of ethephon application at different locations on tapping panel and with different concentrations (clone RRII 105), a new experiment was initiated with RRII 400 series clones (RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430) and RRIM 600 at CES Chethackal to study the stimulation induced biochemical changes in latex and bark samples. Tree girth data was collected and the trees were opened for tapping.

3. Secondary metabolites

3.1. Water relation of latex with reference to the content of inositols and sugars in the latex during drought

A field trial on osmolyte content of laticiferous system and water relation of latex in *Hevea* is maintained at RRII. Plants with uniform girth are under selection for recording test tap yield and water relations in the latex.

4. Ecological impact of rubber cultivation

The greenhouse gas emission potential of rubber plantation and activities in the field was collated. Various sources of CO_2 emission in rubber planting and farm management practices starting from nursery practices, land clearance, field establishment to production of fresh latex were evaluated. In rubber plantations primary farm

Table Phy. 5. **Relative quantification of Sucrose transporters (SUT 1 & SUT 2) and Glutamine synthetase (GS) genes in ethylene stimulated samples of six clones compared to unstimulated control**

Clones	SUT 1	SUT 2	GS
RRII 105	3.93 ± 0.013	4.04 ± 0.097	2.34 ± 0.24
PB 217	3.25 ± 0.09	4.22 ± 0.307	2.8 ± 0.29
RRIM 600	0.691 ± 0.035	2.87 ± 0.291	2.5 ± 0.32
Tjir 1	1.75 ± 0.175	1.24 ± 0.226	1.3 ± 0.09
RRII 33	0.949 ± 0.029	0.52 ± 0.056	0.92 ± 0.16
RRII 38	0.499 ± 0.055	0.55 ± 0.213	0.82 ± 0.11
Unstimulated control	1.0	1.0	1.0

Table Phy. 6. **Potential emission from plantation activities in a life-cycle analysis**

Farm Activities	MT CO_2 equivalent ha^{-1} for life time
Seed bed & nursery preparations	0.08
Tillage and planting operation	3.61
Burning debris	0.29
Fertilizer	12.75
Plant protection measures (Pesticides and Weedicides)	4.99
Transportation of farm inputs	1.51
Crop harvesting	3.63
Total emission	26.86

management practices emit CO_2 significantly. Fertilizer application and fungicides spray are the major sources of emission from rubber plantations. Mechanization of farm management practices also emits considerable level of CO_2 through the use of carbon intensive fossil fuels. Total emission due to various farm activities was estimated to be around 26.9 MT CO_2 ha^{-1} in a life-cycle analysis (Table Phy. 6). Meantime the carbon sequestration potential of mature rubber plantation was worked out. A 30 year old rubber plantation can sequesters and store up to 220 T carbon ha^{-1} in the biomass of trees which is equivalent to 806 MT of CO_2. Thus it can be seen that NR plantations are a net sink of atmospheric CO_2. The net sequestration of NR holdings based on life cycle (30 years) analysis comes to around 777 MT of CO_2 ha^{-1}. Further accounting of emission from primary latex processing unit is progressing.

LATEX HARVEST TECHNOLOGY DIVISION

The Division was actively involved in the research and advisory services on all applied aspects of crop harvesting. The very low and uncertain rubber price, and acute shortage of availability of skilled tapper warrant development, popularization and adoption of measures to reduce cost of production of each kilogram of rubber produced. The programme on popularising low frequency weekly tapping in small holdings was continued. The share of old and senile trees has increased considerably in the recent past and is one of the major reasons for productivity decline year after year. An all-out effort of implementing proper Controlled Upward Tapping (CUT) can be an effective tool to address this issue. Hence focussed research on low frequency controlled upward tapping (LFCUT) was another area of research activity of the division.

In the farm mechanization programme, one model of a mechanised tapping tool was evaluated. Other activities included testing and evaluation of products and training on crop harvesting.

1. Low Frequency Tapping

The Division continued experiments, on farm trials and advisory on Low frequency Tapping. Two RBD experiments on d10 frequency of tapping (BO-1 and BO-2 panel) was initiated during the year.

1.1. Popularising weekly tapping

The programme on popularising weekly tapping in various locations was monitored and continued. Majority of the growers who have joined the programme are continuing weekly tapping with good results (Table LHT 1-3).

Table LHT 1. **Yield performance of clone RRII 105 under weekly tapping**

Location	Plots	Mean yield ($g\ t^{-1}\ t^{-1}$)
Marthandam	1	100
Kanjirappally	3	124
Koottickal	4	83
Thodupuzha	6	72
Mannarkkad	3	77
Thalaserry	3	229
Kothamangalam	1	91
Thrissur	1	100
Changanaserry	3	76
Muvattupuzha	1	83
Pala	1	212
Ernakulam	1	100
Mean	-	112

Table LHT 2. **Yield performance of clone RRII 414 under weekly tapping**

Location	Plots	Mean yield ($g\ t^{-1}\ t^{-1}$)
Thodupuzha	5	113
Kanjirappally	12	55
Koottikkal	7	73
Meenadam	2	66
Thalaserry	2	97
Mannarkkad	1	49
Muvattupuzha	2	77
Pala	2	122
Mean	-	82

Table LHT 3. **Yield performance of clone RRII 430 under weekly tapping**

Location	Plots	Mean yield ($g\ t^{-1}\ t^{-1}$)
Thodupuzha	5	82
Mannarkkad	2	78
Palakkad	2	91
Muvattupuzha	2	82
Mean	-	83

1.2. Low frequency (d10) tapping system in clone RRII 105

1.2.1. *Large scale trial on d10 frequency of tapping in clone RRII 105*

The large scale on farm trial on d10 frequency of tapping initiated in 1987 field during 2014-15 at Kanthimathy Estate, Kulaskharam, Tamil Nadu in clone RRII 105 was continued in the 5th year of BI-1 panel. Once in 20 days stimulation was imposed in 10 tapping blocks (450 trees each). Mean dry rubber yield of 1478 kg ha^{-1} could be obtained under d10 frequency of tapping (Table LHT. 4) Heavy rain fall and climatic constraints leading to loss of tapping days had affected the performance during the year 2018-19.

1.2.2. *Exploratory trial on LFT (d10) in clone RRII 105*

This exploratory trial on d10 frequency of tapping was initiated from April 2004 at CES, Chethackal in field 1987. The trees were tapped under d7 frequency of tapping with monthly stimulation in BO-

Table LHT. 4. **Yield performance of clone RRII 105 to d10 frequency of tapping at Kanthimathy estate, Kulasekharam, Tamil Nadu (Mean of 10 blocks)**

Month	kg block^{-1}	kg tap^{-1}	g t^{-1} t^{-1}
Apr'18	94.3	31.4	73.1
May	70.3	37.9	88.0
June	78.2	38.7	90.1
July	181.6	63.8	148.8
Aug	126.3	51.9	120.9
Sep	119.4	42.0	97.8
Oct	159.9	59.6	138.8
Nov	174.5	65.2	151.8
Dec	162.7	58.8	136.8
Jan'19	149.9	50.0	116.6
Feb	75.9	32.0	74.6
Mar	84.8	32.3	74.2
Total	1478	-	-
Mean	123	47	109

Table LHT. 5. **Monthly yield response of Low Frequency Tapping (S/2 d10) in clone RRII 105**

Months	Yield (kg 400 trees^{-1})	Yield (kg tap^{1})	No. of taps	DRC (%)
Apr'18	236.7	78.9	3	44.3
May	215.7	71.9	3	42.6
June	173.6	57.9	3	37.1
July	177.5	59.2	3	40.0
Aug	141.7	47.2	3	33.8
Sep	159.0	53.0	3	36.2
Oct	187.8	62.6	3	43.0
Nov	174.6	58.2	3	37.3
Dec	144.9	48.3	3	41.4
Jan'19	134.2	44.7	3	44.2
Feb	164.2	54.7	3	41.7
Mar	149.6	49.9	3	39.6
Total	2059.5	52.7	36	40.1
		(mean)		(mean)
Kg tree^{1}	5.1			

2 panel for two years during 2002-2004. From April 2004 onwards the tapping frequency was changed to d10 frequency of tapping with once in 20 day's stimulation. The exploratory trial at CES under d10 frequency of tapping with once in 20 day's stimulation continued to give promising yield even in BI-1 panel during 2018-19 (Table LHT. 2). Controlled Upward Tapping (CUT) (S/3 d10) was practiced for five months and rest of the months trees were tapped in the basal panel tapping (S/2 d10). Yield of 2059 kg 400 trees^{-1} was obtained during 2018-19. It was 5.1 kg tree^{1} year^{1} and 52.7 kg tap^{1}.

1.2.3. *Large scale experiment on Low Frequency Tapping (d10) in clone RRII 105 (Panel BO-1)*

This large scale RBD experiment on d10 frequency of tapping from opening

Table LHT. 6. **Yield response of Low Frequency Tapping (S/2 d10) in clone RRII 105 (Panel BO-1)**

Treatment	Yield	
	(g t^{1} t^{1})	(kg t^{1})
T1- S/2 d6 ET.2.5% Once in 14 days	135.9 ab	7.3 c
T2- S/2 d6 ET 5% 12/y (m)	140.7 a	7.5 c
T3- S/2 d10 ET.2.5% Once in 20 days	131.4 b	4.4 d
T4- S/2 d10 ET. 5% Once in 20 days	131.6 b	4.4 d
T5- S/2 d10 ET.2.5% , 5 % Once in 20 days	129.9 ab	4.3 d

Values followed by common letters are not significantly different

onwards (Panel BO-1) in field 2009 was laid out at Kanthimathy Estate, Kulasekharam, Tamil Nadu trees during 2017-18 in clone RRII 105. The experiment comprised of five treatments and 20 plots (160 trees) and different levels of stimulation comprising of d6 and d10 frequency of tapping. Dry rubber yield was observed to be higher under weekly tapping compared to d10 frequency of (Table LHT. 6).

1.2.4. Large scale experiment on Low Frequency Tapping (d10) in clone RRII 105 (Panel BO-2)

Large scale experiment on Low Frequency Tapping (d10) in clone RRII 105 at CES, Chethackal initiated during July 2017 in field 2002 was continued. Six blocks of RRII 105 were statistically RBD) laid out and imposed yield stimulation under LFT systems. There were six treatments comprising d7 (with monthly stimulation as control) and d10 frequencies of tapping with different levels of stimulation. The experimental design was RBD with four replications (80 trees per plot). Yield under weekly tapping with monthly stimulation (ET.2.5%) was comparable to that of d10 frequency of tapping with once in 20 days stimulation (ET.5%). Yield of 1936 kg 400 trees^{-1} was obtained under d10 frequency of tapping with once in 20 days stimulation (ET. 5%) in the BO-2 (2) panel (Table LHT. 7). Under d10 frequency, monthly stimulation showed significantly lower yield than other treatments. Mean drc under d10 frequency with monthly stimulation (2.5%) was significantly higher than weekly tapping.

12. Demonstration trial on LFT (d7) in clone RRII 105

Two more blocks of demonstration plots on weekly tapping were initiated at Central Experiment Station (CES) in clone RRII 105 during 2016 in two tapping blocks in field 2000. During 2018-19, twelve rounds of yield stimulation were imposed at monthly interval with 2.5 per cent ethephon. Missing of tapping days during April in both the blocks and led to lower yield. Tapping panel of two blocks was changed to BI-1 during 2018-19. Yield of both blocks gave sustainable yield of 1876 (block 1) and 1794 kg 400 trees^{-1} (block 2), respectively during 2018-19 (Table LHT. 8). It was 4.7 and 4.5 kg tree^{-1} respectively during twelve months of tapping. Mean dry rubber content of both blocks was 39.7 per cent. The average yield of both blocks was 4.6 kg t^{-1} year^{-1}.

At CES, Chethackal, another demonstration trial in field 93C under

Table LHT. 7. **Yield response of Low Frequency Tapping (S/2 d10) in clone RRII 105**

Treatment	Yield (kg 400 trees^{-1})	DRC (%)
T1- S/2 d7 ET 2.5% 12/y	2049 a	38.2 c
T2- S/2 d10 ET. .5% once in 10 days	1683 b	37.9 c
T3- S/2 d10 ET 2.5% Once in 20 days	1676 b	40.0 b
T4- S/2 d10 ET 2. 5% once in 20 days	1913 ab	37.7 c
T5- S/2 d10 ET 2.5% 12/y	1370 c	41.7 a
T6 – S/2 d10 ET. 5% 12/y	1698 b	39.7 b

Values followed by a common alphabet are not significantly different by DMRT

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1.2.4. Large scale experiment on Low Frequency Tapping (d10) in clone RRII 105 (Panel BO-2)

Large scale experiment on Low Frequency Tapping (d10) in clone RRII 105 at CES, Chethackal initiated during July 2017 in field 2002 was continued. Six blocks of RRII 105 were statistically RBD) laid out and imposed yield stimulation under LFT systems. There were six treatments comprising d7 (with monthly stimulation as control) and d10 frequencies of tapping with different levels of stimulation. The experimental design was RBD with four replications (80 trees per plot). Yield under weekly tapping with monthly stimulation (ET.2.5%) was comparable to that of d10 frequency of tapping with once in 20 days stimulation (ET.5%). Yield of 1936 kg 400 trees^{-1} was obtained under d10 frequency of tapping with once in 20 days stimulation (ET. 5%) in the BO-2 (2) panel (Table LHT. 7). Under d10 frequency, monthly stimulation showed significantly lower yield than other treatments. Mean drc under d10 frequency with monthly stimulation (2.5%) was significantly higher than weekly tapping.

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At CES, Chethackal, another demonstration trial in field 93C under

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T6 – S/2 d10 ET. 5% 12/y	1698 b	39.7 b

Values followed by a common alphabet are not significantly different by DMRT

weekly tapping with monthly stimulation continued to give promising yield (1937 kg 400 trees^{-1}) during 2018-19. It was 4.8 kg tree^{-1} year^{-1}. With the introduction of CUT under weekly tapping for five months, yield was higher than basal panel tapping yield for seven months.

2. Controlled Upward Tapping (CUT)

2.1. Large scale on farm trial on Low Frequency Controlled Upward tapping (LFCUT) under weekly tapping

The large scale on farm trial on LFCUT with periodic panel change under weekly tapping was initiated during 2016-17 was continued at Kanthimathy estate, Kulasekharam, Tamil Nadu in 12 tapping blocks (8 blocks 1978 mixed clone wand 4 blocks 1962 seedling population). The Low Frequency Controlled Upward Tapping (LFCT) under weekly tapping continued to give promising results. Lower yield was observed in mixed population (with low yielding clones) compared to seedling population. Higher girth of seedling trees has contributed for better yield under CUT in seedling population.

3. Other Experiments

3.1. Response of RRII 400 series clones to yield stimulation

At CES, Chethackal, RRII 400 series clones (RRII 414, RRII 422 and RRII 429) were identified for the experiment in field 2004. The statistical design was completely randomized single tree single plot consisting of more than 100 trees of each clone per treatment. Tapping system adopted in this trial is S/2 d3 6d/7. Three rounds of stimulation (ET 2.5% Pa) was given during 2018-19 in comparison with the unstimulated control trees (50% trees for stimulation and 50% trees

Table LHT. 8. **Monthly yield response of Low Frequency Tapping (S/2 d7 6d/7) in clone RRII 105 (BI-1 panel)**

Months	Block 1 Yield (kg 400 trees^{-1})	Block 2 Yield (kg 400 trees^{-1})	Block 1 DRC (%)	Block 2	No. of taps RC (%)
April'18	43.0	31.2	48.6	45.6	2
May	206.1	160.9	42.3	42.7	4
June	321.7	307.5	39.4	37.0	5
July	342.8	357.6	36.5	35.3	4
Aug	245.8	221.1	35.6	36.1	5
Sep	147.5	113.7	39.3	39.8	4
Oct	110.3	140.9	40.3	41.7	4
Nov	114.2	146.0	37.1	37.7	5
Dec	91.0	82.7	44.5	38.0	4
Jan'19	88.5	97.8	38.2	38.2	4
Feb	96.6	75.1	36.2	37.0	4
Mar	68.3	59.3	38.2	39.5	5
Total	1875.8 (4.7*)	1793.8 (4.5*)	39.7 (mean)	39.1 (mean)	50

*kg tree^{-1}

Table LHT. 9. **Yield performance under LFCUT weekly tapping**

Month	Mixed Clone			Seedling Population		
	kg block^{-1}	kg tap^{-1}	g t^{-1} t^{-1}	kg block^{-1}	kg tap^{-1}	g t^{-1} t^{-1}
Apr'17	78.4	20.2	47.0	81.4	20.3	47.3
May	66.4	27.0	62.7	96.5	35.1	81.6
June	63.8	25.6	59.5	53.6	45.6	106.0
July	179.3	43.4	101.0	206.8	54.6	126.9
Aug	106.8	37.4	87.0	183.0	48.9	113.7
Sep	128.4	30.2	70.2	167.6	44.5	103.4
Oct	132.9	36.7	85.5	165.3	38.5	89.6
Nov	124.3	33.1	77.1	202.1	50.5	117.5
Dec	167.3	41.8	97.3	301.0	67.4	156.8
Jan'18	144.8	32.3	75.1	217.9	47.7	111.0
Feb	99.6	24.9	57.9	135.3	36.1	83.9
Mar	114.4	20.2	70.8	144.1	36.0	83.8
Total	1398	-	-	1955	-	-
Mean	131	32	74	163	44	102

unstimulated, for each clone). In clone RRII 422, stimulated trees showed significantly higher yield than unstimulated trees. No significant yield increase was noticed in stimulated trees of clones RRII 414 and RRII 429 (Fig. LHT. 1). Higher yield was observed both stimulated and unstimulated trees of clone RRII 429 than other two clones of RRII 414 and RRII 422 in BO-2 (3) panel.

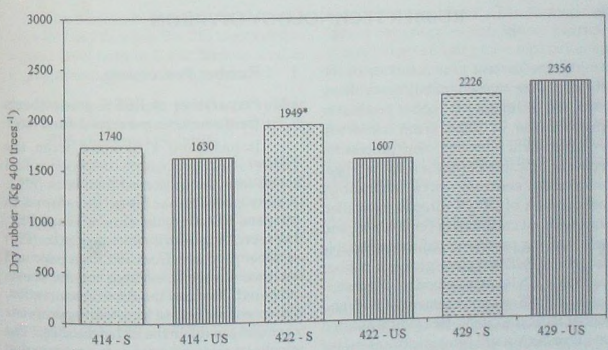


Fig. LHT.1. Cumulative yield performance of RRII 400 series

In clone RRII 422, annual mean drc was 3 per cent higher in unstimulated trees than stimulated trees. TPD percentage was higher in stimulated trees (28%) than unstimulated trees (15%) of clone RRII 414 than other two clones.

3.2. Response of RRII 430 clone to yield stimulation under LFT

In another experiment at HML, Palapilly Estate, response of RRII 430 clone to yield stimulation under Low Frequencies of Tapping (d3, d4 and d7) was studied from June 2016 (BO-1). The statistical design was RBD with four treatments and five replications. Each replication was consisting of more than 300 trees. There were four treatments comprising of d3 (without stimulation as control), d3 (2/y), d4 (4/y) and d7 (12/y) frequencies of tapping. During the period, yield was recorded from April 2018 to March 2019.

Higher yield was noted under d4 frequency of tapping (61.4 g t^{-1} t^{-1}) with 4 rounds of stimulation followed by weekly tapping (58.0 g t^{-1} t-1). Dry rubber yield was more under d3 frequency of tapping with two rounds of stimulation (46.4 g t^{-1} t^{-1}) compared to unstimulated d3 (41.8 g t^{-1} t^{-1}). 11 per cent yield was increased under d3 frequency of tapping with two rounds of stimulation when compared to control (d3 without stim). The drc values of trees tapped under d7 frequency of tapping with twelve rounds of stimulation showed higher (40.9%) than the d3 frequency of tapping with two rounds stimulation (37.5%). Agroclimatic constraints such as flood had affected the crop performance during the year.

RUBBER TECHNOLOGY DIVISION

In the current year activities of the Division were focused mainly on evolving improved techniques in rubber processing (preparation of RSS4 from ammonia preserved field latex and new process for recovery of rubber from serum in sheet processing centres), latex technology (preparation of DPNR directly from filed latex without creaming or centrifuging and new leaching process to reduce extractable protein in NR surgical gloves), rubber technology (NR Latex carbon black master-batch and silica reinforcement of NR) and rubber recycling (Stable free radical assisted devulcanisation and cytotoxicity analysis of crumb rubber from end of life tyres).

1. Rubber Processing

1.1. Preparation of RSS 4 grade sheets from ammonia preserved filed latex

It has been a demand from the growers and processors to prepare RSS sheets from the preserved filed latex (PFL). A new process has been developed to prepare RSS4 grade sheets from PFL. However, the plasticity retention index (PRI) of sheet prepared from PFL was poor and attained a very low value for the sheets prepared after one month of preservation. Pre-treatment of field latex with appropriate chemical can retain the PRI of the sheet. The results of the pre-treatments are shown in Table Chem. 1 and 2.

Table Chem. 1. **Effect of pre-treatment of LATZ latex on raw rubber properties**

Sample	Untreated			Treated		
	P_0	PRI	MV	P_0	PRI	MV
0 day (Fresh field latex)	64	95	98	65	95	99
7 days	59	37	87	61	57	99
10 days	56	22	80	58	55	94
15 days	51	20	80	54	48	77
30 days	48	18	81	51	48	80
60 days	41	14	74	48	49	73
180 days	38	11	71	39	46	76

Table Chem. 2. **Effect of f pre-treatment of high ammonia latex on raw rubber properties**

Sample	Untreated			Treated		
	P_0	PRI	MV	P_0	PRI	MV
0 day (Fresh field latex)	64	95	98	65	95	99
7 days	60	38	87	63	59	98
10 days	57	21	80	58	56	96
15 days	51	18	80	55	50	80
30 days	44	15	81	51	49	78
60 days	41	12	74	50	48	73
180 days	40	11	71	38	47	74

The results showed that the untreated latex lost its PRI while the PRI is retained to a good level both in LATZ latex as well as high ammonia latex when pre-treated.

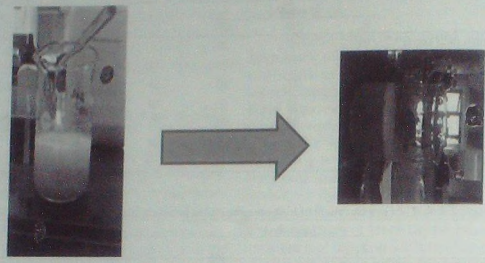
1.2 New and simple process for rubber recovery from acid wash serum in RPS sheet making units

The processing of field latex into rubber sheets generates large volumes of aqueous effluents which contains uncoagulated rubber particles and non-rubber organic materials like proteins, lipids and inorganic salts. The auto flocculation of the rubber particles is not effective and takes several weeks. The discharge of this wash water to local water bodies creates ecological imbalance. In effluent treatment plants the presence of rubber particles creates rubber trapping and subsequent coagulation problems. The formation of larger aggregates of latex particles (flocculation) and its phase separation and floating on the water surface was achieved with the polymeric coagulants under the present study. The clarity of the water beneath could be improved, so that the clear water could be drained from the bottom for further downstream effluent treatment plant (ETP) activities.

2. Latex Technology

2.1. Production of solid deproteinised natural rubber (DPNR-S)

A novel method for production of solid DPNR without centrifugation and creaming was developed last year with deproteinization mixture developed in-house. The pilot scale studies of preparation



Acid wash serum from sheet making RPS units

Serum clarifies and rubber flocculates after treatment

Fig. Chem.1. The new method for flocculation of rubber in acid wash serum from sheet rubber processing units

of 250 kg of solid DPNR was done in a TSR manufacturing factory during the reporting period. The raw rubber properties of the solid DPNR were quiet promising with high PRI (Table Chem. 3).

Table Chem. 3. **Raw rubber properties of solid DPNR**

DPNR Samples	P_o	PRI	MV	N (%)
Batch-1	31	79	61.3	0.08
Batch-2	32.5	78	64.3	0.09
Commercial DPNR (Imported)	31	9	59	0.07

2.2. New leaching process to reduce extractable protein (EP) in surgical gloves

Our studies to minimise the extractable proteins in commercial surgical gloves in Indian market was continued. Commercial gloves were subjected to an offline leaching conditions developed in the laboratory (RRII-M-1) and compared to the normal extraction of the same gloves by ASTM D 5712-10 method. The new method could increase extraction of protein by 37 percent. A further modification of the offline leaching process (RRII-M-2) increased the EP value by 96 per cent than the normal extraction of the same glove by the ASTM procedure. Table Chem. 4 shows the EP values attained in different methods and the percent increase in EP from surgical gloves in the new leaching methods developed in house in comparison to the standard extraction process. The enhanced leaching

Table Chem. 4. **Comparison of extractable protein (EP) values**

Method	EP values of the glove (µg/g)	Percent increase in EP
ASTM D5712-10	150	-
RRII-M-1	206	37
RRII-M-2	295	97

has the potential to be scaled up to online leaching process in future.

3. Rubber Technology : Reinforcement of rubber

3.1. NR Latex carbon black master-batch

Studies on the preparation of NR latex - carbon black master batch were continued. New surfactants (S4 and S5) were used for the preparation of carbon black dispersions and latex-carbon black master-batches were prepared. The samples were compounded as per the formulation given below (Table Chem. 5) and the properties were compared with a control dry mix (Table Chem. 6). The results show that the properties of latex carbon black master-batch are comparable to that of the dry mix except tear strength which is lower for the master-batch. Tear strength of S5 was comparable to that of the control compound. However the flex resistance of the master-batches were considerably better than control compound prepared using surfactant S4.

Table Chem. 5. **Formulation of the mixes**

Ingredients	Phr
Master batch	(Effective NR 100 phr)
ZnO	4.5
Silica	8
Stearic acid	3
TMQ	1
6 PPD	2.75
TBBS	1.6
Sulphur	1.2

Table Chem. 6. **Mechanical properties of master batches with S4 surfactants**

Properties	Control (Dry mix)	Master-batch With S4	Master-batch With S5
Tensile strength, Mpa	29.71	28.71	25.11
Tear strength, N/mm	140	101	145.34
Elongation at break , %	563	578	554
Modulus, 100%, MPa	4.50	4.35	3.52
Modulus, 300%, MPa	14.39	14.12	13.62
Hardness, Shore A	63	63	68
Compression Set, %	35.73	35.56	35.32
Heat build-up, Δ °C	22	21	24
Abrasion loss, mm^3	93	95	118.51
Flex Resistance			
Complete failure, Kcys	180.100	208.520	152.04

3.2. Silica reinforcement of natural rubber

Silica reinforcement of natural rubber (NR) has two aspects. First one is the silanisation of NR by a silane coupling agent and the other one is the chemical bonding of the silanised silica with NR during vulcanisation. In NR, the influence of non-rubber constituents on hydrophobation of silica can be avoided by the use of pre-silanised silica. The issue in silica reinforcement of NR is the poor coupling between the silanised silica and the rubber molecules during vulcanisation. The cause of the poor coupling may arise from the inability of silanes to form sufficient numbers of covalent bond with the NR chains during the vulcanisation process resulting in low M300 and M300/M100 ratio leading to significantly lower abrasion resistance compared to the carbon black filled NR vulcanisates.

A standard vulcanising system comprising N-cyclohexyl-2-benzothiazolyl sulphenamide (CBS), diphenyl guanidine (DPG) and sulphur, which is the widely used cure system in silica reinforced rubbers, is compared with the cure system developed in our lab along with a standard carbon black (HAF) filled NR vulcanisate. On comparing with the standard cure system used for the vulcanisation of NR/ silanised silica and carbon black filled

systems, the four different cure systems developed in our laboratory showed better mechanical properties. Cure system 1 gave an average tensile strength of 27.1 MPa while that of NR/silanised silica cured with standard cure system and carbon black filled NR vulcanisates were 24.8 and 25MPa, respectively. Besides good tensile strength, cure systems I to III showed abrasion resistance comparable to that of HAF filled NR vulcanisates. The results obtained clearly demonstrated the significant role of cure system to attain excellent vulcanisate properties for silanised silica reinforced NR.

4. Rubber Recycling

4.1. Optimisation of stable free radical concentration for mechanical devulcanisation of commercially available ground tyre rubber (GTR) powder

Since the major source of used tyre powder available in the market is ground tyre powder (GTR), optimisation of the concentration of the 4-hydroxy TEMPO (4HT) required for effective mechanical devulcanisation was essential for commercial utilisation of the work. The GTR mixed with 0-10 phr 4HT was mechanically devulcanised in a two roll mill and the efficiency of the devulcanisation process was evaluated in terms of the revulcanisate properties of the corresponding devulcanised rubber (Table Chem. 7).

The impact of adding 4HT on revulcanisate properties is evident even when its concentration is as low as 0.5phr. However more consistent results were obtained at a 4HT concentration of 1-2 phr. Formulation and properties of a typical commercial mat was compared with the results of the above. A number of ingredients used for the preparation of mats can be avoided and the percentage of regenerated rubber (both crumb rubber powder and reclaimed rubber) used can be replaced with devulcanised rubber. Moreover the amount of regenerated rubber used in standard mat formulations can be increased from 71 per cent to 86 per cent with much superior properties using the new devulcanisation technology.

4.2. Cytotoxicity analysis of crumb rubber from end of life tyres

The project was initiated as a collaborative project with M/s. Apollo tyres. The composition analysis of the crumb rubber powder (CRP) from end of life tyres was done using TGA analysis, acetone extraction and ash test.

Table Chem. 7. 4HT Concentarion vs. revulcanisate properties of GTR

Re-vulcanisate properties	4-hydroxy TEMPO, phr							
	0	0.5	1	2	4	6	8	10
Tensile strength, MPa	5.4	6.8	7.1	8.0	7.1	7.4	7.3	7.0
M100, MPa	2.8	3.0	2.8	3.3	3.2	3.1	3.6	3.3
M200, MPa	-	6.3	6.0	7.0	5.8	6.5	7.1	6.1
Elongation at break (%)	189	233	235	226	212	228	205	215
Tear strength, N/mm	17.3	24.2	21.1	24.8	26.2	25.1	25.9	24.5

*Crumb rubber powder- 100g, NR-10g, ZnO-5phr, Stearic acid-2phr, CBS-1phr, S-1.8phr

The TGA analysis shows that the CRP composition has low molecular weight components of 4 per cent, polymer content of 55 per cent, carbon black filler of 32 per cent and silica filler of 8.8 percentage (w/w). The ash content of the CRP from TGA (8.8%) and muffle furnace (9%) matches each other and the total acetone extractable by acetone extraction comes around 13 per cent. The extraction of leachable components from CRP is under progress.

5. Development/advisory work/ project work

Tested and report given for the damaged tyres referred from various consumer disputes redressal forum in the country.

TECHNICAL CONSULTANCY DIVISION

The activities of the Division are designed in such a way that the rubber based units of the country numbering to over 4800 will be able to tap the benefits of the basic and applied research and developmental activities being conducted in ACRT. Technical Consultancy Division is an NABL approved laboratory and has the facility to test over 170 quality parameters of rubber products. Since the laboratory is following ISO 17025 norms for its routine analysis, its test certificates are valid globally and are beneficial to the exporters of rubber products in the country. The Division also accumulates knowledge on industrially important problems by conducting R & D programmes so that the problems in the manufacturing sector can be overcome within the shortest possible time. The major activities of the Division are summarized below.

The services provided are R&D activities of rubber industry (both products and processes), development of new products, testing/certification of rubber products as per relevant national and international standards. The services offered by the division include (i) testing support to industries as per national and international standards *i.e.* ISO, BIS, ASTM, EN, ASRTU *etc.* ii) Product development-demonstration/ practical training for quality improvement (iii) evaluation of chemicals (iv) preparation of project profiles and technical bulletins to entrepreneurs (v) advisory services and (vi) conducting awareness meetings/lectures to entrepreneurs regarding trouble shooting/ cost reduction of factory processes.

The highlights of the projects are given below.

1. Research Projects

1.1. Finger printing of natural rubber latex

Centrifuged latex conforming to IS 5430:1981is essential for the manufacture almost all latex products say gloves, condoms, Balloons *etc.* However very often centrifuged of latex is marketed with impurities either present inherently in the latex or added purposefully in order to reduce the cost of the latex. These added impurities sometimes may not be always reflected in the usual specification testing

of the latex. Hence in order eliminate the traces of such additions and also to fit the latex more specifically to every product, finger printing of latex is carried out using FTIR spectroscopy.

FTIR analysis of NR-skim blend results showed absorption peaks at 1,656 (C=O stretching (amide I)) and 1,539 cm^{-1} (N-H bending of amide II). But its intensity was very feeble in the case of centrifuged latex film, whereas the intensity increased as the skim addition increased. The results showed that as the dose of skim latex increases sharp changes can be seen at 3281 cm^{-1}. Thus skim latex contamination of centrifuged latex can be detected easily.

Similarly blending of SBR latex in minute quantities can be detected by analysing the peaks at 699-700 cm^{-1} referring to the styrene units. The results showed that as the dose of SBR latex increased sharp changes can be seen above 1phr at 700 cm^{-1}.

Thermo Gravimetric Analysis (TGA) of NR/PCC composite showed a peak at 370°C (major peak) mainly attributed to rubber decomposition and two discrete weight loss steps in the temperature ranges of 60-100°C and 400-500°C, corresponding to the loss of water and the degradation of PCC-NR bounded in the composite, respectively.

1.2. Shelf life study of gloves

Shelf-life prediction of rubber medical products is unavoidable since manufacturing industries need to declare the service life of rubber products which is mandatory for several products. Effort was taken to establish the effectiveness of the existing Arrhenius and WLF models to evaluate the shelf-life of surgical gloves. The products were aged at different temperatures to varying periods as per ISO 13320:2005 and properties of importance were measured.

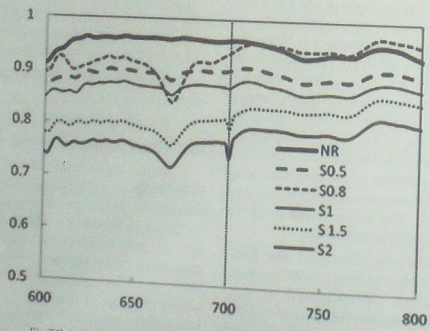


Fig.TC. 1. FTIR spectra of latex films casted with NR-SBR latex blend

1.3. Morphological studies on latex films prepared by creamed prevulcanized latex

In latex product manufacturing, latices having high dry rubber content (concentrated latex) are generally used. During creaming process, the latex gets enriched in rubber content and the non-rubber constituents and other impurities can be separated through the serum. In this study, field latex collected from RRII was prevulcanized and subjected to creaming. Results showed that prevulcanized latex produced by creaming of field latex gave films of better quality and such materials gave products with excellent colour retention.

1.4. Binary blend for tread application

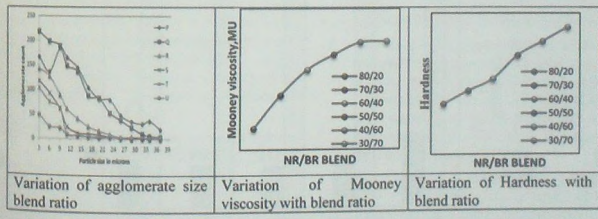
In order to develop cost competitive tread formulations as per ASRTU specifications two grades of carbon black namely HAF and ISAF were mixed with selected blends of NR/PB. The dispersion of carbon black in the blend was analyzed using Dispergrader. It was found that there is a homogenous distribution of carbon black in the blend and the homogeneity increased with BR content. The agglomerate particle size also decreased with BR content as shown below.

The increased Mooney viscosity of the blend with BR content may be the reason for the better dispersion. There is a preferential absorption of carbon black in the BR phase as it is evident from the hardness. The increased dispersion in the BR rich blend might have restricted the movement of the macromolecular chains. This might have led to the increased Mooney viscosity and the hardness.

1.5. Prevulcanization of natural rubber latex

The mechanical and after ageing properties of radiation vulcanized natural rubber latex (RVNRL) are normally low when compared to sulphur prevulcanized natural rubber latex (SVNRL). Therefore, a radiation assisted peroxide crosslinking was suggested as a new method for the vulcanization of NR latex.

Prevulcanization of latex using peroxide was conducted at various irradiation doses (0-16 k.Gy). The result showed that radiation assisted peroxide



Variation of agglomerate size blend ratio | Variation of Mooney viscosity with blend ratio | Variation of Hardness with blend ratio

Fig. TC. 2. Variation of agglomerate size, Mooney viscosity and Hardness with blend ratio

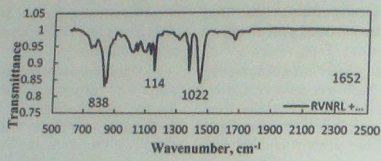


Fig. TC.3. FTIR spectrum of peroxide cross-linked natural rubber

vulcanization gave higher tensile strength and ageing properties. It was found that peroxide crosslinking is a more practical method to reduce the vulcanization dose required for the preparation of prevulcanized latex using γ-radiation. FTIR studies showed that (figure given below) the absorption band due to C-C stretch (1022 and 1147 cm^{-1}) is strong in the new cure system.

1.6. Solvent-based rubber adhesive compounds for tyre re-treading

Rubber-based solution adhesives for tyre re-treading was developed and tested as per ASRTU specifications. Initially the effect of vulcanizing agents on the cure characteristics was studied and optimized the components of the system. The adhesion strength of rubber-based solution adhesives was tested and the role of particle size of carbon black on bonding was investigated. Correlation of the results of the FTIR spectroscopy of different carbon black filled rubber adhesive with their adhesion strength was studied. Effect of carbon black on the crosslink density and swell-index of solvent-based rubber adhesives was also determined. The results (Fig. TC. 4 and Table TC. 1) indicated that both the mechanical properties

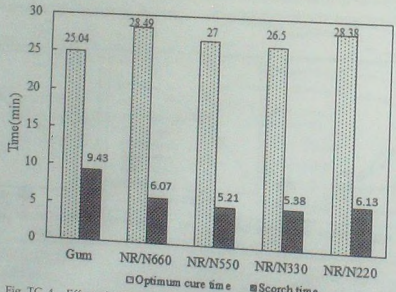


Fig. TC. 4. Effect of different carbon black on scorch and optimum cure time of rubber-based solution adhesives

Table TC. 1. **Effect of different carbon black on mechanical properties of rubber-based solution adhesives**

Sample	NR/N660	NR/N550	NR/N330	NR/N220
Tensile strength (kg/cm^2), Min.	264	229	263	268
Angle tear, kgf/mm, Min.	6.8	4.8	8.4	8.8
Adhesion peel strength, g/cm	2	1.2	1.5	1.7

and adhesion strength of rubber-based solution adhesives were not affected by the type of carbon black used.

It was concluded that general purpose carbon black which are relatively cheap and having high particle size can be used for re-treading applications.

2. Testing and certification

2.1. NABL accredited testing services

The NABL reassessment of the TC laboratory has been carried out successfully from 13 to 14 October 2018. The laboratory follows the ISO/ISE 17025 documentation and national/international testing procedures for its routine services. Products belonging to natural rubber, synthetic rubber, latex, thermoplastics are tested as per the relevant national and international standards (export market). Different types of rubber products tested in the Division are bridge bearings, rubber diaphragms, pre-cured/conventional treads, bonding gum, black vulcanizing cement, tyre flaps, inner tubes, rubber channels/beadings, floor mats, Hawaii soles, sponge rubber, O-rings, bushes, engine mounts, automobile components *etc.* The major latex products tested include examination and surgical gloves, latex adhesives, latex thread, balloons, Foley catheters, condoms *etc.* Purity of rubber chemicals and the quality certification of reclaimed rubber are also tested consistently by the Division. An account of the rubber products tested for the last year is given below.

Table TC. 2. **Number of samples tested and the revenue collected (2018-19)**

No. of clients	416
No. of samples tested	833
No. of parameters analysed	3402
Consultancy letters/e-mail	2385
Trouble shooting of factory process	13
No. of test reports issued	852
Component analysis	54
Total revenue collected (Rs.)	22,64, 356

3. Product development

The division offers services to entrepreneurs as well as existing rubber based industries in the development of rubber products based on both synthetic and natural rubber. In this area, the numbers of services given are categorized as follows (Table TC. 3).

The clients who availed the facilities of the Technical Consultancy division include entrepreneurs from both public and private enterprises.

Externally funded major projects

- *Rubber Products Incubation Centre*

  Based on our proposal and also to promote the innovative ideas of young entrepreneurs, Kerala State Industrial Development Corporation (KSIDC) has sanctioned Rs.50 lakhs for the establishment of Rubber Products Incubation Centre at RRII.
- Prepared and transferred project report for setting up a surgical & examination gloves manufacturing unit for M/s. SPSM Pharmaceuticals, Gujarat and collected Rs.50,000/- towards the consultancy fee.
- Undertaken a project for the

Table TC. 3. **Number of Know-how transferred to industries**

Name of products developed	Numbers
Adhesives	4
Automobile components	11
Expanded rubber sheets and soles	3
Fire resistant mats	1
Latex based dipped and foamed goods	3
Pre-cured tread, bonding gum and tube valve	14
Rubber based agro machinery components	2
Rubber based engineering components for railway, defence, BSF, Kochi Metro *etc.*	2
Rubber moulds	2

development of rubber pots for agricultural purposes sponsored by M/s. Navasakti Trust, Kollom and collected Rs.1.9 lakhs towards the consultancy fee.

- Completed a project report for setting up a automatic balloon manufacturing unit for M/s. Green Tech Balloons LLP, Hyderabad and collected Rs.50,000/- towards the consultancy fee.
- Completed the works for Bharat Petroleum Corporation Limited (BPCL), Kochi to overcome the foul smell associated with the production of rubberised bitumen and collected Rs.1.2 lakhs as consultancy fee.
- Designed an effluent treatment system for their rubber band manufacturing unit of M/s. Sreelakshmi Industries, Pathanamthitta and collected Rs.3,000/- towards the consultancy fee.
- Consultancy services were provided to TSR unit of M/s. Karnataka Forest Development Corporation Ltd., Sullia, and Karnataka for improving the production and collected Rs.47,000/- towards the consultancy fee.

Project profiles/Technical bulletins

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

Advisory services

Matters relating to various aspects like selection of raw materials, dosage of ingredients, redesign of formulation, processing conditions, recent regulation *etc.* were always a subject of concern among the clients. The Division has given appropriate guidance in all these aspects.

ECONOMICS DIVISION

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

1. A road map for attaining self-reliance in natural rubber production in India by 2030

Indian natural rubber (NR) plantation sector supports an industry that is vital to the nation's industrial and economic growth. Sustained extension efforts supported by focused research and development by the Rubber Board in the past more than 60 years helped to establish a robust NR production

base in the country, characterised by a steady expansion in area under cultivation and increase in productivity and total production. This helped India to achieve near self-sufficiency in NR production until 2010. The production consumption gap of NR in India was negligible till 2008-09 (Fig. Eco. 1). The production consumption gap started widening since 2011 due to sharp fall in NR prices forcing the local rubber industry to import almost 50 per cent of their demand from international market. As the Indian economy is expected to maintain its buoyancy in growth, it is estimated that by 2030 the country would require about 20 lakh tonnes of NR per year. Present production is in the range of 6 to 7 lakh tons per year, although there exists the potential to produce about 10 lakh tons a year. Between now and 2030, the country should double its domestic NR production potential to become self-reliant and avoid excessive dependence on import of this critical and strategic industrial raw material. Hence, an action plan was chalked out to expand NR cultivation to more areas, replant old and senile holdings and increase productivity of existing holdings to attain self-reliance in NR production by 2030.

2. Trends and volatility of commodity prices: The case of natural rubber in India during the post-reforms period

The study examined the trends and demand and supply factors influencing the natural rubber (NR) price in India during the period of market and price uncertainties. It is based on the database of 36 years from 1981 to 2016 pertaining to monthly production, consumption, import, stock and price of NR in India. Using Point Analysis, four phases in the trends of price were observed *viz*. Phase I (1981 to 1995), Phase II (1996 to 2005), Phase III (2006 to 2010), and Phase IV (2011 to 2016).

It was observed that the intra-month price had been relatively stable in the second and fourth phases (28% each), whereas it had been highly unstable in the first (42%) and third (31%) phases, indicating that price uncertainty/instability is high when price

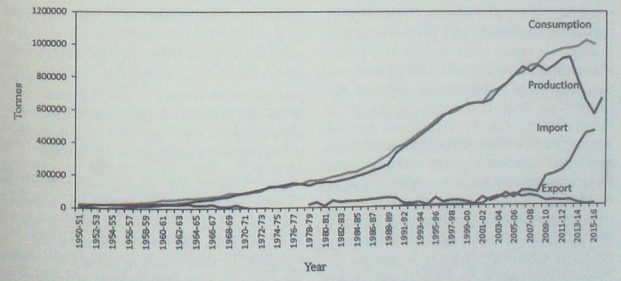


Fig. Eco. 1. Production-consumption gap of NR in India

shows increasing trend and *vice versa*. That is, price had been more volatile during the pre-reforms period during which price had been increasing, whereas it had been less during the post reforms-period during which the extent of fall in price had been on the higher side. The influence of demand and supply factors on NR during 1981 to 2016 is more obvious from Table Eco. 1.

Table Eco. 1. **Factors determined price of NR 1981 to 2016**

Factors/	Coefficient			
Phases	Phase I	Phase II	Phase III	Phase IV
Production	–	–	–	–
Consumption	0.086	0.104	0.46	–
Import	–	–	–	- 0.15
Stock	–	–	0.06	- 0.15
Constant	- 0.49	- 1.53	- 10.39	7.70
R^2	0.66	0.34	0.50	0.75

Significant at < 0.01

While consumption had been the factor that influenced price during the first two phases, it had been the consumption and stock that influenced the price in the third phase and both the import and stock (at manufacturers' level) determined the price during the fourth phase. Historically, regulations of stocks had played vital role in ensuring stable and remunerative prices for commodities, including NR. However, the absence of such regulations and the large-scale import accentuate the stock level during the post-reforms period leaving the domestic price highly uncertain and not remunerative. Therefore, stockholding and import of NR need to be streamlined depending upon the actual demand and supply conditions. It is highly warranted to ensure a remunerative farm gate price to farmers so as to ensure the sustainability of both the NR cultivation and small growers.

3. Cost benefit analysis of natural rubber cultivation

Adopting technology-driven good agricultural practices (GAPs) to increase productivity and reduce cost of production of NR holdings is the key to improve the Benefit Cost Ratio (BCR) and thus ensuring sustained supply of NR in the country. The study attempted cost benefit analysis of NR cultivation for different rubber growing regions *viz*. (i) Traditional region, (ii) North Konkan region and (iii) North eastern region under different cost and yield scenarios. The BCR analyses revealed that by following the traditional standard agricultural practices, rubber cultivation is not profitable under the present productivity and price scenarios, but by adopting cost saving and income enhancing GAPs rubber cultivation could be made sustainable (Table Eco. 2a-c).

The minimum productivity that is needed to get a breakeven BCR in different regions (at the 2016-17 costs and rubber price) were 1920, 1400 and 1600 kg ha^{-1} yr^{-1} in the traditional, NE and north Konkan regions respectively based on old cultivation practices and are above what is realized today. If latest technology driven practices are adopted, the breakeven productivity goes down to 790, 665 and 750 kg ha^{-1} yr^{-1} respectively in the three regions. This clearly shows that rubber cultivation can be made profitable even under the current productivity levels and low price scenario, provided cost-saving/auxiliary income enhancing technologies are adopted in full earnest.

Analysis based only on annual operational costs showed that tapping the rubber trees even during the low price and productivity scenario of 2016-17 is marginally profitable in the traditional (BCR=1.20) and North East (BCR=1.29) regions even with the old cultivation

Table Eco. 2. BCR of rubber cultivation in different rubber growing regions of India under different productivity and cost scenarios

(a) Traditional region

Cost scenario according to cultivation practices	Productivity scenario (kg ha^{-1} yr^{-1})		
	1629[a]	1931[b]	2500[c]
Using standard agricultural practices	0.71	0.85	1.10
Using cost saving and income enhancing GAPs	1.44	1.63	1.98

[a]Annual average productivity in traditional region during 2016-17. [b]Highest annual average productivity obtained in traditional region. [c]Highest productivity obtained in well maintained selected holdings in traditional region

(b) North East region

Cost scenario according to cultivation practices	Productivity scenario (kg ha^{-1} yr^{-1})				
	1257[d]	1270[e]	1750[f]	1931[g]	2500[h]
Using standard agricultural practices	0.75	0.76	1.05	1.16	1.50
Using cost saving and income enhancing GAPs	1.40	1.42	1.85	2.01	2.51

[d]Annual average productivity in North Eastern region during 2016-17. [e]Highest average productivity in North Eastern region. [f]Highest productivity achieved by well-maintained select holdings in North Eastern region. [g]Highest annual average productivity achieved in traditional region. [h]Highest annual average productivity obtained in well maintained selected holdings in traditional region

(c) North Konkan region

Cost scenario according to cultivation practices	Productivity scenario (kg ha^{-1} yr^{-1})				
	1000[i]	1016[j]	1500[k]	1931[l]	2500[m]
Using standard agricultural practices	0.53	0.54	0.79	1.02	1.32
Using cost saving and income enhancing GAPs	1.02	1.08	1.38	1.65	2.01

[i]Annual average productivity obtained in North Konkan region during 2016-17.[j]Highest annual average productivity obtained in North Konkan region. [k]Highest annual average productivity achieved by select holdings in North Konkan region. [l]Highest average productivity achieved in traditional region. [m]Highest annual average productivity obtained in well maintained select holdings in traditional region.

practices. But by adopting cost reducing and income enhancing technologies, the operational profit improves considerably in traditional (BCR=1.64) and North East (BCR=1.99) regions.

The analysis shows that at the current level of price, productivity and cost of production, NR harvesting can still be made profitable by adopting the latest cost saving and income enhancing technologies. But lesser adoption of technological innovations by the grower community has adversely affected their competitiveness. Creating awareness among growers about the latest technological developments and nudging them for adopting the technologies alone can make the growers successfully compete globally and make NR cultivation profitable.

4 Study on the effectiveness of pollination support through bee keeping

This study examines the effectiveness of the "Pollination Support through Bee Keeping" programme being implemented in Kerala by State Horticulture Mission (SHM)-Kerala, during the 2010-11, 2011-12 and 2012-13. It was based on three-stage proportional stratified sampling survey among 426 beneficiaries of SHM conducted during January to April 2017 across three

regions (south, central and north) in Kerala. The collected data were analysed using descriptive statistics.

The results show that, beneficiaries of SHM programme become beekeepers mainly on account of; (1) persuasive and logical training on beekeeping and (2) availability of beekeeping equipment at fair and subsidised rates. However, of the total beneficiaries, less than 34 per cent of beekeepers (8% in south, 7% in central and 19% in north) are still continuing beekeeping while 66 per cent have abandoned it mainly on account of; (1) absence of extension services after the distribution of equipment, (2) distribution of beekeeping equipment not at ideal times, (3) unawareness of beekeepers on seasonal as well as off-seasonal beekeeping management practices.

Only 21 per cent of beneficiaries had sufficient honey for marketing and the entire honey was marketed as raw honey *i.e.* without any value addition. Among the various factors, higher cost of production, absence of assured remunerative and stable prices, exploitation by middlemen, absence of organised market and public support under the oligopsonistic market structure were observed to have constrained the marketing of honey among the beekeepers. Higher cost of production and investment, lack of capital, competition among beekeepers, dominance of branded honey in the output market, difficulty in processing Agmark and BIS certified honey, prevalence of credit sales and absence of institutional support were reported to be affecting the value-addition of honey products among beekeepers across regions. Thus, the effectiveness of the Pollination Programme ends with the distribution of beekeeping equipment. That is, the Programme has conceived only the demand side aspects of beekeeping, but undermined the importance of supply side aspects which are critical from the perspective of beekeepers, especially the small ones.

Therefore, in order to achieve the perceived objectives, the establishment of Self Help Groups (SHGs) like the Kasargod Rural Development Society and the Elavampadam Rubber Producers' Society at grass root level may be considered under the Horticorp. Such SHGs can address not only the issues of commercial exploitation of honey and its products, their price and marketing, and processing and marketing of value-added honey products, but the underutilisation and scale economies of modern honey processing plant of the Horticorp as well. If the scattered beekeeping promotional activities and related manpower of institutions like State Agriculture Department, Khadi and Village Industries Commission (KVIC), Khadi and village Industries Board (KVIB), Rubber Board *etc.* are unified and brought under one agency the execution and monitoring of SHGs would become a reality *vis-à-vis* the very purpose of the Pollination Programme.

5. Policy advisories to the Rubber Board/Ministry of Commerce and Industry

- Associated with the Working Group for the estimation of production/ economic loss in rubber plantation sector due to excessive rain and subsequent natural calamities and in the preparation of the report "Damage/loss in rubber plantation sector due to excessive rain and subsequent natural calamities" which was submitted to Government of India and Government of Kerala.
- Associated with the committee in the estimation of the cost of cultivation/

production of natural rubber for 10 rubber growing regions/states in India for the year 2017-18.

6. Estimation of Rubber wood upset value

Estimated upset value of rubber trees in *Hevea* Breeding Sub-Station (HBSS) Kadaba, RRS Dapchari, RRS Dhenkanal, RRS Padiyoor, Central Experimental Station, (CES) Chethackal, State Farming Corporation of Kerala (SFCK) Limited Punalur, Rehabilitation Plantation Limited (RPL) Punalur and Estates of Kodungoor Devaswom Board.

7. Research Guidance/Internship

Institutes	Number of students underwent internship/ received research guidance in the Division
NIT, Trichi	1
Stella Maris College, Chennai	3
St. Thomas College, Ranni, Pathanamthitta	1
Loyola College, Chennai	2
TERI (The Energy and Resources Institute) School of Advances Studies, Delhi.	1

QUALITY CONTROL DIVISION

The Central Quality Control Laboratory is undertaking various tests for raw natural rubber, chemicals, fertilizers, water, effluent water *etc.* which is beneficial to the growers, processors and the general public. Quality Control Division jointly with Bureau of Indian Standards implements and monitors ISI marking process for the processed rubber in the country and conducts inspections to such units. The division plays a major role in the quality enforcement of processed natural rubber, quality of imported and exported rubber and the issue of NOC for import.

1. Commercial Testing Section

The Division has conducted commercial testing on a charged basis. Details of samples tested during the period are given below.

An amount of Rs.25,23,309/- was collected as testing fee during the year.

2. Specification section

2.1. BIS scheme of testing and inspection

The officers of BIS and Rubber Board (Specification Officers) conduct periodic inspection to ensure proper maintenance of quality in the processing units. Inspection was carried out, jointly with BIS, at "The Rubber Company", Thambalakad for awarding the BIS license.

Inspections were conducted during the period under BIS scheme and an amount of Rs. 38,73,608/- was received as share of marking fee from BIS.

Type of samplesTesting details during the FY 2018-19

	Number of samples	Number of parameters
Field latex	6167	6192
Dry rubber	1997	9346
Concentrated latex	189	1317
Water	486	3946
Effluent	114	926
Chemicals	195	427
Total	9148	22154

2.2. Quality control inspections at rubber processing units

Random surprise inspections were conducted at the rubber processing units for checking the quality of processed block rubber/concentrated latex. Information on such inspections conducted and the samples drawn is given below.

No. of quality control inspections conducted	:	133
Dry rubber	:	39
Latex	:	94

3. Quality enforcement for TSR

Under the rule 48 of the Rubber Rules 1955, every processor shall grade and market his products in conformity with such standards as are specified by the Bureau of Indian Standards from time to time. Further, the TSR purchased, sold or otherwise acquired or disposed of or possessed by any owner of estate, dealer, processor, manufacturer, importer and exporter shall fulfill the aforesaid norm.

Two processing units were compounded for violation of quality norms during the financial year and an amount of Rs.60,000/- was collected from these units.

4. Import of natural rubber

It is mandatory that natural rubber imported to India shall conform to Indian Standard Specifications. NOC for import is issued by Rubber Board. Details of NOC issued for import of NR during 2017-18 are given below.

Type of NR	Quantity (MT)
Block Rubber	477187.252
Sheet Rubber	95612.284
Crepe Rubber	256.0
Latex	6053.56
Others	34.5
Total	579143.566
No. of consignments of import	5247

70 inspections/analysis of consignments were done during the year. None of the consignments failed to conform to the BIS standards.

5. Export of natural rubber

Rubber Board conducts quality checking to ensure the quality of rubber exported from the country. Six latex (Cenex) samples were analyzed at Central Laboratory, before they are exported

6. Other Activities

6.1. Training programmes of Rubber training Institute (RTI)

6.2. Training programmes to University Students/ industry personnel.

6.3. Advisory work

CENTRAL EXPERIMENT STATION, CHETHACKAL

The Central Experiment Station, Chethackal established in 1966 is situated at a distance of about 56 km from Kottayam with a total land area of 254.76 ha. The Station meets the research needs of various disciplines like crop improvement, crop management, crop protection, crop physiology, latex harvest technology and meteorological observation. The priority areas of experimentation at present are breeding for high yield and other beneficial secondary characters with special emphasis on disease and drought tolerance, evaluation of clones developed conventionally, nutrition studies, intercropping systems, reduction in cost of cultivation, low frequency tapping systems, *etc.* and the total number of trials in the station are 134.

During the reporting period, the total number of tapping days available was 299 and 55 tappers were engaged for tapping. The total crop realized was 121 MT. The total man-days engaged were 42,150 days. The Station having 158 permanent workers is managed by Officer-in-charge with 30 staff for office administration, farm management, dispensary, security and canteen. The dispensary attached to the Station caters to the medical needs of the workers. The total patients attended to during the period under report were 3455. One medical camp for workers arranged by Homeopathic Centre, Naranamoozhy and one N.S.S. camp for students from local Higher Secondary School, Edamury were also conducted during this period. The total IEBR raised during the reporting period was Rs. 2,48,75,107/-.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

1. Crop Improvement

1.1. On-farm evaluation of selected ortets of *Hevea*

Evaluation of five genotypes (3 primary ortet selections *viz.* RRSG 9, RRSG 3 and RRSG 1 and two clones *viz.* RRIM 600 and RRII 429) in large scale was continued in Morigaon, Assam. Girth of ortets after three years of planting was at par with RRIM 600. Incidence of powdery mildew disease was severe in RRSG 1, RRSG 3, RRSG 9 and RRIM 600. *Oidium* infection was mild in RRII 429. Even in bulk area, incidence of powdery mildew disease in RRII 429 was lesser than RRIM 600. This is the first time that such variation in powdery mildew disease under undusted condition was observed.

1.2. On-farm evaluation of potential clones / ortets under the agro-climate of Arunachal Pradesh

Two clone/ortet evaluation experiments were initiated under the agro-climate of Namsai, Arunachal Pradesh. In experiment I, 11 promising clones *viz.* RRIM 600, RRII 208, RRII 429, RRII 417, RRII 430, RRII 422, RRII 105, PB 235, PB 260, SCATC 88/13 and Haiken 1 were growing well. In

experiment II, planting of RRSG 1, RRSG 9, RRSG 8, RRSN 1, RRSN 47, RRSN 69, RRSA 114, RRSA 585, RRSA 315, RRSA 114, RRSA 585 and RRSA 315 along with check clones RRIM 600 and RRII 429 was completed and the survival rate was 83 per cent.

2. Crop Management

2.1. Soil fertility mapping in Assam, Arunachal Pradesh, West Bengal, Manipur, Mizoram and Nagaland

RRII has undertaken a detailed survey of rubber growing soils of North-East India with an objective to bring the entire rubber area in the country within the ambit of soil fertility assessment based online fertilizer recommendation system. A total of 662 soil samples were collected from the four states *viz.* Assam (424), Arunachal Pradesh (92), Manipur (66) and West Bengal (80). Soil sample collection has been completed in three states *viz.* Arunachal Pradesh, Manipur and West Bengal. Steep and unreachable terrains were impediments towards collection of soil samples in Arunachal Pradesh and Manipur states. Location coordinates of all the soil samples were recorded using GPS device along with data related to cultural practices of each location.

3. Crop Physiology

3.1. Induction of early flowering in *Hevea* towards reduction of breeding cycle in elite selections in Assam

To induce early flowering in young *Hevea* plants doses of growth retardant, paclobutrazol were given to clone RRII 429 at Sarutari Farm, RRS, Guwahati with an objective to facilitate easier hand pollination, synchronous flowering and reduction of breeding cycles in elite selections/clones.

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The major on-going programmes of the Station are evaluation of clones, crop management, latex harvesting and ecosystem study. Advisory services on discriminatory fertilizer recommendation to growers and latex analysis for dry rubber content are being continued.

1. Crop Improvement

The development of location specific clones, evaluation of promising clones and the standardization of DUS norms are being continued under crop improvement programmes.

1.1. Development and evaluation of clones

The crop improvement programmes were undertaken for development and evaluation of clones for this region. One hundred and thirty one selected clones *viz.* 20 hybrids, 21 OP progenies and 90 half-sib progenies are under evaluation in six clonal and seedling nurseries.

Yield performance study of clones is undertaken in two On Farm Trials and two Large Scale Trials. In the first large scale trial, DD/6/5 showed highest girth (28.1 cm) during third year of planting compared to

control RRIM 600 (21.3). In another LST (G x E) trial, yield was higher in clone RRII 429 (68.5 g t^{-1} t^{-1}) followed by RRII 422 (65.6 g t^{-1} t^{-1}) and control RRIM 600 (53.1 g t^{-1} t^{-1}).

High yielding clones are under evaluation in two OFTs and RRII 429 recorded the highest mean yield (35 g t^{-1} t^{-1}) in an OFT at Pathalia, which was higher than control clone RRIM 600 (33.5 g t^{-1} t^{-1}) in the fifth year of tapping. Clone RRII 429 showed the highest girth (63.1 cm) in another OFT at Hirapur.

In the GxE trial, 45 pipeline clones and four check clones were under evaluation for adaptation to cold conditions based on their yield performance. Response of these pipeline clones to stimulation under cold conditions was also assessed. Based on overall yield, clones P 21, P 107, P 102 and RRII 430 were found promising high yielders. Among the 45 pipeline clones, 13 clones performed better than the popular clone RRIM 600 whereas 20 clones were found better than clone RRII 105.

Standardization of distinctness, uniformity and stability (DUS) testing norms in 57 clones is also in progress. A germplasm garden having 213 wild *Hevea* accessions, source bush nurseries and a breeding orchard are being maintained in the Station.

2. Crop Management

2.1. Soil fertility mapping

Preparation for a web based soil fertility map for rubber growing soils of Tripura was initiated for online fertilizer recommendation *vis-à-vis* site specific nutrient management for natural rubber. Total 664 composite soil samples at the depth of 0-30 cm were collected, covering 93 per cent of rubber growing areas of Tripura. GPS coordinates of the sampling sites and the field details were also recorded. Analysis of these soil samples showed that 61 per cent of these samples were medium in OC content, 89 per cent were low in available P and 48 per cent recorded low available K. Increased soil acidity (pH$_{H_2O}$ < 4) under rubber soils with higher proportion of sand (%) in the surface soil under mature rubber plantation were observed in most of the sites.

2.2. Soil and water conservation

In silt pit experiment, a significant increase in yield was recorded due to imposition of silt pit during second year of tapping. Plants under silt pit block registered an annual yield of 1306 kg ha^{-1} while the plants without pit registered an annual yield of 1171 kg ha^{-1}. Soil recovered by adopting silt pits under mature rubber plantation (220 pits ha^{-1}) was 3.42 tonnes ha^{-1}. Analysis of these soils showed a higher proportion of clay (24.1 %) in the pit soil than that of field soil (18.8%). The results indicated that huge loss of finer fractions of soil took place in the sloping land of Tripura.

2.3. Development of cropping system and management practices

In the cropping system model, the girth of 10 year old rubber in the intercropping plots was 60.3 cm and 60.7 cm respectively for Model I and II. In monocropped plots the girth was 60.8 cm and 61.3 cm respectively for Model I and II, which is on par. The yield of rubber for intercropped area was on par with yield for monocrop area in Model I (Paired row system) (1720 kg ha^{-1} and 1784 kg ha^{-1}, respectively) and in Model II (Rectangular planting) (1710 kg ha^{-1} and 1752 kg ha^{-1}, respectively). In Model I, pineapple was continued till 10th year and yield obtained was 662 kg ha^{-1}.

The growth of plants showed no difference in pits of larger dimensions

compared to smaller dimensions pits in seventh year after planting. The mean girths were 50.3 cm for normal pit and 49.1 cm and 48.3 cm for two reduced size pits, respectively. This trial was carry forwarded as on-farm trial on reduced pit size at Twaisaplang-I block plantation unit and girth recorded after fourth year of planting were 33.8 cm and 33.5 cm for normal and reduced pit size, respectively.

Results of experiment on specific package of practices, showed that the mean girth of rubber in vertical and conventional mulching is statistically on par but it is superior to control (girth is 50.9, 50.11 and 46.5 cm for conventional mulching, vertical mulching and control, respectively).

3. Latex harvesting study

In clone PB 235, d3 system of tapping continued to show higher yield compared to d4 and d7 systems. Yield stimulation study in different RRII 400 series clones are being continued under two frequencies of tapping *viz*. d2 and d3. Yield of d3 system of tapping with stimulation and d2 system with tapping rest were comparable. Clone RRII 429 continued to be the highest yielder among the clones.

The experiment on effect of planting density on d3 tapping systems is being continued with clone RRII 429. Higher yield was observed in high density planting compared to lower density. In block trial experiment, d3 system showed higher yield compared to d7 system of tapping in clone RRIM 600.

4. Ecosystem study

Assessing the invasive potential of four indicator plants differing in biogeographic origin in NE India with respect to climate of 2000 and future climate 2050 was made using ecological niche modelling approach. Favourable bioclimatic variables such as seasonality of precipitation and temperature, mean temperature of the warmest quarter and mean temperature of the coldest quarter are among the important factors responsible for invasion of non-native species in North-East region. *Urena* and *Imperata* species can be broadly considered as native species in North-East region of India. These plants showed lesser invasion in this region with respect to 2000-climate compared to other two non-native species. The set of major contributing bioclimatic factors responsible for distribution of these two native species remains more or less constant between 2000 and 2050-climate. Though *Hevea* and *Ageratum* species share the same biogeographic origin, the set of major contributing bioclimatic factors responsible for distribution of *Ageratum* are different with respect to two climate scenarios indicated the robustness of the species to invade climatically diverse regions. These findings of study will be useful for better understanding the invasion process of several species with different biogeographic origin.

Precipitation during drying months found to be most critical in NE region in deciding the *Hevea* distribution irrespective of GCMs and RCPs. Studies on the extent of climate change in NE region found that the projected annual average temperature would be ranging from -6.0 to 28.2 °C in 2050 indicating climate warming.

In ecological study, eight weed species in the tea rows, 14 weed species in the rubber rows and 23 species of weeds in unmanaged rubber plantation were confirmed over three seasons. Studies on influence of rubber on soil health found a gradual increase in clay (%) down the profile under all land use systems.

5. Advisory work

Discriminatory fertilizer recommendation based on soil analysis was offered to seven growers in the state of Tripura. A total of 169 latex samples were analyzed for DRC.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

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

1. Crop Improvement

1.1. Poly-cross progeny evaluations

In the 2008 poly-cross progeny evaluation trial, a new set of Clonal Nursery Evaluation Trial-2014 has been started at the Rubber Board campus, Dakopgre, Tura in two designs in RBD. Plants are maintained in the field. In the another set of 2011 populations, a total of 34 top yielders were selected on the basis of growth performance and juvenile yield which are being maintained in the field for further evaluation.

1.2. Half-sib progeny evaluation Trial (2008 and 2009)

Populations selected on the basis of growth performance and juvenile yield are maintained in the field for further evaluation.

1.3. Clonal Nursery Evaluation Trial (2010)

The experiment was completed during the year.

1.4. On-farm evaluation of selected clones

Three on farm trials were started during 2009 and 2010. Trial-I includes blocks of six clones, *viz*. RRII 417, RRII 422, RRII 429, PB 235, RRII 203 and RRIM 600, in Mendipathar (North Garo Hills) and Trial-II includes four clones *viz*. RRII 417, RRII 422, RRII 429 and RRIM 600 in Bolchugre, West Garo Hills. In the North Garo Hills, 3rd year tapping was started during the month of June, 2018 onwards (Table Tura 1). Preliminary data on yield indicates highest mean annual yield ($g\ t^{-1}\ t^{-1}$) in RRII 429 (47.9) followed by RRIM 600 (45.2) and RRII 422 (43.6) and minimum yield ($g\ t^{-1}\ t^{-1}$) in RRII 203 (32.4). Maximum girth (cm) was recorded in RRII 203 (59.3) followed by PB 235 (57.2) and RRII 429 (57.0) while minimum girth was recorded in RRII 422 (52.4) (Table Tura 2).

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

The promising seedlings were screened on the basis of test tap yield among polyclonal seeds which were collected from four locations in the NE region *viz*. RES Nagrakata, RRS Agartala, RRS Guwahati and RRS Tura and the selected bud woods are maintained in the nursery for further study.

1.6. Nursery evaluation of poly-clonal seedlings-Trial-2013 and 2014

The polyclonal seeds collected from Polyclonal Seed Garden, Mizoram were field planted during 2013 at two locations of the RRS, Ganolgre farm and one location

Table Tura 1. **Monthly mean yield of various clones in OFT Mendipather (2018-19) (3rd year yield)**

Clone	Monthly mean yield (g t^{-1} t^{-1})								
	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Average yield
PB 235	39.9	37.8	39.8	39.2	53.6	57.8	44.8	27.5	42.6
RRII 417	23.5	23.4	30.5	25.5	43.2	54.5	45.3	28.6	34.3
RRII 429	47.5	41.1	48.6	43.3	57.4	60.1	50.1	35.2	47.9
RRII 422	41.2	43.6	46.5	44.3	48.8	50.1	42.9	31.6	43.6
RRII 203	24.2	27.6	28.7	27.7	37.8	41.7	35.2	36.6	32.4
RRIM 600	41.3	36.6	38.8	40.5	49.9	65.2	55.3	33.7	45.2
Mean									41.0

Table Tura 2. **Growth of different clones in OFT Mendipathar, North Garo hills**

Clones	Girth (cm) at 150 cm from bud union		
	Field 1	Field 2	Mean
PB 235	57.8	56.6	57.2
RRII 417	58.1	51.2	54.7
RRII 429	59.9	54.0	57.0
RRII 422	52.5	52.3	52.4
RRII 203	60.2	58.3	59.3
RRIM 600	56.3	53.2	54.8
Mean			55.9

at R.B. campus, Dakopgre, Tura. On the basis of test tap yield, top 25 best performing progenies were selected and their budwood nursery is being maintained at Rubber Board campus, Tura among which 5 progenies were selected at RRS, Ganolgre farm for further evaluation.

1.7. Germplasm Arboretum at Teksragre Farm

In order to maintain the 1st, 2nd, 3rd and 4th set of Germplasm Arboretum under the agro-climatic condition of Garo Hills of Meghalaya 78 polybag plants (belonging to 26 Accessions) for 4th set of Germplasm were planted in the field during May, 2018. Eight hundred and seventy two budded stumps belonging to 123 accessions and 5 control clones for the 5th set of Germplasm were planted in polybag nursery during March, 2019. Field preparation work for planting is in progress. All the plants were affected by the hailstorm during May, 2018.

2. Crop physiology/Latex Harvesting Technology

2.1. Effect of Low winter temperature on yield of rubber at high altitude

Severe low winter temperature is one of the main factors for depression of yield and per cent dry rubber content in *Hevea* under the agro-climatic condition of Garo Hills. The annual mean yield (32.2 g t^{-1} t^{-1}) and DRC (34.0 %) for reporting year was recorded. Low temperature adversely affected the yield and DRC. Early defoliation and refoliation was observed and during winter DRC ranged from 28.2-29.3 per cent. Lowest soil moisture content recorded in the months of February and March also.

2.2. Shallow tapping

There was no significant difference between treatments. Maximum annual mean yield was recorded in normal tapping system (29.1 g t^{-1} t^{-1}) followed by normal continuous tapping (29.0 g t^{-1} t^{-1}) and LFT + normal tapping (27.4 g t^{-1} t^{-1}) and lowest was in shallow + normal tapping system (25.0 g t^{-1} t^{-1}). Annual mean DRC was low (33.9 %) in normal continuous tapping and high (34.6 %) in shallow + normal tapping system.

Normal continuous tapping system showed higher TPD (7.5 %) followed by the shallow + normal tapping system (7.0 %) and LFT + normal tapping (5.2 %) and minimum was in normal tapping system (5.0 %).

2.3. Location specific stimulant application

Ethylene induced stress response in the tapping panel of the *Hevea* trees was initiated to reduce the stress in tissues in the tapping panel. In RRIM 600 six treatments were adopted with bark applications of 5 per cent ethaphon. Results showed that maximum annual mean yield (42.7 g t^{-1} t^{-1}) and low DRC (33.6 %) was recorded in T3 (Bark application of 5 per cent ethaphon at 150 cm above from bud union and near bud union and minimum yield (29.8 g t^{-1} t^{-1}) and high DRC (34.3 %) was recorded in T6 (Unstimulant trees). There was no significant difference in DRC between treatments.

3. Crop Management

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

Soil samples were collected each month at the depth of 0-15 cm, 15-30 cm and 30-60 cm for soil moisture study. Soil moisture content increased with increase in depth. Maximum was in August/September and minimum was in Jan/Feb. Annual mean was 24.1, 25.1 and 26.0 per cent, respectively.

3.2. Analytical/advisory work for fertilizer recommendation

During the year, 16 soil samples collected from the rubber growing areas indicated that the O.C. content was at the medium range (0.81-1.32 %) in the surface soil (0-30cm), available phosphorus was at low range (2.4-6.9 mg kg^{-1}) and available potassium was at medium range (76.0-96.5 mg kg^{-1}). The soil is acidic in nature with pH ranging from 4.4-5.6 and fertilizer recommendation was given accordingly.

3.3. Evaluation of soil fertility status and mapping soil fertility in Meghalaya

Initiated the new project and collected 210 composite soil samples (0-30 cm depth) from the rubber growing areas of Garo hills of Meghalaya (West, and South-west district) using GPS system. All the soil samples were air dried and processed and transferred to RRII, HQ for analysis.

REGIONAL EXPERIMENT STATION, NAGRAKATA, W. BENGAL

1. Crop Improvement

1.1. Evaluation of clone

Twenty six promising clones were evaluated under the agroclimate of sub-Himalayan West Bengal. At 27th to 28th year of planting, girth of RRIM 612, RRII 118, RRIM 605, RRIM 703, SCATC 93/114 and PR 107 was significantly higher than the check clone RRIM 600. In terms of yield for 2018-19, PB 280, PR 261 and SCATC 88/13 were significantly higher than RRIM 600 (Table Nag. 1).

1.2. Evaluation of germplasm

Performance of 21 germplasm accessions under the agroclimate of Nagrakata, West Bengal was evaluated.

Table Nag 1. Pattern of yield in different clone trials

Trial I and II	Yield ($g\ t^{-1}\ t^{-1}$)	Trial III	Yield ($g\ t^{-1}\ t^{-1}$)	Trial IV	Yield ($g\ t^{-1}\ t^{-1}$)
GL1	28.8	PB 310	36.5	GT 1	40.2
PB 260	29.9	RRII 208	37.4	RRIM 600	40.8
RRIM 612	30.3	PR 107	37.6	RRIM 703	41.16
PB 5/51	31.5	PB 235	37.7	HK 1	41.4
RRII 105	31.5	RRIC 102	38.3	RRII 300	42.0
RRII 308	35.6	PB 86	38.6	SCATC 88/13	44.0 *
RRIM 605	35.7	RRII 203	38.6	PR 261	44.1 *
SCATC 93/114	36.2	RRIC 104	38.8	PB 280	48.8 **
RRII 118	36.2	PB 311	39.1	CD (Pd"0.05)	2.7

* Significant at 0.05 per cent level; ** Significant at 0.01 per cent level

Maximum girth was recorded in RO 2629, MT 44, RO 5430, RO 2635, MT 196, MT 2229, AC 619, RO 5557, RO 2890, RO 3172 and RO 5348. Yield of AC 763 showed significantly higher value in comparison to check clone RRII 105. In general, the performance of Mato Grosso was better than Acre and Rondonia accessions.

1.3. Performance of polyclonal seedlings

Since 1990, study on performance of polyclonal seedlings was under progress in Nagrakata in a block of 240 trees in CRD. After 27 years of planting, mean girth of population was 79.3 cm. The average block yield of population was 28.5 $g\ t^{-1}\ t^{-1}$ where 36 per cent plants had yield above average. 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

Half sib progenies were raised from seven different clones in 2014. Juvenile yield of seedlings raised from seeds of SCATC 88/13 during non-winter period showed highest yield followed by that from RO 5363; number of plants having above average juvenile yield also showed same trend (Table Nag. 2). During winter period, the average juvenile yield of SCATC 88/13 progenies was higher than that of RRIM 600, followed by RRII 417 and RRII 429. Number of seedling plants showing above average juvenile yield was also higher in SCATC 88/13. The potential of half-sib progenies of SCATC 88/13 was prominent from this study.

1.5. Performance of new generation clones under the agroclimate of sub-Himalayan West Bengal

Girth and yield of five promising new generation clones under cold agroclimate of sub-Himalayan West Bengal were similar to that of RRIM 600. However, maximum yield of RRII 422 was followed by RRII 429 and RRII 417. RRII 414 had lesser yield.

3. Crop Physiology

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

Mean yield of the population was 34.6 $g\ t^{-1}\ t^{-1}$ and average yield of seedlings raised from seeds collected from four different places were similar ranging from 2.5 $g\ t^{-1}\ t^{-1}$ to 117.6 $g\ t^{-1}\ t^{-1}$. Twenty promising ortet mother plants showed above 60 $g\ t^{-1}\ t^{-1}$ yield during 2nd year of tapping.

Table Nag 2. **Growth parameters in half-sib progenies**

Progeny	Yield ($g\ t^{-1}\ 10\ t^{-1}$)		Per cent of plants showing above average yield		Number of plants showing above 60 $g\ t^{-1}\ 10\ t^{-1}$	
	Non-winter	Winter	Non-winter	Winter	Non-winter	Winter
RO 5363	22.3	42.0	24.1	25.0	8	18
RRII 208	19.8	42.9	38.9	43.5	3	30
RRII 417	19.1	52.5	29.6	38.0	4	32
RRII 422	16.1	37.9	33.3	37.0	1	20
RRII 429	19.5	50.9	38.0	37.0	2	31
SCATC 88/13	30.3	61.5	28.7	38.9	14	42
RRIM 600	13.4	31.6	29.6	29.6	2	13

Table Nag. 3. **Girth and yield of seedling plants**

Mother tree number	Mean yield $g\ t^{-1}\ t^{-1}$	Mother tree number	Mean yield $g\ t^{-1}\ t^{-1}$
RRSN 319	60.9	RRSN 182	63.1
RRSN 185	61.6	RRSN 43	64.3
RRSN 244	62.1	RRS N 252	65.0
RRSN 335	62.4	RRSN 327	67.0
RRSN 124	62.4	RRSNG 113	72.1
RRSNT 200	73.3	RRSNG 390	83.0
RRSNG 112	73.7	RRSNG 248	86.1
RRSNG 384	75.2	RRSNT 69	95.3
RRSNG 118	75.3	RRSNN 146	111.2
RRSNT 391	77.8	RRSNG 41	117.6

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

Growth of all five clones was at par with that of RRIM 600 in high pH soil except RRII 422 which showed significantly lower growth than RRIM 600. In normal soil, growth of all the clones was at par with RRIM 600.

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

Juvenile yield of the check clone RRIM 600 was high in both non-winter and winter periods during non-winter period, it was followed by RRST 24, RRSG 1 and RRST 37 (Table Nag. 4) and during winter period the check clone was followed by RRST 37, RRSG 1 and RRII 429.

Table Nag. 4. **Ortet clone yield during winter and non-winter**

Ortet/Clone	Juvenile yield ($g\ t^{-1}\ 10\ t^{-1}$)	
	Non-winter	Winter
RRSD 1	51.8	142
RRSD 34	16.6	95.3
RRSD 35	43.6	124
RRSD 36	27.5	121.9
RRSG 1	60.7	276.6
RRSG 3	47	165.4
RRSG 9	28.6	210.1
RRSN 1	33.7	141.3
RRSN 47	44.98	165.3
RRSN 69	44	236.5
RRSA 315	28.9	104.5
RRSA 585	39.5	147.1
RRSA 98	30.8	95.6
RRST 24	62.6	146.1
RRST 37	59	281.3
RRST 39	41.6	85.4
RRII 414	44.4	203.2
RRII 417	38.9	238.9
RRII 422	62.4	190.6
RRII 429	55.1	256.8
RRII 430	23.5	126.3
RRII 105	28.5	230.5
RRIM 600	86.8	297.5
CD (P=0.05)	NS	NS

RRSG = Source from Guwahati; RRSN = Source from Nagrakata; RRSD = Source from Dapchari; RRST = Source from Tura

REGIONAL RESEARCH STATION, DAPCHARI, MAHARASHTRA

The mandates of this Station are to develop suitable clones and location specific agro technology for the prevailing drought condition. The experiments on crop improvement (screening of wild *Hevea* accessions and evaluation of clone, polyclone, pipeline clones, selected ortets and wild *Hevea* accessions for growth and yield performance under Dapchari condition), environmental physiology (physiological evaluation of selected ortetes from various agroclimates of India) are being carried out.

1. Environmental Physiology

1.1. Evaluation of environmental stress tolerance and physiological adaptations of cold and drought tolerant ortet selections

The trial started in 2011 to evaluate the physiological and biochemical adaptation potential and common mechanisms involved in cold and drought tolerant traits, using molecular physiology/biochemical tools for ortet selections from cold and drought regions 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. Significant difference in girth was recorded and it ranged from 14.0 cm in Dap 36 to 29.6 cm in RRSA 315. A significant difference in test tapping yield was also observed and it ranged from 17.8 g in Dap 34 to 65.2 g in RRSA 315 (65.82 g).

Table Dap 1. **Growth and yield of selected clones and ortets from different locations**

Clone	Girth (cm)	Test tapping yield (g)
RRII 105	25.6	28.3
RRIM 600	21.7	26.4
RRII 414	29.2	39.5
RRII 417	28.1	43.5
RRII 422	27.1	22.6
RRII 429	27.7	41.6
RRII 430	25.8	42.1
DAP 1	19.5	37.5
DAP 34	21.1	17.8
DAP 35	23.4	35.7
DAP 36	14.0	20.0
RRSA 98	26.6	29.4
RRSA 315	29.6	65.8
RRSA 585	26.0	55.2
NGK 1	22.7	19.0
NGK 47	18.9	26.2
NGK 69	17.6	20.3
GH 1	21.6	23.7
GH 3	22.7	40.0
GH 9	19.7	50.0
RRST 24	21.8	35.0
RRST 37	23.4	35.0
RRST 39	24.7	41.0
DAP	19.5	27.7
RRSA	27.4	50.1
NGK	19.7	21.8
GH	21.2	37.9
RRST	23.3	37.0
Mean	23.6	37.5
S.E.	2.3	8.1
CD (P=0.05)	6.7	23.4

2. Crop Improvement

The objective of crop improvement research is to identify and to develop drought tolerant clones for their adaptability and stability to the agro climatic condition of Maharashtra. A total of 12 experiments are being conducted to evaluate the growth and yield performance of clone, pipeline clones, hybrid clones, ortets, wild *Hevea* accessions, half sib progeny, polycross seedlings, different root stock plants and root trainer plants with the objective to

identify drought tolerant clones for their adaptability and stability to the agro climatic condition of Maharashtra.

2.1. Large scale trials

2.1.1 *Evaluation of selected ortets*

The trial started during 2008 to evaluate the growth and yield performance of ortets selected from polycross seedling planted at this station with control clones showed non-significant difference in girth. Girth among the ortets and check clones ranged from 31.1 cm in OS 42 to 41.6 cm in OS 236. Among the check clones, RRII 430 recorded higher girth (39.5 cm) while lowest girth was noticed in clone RRIM 600 (33.8 cm). All ortets were superior in girth than clone RRII 105 except ortet OS 42 and OS 8 (32.9, 31.1 cm). Significant difference was noticed in test tapp yield which ranged from 25.0 g t^{-1} t^{-1} in OS 317 to 146.5 g t^{-1} t^{-1} in OS 35. All clones are at par with OS 35 except OS 173 (126.1g t^{-1} t^{-1}). Among the check clones RRII 430 gave highest test tap yield of 72.9 g t^{-1} t^{-1} while clone RRII 105 recorded 45.4 g t^{-1} t^{-1} only. More than 25.0 g t^{-1} t^{-1} yield was seen in all ortets and check clones except OS 317 (Table Dap. 2).

Table Dap 2. **Growth and yield of ortets selected from various locations of India**

Clone	Girth (cm)	Yield (g t^{-1} t^{-1})
OS 1	40.4	95.6
OS 8	32.9	115.0
OS 34	38.3	73.1
OS 35	39.1	146.5
OS 36	40.4	57.2
OS 37	37.1	116.6
OS 42	31.1	43.1
OS 111	35.4	87.4
OS 135	36.7	77.1
OS 136	33.4	82.8
OS 173	41.1	126.1
OS 216	39.4	70.1
OS 236	41.6	103.6
OS 317	33.5	25.0
RRII 105	35.9	45.4
RRII 208	36.5	91.0
RRII 430	39.5	72.9
RRIM 600	33.8	39.4
SEm±	2.3	16.5

2.1.2. *LST with 20 hybrids evolved for drought tolerance under drought (2015)*

The objective was to evaluate growth and yield potential of 20 hybrid clones. The experiment is in initial stage.

2.2. Further field evaluation trial

A total of three experiments comprising of 25, 47 and 11 selections are being evaluated since 2007, 2010, 2014 and are at initial stages.

2.2.1. *Field evaluation of selected Hevea clones for drought tolerance (2007)*

Trial was laid out using 25 potential drought tolerant wild *Hevea* accessions for drought under drought prone climatic (Dapchari) condition along with five HP clones and RRII 105, RRIM 600, Tjir 1, RRII 430 and RRII 208 as check clones in Augmented RBD to confirm their drought tolerance potential. The accessions showed a wide variability for all characters studied, during 11 summer periods from 2008 to 2018 and four wild accessions higher girth than the proven drought tolerant clone RRIM 600. MT 40 recorded maximum girth at 9th year. RRII 430 and RRII 414 showed significantly better growth than RRIM 600 in this region. Hybrid 93/270 recorded highest girth.

2.2.2. *Small scale field evaluation trial of selected wild accessions for drought tolerance (2010)*

Experiment was initiated with 47 selections from wild *Hevea* accessions along with four check clones (RRII 105, RRII 208, RRII 430, RRIM 600) to confirm the drought

tolerance potential of selected seven wild accessions from preliminary field screening.

2.2.3. Field evaluation of selected wild accessions for drought tolerance (2014)

Trial with in rectangular lattice design was laid out with 11 wild *Hevea* accessions along with two check clones (RRII 105 and RRIM 600) to evaluate juvenile and mature performance under drought condition.

2.3. Clonal nursery evaluation

A total of five clonal nursery evaluations are in initial stages for evaluation of half sib progeny, polycross, half sib progeny of prepotent clones, pipeline clones, identification of reliable juvenile and mature characteristics for clone identification (50 divergent clones) and standardization of distinctiveness, uniformity and stability (DUS) testing norms for evolving specific guidelines for varietals registration.

2.3.1. Germplasm screening

In the trial laid out in 2003 for screening 130 wild *Hevea* accession for drought along with RRII 105, RRIM 600, Tjir 1 in Augmented block design, Mato Grosso accessions were found superior for all growth characters studied than those from Randonia and Acre provenances. Twenty five potential drought tolerant accessions were identified based on 3-4 years field performance and further detailed studies are in progress.

2.3.2. Clonal nursery evaluation of promising Hevea clones (half sib progeny of prepotent clones) in hot spot areas for drought tolerance

The trial started in 2010 to evaluate the clones in a clonal nursery and advance the potential ones showing drought tolerance along with higher yield to LST and PCE to reduce the breeding cycle was continued.

2.3.3. Clonal nursery evaluation of pipeline clones (2011)

Continued the trial laid out in 2011 with 50 pipeline clones and two check clones (RRII 105 and RRIM 600) to identify drought tolerant clones for their adaptability and stability to this agro climatic condition of Maharashtra. Clone responses for field establishment were assessed. Recording of all growth, biochemical and physiological parameters are being carried out.

2.3.4. Evaluation of half sib progeny of clones in nursery (2011)

The trial for selection of primary ortets to study the growth and yield performance of polyclonal seedling at early stage in the nursery and to examine the scope for the early selection based on dependable juvenile traits under rainfed condition was continued. The evaluation of poly cross progeny through nursery screening of the seedlings obtained from open pollinated polyclone evaluation trial is a step towards the nursery selection and identification of few locally adapted superior clones. The test tapping was done in peak winter and peak summer season. The highest rate of survival (47%) was noticed in half sib progenies from clones RRII 6 and RRIC 102 while lowest rate of survival (33%) was observed in progenies of clone PB 311. Half sib progenies from clone PB 311 gave maximum selection (49.2%) minimum selection were observed from half sib progenies from PB 260 (17.3%). The half sib progenies from RRIC 102 and RRII 6 also gave maximum selection of 40 and 41 per cent, respectively. No significant difference was noticed in all the growth parameters studied except for number of whorls per half sib progenies from various clones. The test tap from half sib of RRII 105 ranged from 5.0 to 34.9 g t^{-1} t^{-1}. The highest selection mean test tap yield was recorded in half sib progenies of clone RRIC

102 (24.7 g t^{-1} t^{-1}) followed by RRII 208, PB 311 (10.1, 10.0 g t^{-1} t^{-1}, respectively).

2.3.5. ***Development of drought tolerant root stock for the non-traditional areas***

Continued the trial for developing drought tolerant root stocks for the non-traditional area by evaluating the drought tolerance capacity of seedlings from drought tolerant clones and trees grown in drought prone non-traditional areas.

REGIONAL RESEARCH STATION, DHENKANAL, ODISHA

The Station continued its research activities for identifying clones suited to the dry sub humid climate region.

1. Crop Improvement

The six clone evaluation trials are in progress. The trials were laid out to screen and evolve the most suitable and high yielding clones under the dry sub humid climate.

Clone evaluation

In Trial 1 (1987), the elite clone RRIM 600 recorded highest mean yield of 46.4 g t^{-1} t^{-1}. GT 1 recorded the lowest yield (40.1 g t^{-1} t^{-1}). GT 1 (85.4 cm) has recorded significantly higher mean girth over RRII 105 and RRIM 600. In terms of growth, all the three clones performed well in this region (Table Odi. 1).

Table Odi 1. **Growth and yield performance of elite clones**

Clone	Yield (g t^{-1} t^{-1})	Girth(cm)
RRII 105	45.6	77.2
RRIM 600	46.4	81.2
GT 1	40.1	85.4
CD (P=0.05)	—	4.77

In another trial (1990), RRII 208 (82.2 g t^{-1} t^{-1}), SCATC 88-13 (75.7 g t^{-1} t^{-1}) and RRIM 600 (79.5 g t^{-1} t^{-1}) were found superior in terms of yield. SCATC 93-14 recorded comparatively lower yield (49.0 g t^{-1} t^{-1}). However, SCATC 93-14 recorded highest girth (98.1 cm) followed by SCATC 88-13 and PB 310 (Table Odi. 2).

Table Odi. 2. **Growth and yield performance of clones**

Clones	Yield (g t^{-1} t^{-1})	Girth (cm)
Haiken 1	59.1	87.1
RRIM 600	79.5	88.9
RRIM 701	55.9	91.3
RRII 5	64.4	91.2
SCATC 88-13	75.7	97.5
SCATC 93-14	49	98.1
PB 310	62.5	91.8
RRII 208	82.2	91
PCK 1	66.9	90.6
RRII 300	65.4	91.6
CD (P=0.05)	13.01	—

In the 1991 experiment, significant difference in yield was observed among the clones. RRII 208 (93.4 g t^{-1} t^{-1}), RRIC 102 (71.9 g t^{-1} t^{-1}) and RRIM 600 (68.2 g t^{-1} t^{-1}) recorded highest yield. Polyclonal seedling population (54.8 g t^{-1} t^{-1}) yielded low though having better growth and adaptability under the prevailed stress conditions.

In the modern clones trial (2000), highest mean yield was observed in RRIM 600 (60.3 g t^{-1} t^{-1}) and IRCA 109 (57.2 g t^{-1} t^{-1}). The lowest mean yield was recorded in RRII 51 (33.1 g t^{-1} t^{-1}). Highest growth in terms of girth was observed in RRII 300 (70.7 cm).

An On-farm trial with nine clones *viz.* RRII 105, RRII 208, RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, RRIM 600 and SCATC 88-13 was proposed and initiated during March 2018. Polybag plants were maintained and only 36 per cent of the plants survived after one year.

1.2. Polyclonal ortet evaluation

Ortet clones DNKL 3 and DNKL 4 attained comparatively higher yield and were almost at par with the high yielding clone RRII 208 in the region. The ortets DNKL 6 (44.5 cm) followed by DNKL 8 (44.0 cm) exhibited comparatively better girth attainment.

REGIONAL RESEARCH STATION, PADIYOOR, KERALA

Studies aimed at identification of locally adaptable clones suited for commercial cultivation in the region, evaluation of clonal variations to drought / disease incidence and development of suitable agro-management techniques for reduction of gestation period in rubber are the major thrust area of research activity in the Station. A source bush nursery of promising clones/selections is maintained in the station for breeding programs.

1. Crop Management

1.1. Response to applied fertilizers in high yielding clones

The treatments comprised of three clones (RRII 105, RRII 414 and RRII 429) and four levels of applied fertilizers (recommended dose, twice and thrice the recommended dose and a zero fertilizer as control). Clonal differences in yield were not significant (Table Pad. 1).

Table Pad. 1. **Effect of applied fertilizer on yield**

Treatment	Yield (g t^{-1} t^{-1})		
	RRII 105	RRII 414	RRII 429
D1	63.4	58.3	64.7
D2	68.7	67.8	60.2
D3	64.3	70.7	51.4
Control	49.0	61.6	53.3
CD (P=0.05)		NS	

2. Crop Improvement

2.1. Large scale evaluation of clones

Mean annual yield in the renewed bark panel was found highest in IRCA 130 (Table Pad. 2) at 59.8 g t^{-1} t^{-1} and was on par with PB 314 (51.2 g t^{-1} t^{-1}) and RRII 105 (53.4 g t^{-1} t^{-1}). IRCA 130 recorded highest summer yield (38.9 g t^{-1} t^{-1}) and was significantly superior to that of PB 255 (27.8 g t^{-1} t^{-1}) and RRII 105 (27.1 g t^{-1} t^{-1}).

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

The average annual yield BO-2 panel

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

Clones	Mean yield (g t^{-1} t^{-3})	
	Annual yield	Summer yield
PB 255	46.5	27.8
PB 314	51.2	23.8
PB 330	45.8	22.5
PB 28/59	35.7	17.0
RRIM 703	31.0	15.4
IRCA 18	45.2	23.5
IRCA 109	34.9	23.5
IRCA 111	32.5	21.9
IRCA 130	59.8	38.9
IRCA 230	38.9	18.5
RRII 105	53.4	27.1
CD (P=0.05)	12.0	6.9

(2 years) and summer yield of clones RRII 203, RRIC 100 and PB 86 and the ortets P 213, P 270 and Iritty were on par and significantly superior to that of RRII 105 and other clones/ortets tested.

Table Pad. 3. **Yield performance in high altitude area, Ambalavayal**

Clones	Mean yield (g t^{-1} t^{-3})	
	Annual yield	Summer yield
RRII 105	24.8	23.3
RRII 203	52.8	45.7
RRIC 100	63.5	41.7
RRIC 102	12.0	7.0
PB 86	67.6	63.3
P1	28.6	32.6
P 2	23.8	26.0
P90	31.4	28.4
P121	29.7	31.6
P155	29.8	29.7
P213	55.2	50.2
P270	54.6	64.7
P280	27.7	31.5
P296	27.1	26.6
Iritty	58.7	48.2
CD (P=0.05)	34.5	30.1

HEVEA BREEDING SUB-STATION, KADABA, KARNATAKA

Hevea Breeding Sub-station (HBSS) with a research farm at Nettana was established in 1986. The major constraints in commercial cultivation in this region is drought in summer months and occurrence of *Phytophthora* and *Corynespora* leaf diseases. The research programmes in the Station are envisaged to identify clones tolerant to different biotic and abiotic stress factors and to identify locally adapted clones for South Konkan region. The Station has a source bush nursery of 106 clones for generating nucleus planting material and a well-established Class B Agro-meteorological Observatory. There are five ongoing trials in the Station.

Table Kad. 1. **Comparison of yield from large scale clone evaluation**

Clones	Yield (g t^{-1} t^{-3})	Clones	Yield (g t^{-1} t^{-3})
PB 260	62.2	Tjir 1	41.6
HP 223	88.4	Gl 1	43.3
Mil 3/2	54.9	Hil 28	51.5
HP 204	43.7	GT 1	57.6
HP 185	72.3	PB 235	76.0
PB 217	64.9	HP 187	59.0
RRII 105	57.8	SEd	11.0
HP 372	92.8	CD (P=0.05)	22.5
PB 311	63.3		

1. Large scale clone evaluation trial (1990)

In LST 1990 trial, HP 372 recorded higher yield than other clones but was on

par with HP 223. All the other clones recorded comparable yield with each other. The lowest yield was registered by Tjir 1 (41.6 g t^{-1} t^{-1}).

2. Small scale clone evaluation trial (1991 A)

In 1991, three SSTs were planted *viz.* 1991 A (36 clones), 1991 B (13 clones) and 1991 C (13 cones). In the trial 1991 A, RRII 203 (88.9 g t^{-1} t^{-1}) recorded superior yield followed by PB 280 (87.0 g t^{-1} t^{-1}) and PB 235 (84.4 g t^{-1} t^{-1}) and were significantly higher than RRII 105 (35.5) g t^{-1} t^{-1}). The lowest yield was recorded by RRIM 701 and all other clones were recorded comparable yield with each other.

Table Kad. 2. **Comparison of yield from small scale evaluation trial 1991 A**

Clones	Cup lump yield (g t^{-1} t^{-1})	Clones	Cup lump yield (g t^{-1} t^{-1})
RRIC 36	50.1	PB 255	59.3
PB 314	72.5	RRIM 703	26.5
SCATC 88	56.5	PB 310	56.6
PB 312	81.3	PB 217	67.8
RRIC 104	20.8	RRIM 701	25.1
RRIC 100	44.3	PB 5/60	52.3
O 63	38.3	WARRING 4	8.3
KRS 163	48.5	RRIM 605	37.2
LCB 1320	54.6	CH 4	33.1
AVROS 352	24.3	CH 26	44.0
P 46	20.2	RRII 6	70.8
RRIM 501	24.6	RRIM 600	52.6
PB 235	84.4	KRS 25	21.2
RRII 105	35.5	SCATC 93-11	19.7
RRII 203	88.9	HAIKEN 1	24.7
PB 260	16.6	GT 1	56.3
RRII 300	67.4	KRS 128	47.4
PB 311	56.6	SE.d	14.5
PB 280	87.0	CD (P=0.05)	29.5

3. Small scale clone evaluation trial (1991 B)

Among the 13 clones evaluated in the trial 1991 B, RRII 5 (82.8 g t^{-1} t^{-1}) recorded highest mean yield and was significantly higher than RRII 105 (29.6 g t^{-1} t^{-1}).

Table Kad. 3. **Comparison of yield from small scale evaluation trial 1991B**

Clones	Yield (g t^{-1} t^{-1})	Clones	Yield (g t^{-1} t^{-1})
RRII 208	36.5	RRII 5	82.8
CH 3	42.1	AVROS 255	24.0
RRII 3	45.6	PB 5/139	29.6
CH 2	33.2	NAB 17	56.7
GT 1	54.0	RRII 105	29.6
RRII 118	67.3	SE.d	16.9
RRII 308	34.5	CD (P=0.05)	34.9
RRIC 102	21.9		

4. Small scale clone evaluation trial (1991 C)

No significant difference was observed among the clones with respect to yield. But among the clone evaluated PR 261 recorded numerically higher yield than the other clone.

Table Kad.4. **Comparison of yield from small scale evaluation trial 1991 C**

Clone	Yield (g t^{-1} t^{-1})	Clone	Yield (g t^{-1} t^{-1})
PB 5/51	32.5	HP 83/224	46.6
HP 83/225	37.4	PR 255	40.1
HP83/226	27.5	PIL B/84	32.5
GT 1	42.3	AVROS 49	34.8
RRIC 105	35.2	PR 261	71.1
PB 28/59	56.2	SE.d	23.05
RRIM 600	37.2	CD (P=0.05)	NS
CH 2	28.7		

5. Large scale trial (2000)

The large scale trial for evaluation planted in the year 2000 consists of hybrids RRII 403, RRII 407, RRII 414, RRII 422, RRII 429 and RRII 430 and their parents *viz.* RRIC 100 and RRII 105. Significant difference in yield was observed between the clones tested and the highest mean yield was recorded for the clone RRII 430 (82.6 g t^{-1} t^{-1}) compared to check clone RRII 105 recorded 33.8 g t^{-1} t^{-1}.

Table Kad. 2. **Large scale trial (2000)**

Clone	Mean yield over seven years (g t^{-1} t^{-1})
RRII 422	32.5
RRII 414	58.1
RRII 403	38.1
RRII 105	33.8
RRIC 100	54.1
RRII 429	48.4
RRII 407	32.7
RRII 430	82.6
SEd.	9.6
CD (P=0.05)	20.7

HEVEA BREEDING SUB STATION, THADIKKARANKONAM, TAMIL NADU

1. Genetic Improvement

1.1. Conventional breeding

The four projects *viz.* clone evaluation, hybridization and clonal selection, new generation polyclonal seed garden and participatory clone evaluation were under progress at the Station during the year 2018-19. The highlights of the results of these projects are furnished hereunder.

1.1.1. Clone evaluation

A block trial was planned for evaluating the commercial yield performance of the high yielding clones of the region *viz.* PB 255, PB 314, IRCA 109, IRCA 111, RRII 203, RRII 414, RRII 417, RRII 422, RRII 430 and RRII 105 during October 2018 at the Maruthamparai Unit of Chithar Division of Arasu Rubber Corporation (ARC) Ltd.

1.1.2. Hybridization and clonal selection

The research farm at Paraliar constitutes two breeding orchards comprising of 51 parental clones which were properly maintained. During the reporting year, the flowering was very sparse due to the greater day temperature followed by summer showers. Though initially 17 fruits were realised from two parental combinations, the same did not survive till the maturity owing to the intermittent summer showers, in spite of undertaking precautionary measures.

1.1.3. New generation polyclonal seed garden

The seed garden at New Ambady Estate was maintained well. Around 1000 polyclonal seedlings obtained from the seeds collected from the polyclonal seed garden have been raised for seedling nursery evaluation. Two selections were pollarded from the previous seasons' seedlings based on test tapping data.

1.1.4. Participatory clone evaluation experiments

The annual growth of 11 pipeline clones and the three check clones planted at

Tharuvaiyar Estate were recorded and the data tabulated. The clones P 10 (56.5 cm) and P 21 (56.6 cm) were more vigorous based on the data after 10 years of growth. The trial was opened for tapping during June 2018. The preliminary yield data over eight months of tapping indicated that clones P 021 (88.6 g t^{-1} t^{-1}) and P 076 (81.9 g t^{-1} t^{-1}) were promising.

Similarly, assessment of annual girth of the 11 trial clones in the OFT planted at Bethany Estate in 2012 indicated that the trial clones P 102 (58.3 cm), P 49 (55.4 cm) and P 104 (53.7 cm) as superior clones than the rest of the clones in terms of relative growth. The trial was opened for tapping during September 2018. The preliminary yield data over five months of tapping indicated that the clones P 066 (55.4 g t^{-1} t^{-1}) and P 049 (110) (48.0 g t^{-1} t^{-1}) were promising.

The annual girth recording for the OFT planted during 2016 at Chithar Division of ARC Ltd was initiated during 2018. Among the 16 trial clones planted, the clones P 202 (17.6 cm) and P 215 (16.4 cm) were found relatively more vigorous. Among the 3 control clones RRII 422 was relatively more vigorous (16.5 cm).

LIBRARY AND DOCUMENTATION

The Library and Documentation Centre attached to Rubber Research Institute of India is well maintained with a collection of 22998 books, 24320 bound volumes of periodicals, 6008 standards, 1563 reprints, 191 Theses/ Dissertations and 1200 Microfiche/Microfilms. Subject bibliographies and computer based bibliographic databases of all books, research articles, standards, theses and reprints are also accessible to the users.

Library continued the information and literature support to its in-house and institutional users by providing reference services, current awareness services and reprographic services. Forty five books and three standards were added to the stock during the year. Received and registered 262 issues of journals as subscription/exchange.

Published *Cumulative Index to Rubber Science* Vol. 1-30 and compiled information bulletins *Documentation List* (1-4) 2018, *New Additions List* 2018 and *Staff Publications list* 2018-2019. Databases were updated with the details of 45 books and 2 standards, 211 articles, 5 theses, 601 reprints, 299 bound journals and 856 Malayalam articles. Bound and accessioned 517 volumes of journals, circulated 585 books, technically processed 432 books, filed 2038 press clippings of relevant articles and provided 4807 photocopies. Library membership was issued to 63 users and extended library services to 131 outsiders.

As a part of sales promotion of RRII publications, library organized the sale and distribution of 656 copies of the journal *Rubber Science* and 202 other publications including RRII Annual Report.

SCIENTIFIC ADVISORY COMMITTEE RECOMMENDATIONS

1. Upgraded the clone RRII 429 to Category I from Category II of the planting recommendation for North East India and proposed for release for wide scale planting in the region.

2. Fertilizer recommendation for mature rubber in traditional rubber growing regions revised from the current rate of 30:30:30 to 30:20:30 kg NPK $ha^{-1}year^{-1}$.

ANNUAL EXPENDITURE

Expenditure at a glance (2018-19)

Head of Account	Expenditure (Rs. In lakhs)
Plan	
Rubber Research other than NE Region	2,132.37
Rubber Research - NE Region	413.97
Total	**2,546.34**

PUBLICATIONS

RESEARCH ARTICLES

Abraham, J. and Jessy, M.D. (2018). Soil CO_2 flux measurements from a mature natural rubber plantation. *Rubber Science*, **31**(3): 196-207.

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Research divisions and functions

The major research divisions are Agronomy/Soils, Biotechnology, Botany, Climate Change & Ecosystem Studies, Germplasm, Latex Harvest Technology, Plant Pathology, Plant Physiology, Rubber Technology, Technical Consultancy 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 another culture systems for propagation and development of transgenic plants incorporating agronomically important genes for improvement of *Hevea* are the important areas in which the Biotechnology Division is engaged. The Advanced Centre for Molecular Biology and Biotechnology (ACMBB) is a functional grouping of scientists working in the areas of Molecular Biology, Biotechnology, Genome Analysis, Molecular Physiology and Molecular Pathology. 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 Climate Change & Ecosystems Studies Division is pursuing studies on climate change process in traditional and non-traditional rubber growing regions of India and developing information system on rubber cultivation using remote sensing (RS) platform to identify area under rubber cultivation and suitable area where rubber plantations can be extended. 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 Rubber Technology Division and Technical consultancy Division together forms the Advanced Centre for Rubber Technology (ACRT). The Economics Division undertakes studies on economic aspects related to rubber plantations.

The research supporting sections includes Library and Documentation, Instrumentation, Statistics, Computer and Maintenance Wing. There is also a small experimental farm of 33 ha. at the headquarters of RRII.

Central Experiment Station

The 255 ha. Central Experiment Station at Chethackal (Ranni), 50 km away from Kottayam, was started in 1966. Field trials laid out by the research divisions cover almost the entire area.

Regional Research Stations

RRII has established a North-Eastern Research Complex with headquarters at Agartala having regional research stations at Agartala in Tripura, Guwahati in Assam and Tura in Meghalaya. The RRII has also set up regional research establishments at Dapchari (Maharashtra), Dhenkanal (Orissa), Nagrakata (West Bengal), Thadikarankonam (Tamil Nadu), Kadaba (Karnataka) and Padiyoor (Kerala).

Regional soil testing laboratories have been established at 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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