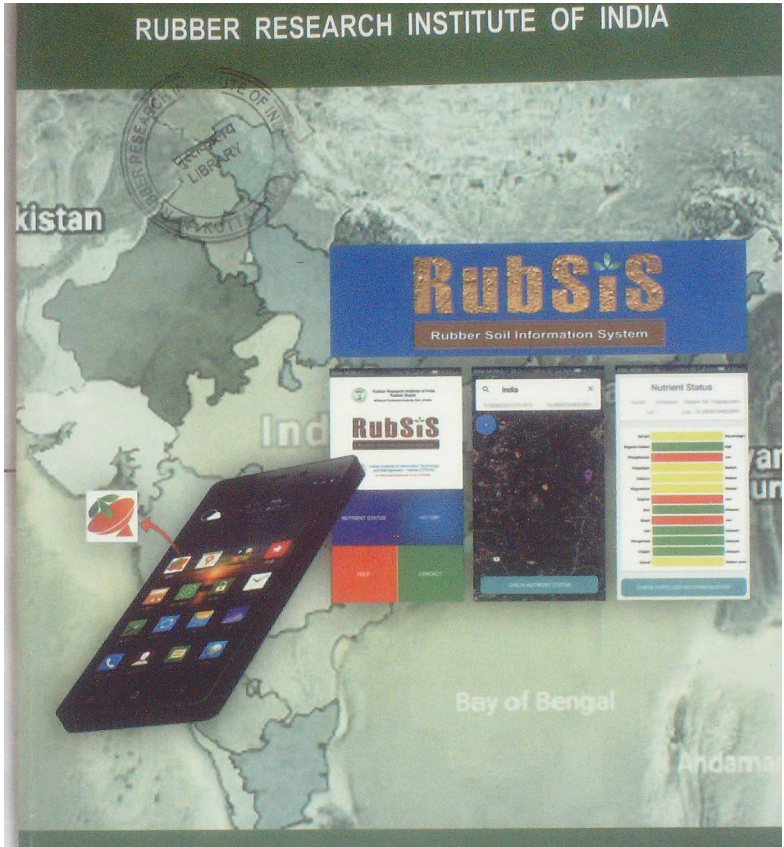
RUBBER RESEARCH INSTITUTE OF INDIA



ANNUAL REPORT

2016 - 2017

Rubber Research Institute of India

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

Location

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

Organization

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

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ANNUAL REPORT
2016-2017

RUBBER RESEARCH INSTITUTE OF INDIA
RUBBER BOARD
(Ministry of Commerce & Industry, Government of India)
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THE RUBBER BOARD

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

Organization

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

Chairman
Dr. A. Ajith Kumar IAS

Rubber Research Institute of India
Dr. James Jacob
Director

Crop Improvement
Dr. Kavitha K. Mydin
Joint Director

Botany
Deputy Director (Vacant)

Biotechnology
Dr. A. Thulaseedharan
Joint Director (up to 30-11-16)

Genome Analysis
Dr. Thakurdas Saha
Principal Scientist

Germplasm
Dr.V.JayasreeMadhavan
Principal Scientist

Crop Management
Joint Director (Vacant)

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

Fertiliser Advisory Group
Dr. Mercykutty Joseph
Principal Scientist

Crop Physiology
Climate Change and Ecosystem Studies
Dr. R. Krishnakumar
Joint Director (up to 30-05-16)

Plant Physiology
Dr. K. Annamalainathan
Joint Director

Crop Protection
Mr. Sabu P. Idicula
Joint Director

Economics
Dr. Tharian George K.
Joint Director (up to 30-05-2016)
Toms Joseph
Joint Director

Latex Harvest Technology
Dr. K.U.Thomas
Joint Director (up to 31-12-2016)

Rubber Technology
Joint Director (Vacant)
Deputy Director (Vacant)

Technical Consultancy
Dr. Siby Varghese
Joint Director

Central Experiment Station, Chethackal
Dr. P. Mallinath Priyadarshan
Deputy Director (up to 30-05-2016)

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Dr. Gitali Das
Deputy Director

Regional Research Station, Agartala
Dr. Sushil Kumar Dey
Joint Director

Regional Research Station, Nagrakata
Deputy Director (Vacant)

Regional Research Station, Tura
Deputy Director (Vacant)

Regional Research Station, Dapchari
Dr. Meena Singh
Senior Scientist (Officer-in-charge)

Regional Research Station, Dhenkanal
Dr. Bal Krishan
Senior Scientist (Officer-in-charge)

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

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

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

Hevea Breeding Sub-station
Dr.M.Suryakumar
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Deputy Secretary

Finance and Accounts
CA. Zachariah Kurian
Joint Director

Instrumentation
Dr. Thomas Baby
Deputy Director

Library and Documentation Centre
N. Latha
Documentation Officer

Statistics and Computer
B. Biju
Assistant Director (Systems)

PREFACE

I am very pleased to note that the Institute has made some remarkable achievements during 2016-17. Rubber Soil Information System or RubSIS, the online fertilizer recommendation system for rubber growers developed by RRII is a pioneering effort. RubSIS combines good science and cutting edge technologies, including geospatial technology, ICT, Soil Science and Agronomy to develop a product that is highly useful to rubber growers with practically no cost or effort by the growers. Successful completion of RubSIS is also a good example of multi-institutional collaboration. I urge RRII to complete the RubSIS program for North Eastern states in the shortest possible time.

During this year, RRII 208, a high yielding, cold/disease tolerant clone was released for commercial cultivation in the North eastern region. This is the first time Rubber Board has released an exclusive clone for this regions where natural rubber cultivation has great potential. A large number of high yielding clones were imported to India as part of the multilateral clone exchange initiative of the International Rubber Research and Development Board during the reporting year. It is a matter of satisfaction that 22 promising pipeline clones were planted at seven locations in Kerala, Karnataka and Tamil Nadu during this year as part of phase V of the farmer participatory clone evaluation program. The remarkable progress made in genomics and genetic markers associated with beneficial Agronomic traits should not go unappreciated. The whole genome sequencing project should be completed at the earliest which will be a great boon to crop improvement research and this will remain a top R&D priority as new challenges to rubber cultivation continue to emerge.The next big breakthrough will be made by RRII in this particular area of research in the next one year.

There should be increased focus on rubber products manufacturing, particularly in non-tyre products in the MSME sector. I appreciate the discussions initiated with the

Kerala State Agriculture Department and the Kerala State Industrial Development Corporation. A national seminar on rubber products should be organized to bring technology research at RRII closer to the industry. The Technical Consultancy Division of RRII bagged NABL accreditation which has been a long felt need and this will certainly help to serve the rubber products manufacturing industry better.

The latest findings on making good quality block rubber from low ammonia preserved latex, preparation of high quality solid deproteinized natural rubber, mixing of silica with natural rubber latex, progress made in devulcanization *etc.* deserve further work to scale up and transfer the technologies to the industry.

I am happy to learn that the core areas of research activities are progressing despite a large number of vacancies in scientist's posts and budget cuts. I urge the RRII community to continue to show resilience in the face of adversities and do your best for the sector. I firmly believe that good science can make a positive difference to the lives of people, be that rubber growers, processors, traders or rubber products manufacturers. Reasons for the less than impressive performance of several Rubber Producers' Societies, socio-economic impart of natural rubber cultivation in the tribal belts/LWE affected regions of the country, reasons for poor adoption by growers of several cost-saving recommendations made by RRII *etc.* are some key areas that require immediate examination and remedial actions. Much better convergence between RRII and the Rubber Production Department of Rubber Board is needed to take the R&D findings to the rubber growers. Indian rubber industry, especially the natural rubber production sector is passing through a challenging phase and scientists and extension personnel need to equip themselves better to continue to serve the growers during these difficult times.

Kottayam
15.11.2017

A. Ajith Kumar IAS
Chairman & Executive Director

DIRECTOR'S REVIEW

This year was marked by the Institute crossing some major milestones. One was launching of the online fertilizer recommendation for rubber growers (Rubber Soil Information System, RubSIS), a first of its kind in the country by the Honorable Minister of Commerce and Industry on 23 January 2017. Equally remarkable was the release of the first Indian clone for cultivating in the North eastern states. After elaborate field trials that lasted about a quarter century, the first high yielding indigenously developed clone for exclusive cultivation in the cold-prone North eastern states of India was released during October 2016. Obtaining NABL accreditation for the Technical Consultancy Division of RRII was another significant milestone that RRII could achieve during 2016-17. The first ever census of rubber tappers of Kerala was conducted and it was found that there was a serious deficiency in the availability of skilled tappers. Detailed survey of commercial yield in Tripura state clearly indicated the promising yield potential in the state. This year the Quality Control Division functioning under the P&PD Department was brought under the control of RRII.

RubSIS combines principles of Agricultural Science, Geo-spatial Technology and Information and Communication Technology. RubSIS was conceived entirely by RRII and executed in collaboration with IIITM-K, Thiruvananthapuram which developed the required software and ICAR, NBSS&LUP, Regional Centre, Bangalore which assisted in soil sample collection and analyses. A significant recommendation made during the reporting year was skipping of fertilizers in mature rubber plantations with high organic carbon status and deep soils. Rising soil acidity with the pH often going well below five was a major concern and a recommendation was approved to apply powdered shell lime or dolomite. Application of zinc and boron was also found necessary as these micronutrients were increasingly becoming deficient in rubber soils.

As of now RRII has a total of 281 promising pipeline clones which is perhaps the largest such collection anywhere in the world. With the planting of the 5th phase of the farmer participatory clone evaluation trial involving 22 pipeline clones at seven locations, we have now a total of 92 pipeline clones planted at 44 locations. This is a highly ambitious field evaluation programme and we may get some new clones for future release from these trials.

During the reporting year a total of 2400 hand pollinations were performed in 21 parent combinations resulting in the production of 236

fruits. The success of fruiting ranged from 0 to 9.26 per cent with a mean of just 2.88 per cent which is a major disadvantage in bi-parental mating in rubber trees. Increasing the sample size of seeds (by collecting large number of seeds produced through open pollination among a large number of elite clones) and evolving fast, efficient screening tools for early selection of promising seedlings will be the future of rubber tree breeding. Developing molecular markers, including genic markers associated with useful agronomic traits should be a research priority that the Breeders, Physiologists and Molecular Biologists should together pursue.

Out of the more than 3500 wild accessions of *Hevea*, 277 have been found to have promising potentials for various useful traits and these are under different stages of field evaluation. Twelve of these have been successfully utilized for hybridization with Wickham clones and the F1s are under field evaluation.

Agrobacterium mediated genetic transformation of *Hevea* using various genes of agronomic importance (*eg.* stress tolerance, yield improvement *etc.*) were continued. Protocols for developing antibiotic marker-free transgenic *Hevea* and somatic embryogenesis from RRII 414, RRII 105 and RRII 430 explants were attempted. After obtaining NOC from Assam Government, two lines of MnSOD transgenic plants were multiplied by bud grafting and these were transferred to RRS, Guwahati and maintained there in a protected nursery for planting in the field after obtaining clearance from Govt. of India.

Significant progress was made in the development, optimization and validation of molecular tools in genetic diversity analyses, genetic linkage mapping, genetic markers for stress tolerance, cloning and characterization of agronomically important genes *etc.* An important and interesting finding was the marked allele loss in the modern cultivated Asiatic (Wickham) clones compared to the wild accessions as studied using SSR markers.

Severity of abnormal leaf fall (ALF) disease during 2016 monsoon season was relatively less. RRII 429 showed excellent leaf retention during ALF disease season whereas RRII 414 was slightly more susceptible. Trees crown-budded with Fx 516 had better timber volume and practically no ALF infection. Attempts to make crown-budded plants in root trainers in the nursery have not become fully successful as of now. Root trainer cups were being modified to address this issue. A quick leaf wilt bioassay protocol using crude toxin of *Corynespora* was developed and this protocol could distinguish between susceptible and tolerant clones. An *in vitro* spore inoculation test was also developed to detect the clonal susceptibility to *Corynespora*.

It was an interesting finding that there was lack of synchronization between photosynthetic carbon assimilation and transpiration in response to variations in ambient light levels. Transpiration

decreased at a much slower rate than photosynthesis decreased when the ambient light intensity was reduced. This lack of synchronization has major implications for water use efficiency at the ecosystem level. Based on measurements of canopy temperature during summer season, 14 wild accessions were identified with better intrinsic drought tolerant capacity. Expression of a 23.8 kDa protein in the chloroplasts in response to water deficit stress and high light intensity was prominent in drought tolerant clones. Studies on quantitative gene expression analyses of ethylene receptors and other genes involved in signaling pathways are in progress.

New projects were initiated to estimate the carbon footprint of rubber production and assess agro-climatic stability of rubber cultivation in the Left Wing Extremism affected areas and North Eastern states. Vegetation Temperature Condition Index (VTCI) of rubber growing areas was estimated using satellite-derived data which acted as a reliable proxy estimate for occurrence of drought stress at the ecosystem level. During September 2016 most parts of the rubber growing regions of Kerala and Kanyakumari district of Tamil Nadu experienced widespread drought compared to the same time of the previous year. It is pertinent to note that September is a highly unlikely time for drought to occur in Kerala. Summer of 2016 witnessed heat wave conditions in Kerala which was the first time heat wave was declared in Kerala by the India Meteorological Department.

Yield performance of clone RRII 105 under weekly tapping in small holdings continued to be good. The yield tree^{-1} yr^{-1} ranged from 3.4 kg to 6.6 kg with a mean of 5 kg.

A compendium on product-wise tariff policies of India on rubber and rubber products under various regional trade agreements was published. The impact of regional comprehensive economic partnership on India's rubber and rubber products was analyzed comprehensively. A study was made to evolve projects for rejuvenating the Rubber Producers' Societies in Kerala. Despite clear advantages, adoption rate of weekly tapping was extremely poor. A major census of rubber tappers of Kerala was conducted and it was found that there was a serious deficiency in the availability of skilled tappers. A large scale study on commercial yield performance of rubber in Tripura indicated the promising yield potential in Tripura.

During 2016-17, the Quality Control Lab tested 29343 parameters in 11721 samples of field latex, dry rubber, cenex, water, effluents *etc.* In addition, it also conducted 65 inspections for import of rubber and issued NOC for import of more than 4.34 lakh tonnes of natural rubber.

There were significant achievements made in the processing and products development research. A novel method for the production of solid deprotenized natural rubber from the field latex was developed. Cytotoxicity due to residual accelerators present in gloves was evaluated. An efficient and cost effective method for

recovery of good quality rubber from skim latex was developed. A stable free radical was identified for efficient devulcanization of used rubber products. During the reporting year 5104 parameters were tested in 1051 samples supplied by 511 clients (processors/product manufacturers). Know-how for eight different products was transferred to more than ten entrepreneurs.

Scarcity of funds and scientists in various disciplines had a serious impact on the R&D outcome during the reporting year. Despite these difficulties, we could publish three issues of *Rubber Science* with a total of 30 articles. In addition, 12 research articles, 45 conference papers, 26 popular articles, one book, 11 book chapters, one monograph and one working paper were published. A total of five PhD theses and 11 other dissertations were also made. The IEBR collected during the year was Rs. 4.1 Cr and the total expenditure incurred for research schemes was only Rs. 49 lakh.

Dr. James Jacob

AGRONOMY / SOILS DIVISION

Development and validation of newer agro-management techniques that will sustain profitable rubber cultivation in the country is the thrust area of research of the Agronomy/Soils Division. Specific attempts are being made to disseminate the accumulated knowledge and wisdom from current and past long term experiments, to the farming community. Ensuring profitability of rubber farming through reducing the input cost and crop diversification as well as assessment and monitoring the rubber ecosystem are also primary areas of research. Various experiments with these objectives were continued.

In the fertilizer advisory area, Division entered a new horizon with online fertilizer recommendation for rubber plantations in South India. Online fertilizer recommendation (Rubber Soil Information System-RubSIS) developed in collaboration with Indian Institute of Information Technology and Management-Kerala (IIITM-K) and ICAR-NBSS&LUP, Bengaluru by overlaying different soil fertility parameters and soil depth and following the guidelines of discriminatory fertilizer recommendation was launched by Union Minister of Commerce and Industry on 23-01 2017. Gravel content which is devoid of any nutrient supplying property was considered prior to the evaluation of soil fertility assessment. Fertilizer advisories were also modified considering the geo-referenced spatial variability in soil fertility status mapped by geospatial analyses, so as to avoid over/under/unbalanced manuring. Skipping fertilizers in mature rubber fields with deep soil and high organic matter content was recommended to farmers based on the results from long term field experiments. Liming in very strongly/extremely acidic soils with low available calcium status and supplementing zinc and boron in areas delineated as low in their status were also advised to growers.

1. Nutrient Management

The field experiment at CES, Chethackal to study the effect of long term use of chemical fertilizers and organic manures on growth and yield of rubber and on soil properties was continued. The residual effects of treatments were studied without applying chemical fertilizer and FYM. Rubber growth was superior for the combination treatment, (25 % chemical fertilizer + 75 % FYM) compared to standard practice as observed earlier (mean girth 67.1 and 63.0 cm, respectively). However, no significant difference in yield among treatments could be observed. Another field trial was initiated at SFCK, Chithalvetty Estate to study the effect of a branded organic manure 'Geo green' on growth of young rubber.

To study the effect of supplementing secondary and micronutrients on growth of rubber plants, field trials were initiated at Thamarasserry, Palappally and Cheruvally estates during 2011. In all the three locations, no significant difference was observed on girth of the plants between the treatments six years after planting.

The field experiment at Thanneermukkom, Cherthala, to study the performance of rubber in coastal sandy soil was continued. Rubber plants attained tappable girth by the seventh year. Mean yield during the second year of tapping was 50 g $t^{-1}t^{-1}$. This study showed that by adopting appropriate agro-management practices, growth comparable to that in conventional rubber growing soils can be achieved in coastal sandy soils also.

A new nursery experiment was initiated to revise the current fertilizer recommendation for root trainer rubber plants. The treatments comprised combinations of different fertilizer doses, sources and application methods. Preliminary results indicated the superiority of foliar spraying of fertilizers along with Mg and Ca and integrated management for the growth of root trainer rubber seedlings.

In the rhizosphere field study, the exchangeable Al and exchangeable acidity were lower and CEC was higher in the rhizosphere soils than the bulk soils in majority of locations. Rhizosphere chemistry study conducted in rubber seedlings in soils having three distinct pH, the root growth of rubber seedlings was affected when pH was 4.4 with exchangeable Al and exchangeable acidity above 2.2 cmol (+)kg^{-1}. The aluminium content in the root and shoot was high in low soil pH.

The study on leaf nutrient status of low and high yielding rubber trees in clone RRII 105 was continued.

2. Soil and water conservation

The experiment on evaluation of biological/ vegetative hedges for soil and water conservation in rubber plantation was continued. The establishment of hedges had no significant effect on growth of rubber over a period of seven years compared to plots without hedges. From fifth year onwards, significant difference between treatments was not observed in soil erosion.

3. Intercropping and cropping systems

The field experiment initiated to develop a multi-species rubber-based cropping system for Tamil Nadu region was in progress. Six years after planting, growth of rubber was significantly higher in all intercropped plots (except that of cocoa) compared to monoculture.

The experiment initiated at CES, Chethackal in 2001 to evaluate the feasibility of growing perennial intercrops with rubber was also in progress and the growth and yield of rubber continued to be not influenced by cultivating coffee as intercrop in normal system of planting. In the paired row system of planting also growth and yield of rubber was not influenced by intercropping *Dracaena massangeana* continued to perform well in mature rubber plantation and leaves could be harvested once in 45-60 days.

Experiment on inter planting of rubber with timber trees *viz*. teak, wild jack and mahogany was continued. The block trial to study the effect of retaining pineapple as intercrop on performance of rubber and soil properties is in progress. No significant difference was observed in girth and girth increment between the treatments *viz*. rubber with pineapple and rubber without pineapple. Effect of cocoa and coffee on soil pH when grown as intercrops in rubber plantation was studied at CES Chethackal, Malankara and Kaliyar Estates. Soil pH was more in the root zone of cocoa than that of coffee.

4. Ground cover management

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 at CES, Chethackal laid out during 2012 was continued. The growth of rubber plants was adversely affected significantly by allowing natural flora to establish with minimal weeding. The highest weed count was associated with rubber + natural cover with 1 m^2 weeding. The dry matter production (DMP) was highest in *Mucuna* established plot followed by natural cover with 1 m^2 weeding. A stable pH was maintained throughout the immaturity period for rubber with natural flora

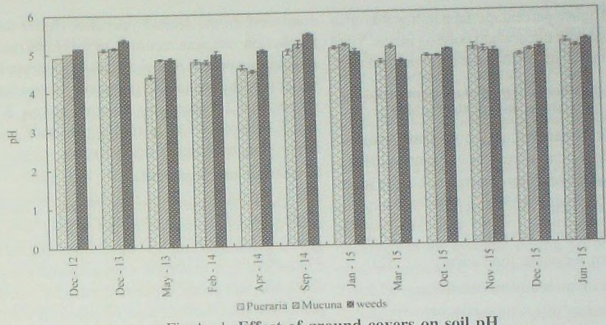

Fig.Ag.1. **Effect of ground covers on soil pH**

while slight acidification was noticed in the early years of immaturity in plots with legume covers (Fig. Ag.1).

The field trial initiated at CES, Chethackal for the evaluation of *Calopogonium caeruleum* was continued. An observational trial to find out the feasibility of establishing *C.caeruleum* under partial shade was initiated at Kaliyar Estate.

5. Planting techniques

The experiment to evaluate different planting designs was in progress at Cheruvally estate. It was observed that in the modified planting designs, the canopies of rubber trees were assymmetrical in growth pattern. Girth of plants in the twin planting system was superior to control. In the paired row planting system, an area with width 2-2.5 m was available between two paired rows without shading for cultivation of intercrops even eight years after planting.

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

The field experiment laid out at Malankara Estate, Thodupuzha during 2005 with the objective of evolving an agronomic package to reduce the immaturity period of rubber was continued. The growth and yield of rubber under integrated management continued to be the highest and all the treatments recorded significantly higher girth and yield than control. The experiment was concluded.

The field experiment initiated at CES, Chethackal during 2008 to evolve an agronomic package to reduce the immaturity period of rubber starting from selection of planting material onwards in which the treatments included combinations of two types of planting materials and two management options was in progress. Direct-seeded green budded plants under integrated management were significantly superior to all others and yield recording was initiated in the trial area.

The field experiment investigating the comparative field performance of one-whorl, two-whorl and three-whorl polybag and root trainer rubber plants initiated at CES, Chethackal during 2008 was continued. Growth of three-whorl polybag plants was significantly superior to all other types of planting materials (Table

Ag.1). Higher percentage tappability was also associated with three-whorl polybag plants. Yield recording was initiated in the experimental area.

Table Ag. 1. **Effect of type of planting material and its stages on growth of rubber**

Treatments	Girth (cm) (January 2016)
Polybag plants (one whorl)	52.6
Polybag plants (two whorl)	52.8
Polybag plants (three whorl)	55.4
Root trainer plants (one whorl)	52.0
Root trainer plants (two whorl)	52.9
Root trainer plants (three whorl)	52.7
SE	0.6
CD (P=0.05)	1.9

7. Rubber growing soils

The experiment to assess the contribution of root respiration towards soil respiration was continued. In the second year also, higher soil respiration rate was recorded in rubber-banana system than mature rubber and rubber-pineapple systems. The mean soil respiration in mature rubber was 2.5 µmols m^{-2} s^{-1} while it was 2.7 and 5.1 µmols m^{-2} s^{-1} in rubber-pineapple and rubber-banana systems, respectively.

8. Stress management

The feeler experiment on'efficacy of latex coated mulch materials in preventing weed growth in immature rubber' was continued. Latex coated low cost materials were prepared using newspaper and gunny, applied as mulch for young plants and observations were recorded on weed growth and soil moisture. Results showed that latex coated newspaper and latex coated gunny were effective in preventing weed growth and conserving soil moisture. Both were durable for 5-6 months in the field and the durability can be improved by increasing the thickness of latex coating.

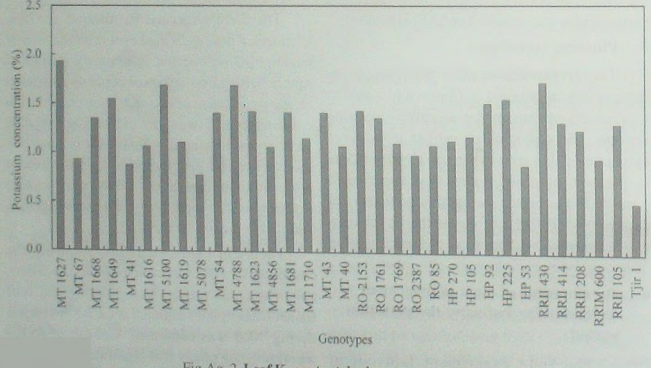

Fig.Ag. 2. **Leaf K- content during stress period**

9. Environmental aspects of rubber cultivation

The experiment to assess the soil nutrient changes in continuously cultivated rubber fields was continued. The soil samples collected from the two fields in RRII, at the end of the second cycle of cultivation were analyzed for pH, OC, available P, K, Ca and Mg. The values were compared with the values obtained at the beginning of the cultivation and after eight years of growth. It was observed that at the end of the second cycle, OC, available P and K were improved in soil. Available Ca and Mg were maintained while pH did not vary considerably. The study indicated that the chances of soil nutrient depletion in rubber plantations are more during the immature phase. The build-up of soil OC towards the end of the cultivation cycle (Fig.Ag.3) indicates the possibility of considering natural rubber as a crop that can sequester soil carbon.

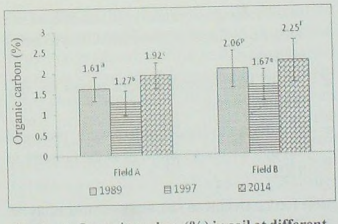

Fig.Ag. 3. **Organic carbon (%) in soil at different stages of rubber growth**

The study on assessment of water quality in watersheds dominated by rubber, tea and cardamom plantations was continued. Samples of ground water and surface water from selected sites in each watershed were collected during the pre and post-monsoon seasons, and analysed for different physico-chemical parameters. Quality parameters such as total hardness, total dissolved solids and nitrate content were significantly higher in water samples from tea and cardamom watersheds than rubber watershed. However, all these parameters were within the BIS regulatory limit in all three systems. Dissolved oxygen content of surface water in rubber watershed was significantly higher than that of tea and cardamom, indicating better health of water resources in rubber watershed. A drop in dissolved oxygen content of surface water was observed in all systems during pre-monsoon season compared to post-monsoon season. Pesticide residues in the water samples collected during pre-monsoon season (organo-chlorines, organo-phosphates and pyrethroides) were below the level of quantification in all three systems. Contents of heavy metals (lead, cadmium, chromium and copper) were within permissible limits in all three systems. Bacteriological analysis showed that water samples from all the three watersheds contained total coliforms and *E.coli* above the limit specified for drinking water.

10. Soil fertility mapping and soil health monitoring of traditional rubber growing regions of Kerala, Tamil Nadu and Karnataka (Collaborative project of RRII with ICAR-NBSS & LUP, Bengaluru and IIITM-K, Thiruvananthapuram)

The entire idea of RubSIS was conceived by RRII and the project was implemented in collaboration with ICAR-NBSS & LUP, Bengaluru and IIITM-K, Thiruvananthapuram. Methodology for soil sample collection and analysis of soil fertility parameters were finalized by RRII in consultation with ICAR-National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Bangalore. Entire soil samples collected from rubber growing regions in South India during 2013 were geo-referenced and soil sampling was done by RRII. Soil sample analysis

was shared between RRII (2000 no.) and NBSS & LUP (9000 no.). The software for online fertilizer recommendation (Rubber Soil Information System-RubSIS) was developed by the Indian Institute of Information Technology and Management-Kerala (IIITM-K) using different soil fertility parameters and soil depth and following the guidelines of discriminatory fertilizer recommendation. 'RubSIS', the interactive web-based ICT tool makes the rich geospatial soil fertility knowledge-base available to the farmers and other stakeholders. The process involved migration of geostatistically derived thematic information of 13 soil fertility parameters to a smart Geospatial Content Framework. Several dynamic Web Map services such as Digital Globe, Google Map and Open Layers were integrated to facilitate thematic backdrops so as to enable easy identification of farms on the Map Interface. 'RubSIS' was launched by the Hon. Minister for Commerce and Industry on 23rd January 2017 and has been uploaded for entire rubber growing regions of South India. 'RubSIS' will be translated to local languages and a Mobile App will be developed. Feedback of growers on 'RubSIS' also will be collected. It is also proposed to undertake soil fertility mapping and online fertilizer recommendation (RubSIS) of rubber growing regions of North-East India. All these were directed by the Ministry while RubSIS was launched.

FERTILIZER ADVISORY GROUP

The Fertiliser Advisory Group operates through the Central Soil and Leaf Testing Laboratory at RRII and the seven Regional Laboratories in Kerala and offers recommendations on fertilizer application both to large estates as well as small growers on the basis of analysis of soil and leaf samples. Advices on fertilizer use were provided during the visit of the growers to the laboratory or as clarifications on telephonic enquiries or email queries. The major activity of the regional laboratories were dry rubber content estimation of the latex samples.

- Offered site specific fertilizer recommendation to 700 individual fields from19 large estates on the basis of analysis of 349 leaf samples and 578 soil samples.
- 40 leaf samples and 443 soil samples from small holdings were analyzed and offered site specific fertilizer recommendations to small holders.
- 79139 latex samples were tested for dry rubber content.
- 71 Advices to small holders were provided through telephone and visit of the farmers to RRII.

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

Parameter	Number	Testing fees (Rs.)
Soil	1021	1,48,416.00
Leaf	389	1,23,291.00
DRC of latex samples	79139	46,69,547.00
Total Revenue		49,41,254.00

CLIMATE CHANGE AND ECOSYSTEM STUDIES DIVISION

One of the major thrust areas of research in the Division includes development of information system on rubber cultivation using satelite based remote sensing platform to identify area under rubber cultivation and identify suitable areas where rubber cultivation can be extended in the country. Agro-met data base management from the traditional and non-traditional rubber growing regions is regularly updated to create a robust date base. Studies were undertaken on biodiversity conservation and impact of extreme weather events on rubber cultivation. Studies were also initiated to identify climate suitability for rubber cultivation in left wing extremism affected (LWE) and NE India regions.

Estimation of rubber plantation area in West Bengal and waste land suitable for NR plantation in Assam was completed using satellite based remote sensing. Estimation of vegetation temperature condition index (VTCI) of rubber growing areas of Kerala and Kanyakumari district of TN indicated unseasonal drought in NR areas during September 2016. Enough datasets were prepared in GIS platform for the development of an online portal for fertilizer recommendation known as Rubber Soil Information System (RubSIS). Weather conditions of traditional and NE regions were analyzed in the context of changing climate conditions. The climatic suitability for rubber cultivation in ten LWE affected states in India was analyzed.

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

The project on developing rubber plantation information system using satellite based remote sensing and GIS was continued since 2010. Estimation of rubber plantation area was completed in West Bengal using Landsat satellite data for the year 2016. NR holdings were very low in this state. Very scanty patches of NR area were mapped in Jalpaiguri, Alipurduar and lower reaches of Darjeeling districts (Ganeshpur area near Siliguri). Among the districts, distribution of rubber plantations was the highest in Jalpaiguri, followed by Alipurduar and Siliguri (Darjeeling district).

2. Estimation of vegetation temperature condition index (VTCI)

Satellite-derived land surface temperature (LST) and normalized difference vegetation index (NDVI) were analyzed to estimate VTCI of rubber growing areas of Kerala and Kanyakumari during September 2015 and 2016. The main objective was to understand intensity of unseasonal drought in NR areas from VTCI data. Results indicated that during September 2016 most parts of the NR plantations of Kerala state and Kanyakumari district of Tamil Nadu state experienced widespread drought compared to same period of the previous year (2015). Around 29 per cent of the NR holdings experienced drought in September 2016, whereas during previous year this was only 19 per cent. In September 2016, severe drought was observed in natural rubber holdings of Kanykaumari and Thiruvananthapuram districts followed by parts of Ernakulam, Idukki, Malappuram and Northern districts of Kerala.

3. Identification of potential areas for rubber cultivation in NE India using satellite data, long term climatic data and soil fertility status

Mapping of rubber plantations and wastelands suitable for NR cultivation in Assam was completed. Multi resolution satellite images of Indian Remote Sensing satellites (IRS) were used for identification and estimation of spatial extent of NR and wastelands suitable for its cultivation. Temporal and multi-resolution satellite images of IRS R I and II, L-3, L-4, Cartosat PAN were used for the study. Mapping of the wastelands was carried out without interfering areas under food crops, forests, steep valleys and lands suitable for cultivation of food crops. Spatial distribution of rubber plantation in Assam was 16,872 ha and additionally about 24,783 ha of wastelands suitable for NR cultivation have been estimated as on 2011-2012 stand. Karimghanj district of Assam has the highest extent of wastelands (6969 ha) followed by Karbi Anglong (6052 ha), Kamrup (4525 ha) and Goalpara (2988 ha) districts. These four districts alone accounted for about 63.5 per cent of total rubber cultivation and 82.8 per cent of total wastelands estimated from the state. Other moderately suitable districts *viz.* Dima Hasao, Hailakandi, Dhubri, Kokrajhar and Jorhat together comprises 12.1 per cent of total wastelands available for NR. In general, standing crop and wastelands suitable for cultivation were higher in southern and southwest districts compared to northeast districts of Assam. Estimated wastelands can be prioritized for suitability in terms of soil and climate variables. The outcome may serve as a geo-spatial decision support system for planners in NR sector.

4. Development of WebGIS enabled RubSIS *(Collaborative work with Agronomy and Soils Division)*

WebGIS based Rubber Soil Information System (RubSIS) was developed in colloboration with Indian Institute of Information Technology and Management (IIITM-K), Kerala. As a major part of contribution from the CC&ES Division necessary data sets were prepared in GIS environment. Projection, cell size, lay outs, file format *etc.* have been set uniformly to all GIS data layers required to develop WebGIS based RubSIS. Geostatistical analysis was performed to generate prediction maps of soil fertility parameters. Soil nutrient values were krigged in GIS environment to generate spatial variability maps of the nutrients and valid prediction surface maps were generated for each soil fertility parameter with a cell size of 30m. Then the district-wise soil fertility maps (total number - 352) of NR growing regions in Kerala, Tamil Nadu, Karnataka, Maharashtra and Goa were integrated with soil depth and discriminatory soil fertilizer recommendations of the Rubber Board to develop RubSIS portal by IIITM-K, Thiruvananthapuram.

5. Estimation of area under rubber in TTAADC zones of Tripura *(Collaborative work with Economics Division)*

With an objective to estimate the extent of rubber area falling under the Tribal Area Autonomous District Council zones (TTAADC), overlay analysis was carried out with satellite-derived rubber plantation map of Tripura. It was found that as much as 43.8 per cent of the area under rubber cultivation in the state of Tripura was coming under the TTAADC zones as given in Table 1 (based on satellite data of 2011-2012). The total area under rubber (three and above three years old) under TTAADC was 21,104 ha (as of 2011-2012). The largest extent of area was in Sepahijala followed by South Tripura district.

Table CCES. 1. **Extent of area under rubber in TTAADC zones of Tripura**

Districts	Rubber area under TTAADC (ha)	Per cent of the total area under rubber in the state
South Tripura	5660	11.7
Dhalai	663	1.4
Khowai	1764	3.7
North Tripura	846	1.8
Sepahijala	5886	12.2
Unakoti	493	1.0
West Tripura	2316	4.8
Gomati	3476	7.2
Total	21104	43.8
Total area under rubber in Tripura :		48037 ha

6. Identification of suitable areas for natural rubber cultivation in the Left Wing Extremism affected (LWE) states in India

The study attempted to identify the level of variation in climatic suitability for NR with respect to the most optimum location (considered to be Kottayam, Kerala) and also with respect to minimum tolerable climatic limits for the crop.

Initially seven relevant monthly climatic parameters *viz.* maximum temperature, rainfall, rainy days, potential evapotranspiration, vapour pressure deficit, aridity index and hot degree days were tested for 30 districts of Odisha state. The state comprising more than 6000 acres of NR plantation with district level was chosen as the smallest unit. These data, obtained for a period of 1901-2002 (India Water Portal Website), were tested based on the Area-Under-Curve (AUC) (trapezoidal rule) after plotting annual graphs with mean monthly data. Climatic suitability in Odisha was evaluated based on the percentage differences in AUCs of each district of Odisha with that of Kottayam. Percentage differences in AUC were also compared with <10 and <20 per cent favourability from Kottayam AUC. These comparisons showed that none of the districts in Odisha could be classified as suitable even when compared up to 20 per cent less favorability from that of Kottayam, where rubber is successfully cultivated as a rainfed crop.

The above analysis also showed that a single parameter, Aridity Index, (derived as ratio of monthly rainfall and potential evapotranspiration) could be used to describe the climatic suitability of a place for NR cultivation. The maximum favourable percentage AUC out of 288 districts was obtained for Jalpaiguri district of W. Bengal (57%). The analysis showed that after reduction in favorability threshold even up to 42 per cent from that of Kottayam, no district was found to be suitable within the 10 LWE affected states. Suitability of LWE districts was also categorized based on the maximum tolerance limit of rubber in respect of five important parameters for growth and yield of rubber *viz.* annual rainfall (>1500mm), maximum temperature (<36^{0}C), minimum temperature (>10^{0}C), number of rainy

months (>6) in a year and annual aridity index (>0.5). In all these analyses, mostly, districts from the state of W.Bengal were found moderately suitable in the LWE affected region. Favourable crop-water relation parameters could not be identified for most districts in the LWE affected states, which indicated that rubber could only be grown with adequate life-saving irrigation.

7. Climate resource characterisation of rubber growing areas

Interpretation of data on T_{max} and T_{min} profiles obtained from eight locations of traditional and non-traditional areas was made. The yield profile of NE region was analyzed in respect of monthly/seasonal yield differences and meteorological parameters.

Rubber yield during SW monsoon period was low in Agartala (Tripura) compared to Padiyoor (Kerala). It was in the range of 26.0-36.0 g $t^{-1}t^{-1}$ in Agartala during SW monsoon period (June to September) whereas it was 31-76 g $t^{-1}t^{-1}$ in RRS, Padiyoor for the same period. In Agartala, a decline in morning relative humidity (85 to 90%) with warming T_{max} and T_{min} was observed during June to August. A better yield profile during SW monsoon in Padiyoor was supported by morning RH also which remained >94 per cent. A reverse order was noticed in the case of November–January period. Decline in rubber yield during the peak yielding season at Padiyoor may be due to a reduction in turgor pressure which is directly controlled by morning temperature and RH.

The analysis of yield profile in NE region supports the hypothesis that high temperature during SW monsoon can also be a limiting factor; however, it needs to be investigated further. Humidity profile supports the inference of the temperature profiles in these two stations.

8. El Nino/La Nina episodes and regional climate of rubber growing areas

This new project was initiated during the reporting period (2016-17). The association of El Nino indicators with SW and NE monsoon rainfall of Kerala state was analyzed. SW monsoon rainfall is correlated negatively (-0.26*, -0.28* and -0.26*) with the oceanic nino index (ONI) of August, September and October, respectively. In the case of overall India South West monsoon rainfall is correlated negatively (-0.28*, -0.41**, -0.53**, -0.58**, -0.59** and -0.58**) with the ONI of May, June, July, August, September and October.

Change in the temperature time series was associated well with the strong El Nino event of 1998. T_{max} series of RRII showed that a significant change during 1998 (31.6 to 32.1^{0}C), an El Nino year with 100. Time series of all India maximum temperature also showed a warming phase during 1998 with 100 per cent confidence level.

The association of El Nino occurrences with the productivity of plantation crops was analysed. Productivity of all the crops were studied *i.e.* rubber, coconut, pepper, tea and coffee suffered a yield drop either during the strong El Nino year (1997- 98) or in the subsequent year. Among the crops studied impact was significantly evident for coconut irrespective of the intensity of the El Nino event.

9. Weather conditions in the NR growing regions for the year 2016

Global temperatures have continued to rise, making 2016 the hottest year on the historical record and the third consecutive record-breaking year. The peak of the most recent El Niño occurred during the winter of 2015, and temperatures were dramatically higher than

normal. It began to subside over the course of 2016. The overall effect was raised temperatures and reduced annual rainfall in many of the rubber growing locations in the traditional belt. Twice daily data from six agrometeorological stations *viz*. Kottayam, Chethakal, Padiyoor, Nettana, Dapchari and Vaikundam are being maintained in RRII. The principal meteorological station in Kottayam recorded an annual rainfall of 1578 mm over 107 rainy days, being 53 per cent of the normal rainfall of 2980 mm. The state as a whole received 26 per cent less rainfall, being attributed to the El Nino effect. Rainfall during the SW monsoon period was lower than the normal for all traditional rubber growing regional stations. Nettana (Karnataka) received the highest share of 80 per cent of the normal rainfall amongst the rubber research stations. The highest annual maximum temperature (T_{max}) was recorded in Vaikundam (34.6^{0}C) and the lowest minimum in Dapchari (Maharastra). The last week of March recorded T_{max} above 40^{0}C in Dapchari. Mean monthly T_{max} for Padiyoor registered above 36.0^{0}C during the late winter and pre-monsoon seasons.

Among the NE India region West Tripura recorded an annual rainfall of 1814 mm during 2016. Monsoon months June, July, August and September registered a marked decline of 60, 12, 40 and 52 per cent rainfall, respectively compared to the three decadal average. In October a decline of 48 per cent and in November an abrupt rise of 372 per cent rainfall was noticed in W. Tripura. Karimganj district, having the largest share of NR area in Assam, recorded an annual rainfall of 3953mm in 2016. Rainfall during June and July showed a decline of 31 and 24 per cent, respectively. Post monsoon month of November registered a hike of 179 per cent of rain followed by 168 per cent during April. Goalpara district recorded an annual rainfall of 2437mm with a decline of 42, 26 and 44 per cent rain during June, July and August, respectively. West Garo hills in Meghalaya received an annual rainfall of 1854mm. A decline of 54, 41 and 59 per cent of rainfall was noticed in August, September and October, respectively during 2016.

10. Vegetation dynamics in rubber plantation ecosystems

Spreading of rubber plantation in Kerala has altered over the decades the natural vegetation pattern and ecosystem services along the midlands. A new project was initiated in the reporting period with the major objective to study spatial and temporal variations in native vegetation structure and composition in rubber plantation ecosystems and the influence of environmental factors on vegetation dynamics. The study has been initiated in the experimental plots located at CES, Chethackal. Long term regular vegetation analyses will be conducted following standard analytical sampling methods.

BOTANY DIVISION

The year under report saw the initial yield results of farmer participatory clone evaluation trials coming in as well as completion of the recent IRRDB initiative of multilateral clone exchange among IRRDB member countries. A total of 39 promising clones evolved in IRRDB member countries *viz*. Vietnam, China, Thailand, Brazil, Indonesia, Philippines, Cote de Ivoire and Srilanka were introduced to India. Programmes for the development of clones possessing high rubber yield, and dual purpose clones via conventional methods of hybridization, polycross breeding and ortet selection were continued. The fifth phase of the farmer participatory clone evaluation programme with 22 pipeline clones was laid out with the establishment of the central large scale evaluation trial at CES, Chethackal and satellite on farm evaluation trials at six locations in Kerala, Karnataka and Tamil Nadu. Studies on propagation with special emphasis on root stock were undertaken. Studies on response to stimulation in pipeline clones were continued. Further addition to the list of clones in the pipeline raised the total number of promising pipeline clones to 281.

1. Evolving high yielding clones for the traditional area

1.1. Hybridization and clonal selection

Out of 34 hybrid clones under evaluation in the 1995 SST A, 31 clones showed significantly higher girth. Response to stimulation was observed in 11 high yielding clones selected from these 34 clones. When yield under pre-stimulation and stimulation was recorded during May, September and November, all the high yielders exhibited good response to stimulation with an increase in yield ranging from 58.8 to 126.2 per cent (Table Bot. 1), among which only two clones were found better than RRII 105.

Table Bot. 1. **Response to stimulation in selected high yielders**

Sl. No.	Clone	Pre-stimulation on yield ($g\ t^{-1}\ t^{-1}$)	Yield on stimulation ($g\ t^{-1}\ t^{-1}$)	% increase
1	89/7	58.3	113.1	91.7
2	89/27	48.9	86.8	77.2
3	89/64	24.9	50.5	101.7
4	89/79	56.8	88.2	58.8
5	89/95	58.3	96.6	65.5
6	89/124	58.3	103.7	76.5
7	89/145	68.3	147.5	126.2
8	89/243	38.9	75.4	109.9
9	89/308	68.9	127.7	90.6
10	89/309	57.2	90.3	69.9
11	89/349	54.4	99.4	85.4
12	RRII 105	40.3	79.5	105.2

In the SST 1995 B, yield performance of WxA hybrids (1990 hybridization) was monitored during 13th year of tapping. Selections continued to show superior performance in terms of yield compared to parental check clone RRII 105 (Fig. Bot.1). Top selection 90/10 (RRII 105 x RO 142) showed maximum yield of 80 g t^{-1} t^{-1} followed by 90/271 compared to check clone RRII 105 (47 g t^{-1} t^{-1}). Mean yield over 13 years also established superior performance of the above selections.

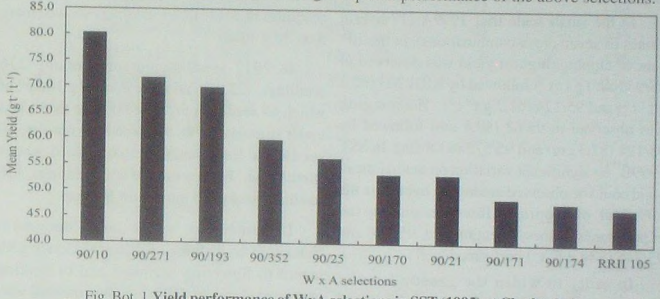

Fig. Bot. 1. **Yield performance of WxA selections in SST (1995) at Chethackal**

In SST 1995 C of 17 W x A hybrid clones, highest girth was recorded in 90/55 (99.6 cm) followed by 90/109 (90.4 cm) and 90/129 (88.3 cm). Highest yield was recorded in 90/109 (54.6 g t^{-1} t^{-1}) followed by 90/55 (54.3 g t^{-1} t^{-1}) and 90/94 (50.4 g t^{-1} t^{-1}).

In the SST 1998 A, (11 high yielding clones selected from 16 clones), all the high yielders showed response to stimulation with an increase in yield ranging from 54.7 to 75.9 per cent with two clones performing better than RRII 105 (Table Bot. 2).

Table Bot. 2. **Response to stimulation in selected high yielders**

Sl. No.	Clone	Pre-stimulation on yield (g t^{-1} t^{-1})	Yield on stimulation (g t^{-1} t^{-1})	% increase
1	93/2	46.5	80.5	75.9
2	93/5	39.4	65.8	64.1
3	93/17	42.0	65.7	54.7
4	93/37	74.5	119.0	63.1
5	93/39	43.5	68.5	56.7
6	93/45	51.9	85.2	64.0
7	93/48	43.7	70.3	58.6
8	93/172	56.3	89.9	61.1
9	93/248	52.4	89.4	70.3
10	93/250	65.1	108.9	67.1
11	93/263	55.6	89.7	61.2
12	RRII 105	39.3	64.2	67.8

In SST 1998 B, (response to stimulation in one high yielding clone selected from 5 clones) 61.4 per cent increase in yield on stimulation was observed while the check clone RRII 105 responded with 62.9 per cent yield increase.

In the small scale trial 1999A (17 hybrid clones of seven cross-combinations), in the 10th year of tapping, highest yield was observed in 95/95 (64.0 g t^{-1} t^{-1}) followed by RRII 203 (62.3 g t^{-1} t^{-1}) and 95/124 (61.2 g t^{-1} t^{-1}). Highest girth was observed in 95/62 (89.4 cm) followed by 95/124 (87.3 cm) and 95/575 (83.8 cm). In SST 1999B, no significant variation on annual mean yield could be observed among the hybrids in the 10th year of tapping. However, among the experimental clones, selections *viz*. 95/306 and 95/346 (>44 g t^{-1} t^{-1}) performed well.

In order to widen the genetic base of breeding populations and to develop improved clones by crossing promising Wickham x Amazonian hybrids (WxA) with the RRII 400 series clones and RRII 105, progenies developed between 2011 and 2014 were planted in seedling nurseries along with half-sibs from female parents. Female parents used were RRII 105, RRII 414, RRII 429, RRII 430 and male parents include three WxA hybrids *viz*. 95/10, 95/34 and 95/274. A total of 353 hybrid seedlings were obtained from 14703 hand pollinations and these are maintained along with 1000 half-sib seedlings in the seedling nursery. Out of 275 test tapped seedlings in the 3rd year of planting (2012 HP), five half sib seedlings yielded more than 100 g tap^{-1} and four hybrids as well as 21 half-sib seedlings yielded more than 50 g $10tap^{-1}$. Seedlings planted in 2013 were also test tapped in which three half-sibs yielded more than 50 g $10\ tap^{-1}$ (two from RRII 414 and one from RRII 105). Out of the 175 HP and 302 half-sib seedlings, 18 and 45 seedlings, respectively showed more than 20 g tap^{-1}. Out of the 200 seedlings in 2014 HP, 14 seedlings showed more than 30 g $10tap^{-1}$.

In 2012 seedling nursery, among 276 seedlings, 122 yielded more than 30 g $10tap^{-1}$ of which 65 seedlings exhibited double their yield under stimulation. In 2013 seedling nursery, 50 seedlings had doubled their yield under stimulation. From a total of 350 seedlings, 126 seedlings had yield more than 30 g tap^{-1}.

In order to study and document characteristic features of different clones with regard to flowering attributes and to develop improved clones by HP, a breeding orchard with 48 elite clones was maintained at RRII. Flowering was observed in 26 clones during normal flowering season within a window period from February to March. Clones with synchrony in flowering were identified which will be useful in the selection of parents for crossing. Observations regarding wintering pattern, off seasonal flowering, flower initiation, maturation of male and female flowers and duration of fruit maturity were recorded for preparing a database for planning future breeding programmes. HP was carried out by selecting suitable parent cross combinations. A total of 8274 hand pollinations were carried out involving 22 cross combinations (Table Bot. 3). 236 hybrid fruits were obtained from the crosses with a mean final fruit set success of 2.88 per cent.

Table Bot. 3. **Details of hand pollinations performed in clones available in the Breeding Orchard**

Sl. No.	Cross combination	Number of pollinations	Hybrid fruits obtained	Success (%)
1	RRII 414 x PB 330	490	8	1.63
2	RRII 414 x RRIM 600	693	13	1.88
3	RRII 414 x IRCA 18	1819	43	2.36
4	RRII 417 x IRCA 18	126	6	4.76
5	RRII 430 x IRCA 18	323	9	2.79
6	RRIC 100 x IRCA 18	1166	72	6.17
7	Gl 1 x IRCA 18	225	3	1.33
8	Tjir 1 x IRCA 18	356	3	0.84
9	IRCA 18 x Tjir 1	465	11	2.37
10	IRCA 18 x Gl 1	524	27	5.15
11	IRCA 109 x RRIC 100	108	10	9.26
12	MO 7 x IRCA 18	112	0	0.00
13	IRCA 18 x RRII 414	923	21	2.28
14	IRCA 18 x RRII 430	21	1	4.76
15	IRCA 109 x RRII 414	36	1	2.78
16	PB 217 x RRII 118	264	1	0.38
17	MO 7 x DR 2	232	2	0.86
18	DR 2 x MO 7	65	4	6.15
19	PB 217 x DR 2	160	1	0.63
20	RRII 430 x PB 255	25	0	0.00
21	PB 260 x RRII 118	130	0	0.00
Total		8274	236	Mean 2.88

1.2. Ortet selection

Ortets selected from south Andamans after screening one of the oldest surviving seedling populations of *Hevea* (source GG1 and GG2 seedlings), were assessed in clonal nursery trial (2012) at Chethackal. Out of 11 ortets, four accessions *viz.*, 104/10, 99/25, 103/7 and 87/3 were found superior in terms of yield on test tapping when compared to RRII 105 and these were comparable to RRII 430.

In the 1995 SST of ortet clones, ethephon application to study the response to stimulation was initiated. Ortets O72 and O73 were consistently superior throughout the trial period and recorded significantly superior yield of 73 to 78 g t^{-1} t^{-1} over RRII 105 (48 g t^{-1} t^{-1}). The

check clone RRII 203 recorded higher yield (73 g t^{-1} t^{-1}) when compared to RRII 105.

2. Evaluation of clones

2.1. Large scale evaluation

In the first two large scale evaluations of RRII 400 series clones at CES, which were in the sixteenth year of tapping, clones RRII 417 and RRII 55 in Trial 1 and RRII 430, RRII 410 and RRII 52 in Trial 2 performed better than RRII 105 with a mean annual yield of >70 g t^{-1} t^{-1} under stimulation. The yield of check clone RRII 105 under stimulation was 69 and 64 g t^{-1} t^{-1} in Trial 1 and Trial 2, respectively.

RRII 414 and RRII 430 exhibited maximum total latex volume as well as dry rubber yield both in summer and peak yielding seasons when tapped in the renewed bark.

Total latex volume was lesser irrespective of the clone during summer compared to peak season. The drop in total latex volume during summer ranged from 39.5 to 64 per cent and 36.6 to 60.9 per cent in Trial 1 and Trial 2, respectively. Clones responded differently to summer stress. The highest drop in summer latex volume was recorded in clone RRII 430. Among the high yielders, the drop in RRII 422 was comparatively lesser. The drop in latex volume generally showed a decreasing trend from high yielders to low yielders and it was more in high yielding clones and less in low yielding clones.

When virgin and renewed panels were compared, the total volume of latex was lesser in renewed panel compared to virgin panel in both the trials barring very few exceptions (Table Bot 4&5). In trial 1, very high yielding clones such as RRII 417, RRII 403, RRII 429, RRII 414 and RRII 105 maintained its high yield in the renewed panel as well. But some of the best performing clones in virgin panel such as RRII 55, RRII 407 and RRII 402 failed to continue its good performance in the renewed panel. Hence, these clones cannot be recommended for commercial cultivation although they were found high yielders in the initial years (virgin panel). In trial 2, RRII 427, the highest latex yielding clone in virgin bark failed to show the same performance in the renewed bark justifying the non-recommendation of that clone for commercial cultivation. Status of clones with regard to latex volume and DRC during early mature (virgin panel) and late mature (renewed panel) phase were comparable in majority of the clones. The present study proved the superiority of RRII 430, RRII 417, RRII 422 and RRII 414 in the long run as well.

Table Bot.4. **Total latex volume & DRC in the 4th year in virgin as well as renewed bark (mean of summer & peak seasons) – LST I**

Clone	Total latex volume (ml)		DRC	
	Virgin panel	Renewed panel	Virgin panel	Renewed panel
RRII 446	93.7b	67.9e	41.3	40.1 ab
RRII 55	206.0a	123.2bc	42.0	40.3 ab
RRII 54	75.0b	81.1de	41.7	37.7b
RRII 417	219.0a	179.4ab	43.3	39.0b
RRII 407	178.3 a	125.8bc	38.3	36.4b
RRII 403	194.7 a	168.3 ab	43.3	44.9a
RRII 449	73.7b	97.1cd	41.3	39.5ab

RRII 429	201.0 a	150.0 ab	39.0	38.1b
RRII 402	207.3 a	112.0 bc	42.7	44.5 a
RRII 453	105.7b	72.3e	39.0	36.7b
RRII 414	219.3 a	209.7 a	43.3	41.2 ab
RRII 105	222.7 a	172.9 ab	43.7	40.7 ab
GM	166.4	129.9	41.7	40.1
CV%	25.7	44.65	12.8	10.3

 # Duncan's Multiple Range Test (DMRT). Averages followed by the same letter do not differ statistically between themselves at 5 per cent level of probability.

Table Bot.5. **Total latex volume & DRC in the 4th year in virgin as well as renewed bark (mean of summer & peak seasons) – LST II**

Clone	Total latex volume (ml)		DRC	
	Virgin panel	Renewed panel	Virgin panel	Renewed panel
RRII 454	130.2d	80.0cd	36.2	34.5d
RRII 430	181.0bc	182.5 ab	40.4	48.4 a
RRII 434	71.0e	66.4d	39.0	36.6cd
RRII 427	249.0 a	87.0cd	38.2	39.4bc
RRII 53	69.4e	61.7d	40.2	40.2bc
RRII 422	210.7 ab	120.2bc	42.3	44.7 ab
PB 330	130.2d	186.7a	40.4	46.7 a
RRII 410	118.5d	134.5 ab	39.4	46.9 a
RRII 52	143.0cd	117.2cd	38.0	36.3cd
RRII 105	217.1 ab	168.1 ab	40.9	43.7 ab
GM	152	120.44	39.5	41.75
CV%	16.57	30.46	8.24	6.68

 # Duncan's Multiple Range Test (DMRT). Averages followed by the same letter do not differ statistically between themselves at 5 per cent level of probability.

In the 1994 LST of 12 indigenous and exotic clones at CES, yield in the 12th year of tapping and girth in the 22nd year after planting were recorded. Among the clones, highest girth was recorded in 86/120 (100.2 cm) followed by O 65 (95.5 cm) and 55/180 (81.6 cm). Highest yield was recorded in RRIM 722 (66.1 g t^{-1} t^{-1}) followed by O 70 (64.9 g t^{-1} t^{-1}), O 65 (63.1 g t^{-1} t^{-1}), RRIM 728 (55.7 g t^{-1} t^{-1}) and 86/44 (54.0 g t^{-1} t^{-1}).

Thirteen hybrid clones were evaluated in LST (1996B) in RRS, Padiyoor. Four clones *viz.* 86/468, 86/597, 86/613 and 86/674 showed comparable yield with RRII 105 in the BO 1 (50-60 g t^{-1} t^{-1}) and BO 2 panels and over eight years of tapping. 86/468 was the highest yielder over eight years of tapping. Clones 86/613 (P 015) and 86/674 (P 040) are now included in farmer participatory trials in various locations.

In LST 1999A, nine clones were evaluated for growth and yield performance. Yield of the clones in the 7th year of tapping and girth of the clones in the 17th year after planting were recorded. Of all the nine clones screened, highest girth was recorded in clone 4 (75.1 cm) followed by clone 12 (70.7 cm) and RRII 105 (63.7 cm). Maximum yield was recorded in clone 4 (50.3 g t^{-1} t^{-1}) followed by RRII 105 (48.1 g t^{-1} t^{-1}) and clone 12 (44.1 g t^{-1} t^{-1}).

In a large scale evaluation of nine promising ortets selected from Cheruvally estate, six ortets showed significantly superior girth compared to the check clone RRII 105 (Table Bot.6).Mean yield in the first four years of tapping ranged from 13.9 to 44.8 g t^{-1} t^{-1} with RRII 105 having 36 g t^{-1} t^{-1} and Ortet Cy 72 being the highest yielder. Mean yield in the first four years of tapping showed that dry rubber yield of five ortet clones are comparable to the check clone RRII 105 (Table Bot. 8).

Table Bot.6. **Girth and yield of ortet clones selected from HML Cheruvally**

Sl. No	Clone	Girth (cm)	Yield (g t^{-1} t^{-1}) (Mean of first four years)
1	Cy 18	60.7 bc	30.5 cd
2	Cy 30	60.5 bc	42.8 ab
3	Cy 31	56.8 cd	13.9 e
4	Cy 35	62.0 b	33.3 bc
5	Cy 41	68.2 a	37.9 ab
6	Cy 43	58.2 bc	39.5 ab
7	Cy 48	70.7 a	26.6 d
8	Cy 68	50.3 e	26.3 d
9	Cy 72	55.8 d	44.8 a
10	RRII 105	54.5 de	36.0 ab
	GM	56.7	33.2
	CV (%)	4.78	15.97

In the LST of Ortets selected from Mundakkayam and Koney Estates, in terms of yield, control clone RRII 105 (53.4 g t^{-1} t^{-1}) was found superior with ortets MO7, KO9 and RRII 430 (44.1, 42.2 and 45.6 g t^{-1} t^{-1}, respectively) exhibiting yield at par with it.

2.2. On-farm evaluation

2.2.1.OFT of RRII 400 series clones in small holdings

Growth and yield performance of RRII 400 series clones was evaluated across various small holdings in the Malabar Region at Ottapalam, Tichur, Wandoor and Mannarakadu, and overall performance across locations was assessed. Based on performance over years, RRII 414 (56 g t^{-1} t^{-1}) at Tichur (comparable with RRII 105; 60 g t^{-1} t^{-1}), RRII 414 at Nilambur, RRII 417 at Ottapalam and RRII 429 at Mannarkadu performed well, indicating local adaptive tendency of the clones. Also, based on data across locations, bark thickness of RRII 400 series clones was comparable with that of RRII 105.

2.2.2.OFT of RRII 400 series clones in large estates

The performance of RRII 400 series clones was assessed in an on-farm trial at Chemoni Estate (Thrissur District). During 2nd year of tapping, RRII 429 showed a yield of 54 g t^{-1} t^{-1} followed by RRII 430 (51 g t^{-1} t^{-1}). Clones *viz.* RRII 414 and RRII 417 performed well with 40 g t^{-1} t^{-1} compared to RRII 105 (31 g t^{-1} t^{-1}).

2.2.3. OFT of pipeline clones in Karnataka

To evaluate the performance of promising pipeline clones at South Karnataka Region, an on-farm trial was laid out with 14 top yielding experimental clones selected from Participatory Clone Evaluation phases 1, 2 and 3 along with seven control clones at KFDC, Karnataka. In the second year of planting, seven pipeline clones showed better growth compared to RRII 105.

One of the pipeline clones (P 102) had a growth that can be equated with RRII 430, the most vigorous check clone.

3. Polycross progeny evaluation

In the observational trial of polyclonal seedlings (2005) at Chethackal, growth and yield performance of polyclonal seedlings and RRII 105 was compared in 4th year of tapping. Progeny of PB 242 followed by those of PB 215 and PB 217 showed higher yield of more than 60 g t^{-1} t^{-1} compared to the mean yield of check clone RRII 105 (28.2 g t^{-1} t^{-1}). The frequency distribution in yield of progenies showed some skewness but indicative of normal distribution (Fig. Bot. 2).

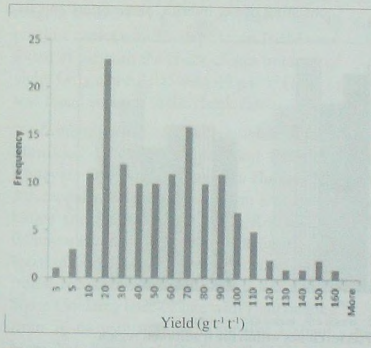

Fig. Bot.2. **Frequency distribution of progenies in terms of yield in the 4th year of tapping**

Under the experiment on estimation of out-crossing rate among parent clones in polyclonal gardens of rubber, progenies were evaluated for growth and yield performance at CES, Chethackal. Seventeen progenies showed more than 50 g t^{-1} 10 t^{-1}. Fifty three progenies showed more than 35 g t^{-1} 10 t^{-1}. Growth of progenies ranged from 7-22 cm.

4. Genetic studies and investigations on genotype x environment interactions

4.1. Genetic parameters

In order to assess genetic parameters of wood traits, wood core samples were collected from full-sibs and parents and heritability and genetic correlation of growth and fibre traits were estimated. Fibre width, fibre wall thickness had very high heritability (70 and 50%) while girth (35%) and specific gravity (33%) had moderate heritability.

4.2. Genotype x environment interaction studies

Growth and yield of pipeline clones was assessed under cold and drought conditions at Agartala and Dapchari, respectively. Under drought conditions (at Dapchari), P 102 (26 cm) and P 26 (22 cm) performed better than RRII 208. Fourteen pipeline clones were found better than RRII 430. In terms of test tap yield, P 20 topped yield along with RRII 430 and (50 g t^{-1}15 t^{-1}) and eleven other clones performed better than RRII 105. At Agartala, under cold condition, pipeline clones P 107, P 102, RRII 429 and P 21 (34 cm) showed maximum girth while 10 clones were found better than RRII 105. In terms of test tap yield, P 21 and RRII 430 (138 g t^{-1} 15 t^{-1}) topped with highest yield. The above observations indicated local adaptive nature of pipeline clones under contrasting environs.

An attempt was made to estimate Genotype x environment interaction for growth, yield and clear bole volume within the traditonal region with respect to RRII 400 series clones. Three locations *viz*. Kanyakumari, CES, Chethackal and Padiyoor were selected. Significant differences between clones, sites and clone x site interactions could be observed. Central Kerala gave superior

yields compared to Kanyakumari and Padiyoor. Kanyakumari showed least variation in growth. Padiyoor showed poor growth and high variability. RRII 414 was superior in terms of timber yield and RRII 430 was superior in terms of latex yield in two out of three locations. RRII 422 was second to RRII 430 in terms of stability. RRII 417 and RRII 422 performed better than RRII 414 across locations. RRII 414, RRII 417 and RRII 429 showed high varaibility for growth and yield. The study also revealed the extent of intraclonal variability for growth and bole volume. CV for growth varied from 9-24 per cent and CV for bole volume was to the tune of 40 per cent. Mean yield over nine years across three locations is given in Figure Bot.3.

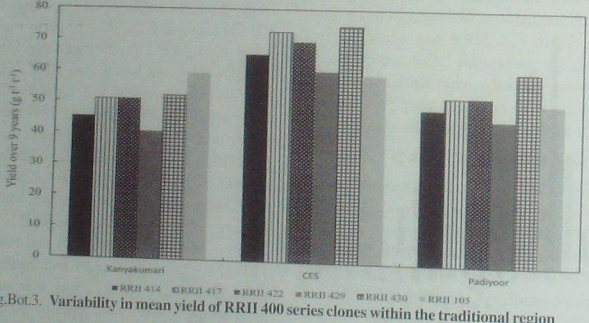

Fig.Bot.3. **Variability in mean yield of RRII 400 series clones within the traditional region**

4.3. Physiological evaluation for drought /cold tolerance in pipeline clones

One batch of 48 pipeline clones were screened for intrinsic traits of drought and cold tolerance. In terms of CO_2 assimilation, the pipeline clones P20, P73 and P57 were superior to the tolerant check clones under drought conditions with least inhibition in CO_2 assimilation. In terms of transpiration and photosynthetic rate, the clones P20 and P73 performed better while P20, P21 and P73 displayed least membrane injury and stayed green under drought stress. In terms of overall performance, P20 and P73 were found superior in drought prone region. Under cold stress, CO_2 assimilation of P078, P083, P01 and P101 was at par with the check clones RRII 429 and RRII 208. Photosynthetic rate was better in P01, P71, P83 and P76. In general, clone P01 was found superior under cold stress in terms of parameters investigated.

5. Participatory evaluation of rubber clones in the pipeline

5.1. Source bush nurseries of pipeline clones

In the current year, 14 newer clones consisting of six hybrids, three ortets and five half sibs from clonal nursery evaluations were added to the pipeline list. Source bushes of these clones were established. The total number of pipeline clones at present is 281, comprising 165 hybrids among which 57 were evolved by polycross breeding and 59 were evolved by ortet selection.

5.2. Phase 1 trials

Under Phase 1, 20 pipeline clones and three checks *viz*. RRII 105, RRII 414 and RRII 430 are under evaluation in 13 field trials at 12 sites since 2008. The two Central Large Scale Trials under Phase 1 of the PCE project were in the first year of tapping. Preliminary results on yield showed significant clonal variation in both trials. The three check clones RRII 105, RRII 414 and RRII 430 with 46-48 g t^{-1} t^{-1} in LST 1 were at par in terms of annual mean yield ranging from 29-40 g t^{-1} t^{-1} in LST 2 in which RRII 414 and RRII 430 were found superior to RRII 105. RRII 414 was found superior in terms of summer yield in both the trials. Eight pipeline clones with yield ranging from 34-49 g t^{-1} t^{-1} in Trial 1 and six pipeline clones with 33-39 g t^{-1} t^{-1} in Trial 2 were found at par with the check clones in terms of yield. Only clone P 015 with 49 g t^{-1} t^{-1} (Trial 1) was found superior to the check clone.

Among the satellite On Farm Trials, those in Manikkal Estate and Kootickal Estate in Kottayam Dt., Mooply Estate in Thrissur Dt., Athirappally Estate in Ernakulam Dt., Be Be Estate and Shaliacary Estate in Kollam Dt., Balanoor Estate in Malappuram Dt. and Calicut Estate in Kozhikode Dt. have come under tapping. Initial results demonstrated RRII 430 as the highest yielding check clone with yield ranging from 50-64 g t^{-1} t^{-1} in Athirappally, Calicut, Manikkal, Kootickal, Be Be and Shaliacary estates while RRII 105 with 52 g t^{-1} t^{-1} was the highest yielding check clone in Mooply estate. Among the pipeline clones, in terms of yield compared to the check clones, P 066 (Kootickal with 55 g t^{-1} t^{-1} and in Shaliacary with 64 g t^{-1} t^{-1}), P 068 (51 g t^{-1} t^{-1} in Mooply), P 067 (51 g t^{-1} t^{-1} in Athirappally and with 50 g t^{-1} t^{-1} in Manikkal), P 088 (63 g t^{-1} t^{-1} in Calicut) and P 026 (52 g t^{-1} t^{-1} in Be Be Estate) were found promising.

5.3. Immature trials under Phases 2 to 5

The Phase 2 trials laid out with 14 pipeline clones and three check clones in 2010 at nine locations and Phase 3 trials laid out in 2012 with 12 pipeline clones and three check clones at seven locations were monitored for growth, tappability and disease incidence periodically. The Phase four trials were laid out with 25 pipeline clones and four checks in 2014 in eight locations. These were monitored for early establishment and disease incidence. In the year 2016, the phase 5 trials were laid out with 22 pipeline clones and three check clones in seven locations in Kerala, Karnataka and Tamil Nadu.

6. Breeding for other specific objectives

6.1. Breeding for drought tolerance

The small scale trial (1998 A) consisting of 15 hybrids (progenies of hybridization in 1993 between high-yielding and drought tolerant clones) was assessed for growth and yield performance in the 11th year of tapping. There was significant variation in annual mean yield among the experimental clones. Top selections *viz*. 93/216 (63 g t^{-1} t^{-1}) and 93/214 (70 g t^{-1} t^{-1}), exhibited better yield than RRII 105 (39 g t^{-1} t^{-1}). In general, hybrids of family RRII 105 x RRIC 52 showed better yield performance in terms of annual mean and summer yield.

In two other 1998 SSTs under evaluation for drought tolerance, annual girth (18th year of planting) and mean yield in the BO 2 panel over first four years is given in Table Bot 8. In the SST 1998B, Clone 93/58 showed significantly superior growth and yield than RRII 105 in the long term. In the SST of ortets selected from drought prone region (Dapchari), Dap 111 and Dap 236 continued to yield more than 90 g t^{-1} t^{-1} in the BO 2 panel (Table Bot 7). Selected clones were subjected to study the response to stimulation.

Table Bot.7. **Girth (18 YAP*) and yield of clones in BO 2 panel (g t^{-1} t^{-1}) in the evaluation of hybrids (Trial 1) and ortets (Trial 2) for drought tolerance**

Trial 1			Trial 2		
Clone	Girth (cm)	BO 2 panel (over 4 yrs)	Clone	Girth (cm)	BO 2 panel (over 4 yrs)
93/75	61.1cd	46.1cde	Dap 95	68.7 abc	60.2de
93/130	71.4cd	44.1de	Dap 236	73.5 ab	95.9a
93/272	83.9ab	61.2bcd	Dap 1	59.7 cd	47.1de
93/82	61.1d	30.0e	Dap 216	71.6 ab	61.3cde
93/110	59.5d	54.0bcd	Dap 42	63.1 bcd	42.9e
93/58	90.9a	80.2a	Dap 136	55.2 d	54.7de
93/92	74.1bc	63.6abc	Dap 173	67.1 bc	83.2abc
93/185	85.2ab	50.1cd	Dap 35	70.8 abc	50.4de
RRII 105	67.7cd	70.4ab	Dap 111	73.6 ab	93.1a
			Dap 37	71.6 ab	85.5ab
			Dap 317	66.4 bc	54.7de
			RRIM 600	70.5 abc	67.9bcd
			RRII 105	79.9 a	82.3abc

*Year after planting

In SST 1999 A, (13 hybrids 17th year after planting in the 10th year of tapping), highest girth was recorded in 94/23 (84.1 cm) followed by 94/44 (83.8 cm) and 94/90 (82.1 cm). Highest yield was recorded in 94/23 (67.2 g t^{-1} t^{-1}) followed by 94/101 (64.4 g t^{-1} t^{-1}) 94/50 (54.1 g t^{-1} t^{-1}) and 94/44 (49.5 g t^{-1} t^{-1}).

In order to develop drought tolerant clones for the non-traditional area, the drought tolerance capacity of the selected progenies from a cross between high yielding parent (RRII 105) and a drought tolerant parent (PB 280) and also its reciprocal (PB 280 x RRII 105) was evaluated in a clonal nursery trial in the drought prone area (RRS, Dapchari 40 trial clones along with nine control clones).Girth of 16 experimental clones were found superior to the top most check clone RRIM 600 of which nine clones (58, 31, 38, 64, 83, 37, 78, 100 and 29) were found high yielders in the seedling nursery evaluation.

6.2. Breeding for disease tolerance

Some of the hybrids with better yield [24 g t^{-1} 10 t^{-1} (RRII 414 x Fx 516); 18 g t^{-1} 10 t^{-1} (RRII 430 x Fx 516), and 11 g t^{-1} 10 t^{-1} (RRII 105 x Fx 516)] were also found free of fungal diseases. The hand pollination programme was continued using RRII 400 series (♀), Fx 516 (♂), resistant Rondonian accession (♂) and other tolerant species (♂) at CES, Chethackal and using RRIM 600 (♀) and RRII 414 (♂) in the breeding orchard. Open pollinated progenies and half-sibs of Fx 516 were assessed for yield and *Corynespora* tolerance. It was possible to recover high-yielding selections with yield up to 40 g t^{-1} 10 t^{-1} indicating better posibility for further selection. Some of the half-sibs were found highly tolerant to *Corynespora* through *in vitro* leaf bio-assay.

7. Anatomical investigations

A study was initiated to assess pipeline clones for their wood traits. Among the first set

of pipeline clones studied, clone 180 had maximum fibre length; clones did not differ in fibre width.

8. Molecular breeding

8.1. Genetic linkage mapping using stress responsive SSR markers

The work was initiated. Preliminary study like PCR using template DNA from five progenies (of *Hevea brasiliensis* and *Hevea benthamiana*) was performed and its amplification was confirmed. Further standardization is in progress.

9. Studies on propagation

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 as against the 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 seeds of drought tolerant clone RRIM 600 and drought susceptible clone RRII 105 were collected from each location. Seeds of (drought tolerant) clone RRII 203 were also collected from Orissa. The plants were maintained under rain fed condition. It was interesting to note that seedlings from drought prone locations exhibited better growth during summer (Table Bot.8) indicating that exposure to drought may contribute to the production of drought tolerant offsprings. During summer, the increase in height of seedlings from traditional areas (Paraliar and Chethackal) was less than 35 per cent as compared to more than 41 per cent growth in seedlings from drought prone areas (Nettana, Dapchari and Dhenkanal).

Table Bot.8. **Source location-wise growth of seedlings**

Location	Seedling height (cm)		Per cent increase
	Pre-summer	Post-summer	
Dapchari	47.5 b	67.1 b	41.1
Dhenkanal	47.3 b	68.4 ab	44.8
Nettana	41.6 c	64.0 c	53.6
Chethackal	55.2 a	69.9 ab	26.7
Paraliar	57.0 a	76.9 a	34.9
CV	9.0	13.5	

Bud-grafting of the drought survived seedlings at Dapchari was done using drought susceptible clone RRII 105 as scion. Budgrafting of the unselected seedlings at Central Nursery Karikkattoor was also done using clone RRII 105. Both the drought survived and control plants were planted in the field at RRS Dapchari for further evaluation of the drought survived stock (as against the unselected stock).

10. International Clone Exchanges

Five RRII clones *viz*. RRII 414, RRII 417, RRII 422, RRII 430 and RRII 429 were provided to ten IRRDB member Institutes under the IRRDB multilateral clone exchange programme. The ten countries with whom successful clone exchange had been effected are: China, Vietnam, Thailand, Cambodia, Indonesia, Cote de Ivoire, Philippines, Myanmar, Sri Lanka and Ghana (CIRAD). Exchange of budwood took place in a period of two years between August 2014 and December 2016. Failure due to damage of budwood on transit was experienced in three cases *viz*. for clone USM 1 from Philippines, clones RRISL 203 and RRISL 219 from Sri Lanka and clones IRCA 41, IRCA 825, IRCA

317 and IRCA 733 from Cote de Ivoire. Efforts are being taken to reimport these clones from their respective countries. Once sufficient source bushes of the clones are established, large scale evaluations will be laid out. Clone exchanges with Malaysia and Nigeria are yet to be initiated.

A total of 39 clones have been imported (Table Bot. 10) under the MCE program of which seven have failed. The 32 clones successfully multiplied have been established in a source bush nursery at the Central Experiment Station of RRII. Nursery screening of the clones for biotic and abiotic stress tolerance has been initiated. Of the seven failures, one clone, USM 1 from Philippines has been reimported and successfully multiplied.

Table Bot.9. **International budwood exchanges among countries**

Country	Date		Clones exchanged
China	September 2014	Clones given	RRII 417, RRII 429, RRII 430, RRII 203, RRII 208
	September 2014	Clones received	Reyan 7-20-59, CATAS 7-33-97, Hongshan 6-7-15, Zhanshi 8-67-3, Baoting 936.
Vietnam	August 2015	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, RRII 203, RRII 208
	December 2014	Clones received	RRIV 1, RRIV 5, RRIV 103, RRIV 106, RRIV 107, RRIV 109, RRIV 114, RRIV 124
Thailand	November 2014	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	November 2015	Clones received	RRIT 226, RRIT 251, RRIT 408, RRIT 3604, RRIT 3904
Cambodia	October 2015	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	September 2015	Clones received	FDR 4575, FDR 5788, CDC 312, PMB 1, FDR 5665
Indonesia	November 2015	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	October 2015	Clones received	IRR 5, IRR 104, IRR 119
Cote de Ivoire	July 2016	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	August 2016	Clones received	IRCA 41, IRCA 317, IRCA 331, IRCA 733, IRCA 825
Philippines	August 2016	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	August 2016	Clones received	USM 1
Myanmar	August 2016	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	September 2016	Clones received	ARCPC 6 (22), ARCPC 2 (4)
Srilanka	August 2016	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	October 2016	Clones received	RRISL 208, RRISL 211, RRISL 203, RRISL219, RRISL2001
Ghana	November 2016	Clones given	RRII 414, RRII 417, RRII 422, RRII 429, RRII 430
	December 2016	Clones received	FDR 5597, FDR 5802, LDC 56, MDX 624, CD 1174

An International *Hevea* Clone Museum was also established in RRII Main Campus with an aim to make a repository of high-yielding and disease resistant clones imported under clone exchange during 2014-2017.

11. Arboreta

An arboretum of popular *Hevea* clones (2014) consisting of 55 clones and arboretum of assorted forest plant species (2014) consisting of 63 species along with *Hevea*, were maintained at RRII Main Campus.

GERMPLASM DIVISION

The genetic resources collection of *Hevea* maintained at RRII includes 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 all the three gene pools, the Division focuses on the wild germplasm collection- its conservation, agronomic evaluation, screening for diseases, drought and cold stress resistance, timber latex traits and utilisation in crop improvement. 277 wild accessions showing potential for various traits so far, are now in various stages of further evaluation. Twelve of these have been utilized for hybridization with Wickham clones to improve productivity as well as broaden the genetic base of the crop.

1. Introduction, conservation and documentation

1.1. Domesticated genepool (Wickham collection) from secondary centers

The 183 Wickham clones belonging to this gene pool 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 budwood 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.

Fifty one introduced clones are being conserved in Germplasm Garden 77. In Germplasm Garden 92 comprising the five introduced IRCA clones, IRCA 130 (59.5 g $t^{-1}t^{-1}$), IRCA 111 (52.9 g $t^{-1}t^{-1}$) and IRCA 230 (49.6 g $t^{-1}t^{-1}$) recorded highest yield, while IRCA 130 (84.3 cm), and IRCA 111 (82.3 cm) recorded highest girth compared to the check clone RRII 105 (72.9 cm). In the Germplasm Garden 94, statistically significant clonal differences were recorded for girth and yield. In the 14th year of tapping, among the 20 clones, RRIM 609, RRIC 100 and RRII 23 were superior to the remaining clones (105.6-79.9 g $t^{-1}t^{-1}$) for yield. (control) RRII 105 had an average yield of 43.1 g $t^{-1}t^{-1}$. The most vigorous clones were RRII 23, PB 255 and RRIC 100 (107.0-102.7 cm), compared to the popular clone RRII 105 (79.6 cm).

1.2. 1981 IRRDB wild germplasm

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

1.2.1 Conservation nurseries

3576 accessions have been reestablished 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. The large number of potentially useful accessions identified during the characterization and preliminary evaluation in the juvenile stage, are being put into Further Evaluation Trials. All accessions showing potential for yield, yield contributing traits like latex vessels, disease and drought tolerance traits, are being established in a separate working collection. The second set of 67 wild accessions has been planted in polybags for the Germplasm Working Collection Source Bush Nursery.

1.2.2 Arboretum

The arboretum established at Teksragre farm, Tura, Meghalaya, is intended to serve primarily as an insurance collection for the entire genepool of *Hevea* currently available in the traditional area, as well as facilitating free cross pollination and genetic mixing between the different genepools. This year, the third set comprising 173 accessions were field planted. These include 164 wild accessions and nine modern clones. So far, a total of 442 wild and Wickham accessions have been established here. The fourth set of 82 wild accessions and 10 Wickham clones was multiplied at the Central Nursery in Kerala and established in the polybag nursery at Tura, Meghalaya. Another arboretum established earlier and comprising of 120 accessions, is being maintained at Central Experiment Station, Chethackal.

1.3. Other *Hevea* species

An arboretum of all the available *Hevea* species was established in 2006 at Central Experiment Station, which 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 withfive 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 accessions AC 3131, AC 552, RO 2136, RO 1313, AC 567, AC 1964, AC 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 W x A hybridization programmes at RRS, Padiyoor.

3. Further evaluation and selection

Detailed evaluation of selections from preliminary evaluations are carried out in clonal nurseries 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 being evaluated in field trials (FETs) at normal spacing.

3.1. Clonal nursery evaluation

The clonal nursery planted in 2010 at Central Experiment Station comprising 15 wild accessions in RBD at 2.5 x 2.5 m spacing, was subjected to two rounds of test tapping. The high yielding accessions AC 2027 and MT 1056 which gave half the test tap yield of check clones RRII 430 and RRII 414 were selected from this clonal nursery for future crop improvement programmes irrespective of the test tap yield, as they represent an entirely different genepool.

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, are subjected to detailed evaluation in FETs in statistically laid out trials at normal spacing. There are currently six FETs comprising 117 accessions.

Out of 22 wild accessions in the FET 2003, RO 2629 (87.3 cm), MT 2233 (76.6 cm), AC 626 (73.1 cm) recorded the highest annual girth as compared to the check clones, while RO 2629 (47.4 g t^{-1} t^{-1}), AC 4149 (46.2 g t^{-1} t^{-1}), AC 716 (35.6 g t^{-1} t^{-1}) recorded the highest yield.

The FET 2005 comprising 22 wild accessions and three controls was opened for regular tapping in S2 d/3 system last year, when six clones showed 70 per cent tappability. Another four clones came into tapping this year. Yield data was not statistically analyzable as only 10 accessions had reached 70 per cent tappability for tapping. Among these, clone averages were highest for AC 2004 with 39.4 g t^{-1} t^{-1}, followed by MT 43 and MT 4788 (24.4-22.1 g t^{-1} t^{-1}) while RRII 105 and PB 260 gave 38.8 and 34.4 g t^{-1} t^{-1} respectively. The overall tappability of the trial was 63.6 per cent. Clonal differences for girth were statistically significant, with AC 2004 recording the highest girth (66.9 cm). This was on par with the best control PB 260 (63.8 cm), and MT 43 (60.1 cm) (Table Germ.1).

Table Germ.1. **Cumulative performance of selected accessions in FET 2005**

Clone		Girth 2015 (cm)	Tappability 2015 (%)	Average DRY 2015 (g t^{-1} t^{-1})	Girth 2016 (cm)	Tappability 2016 (%)	Average DRY 2016 (g t^{-1} t^{-1})
AC	2004	61.7	93.8	31.7	66.9	93.8	39.4
PB	260	57.9	86.7	33.0	63.8	93.3	34.4
MT	2217	52.3	81.8	11.2	59.1	100.0	15.0
MT	43	54.3	78.6	17.7	60.2	92.9	24.4
MT	4788	53.6	76.9	25.2	57.1	92.3	22.1
RRII	105	53.9	75.0	40.9	57.5	100.0	38.8
RO	2255	52.2	66.7		56.7	80.0	11.3
MT	185	52.0	60.0		59.0	80.0	11.4
MT	192	47.2	56.3		52.7	75.0	12.6
RO	2731	50.5	50.0		53.5	83.3	10.8
CD (P= 0.05)		6.03			6.83		

DRY -Dry rubber yield

In FET 2008, RO 2846 (62.2 cm), MT 200 (61.3 cm), AC 176 (59.9 cm), AC 4104 (56.9 cm) recorded the highest girth in the eighth year of growth as compared to the check clones RRII 105 and RRII 208 (52.5 & 37.1 cm). Among the 13 accessions in FET 2010 at CES, Chethackal the highest girth was recorded by RO 1769 (36.8 cm) followed by AC 3146 (36.6 cm) and the check clone RRII 430 recorded the highest girth of 44.6 cm. Another set of 22 selected wild accessions along with three control clones are in the fourth year of growth in FET 2013 at CES. Among the 22 accessions, 54/585 (22.9 cm), RO 2784 (19.7 cm), MT 1598 (19.4 cm), MT 201 (19.0 cm) recorded the highest girth as compared to the check clones RRII 105 and RRIM 600 (16.8 & 16.1 cm). In a further evaluation trial 2014 at RRS, Dapchari, a set of 12 wild accessions and three controls were monitored in the field conditions.

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, established 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 three selected IRCA clones (IRCA 130, IRCA 111, IRCA 109) and one wild accession (AC 166), is in the seventh year of growth. Girth of the clones was recorded in all the five locations. At Mooply estate, among the test clones wild accession AC 166 recorded the highest girth (51.2 cm) and the lowest girth was in the clone IRCA 109 (35.7 cm). Among the check clones RRII 414 recorded the highest girth (60.0 cm) followed by RRII 430 (59.2 cm). At Malankara estate, among the test clones IRCA 130 (41.9 cm) recorded the highest girth followed by wild accession AC 166 (41.5 cm) and the lowest girth was in the clone IRCA 109 (38.2 cm). Among the check clones RRII 414 recorded the highest girth (56.5 cm). At BC Cheruvally estate, highest girth was recorded by IRCA 130, followed by IRCA 111 and AC 166 as compared to the check clone RRII 105. At Calicut estate, the clone IRCA 130 showed better growth performance followed by IRCA 111, IRCA 109 and the least in AC 166. At Bethany estate, Ullimala, IRCA 109 performed slightly better (47.9 cm) than the remaining test clones (43.4-45.8 cm). The average girth of the three controls were ranged from 48.5-50.2 cm. The mean girth of the covariance plots of RRII 105 ranged from 36.5-50.1 cm.

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 were under evaluation for confirmation of field tolerance to *Corynespora* at Ulickal nursery, Iritty. The hotspot trial is in progress.

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

In the clonal nursery of 40 potential half-sibs of nine clones and seven hybrid seedlings at RRS, Dapchari, the highest average girth was recorded by the family of PB 5/51. Among the three check clones, girth was on par for RRII 430 and RRII 414. There was significant difference in clonal response towards drought stress. The clones were subjected to second round of test tapping for reconfirming their yield potential. Out of 40 half-sibs, 26 recorded test tap yield higher than that of drought tolerant check clone RRIM 600 and among the 7 hybrids, three recorded higher test tap yield than RRIM 600. Among the check clones RRII 430 recorded highest test tap yield (10.8 g t^{-1} t^{-1}) compared to RRIM 600 (7.6 g t^{-1} t^{-1}). Out of 40 half-sib clones, six half-sibs recorded test tap yield higher than RRII 430 and among seven hybrid clones, two clones recorded higher test tap yield than RRII 430. The highest average yield was recorded by the family of PB 242. Twenty six half-sibs and three hybrid clones were selected from this clonal nursery for further detailed field evaluation.

In the clonal nursery at RRS, Padiyoor with 31 potential half-sibs of eight clones and two hybrid progenies, the highest average test tap yield after second round of tapping was recorded by the family of PB 242. Among the four check clones, the highest test tap yield was recorded by RRII 414 (8.6 g t^{-1} t^{-1}) followed by RRII 430 (7.2 g t^{-1} t^{-1}). The test tap yield recorded by check clone RRII 105 was 6.2 g t^{-1} t^{-1} and there were eight half-sib clones with test tap yield higher than RRII 105 out of 31 half-sibs and among this three

recorded test tap yield higher than the highest yielded check clone RRII 414. On the basis of test tap yield, eight half-sibs and two hybrid clones were selected for detailed evaluation.

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. After experiencing nine summer periods from 2008 – 2016, nine wild accessions recorded girth higher than the proven drought tolerant clone RRIM 600. Accession MT 4856 recorded highest girth (45.2 cm) at 9th year under unirrigated condition at Dapchari. The modern clones RRII 430 (46.2 cm) and RRII 414 (45.9 cm) showed significantly better growth than the proven drought tolerant clone RRIM 600 (38.8 cm) under Dapchari conditions. Among the five hybrid clones, 93/270 recorded the highest girth (40.9 cm).

4.2.2. Cold tolerance

Sixty four wild *Hevea* accessions are under evaluation for growth and yield under cold stress in two trials at Regional Experiment Station, Nagrakata, West Bengal. Higher girth was observed in RO 2902, MT 923 and MT 5105 as compared to the check clones SCATC 93/114 and RRIM 600 in Trial 1. In Trial 2, accessions RO 2727, MT 915, and RO 3197 recorded the highest girth compared to that of the controls Haiken 1 and RRIM 600. The variability in 22 clones in this trial for various traits observed in the 14th year of growth is presented in Table Germ. 2. MT 915 showed the highest growth in terms of average girth increment during winter, annual girth increment as well as overall growth, over seven years.

Table Germ. 2.**Mean and range of variability for various growth characters in 22 wild *Hevea* accessions under cold stress in Nagrakata**

Characters	Wild accessions		General	Control		CD
	Minimum	Maximum	mean	RRIM 600	Haiken 1	(P=0.05)
Plant height (m)	14.7 (RO 2948)	19.6 (RO 2727)	17.2	16.7	16.7	1.8
Bark thickness (mm)	2.3 (AC 3293)	5.0 (MT 900)	3.9	4.0	4.5	0.9
Annual girth (cm)- 14th year	34.9 (AC 3074)	92.3 (MT 915)	68.5	66.1	82.6	9.1
Annual girth increment (cm yr^{-1})- over 7 years	1.2 (AC 3074)	5.6 (MT 915)	3.6	2.7	4.6	0.9
Winter girth increment (cm yr^{-1})- over 7 years	0.3 (AC 3074)	1.0 (MT 915)	0.7	0.6	0.8	0.2

5. Screening for timber characteristics

5.1. Field screening

In a timber evaluation trial at RRS, Padiyoor, twenty five genotypes were evaluated for annual girth and monthly yield. MT 941, MT 1032 and AC 650 recorded the highest girth, while AC 707 (58.9 g t^{-1} t^{-1}) and RO 685 (54.9 g t^{-1} t^{-1}) recorded the highest yield as compared to the control clone RRII 118 (28.1 g t^{-1} t^{-1}).

6. Utilisation of *Hevea* germplasm

6.1. Hand pollination programmes

At Central Experiment Station, Chethackal, the six promising hybrids from the 2009 hand pollination programme involving three wild accessions and six cultivated Wickham clones, are being multiplied for further evaluation.

At Regional Research Station, Padiyoor, 29 seedling progenies derived from two cross combinations in 2009, along with 25 OP seedlings of RRII 105, are under evaluation in a seedling nursery. Seven hybrid progenies and five OP seedlings were found to be promising for test tap yield. They were multiplied at Ulickal nursery and a budwood nursery of these high yielding hybrids was raised at RRII in order to generate sufficient bud wood for a detailed field evaluation.

At RRII, growth and test tap yield (2nd round) were monitored in the hybrid progeny of the 2013 W x A HP involving two cross combinations (Table Germ.3). Of the 29 seedlings test tapped, eight showed more than 2.5 g t^{-1} t^{-1} in the third year of growth, including four which showed 3.7 - 8.3 g t^{-1} t^{-1}.

Table Germ. 3. **Performance of HP2013 W x A progeny in the 3rd year of growth**

	RRII 105 x MT 4788		RRII 105 x RO 4599	
	Girth (cm)	TTY 2 (g t^{-1} t^{-1})	Girth (cm)	TTY 2 (g t^{-1} t^{-1})
Number	20	16	14.0	13.0
Average	15.4	2.2	15.4	2.0
Min	5.0	0.3	9.0	0.3
Max	31.0	8.3	25.0	7.0
SD	5.9	2.0	4.1	2.0
CV(%)	38.3	90.9	26.6	96.6

In HP 2014, growth and yield were monitored in the 26 surviving progenies of three W x A combinations (Table Germ.4). Twenty two were test tapped. Two seedlings had more than 2 g t^{-1} t^{-1} and seven showed more than 1 g t^{-1} t^{-1}. The highest yield was 2.9 g t^{-1} t^{-1}. Ten progenies showed good growth of more than 14 cm. The cross AC 4833 x RRII 105 had the highest percentage of high yielders.

Table Germ. 4. **Performance of HP2014 W x A progeny in the 2nd year of growth**

	AC 4833 x RRII 105		RRII 105 x MT 182		RRII 105 x MT 4788	
	Girth (cm)	TTY 1(g t^{-1} t^{-1})	Girth (cm)	TTY 1(g t^{-1} t^{-1})	Girth (cm)	TTY 1(g t^{-1} t^{-1})
Number	8	8	3	3	15	12
Average	16.1	1.57	15.3	0.29	12.0	0.43
Min	12	0.36	12	0.00	8	0.23
Max	19	2.87	19	0.43	15	1.07
SD	2.5	0.87	2.9	0.20	2.0	0.23

The performance of the 149 surviving interspecific progenies of HP 2013 and 2014 are reported in the next section.

In the 2016 HP, 100 hybrids of three W x A combinations, including one *Oidium* tolerant accession, were planted in the seedling nursery.

6.2. Phenotyping of mapping population for QTL identification

The total number of seedlings planted in the nursery from all the HP programs (HP 2009, 2010, 2013 and 2014) was 306, of which 255 are now surviving. The HP 2009 and 20 populations were multiplied for raising a budwood nursery for a field trial, as the nursery phenotyping was complete. Growth and juvenile yield of the HP 2013 and 2014 interspecific progenies was monitored (Table Germ.5). In HP 2013, of the 62 seedlings test tapped in the third year of growth (second round of test tapping), five gave more than 2.5 g t^{-1} t^{-1}, and 20 gave between 1.0 and 2.4 g $t^{-1}t^{-1}$. In the 72 tappable seedlings of the HP 2014 population, in the first round of test tapping, 10 had yield ranging from 1.0-1.8 g $t^{-1}t^{-1}$, in the second year of growth.

Table Germ.5. **Juvenile performance of interspecific hybrids**

	HP 2013		HP 2014	
	Girth (cm)	TTY2 (g t^{-1} t^{-1})	Girth (cm)	TTY1 (g t^{-1} t^{-1})
Number	70	62	79	72
Average	15.7	1.0	14.9	0.6
Minimum	7.5	0.0	7.0	0.0
Maximum	31.0	3.9	20.0	1.8
SD	4.8	0.8	3.1	0.3

7. Other studies

7.1. Studies on alternative sources of natural rubber yielding plants

Seven germplasm accessions from Palakkad Region and four plants multiplied through stem cutting collected from Vaikom region are being conserved at RRII.

BIOTECHNOLOGY DIVISION

The research activities of Biotechnology Division are mainly focused on developing genetically modified *Hevea* plants integrated with different genes with desirable traits. Experiments on genetic transformation were fine tuned for producing transgenic plants with enhanced biotic and abiotic stress tolerance, improved latex yield and antibiotic marker-free transgenic plants. Other research programmes carried out in the Division are: development of efficient protocols for the propagation of elite *Hevea* clones, *in vitro* regeneration of haploid plants and *in vitro* approaches to complement conventional breeding programmes.

1. Development of transgenic plants

1.1. Genetic transformation of *Heveabrasiliensis* for stress tolerance

Agrobacterium mediated genetic transformation was carried out with *HbMnSOD* gene construct using embryogenic callus of *Hevea* (clone RRII 105). Two antibiotic resistant callus lines emerged were selected after performing GUS assay and the cell lines were proliferated. The stress tolerance of the transgenic callus was assessed by incorporating varying concentrations of PEG, NaCl and phytagel in the culture medium. Results revealed that the *MnSOD*

transgenic callus lines were capable of tolerating saline stress (NaCl) up to 150 mM, whereas the non-transgenic callus was unable to grow in medium containing NaCl. Similarly transgenic callus grew well in the medium supplemented with high concentrations of phytagel (0.6%) and PEG (2.5%). The transformed calli were cultured for somatic embryogenesis and pro-embryogenic masses could be produced.

MnSOD gene integration in *Hevea* callus was also attempted with leaf proliferated fresh callus and embryogenic callus generated in the reporting year. Transgenic callus lines emerged from the proliferated fresh callus were selected and cultured individually for somatic embryogenesis. Embryogenic callus was obtained from two cell lines and were maintained for plant regeneration. In another experiment *MnSOD* gene insertion was executed with somatic embryos as the target tissue. *Agrobacterium* infection of somatic embryos by vacuum infiltration for 20 min. at 30 psi vacuum, transient GUS expression was noted after 48 hrs. of infection. GUS expression was also observed in the embryos after three weeks of culture in the selection medium. These embryos were further cultured for plant regeneration.

In another experiment, *MnSOD* gene with FMV34S promoter was integrated in *Hevea* callus. Embryogenic callus of RRII 105 clone as well as zygotic embryo derived embryogenic calli were used as the target tissue. *Agrobacterium* infected calli were periodically cultured over selection medium fortified with kanamycin (300 mg L^{-1}) and cefotaximet 500 mg L^{-1}). After 40-50 days of culture in the selection medium, several antibiotic resistant cell lines emerged from both target tissues. These putatively transgenic callus lines were selected and cultured individually over proliferation medium. Extensive media modifications were made for proliferation of the callus and induction of somatic embryos.

1.2. Confined field trial of MnSOD transgenic plants at RRS, Guwahati

Steps were taken for initiating a confined field trial at the RRS, Guwahati. Two MnSOD transgenic plants (RRII 105) developed earlier and control (RRII 105) somatic plants along with two check clones (RRII 208 and RRIM 600) were multiplied by bud grafting and the budded stumps were transported to RRS Guwahati for field planting and celarence from GoI is awaited.

1.3. Genetic transformation of *Hevea brasiliensis* for yield enhancement

1.3.1. Genetic transformation with *hmgr1* gene construct

Agrobacterium mediated genetic transformation with *hmgr*1 gene construct was carried out with embryogenic callus derived from *Hevea* integument tissue. Numerous antibiotic resistant cell lines emerged were selected, proliferated and cultured for somatic embryogenesis. Embryogenic competency was observed in four callus lines. Gene integration was ascertained in the four callus lines by performing PCR with promoter gene specific primers using the DNA isolated from these lines. Positive amplification was observed in all the cell lines tested and the PCR positive embryogenic calli were cultured over embryo induction medium and embryo induction was achieved from all the lines. Embryos were further cultured for maturation and plant regeneration. The regenerated plantlets were subjected to hardening. Five more transgenic cell lines became embryogenic which were transferred to the embryo induction medium. Embryogenesis was observed in two transgenic cell lines.

Expression studies were continued in the *hmgr*1 gene integrated transgenic plants developed earlier from the zygotic tissue.

cDNA was synthesized from the RNA isolated from the latex samples and RT-PCR was done using gene specific primers. It was noted that the enzyme expression was higher in the transformed plantlets compared to control.

1.4. Genetic transformation with osmotin gene

Agrobacterium infections were carried out with osmotin gene construct for generating new transgenic events. Antibiotic resistant callus lines were developed from both clonal and zygotic explants and the cell lines were cultured for proliferation. In spite of all the media manipulations made, the cell lines failed to proliferate. Repeated *Agrobacterium* infections were attempted with fresh antibiotics using clonal embryogenic callus. Putatively transgenic cell lines emerged were selected and transferred to proliferation medium. The transgenic embryogenic callus (RRII 105), developed from earlier transformation experiments were cultured for somatic embryo induction and the embryos produced are under different stages of development. The osmotin gene integrated transgenic plantlets developed earlier from clonal target tissue were transferred for hardening. Already developed transgenic plants were multiplied through bud grafting for ex vitro evaluation for stress tolerance.

Attempts were also made for developing transgenics pyramided with two genes (MnSOD and osmotin) through repeated transformations. Transgenic calli integrated with osmotin gene was infected with MnSOD gene construct and transferred to selection medium. Results are awaited.

1.5. Genetic transformation of *Hevea* with *ipt* gene

Genetic transformation experiment was carried out with *ipt* gene construct. *Agrobacterium* infection was attempted using proliferated fresh leaf callus and embryogenic callus generated in the reporting year.

1.6. Development of antibiotic marker-free transgenic *Hevea* plants

1.6.1. Synthesis of a binary vector (PNS 15) harbouring MnSOD gene

The first step in the synthesis of the binary vector was cloning of *H.brasiliensis MnSOD* gene. PCR was performed with the DNA isolated from *Hevea* MnSOD transgenic callus using gene specific primers. The *MnSOD* gene fragment was eluted and again PCR amplified with MnSOD gene specific primer containing specific restriction site. The PCR product was eluted, purified and inserted at the multiple cloning site of PNS14 binary vector (synthesized earlier). Restriction enzyme digestion of PNS14 empty cassette and MnSOD PCR product was carried out and the required fragments were eluted, purified and the fragments were subjected to ligation and the ligated product was transformed in *E.Coli* cells following the standard protocol. Molecular confirmation of *MnSOD* gene insertion was ascertained by PCR, restriction enzyme digestion and DNA sequencing. Transformation vector (PNS15) was produced by transforming the *Agrobacterium* strain EHA 105 with the MnSOD plasmid DNA as well as the PCR amplified product following freeze thaw method. The transformed colonies were selected and presence of MnSOD gene in the binary vector was ascertained by restriction enzyme digestion of the plasmid DNA, PCR amplification with *MnSOD* gene specific primer pairs and finally by DNA sequencing. For long term preservation, glycerol stock was prepared and stored at -80°C.

1.6.2. Functional validation of the MnSOD binary vector (PNS15) in tobacco plants

Agrobacterium mediated genetic transformation was attempted with tobacco leaf disc and

callus following vacuum infiltration technique. The infected calli/leaf disc after three days co-culture were transferred to MS basal medium fortified with 2 mg L^{-1} NAA, 0.2 mg L^{-1} BA and antibiotic- 500 mg L^{-1} cefotaxime and 200 mg L^{-1} kanamycin for the stringent selection of transformed callus. The same basal medium containing 0.2 mg L^{-1} NAA and 2.0 mg L^{-1} BA, 500 mg L^{-1} cefotaxime and 100 mg L^{-1} kanamycin was used as the selection medium for leaf disc transformation. The infected target tissues were cultured over fresh selection medium at four weeks interval. Emergence of transgenic callus/shoots and plant regeneration is awaited.

1.6.3. Development of antibiotic marker-free transgenic Hevea brasiliensis plants

Agrobacterium infections were carried out with PNS14 and PNS15 binary vectors using embryogenic callus derived from RRII 105 and zygotic embryo derived from embryogenic callus. The infected calli were periodically cultured over selection containing 300 mg L^{-1} kanamycin and 500 mg L^{-1} cefotaxim. The antibiotic resistant callus lines emerged after 40-50 days of culture were selected and cultured individually over proliferation medium fortified with kanamycin. Proliferation and embryogenesis of the putatively transgenic callus lines is awaited.

1.7. Testing of Hevein promoter efficiency

Hevein promoter: Gus fusion constructs with three different promoter lengths (1817, 850, 303 bp) were used to test their efficacy. *Agrobacterium* infection was attempted employing vacuum infiltration using leaf callus. Transient GUS expression as well as GUS expression in transgenic lines was noted. Promoter expression will be studied in leaves and latex of transgenic plants for promoter specificity and minimal length required for expression.

2. Propagation of elite clones of *Hevea*

2.1. Somatic embryogenesis of *Hevea brasiliensis* from immature anther

2.1.1. In vitro plant regeneration from RRII 414 and 430 clones

An experiment was designed to study the effect of chilling of explants on callus induction. Floral buds were collected from clone RRII 414, surface sterilized, immature anthers were dissected and inoculated on standard callus induction medium as control. In the first treatment, floral buds were subjected to cold treatment at 4^{0}C for different time intervals *viz*. 0, 24, 48 and 72 hours. After cold treatment the explants were surface sterilized, anthers were dissected and cultured over standard callus induction medium. In the second experiment, sterile anthers were inoculated on standard medium and the cultures were kept at 4^{0}C for 0, 24, 48 and 72 hours. After 40-50 days of culture, callus was induced in control medium with a callus induction efficiency of 80 per cent. Cold treatment of the explants couldn't improve callus induction. It was also noticed that the callus induction frequency was reduced to 10 per cent by prolonged cold treatment and the callus growth was very poor (rating 1).

To study the effect of BA on callus induction, kinetin was replaced with BA and four concentrations of BA (0.5, 1.0, 2.0 and 3.0 mg L^{-1}) were included in the callus induction medium. Inclusion of BA showed +ve response on callus induction and 100 per cent efficiency was obtained in callus induction with the four combinations tried. However, at 0.5 mg L^{-1}, callus growth was low (5 rating). When BA level was increased to 2-3 mg L^{-1}, callus growth was also increased and the rating was increased to eight. The embryogenic calli induced were sub-cultured

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on embryo induction medium for embryo differentiation and maintained at dark for four months. It was observed that callus with nodular texture differentiated into embryos and eventually get matured. Mature embryos upon transfer to germination medium, embryos turned green and cotyledons grew and root and shoot apical axes developed. Even though embryo induction frequency was low (30%), most of the embryos developed into full plants. Plantlets with well-developed root and shoot system was transplanted in porting mixture sand:soil:cow dung for hardening.

Work was also initiated to induce somatic embryogenesis from RRII 430. Immature anthers were cultured on standard callus induction medium. After 40-50 days of culture, calli were induced and further cultured on proliferation medium. The proliferated compact callus upon subculture on callus induction medium containing half strength of hormones, friable callus was developed.

2.1.2. Synthetic seed production and plant regeneration

In *Hevea brasiliensis* it is well documented that the hardening success of *in vitro* plants is very low. Therefore preliminary attempts were made to synthesize synthetic seeds for improving the hardening technique. Polyembryony derived zygotic embryos was used as explants and the embryos were dipped first in a homogenous sodium alginate (3%) solution and then in 100 mM anhydrous calcium chloride solution, with constant stirring. Encapsulated embryos obtained were cultured on germination medium and a few embryos germinated were developed into full plantlets. Further optimization of the conditions for producing viable alginate beads (suitable size, shape and texture) is in progress.

2.2. Somatic plant regeneration and acclimatization from RRII 105 clone

In the reporting year, attempts were made for *in vitro* plant regeneration *via* somatic embryogenesis from RRII 105 clone as control plants. Media amendments were made in the various stages of somatic embryogenesis pathway (embryo induction, maturation and plant regeneration) for regenerating healthy plants which could be hardened. The medium optimized for embryo induction was modified ½MS basal medium supplemented with 6.0 per cent sucrose, 2.0 per cent PEG and 1 mg L^{-1} ABA. Experiments were conducted to assess the effect of varying concentrations of growth hormones NAA, BA, Kin, GA_3, IBA and IAA (0.2-2.0 mg L^{-1}) as well as different combinations on embryo maturation and plant regeneration. It was noticed that higher levels of BA and Kin with lower concentrations of NAA and IAA promoted the development of healthy and normal mature embryos. Embryo germination and plant regeneration could be obtained in the plant regeneration medium containing 5.0 per cent sucrose, higher concentrations of BA, Kin, GA_3 and lower levels of IBA and IAA. Plants with long shoot, leaves and good root system could be regenerated.

2.2.1. Hardening of somatic plants

Fully developed plantlets with two whorl mature leaves were planted in pots for hardening. Different hardening treatments were incorporated at various stages of hardening. It was observed that selection of plants is an important criterion for hardening. Healthy plants in the actively growing stage need to be transferred immediately so as to get continued growth. The nutrient absorption of tissue culture plants is found to be impaired after two three weeks of hardening. Foliar spray of nutrient solution is found to be effective for improving the hardening process to some extent.

Experiments in this line are continuing. Around 50 somatic plants were transferred for hardening. Out of which eight plants were hardened in the small polybags and transferred to big poly bags. Around 10 plants are under different stages of hardening.

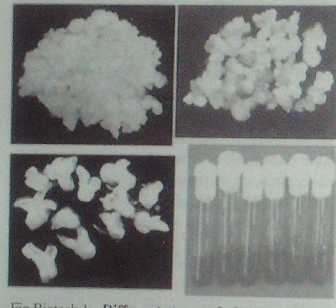


Fig.Biotech.1. **Different stages of plant regeneration via somatic embryogenesis**

Fig.Biotech.2. **Hardening of somatic embryo derived plants**

2.2.2. Somatic embryogenesis from leaf explants

Callus was induced from clones RRII 105, RRII 414 and RRII 430 from leaf cultures initiated during the year. The calli were proliferated and somatic embryogenesis attempted. Embryogenic callus obtained from clones RRII 105 and RRII 414 were proliferated and embryo induction obtained from two embryogenic lines of clone RRII 105 in the earlier standardized medium. Proliferated embryogenic callus of clone RRII 414 also induced embryos with a lower frequency in this medium. Since embryogenic competence of callus varied each year with the explant and clone, slight modifications had to be made in the standardized protocol. Medium modifications were attempted by providing water stress with addition of PEG and higher concentrations of phytagel along with change in concentration and combination of phytohormones. Enhanced embryo induction was observed in medium with addition of PEG and altered phytohormones. Mature embryos of clone RRII 105 obtained from two different embryogenic lines were attempted for maturation and plant regeneration. Embryo maturation and germination could be obtained in the earlier standardized medium.The embryos cultured in germination medium containing IAA and GA_3 after desiccation treatment, showed more vigour. Maturation medium containing IAA (0.4 mgL^{-1}) and GA_3 (0.5 mgL^{-1}) enhanced embryo germination. It was observed that higher concentration of GA_3 was essential for improving germination, though IAA at elevated levels had not much influence. Germinated embryos were tried for plant regeneration in both WPM and MS medium supplemented with combination of phytohormones BA, KIN, GA, TDZ and IBA at different concentrations. Rate of plant regeneration from the two embryogenic lines was different. Successful plant regeneration was obtained in MS medium containing slightly higher concentration of either BA/KIN along with GA and IBA and TDZ at minimal concentrations. After plant regeneration, these were transferred to medium containing ½ MS medium without phytohormones for further growth before attempting for hardening. Continued growth of the regenerated plants was obtained in this medium.

Cultures were initiated with zygotic embryos, axillary nodes and shoot tips of clones and seedlings from bud grafted plants maintained in the glass house. Clonal and seedling shoot tips were given hormone pulse treatment for different time intervals (10, 15 and 20 minutes) where a higher concentration of phytohormones was used. Vacuum infiltration of hormones for different time intervals (5, 10 and 15 min.) was also experimented. After hormone pulse, the shoots were cultured *in vitro* in rooting medium and root induction was obtained. *In vitro* culture response of antigibberellin was observed in cultured seedlings with change in root architecture. Dwarfing and root thickening along with root proliferation which are typical responses of PBZ were observed in *in vitro* developed seedlings and *in vitro* rooted shoots.

3. Development of *Hevea* homozygous haploid plants

3.1. Embryo sac culture for haploid development

Embryo sacs were isolated from mature female flowers of clones RRII 105, 414, 422, 429 and 430 and cultured for callus/embryo induction over different media combinations where the basal media MS, K&M and WPM were fortified with different concentrations and combinations of the growth regulators NAA, 2, 4-D, BA, Kinetin, GA, IBA and IAA. It was observed that in a few combinations callus induction occurred within three months. These calli were proliferated by transferring to another medium with reduced levels of growth regulators. Proliferated calli have been subcultured to embryo induction media for further development.

3.2. Development of haploid plants from mature anthers

Mature anthers of RRII 414 and 422 were inoculated in the culture medium with simultaneous application of stress and temperature shock. Anthers dried up and callusing was observed from the pollen grains on the surface of the anthers of clone RRII 414. The callus was separated and kept for proliferation. The proliferated calli of RRII 430 obtained earlier turned friable in the media supplemented with 2,4-D, NAA and BA.

3.3. Gynogenic haploids in *Hevea brasiliensis*

Unfertilized ovules of FX 516, RRII 414 and 422 were pre cultured and enlarged ovules were dissected and the embryo sac was isolated. The embryo sacs were cultured for callus induction. Callus initiation occurred from the embryo sac of clone RRII 414 and 422. The calli obtained from the embryo sac cells isolated from the pre cultured unfertilized ovules (RRII 105) became embryogenic and proembryogenic masses were also observed. The somatic embryos obtained from the embryo sac calli (RRII 105) developed earlier were cultured for maturation and subsequent plant regeneration. Five plantlets obtained were transferred for hardening.

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

4.1. Induction of polyembryony

For developing new embryogenic cell line from zygotic embryo, immature fruits were inoculated in the already reported medium during the current season. Three new poly embryonic ovules were identified and plants are being regenerated and hardened. Multiple seedlings developed through polyembryony and their bud grafted counter parts were planted in the field for comparison.

Methylation profiling and sequencing among the uniform seedlings and their budgrafted counter parts revealed that methylation changes are happening among the budgrafted plants in the promoter and coding region of important genes

involved in signal transduction. Experiments are progressing in this direction.

Attempts were initiated for the rescue of hybrid embryo after hand pollination, which are otherwise lost due to fungal diseases /endosperm abortion, using the already developed protocol.

5. Development of polyploids in *Hevea brasiliensis*

Anther derived calli of clone RRII 105 were made embryogenic by transferring to embryo induction medium. These embryogenic calli were exposed to colchicine by culturing over media incorporated with colchicne. The calli were kept for different time intervals after which they were transferred to the same medium without colchicine. The newly emerged callus from these treated samples were selected and cultured over callus proliferation medium.

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

Clones susceptible and resistant to *Corynespora cassiicola* were identified and four popular clones were selected and included in each group for further study. Susceptible clones were RRII 105, RRII 203, PB 217 and PB 260 and tolerant clones were RRII 414, RRII 430, GT1 and FX 516.

6.1. Laboratory screening of clones

6.1.1. In vitro pathogenicity test

Callus cultures were raised from susceptible and tolerant clones, using intact anthers. Callus induction was obtained after four to five weeks of inoculation and transferred to the proliferation medium. The proliferatedcalli produced from these clones were exposed to different concentrations of the toxin *cassiicolin*. For this, different volumes (0.2- 2.0 ml) of filter sterilized crude toxin were incorporated in the callus proliferation medium. Proliferated callus was gently macerated for uniform exposure of the cells to toxin and cultured in the toxin enriched medium. Calli inoculated on toxin free medium served as control. All the cultures were maintained at 25±2°C. It was observed that in the presence of 0.8 and 1.0 ml toxin, calli from tolerant clones remained intact, whereas those cultures from the susceptible clones turned black and started drying up.

6.1.2. Detatched leaf technique

Pale green and late copper brown stages of leaves from both susceptible and tolerant clones were collected. Leaf pieces of each clone were vacuum infiltrated with the crude toxin for 15 minutes and later transferred to moistened petriplates for development of necrosis. Leaf pieces vacuum infiltrated with water for 15 minutes served as control. Both control and toxin treated leaves were scanned at 12, 24 and 36 hrs interval for visualization of necrosis. Browning was observed in the leaf pieces of susceptible cultivars whereas those of tolerant cultivars remained green, indicating necrosis in the susceptible cultivars. For better visualization of necrotic areas the eye dropper tool was used so that colour difference between necrotic and non-necrotic areas could be identified.

GENOME ANALYSIS LABORATORY

Our genomic research is mainly focused on four major areas of research complementing classical breeding program for crop improvement in rubber, *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.

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

1.1. SSR marker development from NBS-LRR transcripts (R gene) conferring disease tolerance in rubber

Validation of *in silico* polymorphic SSR loci identified in NBS-LRR transcripts (R genes) from disease transcriptome was carried out. Thirty-four primer-pairs generated for SSR loci were assessed for polymorphisms using three cultivated clones RRII 105, GT1, RRIM 600 and *H. benthamiana* and 18 of them could be used as genic SSR markers.

1.2. Effect of selective breeding in cultivated Wickham clones for latex yield interms of allelic distribution and allele loss over the past 100 years in India

Earlier 56 per cent allele loss was observed in cultivated Wickham clones compared to wild accessions using SSR markers. While comparing with wild sub population from three different provinces, only 28 per cent allele loss was detected between cultivated population and Mato Grosso accessions revealing closeness of cultivated clones with Mato Grosso accessions.

To understand allelic distribution over the past 100 years following selective breeding in rubber, available three generation pedigree of RRII 400 series clones (from parental clones of RRII 105 and RRIC 100 to RRII 400 series clones) were analyzed with SSR markers. Result showed that frequencies of some alleles were reduced considerably (50 - 10%) along with complete loss of alleles, and in some cases the allele frequency was increased (50 - 90%).

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

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

A comparison 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 initiator molecule synthesizing genes like *FDPS* and *GGDPS* were moderately conserved. Interestingly FDPS and HMGR didn't contain non-synonymous SNPs. FDPS also had the highest number of SNPs from non-coding regions.

1.3.2. Phylogenetic analysis of downstream rubber biosynthesis genes

Cis-prenyltransferase (*CPT*) gene: Conceptual translation (aminoacid sequence) of *CPT* gene from *Hevea brasiliensis*, orthologs of *CPT* from other plants species and representative organisms from major domains of life were subjected to phylogenetic analysis to understand their evolutionary relationship and diversity. Simultaneously, DNA based phylogenetic analysis of all the *Hevea CPTs* were also performed to classify them based on their sequence structure similarity. Phylogenetic analysis 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. *Hevea* CPT sequences obtained from the present study were placed away from the previously reported CPTs indicating the presence of a new isoform. Inconsistency in nomenclature of CPTs was identified based on sequence homology studies.

Rubber elongation factor (*REF*) gene: Phylogenetic tree was constructed using the amino acid sequences of *REF* and/ or *SRPP* from related plant species to estimate the uniqueness of *REF* in *Hevea*. The analysis 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, probably evolved for rubber biosynthesis.

1.3.3. SNP marker development

SNP markers from the gene *CPT* (HbCPT 760A/G & HbCPT1438C/T) and *GGDPS* (HbGGP741C/T & HbGGP1173A/G) were tested using HRM genotyping methodology for polymorphisms between the parents (RRII 105 and *H. benthamiana*) of an interspecific mapping population. Only the SNP marker: HbCPT1438C/T was found to be polymorphic and which could be used in segregation analysis of the progeny population for its integration in the linkage map. Markers were also generated from *MVK* (HbMVK2628A/G, HbMVK3297C/T), *PMVK* (HbPMVK1786C/T) and *HMGS* (HMGS3059A/G) genes based on polymorphisms.

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

Construction of a saturated genetic linkage map for *Hevea* spp. with SNP and DArT markers using an interspecific mapping population derived from a cross between RRII 105 *(H. brasiliensis)* x F4542 (*H. benthamiana)* was reported last year, which assembled into 18 linkage groups, thus reflecting the basic chromosome number of *Hevea*. However, this linkage map was generated using proprietary software which is not available in the public domain. Therefore, we made an effort to refine the linkage map with a total number of 7692 filtered SNP markers without any missing genotype using Lep-MAP2 (available in public domain). 18 linkage groups with LOD score 3 were obtained with these filtered data set.

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

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

2.1.1. Genes involved in host tolerance to Phytophthora leaf fall disease of rubber

De novo assembly of leaf transcriptome, derived from control samples of FX 516 (tolerant) and RRIM 600 (susceptible) generated 48912 and 55189 transcripts respectively. Identification of differentially expressed transcripts and their annotation are in progress.

2.1.2. QTL markers for Phytophthora tolerance

QTL mapping was carried out using a saturated genetic linkage map (18 linkage groups), constructed with DArT markers using an interspecific mapping population derived from the cross between *H. brasiliensis* clone RRII 105 (high yielding cultivated clone with moderate level of resistance to *Phytophthora* spp.) and *H. benthamiana* F4542 (high level of resistance to *Phytophthora* spp.). Variation in the levels of resistance among the hybrid progenies with extreme phenotypes indicated the involvement of multiple genes conferring tolerance against the pathogen. Six QTL markers for resistance to *Phytophthora* were identified. The results obtained from the study are important for understanding genetic determinism of disease resistance and for marker assisted selection.

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

2.2.1. NAC domain containing sequence analysis in leaf transcriptome

The NAC proteins are plant-specific transcriptional regulators. They are involved in various biological processes, including both biotic and abiotic stress responses. We reported successful cloning of NAC cDNA from both susceptible (RRII 105) and tolerant (RRIM 600) rubber clones to abiotic stresses. Cloning of the promoter sequence (~2 kb) from both these clones was also carried out for sequence comparison to identify any variation existing in DNA binding motifs between these two clones.

2.2.2. HSP and TCTP sequence analysis (stress responsive genes)

Potential role of Heat Shock Proteins (*HSP*) and Translationally Controlled Tumor Protein (*TCTP*) in abiotic stress tolerance has been identified in many plant species. Therefore, an effort was made to characterise the functional full-length genes belonging to these gene families from stress related transcriptomes of rubber. Transcripts sequences from drought (B), cold (C) and *Corynespora* challenged (T2) transcriptomes were aligned against Heat Shock Protein (*HSP*) and Translationally Controlled Tumor Protein (*TCTP*) sequences of *Arabidopsis thaliana, Hevea brasiliensis, Ricinus communis* and *Populus trichocarpa* using blast homology search. *HSP* and *TCTP* domain containing sequences with full length Open Reading Frame (ORF) were extracted from drought (B), cold (C) and *Corynespora* challenged (T2) transcriptomes. Genes encoding three 17.5 kDa, two 18.2 kDa and one 15.7 kDa HSP family protein were identified. Three translationally controlled tumor protein (*TCTP*) genes were also detected. Cloning is in progress.

2.2.3. Methylation dynamics of Hevea brasiliensis genome

Studies on DNA methylation induced by cold stress 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. Genomic DNA from the growth chamber maintained plants of RRII 105, RRIM 600 and RRIC 100 before imparting the stress, after two weeks of giving stress and one month post stress were subjected to bisulfite treatment in order to

identify methylated cytosines in the promoter region of gene of interest.

The partial promoter region of both *HMGR* and *HMGS* genes from 24 samples were amplified by bisulfite PCR. A total of 77 plasmid samples having bisulfite treated partial promoter region of *HMGR* gene from the clones RRII 105, RRIM 600 and RRIC 100 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.

3. Cloning and characterization of agronomically important genes

3.1. Cloning and characterization of lignin biosynthesis genes involved in phenylpropanoid pathway for timber quality improvement

Altering the lignin monomeric composition (Syringyl to Guaiacyl or S/G ratio) without reducing total lignin content is the effective way to improve desired wood properties. Conferaldehyde - 5-hydroxylase (*CAld5H*) gene is known to play a key role in regulating S/G ratio by channeling the intermediate compounds of guaiacyl precursor to syringyl lignin pathway without any influence on total lignin content. Present study aimed towards molecular characterization of *CAld5H* gene in *Hevea brasilensis*, a promising timber tree species primarily cultivated for natural rubber production. Both genomic sequences and cDNA encoding *CAld5H* were cloned and characterized from RRII 105. The genomic DNA was about 1.93 kb in size with one intron and cDNA was 1.5 kb. Both full-length genomic and cDNA sequences were submitted to the NCBI GenBank under the accession numbers KY930624 and KY930625 respectively. Bacterial expression of *CAld5H* cDNA is in progress.

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

4.1. *Hevea* genome sequencing

Hevea genome sequencing program was launched in RRII to generate a draft sequence of natural rubber (clone RRII 105) using next generation sequencing (NGS) platforms for deciphering the important informations encoded in rubber genome. More than 170X coverage of *Hevea* genome sequence data was generated using cross-platform sequencing technologies. Sequence data were assembled using SOAP denovo and MaSuRCA assembly tools. Better assembly statistics was obtained with MaSuRCA using test data. Therefore attempts were made to assemble the cross-platform sequence data using MaSuRCA assembler. Unfortunately the genome assembly process using MaSuRCA stopped with complete set of sequence data. Therefore, further stringent filtration was performed on entire sequence data, especially on mate-pair (MP) data to reduce the data size for assembly.

4.2. Transcriptome sequencing

Transcriptome sequencing of 15 samples derived from leaf, root, bark and latex of rubber plant subjected to various biotic and abiotic stresses was carried out for achieving better assembly of whole genome, gene prediction and annotation. *De novo* assembly of transcriptomes followed by gene annotation was completed. Complete Open Reading Frames (ORFs) were predicted for RNA-Seq assemblies after removal of ORF with any ambiguity in the sequence (presence of 'N') and also transcripts with multiple ORFs.

4.2.1. *Phytophthora* challenged transcriptome data analysis

Sequencing reads from phytophthora challenged and control samples (RRIM 600) were subjected to gapped read alignment. Using gapped alignment, 61 and 63 per cent of sequencing reads were mapped in control and treatment samples respectively. Mapping data were quantified using eXpress tool and un-gapped mapping data were quantified using RSEM tool. Count data extracted from eXpress and RSEM tools was subjected to differential gene expression analysis using DESeq. Since read counts were obtained from poor mapping data, the "parametric dispersion fit" method was failed in DESeq and "local fit" method was used for measuring gene expression. Screening of differentially expressed genes from DESeq with false discovery rate <0.1 resulted no significant hits due to poor mapping data.

4.2.2. RNA-Seq data analysis of *Colletotrichum* challenged samples

Assembled transcriptome from *Collectotrichum* challenged and control samples were clustered using CD-HIT tool with 90 per cent sequence identity. In total 113004 and 93132 clustered transcripts were obtained in clone RRII 105 and PB 260, respectively. Similarly, assembled transcriptome from *Collectotrichum* challenged samples (COL-105-TP vs COL-260-TP) from clone RRII 105 and PB 260 were clustered using CD-HIT tool with 90 per cent sequence identity. In total 94743 non-redundant transcripts were obtained from the analysis.

RNA-Seq reads from these samples were mapped against the clustered transcriptome data using bowtie tool. Read quantification was carried out using RSEM tool. Read count data from both challenged and control samples were subjected to differential gene expression analysis using DESeq tool. In total, 206 and 388 transcripts were up/down regulated in clone RRII 105 and PB 260 respectively. Similarly, differential gene expression analysis carried was out between *Collectotrichum* challenged samples (COL-105-TP vs COL-260-TP) using DESeq tool. In total 527 transcripts were identified as differentially expressed between RRII 105 and PB 260 clones.

Perl script "seq_extract.pl" was generated to extract transcript sequences from differential gene expression (DGE) analysis results of Collectotrichum challenged and control samples. Nucleotide sequences of around 200 differentially expressed transcripts from Collectotrichum challenged and control samples were extracted using "seq_extract.pl" perl script. The nucleotide sequences were further aligned against *Ricinus communis* protein sequences from Uniprot using blastx homology search.

4.2.3. RNA-Seq data analysis of TPD samples

Assembled transcriptome from control and TPD samples were clustered using CD-HIT tool with 90 per cent sequence identity. In total 21200 non-redundant transcript were obtained after clustering. A total of 21200 non-redundant transcript sequences were subjected to read quantification using control (N1) and treated samples (T1). The quantified data from control and TPD samples will be used for differential gene expression analysis.

4.3. Bioinformatics resource generation

4.3.1. Tools/software/pipelines setup

Latest version of Bio-Linux was installed. Bio-Linux 8 adds more than 250 bioinformatics packages to an Ubuntu Linux 14.04 LTS base, providing around 50 graphical applications and several hundred command line tools. Bioinformatics tools/software/pipeline had been installed and setup in a server at genomics lab.

These tools are being upgraded periodically. Most of these tools were subjected to benchmarking studies to understand their algorithm using publicly available or simulated data. Multiple in-house PERL scripts were generated as and when required for data parsing.

PLANT PATHOLOGY DIVISION

Plant Pathology Division focuses on monitoring the occurrence of pest and diseases, their management through chemical and biological methods, studying the impact of protecting trees against various diseases, evaluation of new clones for disease resistance, identification of genes involved in disease resistance, understanding the etiology of tapping panel dryness, use of beneficial microorganisms for plant growth, disease control, drought tolerance, *etc.* In addition to these areas of research, the Division also takes up testing of spraying equipment, evaluating new generation plant protection chemicals and compounds, water sample analysis to assess load of harmful bacterial population in water, *etc.* Training is also imparted on disease management, maintenance of spray equipment, mushroom culture and apiculture. Advisory work is undertaken through visits and Online Rubber Clinic. For better and speedy advisory service, WhatsApp facility was introduced and 1631 cases were attended through this. There were a total of 4332 users and 7681 hits in Online Rubber Clinic from 29 countries. The water samples tested were 643 during the period under report.

1. Leaf diseases

1.1. Abnormal leaf fall disease

The impact of abnormal leaf fall (ALF) disease on growth and yield of rubber trees varies from clone to clone depending on their level of susceptibility to *Phytophthora* and the severity of disease which depends on prevailing weather conditions. In this regard, the studies undertaken in four modern clones *viz.* RRII 414, RRII 422, RRII 429 and PB 260 have been giving varying results.The severity of ALF disease in general during 2016 disease season was less. Among the clones, leaf fall of 30 to 40 per cent was recorded in unprotected blocks of RRII 414, RRII 422 and PB 260 whereas RRII 429 recorded good retention (> 80%) in unprotected block also. The girth of trees in sprayed blocks continued to be significantly higher in clones RRII 414 and RRII 429, whereas girth increment was higher only in RRII 414. Bark thickness and bark thickness increment (2015-17) did not show any trend between sprayed and unsprayed conditions(Table Path.1).

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

Clone	Girth (2017) (cm)	Girth increment (cm)	Bark thickness (mm)	Bark thickness increment (mm) 2015-2017
RRII 414				
Sprayed	67.7**	2.2**	10.0	0.85
Unsprayed	64.8	1.8	9.7	1.48
RRII 429				
Sprayed	66.4**	1.6	8.3	0.55
Unsprayed	64.9	2.6**	8.9	1.84
RRII 422				
Sprayed	62.7^{NS}	2.8^{NS}	9.1	0.42
Unsprayed	62.8	2.9	8.8	0.35
PB 260				
Sprayed	67.4^{NS}	3.1^{NS}	9.7	0.71
Unsprayed	66.6	3.2	9.6	0.97

The impact of ALF was consistently more in clone RRII 414 recording yield drop in the current year also. The clones RRII 414, RRII 422 and PB 260 registered yield drop of 9.8, 5.8 and 4.4 per cent, respectively in unprotected blocks, whereas the impact of leaving the plots unsprayed was not noticed in clones RRII 429 in the current season also due to low ALF. The DRC did not seem to get influenced by ALF. The tapping panel dryness (TPD) was higher in PB 260 followed by RRII 414 and the lowest in RRII 422 when BO-1 panel was exhausted. Between sprayed and unsprayed areas, no definite trend could be observed (Table Path.2).

Table Path. 2. **Percentage of TPD in crop loss trial in BO-1 panel**

Clone	Field	Total trees (No.)	TPD trees (No.)	TPD (%)
RRII 414	Sprayed	325	67	20
	Unsprayed	315	53	16
RRII 429	Sprayed	329	38	11
	Unsprayed	318	30	9
RRII 422	Sprayed	311	48	15
	Unsprayed	296	51	17
PB 260	Sprayed	339	96	28
	Unsprayed	341	82	24

The crown budding experiment on clone PB 311, located in Malankara Estate, was completed. The trees crown-budded with Fx 516 recorded better girth and timber volume. In another experiment to evaluate the effectiveness of nursery crown budding in clone PB 260 at Central Experiment Station, girth and girth increment were found significantly higher in crown-budded than in control plots (Table Path. 3).The ALF and Phytophthora shoot rot were mild in crown-budded plants whereas, both were moderate in control. The crown-budded trees recorded higher yield, whereas DRC did not show significant variation. TPD was found to be more in crown-budded trees on completion of BO-1 panel.

Table Path. 3. **Growth in PB 260 crown-budded trees**

Treatment/crown	Girth (cm)	Girth increment (cm)
Crown-budded/ Fx 516	72.3**	3.7**
Control/ PB 260	66.7	2.8

The effect of crown modification in clone PB 260 on latex/raw rubber properties was studied. The Plasticity and Mooney Viscosity were found less in crown-budded trees whereas, PRI was higher in crown-budded trees. The properties did not get influenced by summer, monsoon and post-monsoon seasons (Table Path. 4).

Table Path. 4. **Latex properties in crown-budded trees in three seasons**

Parameter	Summer		Monsoon		Post monsoon	
	PB 260 (control)	CB Fx 516	PB 260 (control)	CB Fx 516	PB 260 (control)	CB Fx 516
TS (%)	36.6	36.0	38.2	36.7	38.9	37.2
DRC (%)	33.7	32.7	35.3	33.5	35.8	33.9
NRS (%)	2.9	3.3	2.9	3.2	3.1	3.3
Mg (ppm)	189	160	-	-	-	-
MV, ML at 100°C	67	58	63	53	70	57
P_0	33	29	34	28	36	32
PRI	64	76	85	89	83	88

In another experiment, attempts are being made to develop crown-budded root trainer plants. The initial attempts to develop plants up to the desired height (>10 ft) for crown budding in root trainer cups of size (1000 cc) did not succeed. Therefore, RT cups were modified by giving PVC-pipe attachments of 3" and 4" diameter with a length of 2.5 ft. Plant growth was found satisfactory (Fig.Path. 1).

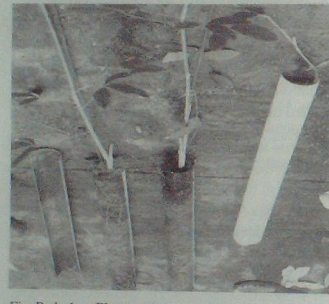


Fig. Path. 1. **Plants raised in modified root trainer cups for crown budding**

Evaluation of the crop loss due to the combined effect of ALF and powdery mildew diseases at RRS, Padiyoor was continued. Powdery mildew disease was mild and abnormal leaf fall disease was moderate to severe. The leaf retention was only 65 per cent in RRII 105 unsprayed area and it was 20, 50 and 40 per cent for the clones RRIM 600, PB 235 and PB 5/51, respectively after the ALF disease season. Overall crop loss in RRII 105, RRIM 600, PB 235 and PB 5/51 for the year was found to be 22, 41, 32 and 48 per cent, respectively.

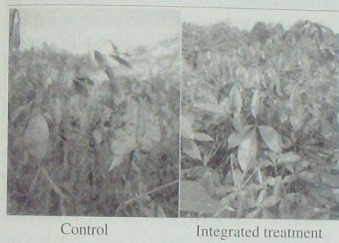
In an effort to identify genes involved in disease resistance for ALF and mapping Quantitative Trait Loci (QTL) for disease resistance, a mapping population of an interspecific cross between *H. brasiliensis* (clone RRII 105) and *H. benthamiana* (clone F4542) generated over six years (2009-2014) by the Germplasm Division of RRII was utilized for the study. Eighty six progeny population derived from this inter-specific cross was used for this study. Phenotyping the mapping population for Phytophthora disease resistance was carried out using *in vitro* challenge inoculation. Lesion size clearly discriminated progenies with varying levels of resistance reactions. Segregation pattern for disease resistance trait in response to *Phytophthora* infection was assessed. Progenies with extreme level of tolerance and susceptibility were identified along with the majority of the population showing moderate level of resistance/ susceptibility. Out of 84 progenies, four (4.8%) were found to be highly resistant and nine (10.7%) were highly susceptible. In the resistant and susceptible category, there were 27 (32.1%) and 10 (11.9%), respectively. Twenty three of the progenies (27.4%) were categorized in the moderately resistant category and eleven (13.1%) in the moderately susceptible group.

1.2. Corynespora disease

The compatibility of antagonistic microbes with recommended fungicides was tested *in vitro*. The antagonist endophytic bacteria 8LK showed tolerance to the recommended fungicidecarbendazim (0.05% *a.i.*). Evaluation of bioagents (endophytic bacteria) and efficacy of these organisms in integrated control against Corynespora leaf fall disease on RRII 105 was carried out at Ulickal nursery. Results showed that integrated control with carbendazim (0.025%) and antagonistic endophytic bacteria was effective and on par with recommended fungicides (Table Path. 6; Fig. Path. 2).

Table Path. 6. **Effectiveness of integrated treatments in Corynespora disease control**

Chemical	Concentration	Disease intensity
Carbendazim	0.05%	2.4
Antagonistic endophytic bacteria (8LK)	1 x 10^6/ml	2.3
Integrated treatment (Carbendazim + Antagonistic endophytic bacteria)	0.025%+1x10^6/ml	1.3
Contol (unsprayed)		4.7
CD (P=0.05)		0.2



Control Integrated treatment

Fig. Path. 2. **Corynespora disease severity between control plants and integrated treatment**

Evaluation of new generation fungicidethiophonate methyl at different concentrations along with recommended fungicides and untreated control was carried out at two locations in Karnataka during 2017 disease season. Among the treatments,thiophanate methyl (0.07%) was found significantly superior to all other treatments in controlling the disease (Table Path 7).

Table Path. 7. **Effect of fungicides on the control of Corynespora disease**

Chemical	Location 1: Sheradi, Karnataka		Location 2: Ichilampadi, Karnataka	
	Concentration (%)	Disease intensity	Concentration (%)	Disease intensity
Carbendazim	0.05	1.3	0.05	1.0
Carbendazim	0.1	1.2	0.1	0.6
Thiophanate methyl	0.07	0.5	0.07	0.5
Thiophanate methyl	0.14	0.4	0.14	0.5
Control (Unsprayed)		4.2	Unsprayed	1.3
CD (P=0.05)		0.2		0.2

In order to study the virulence of *Corynespora*, infected leaves were collected from different rubber growing areas of Kerala and Karnataka during January to March 2017. The virulence of isolates was studied by leaf wilt bioassay using crude toxin and spore inoculation method. The isolates showed high and faster wilting in susceptible clones during *in vitro* leaf wilt bioassay. The twelve tested *Corynespora* isolates were found equally aggressive. *Cas*1, the cassiicolin gene was amplified from the genomic DNA of all isolates tested.

The clone Fx 516 was identified with high level of tolerance to *Corynespora* in spore inoculation/ toxin bioassay method and no infection was observed in Fx 516 plants at Ulickal

nursery during peak disease season. The half sibs of Fx 516 were screened for their tolerance to *Corynespora* using crude toxin and six tolerant plants were shortlisted.

In an attempt to identify QTL markers associated with tolerance to Corynespora leaf disease in rubber, the same mapping population of an interspecific cross between *H. brasiliensis* (clone RRII 105 highly susceptible to *C. cassiicola)* and *H. benthamiana* (F4542 with high level of tolerance to *C. cassiicola*) was utilized. Phenotyping for disease resistance to *Corynespora* infection in the progeny population was carried out using the toxin extracted from the pathogen and performing leaf wilt bioassay.

The study with progenies revealed that out of the 85 progenies tested for their resistance to *C. cassiicola*, nine were highly resistant (10.6%) and 18 were highly susceptible (21.2%). The remaining 58 progenies (68.2%) belonged to moderately resistant/ susceptible category.

In order to identify the QTL markers for Corynespora disease, genotypic and phenotypic data for disease resistance derived from the progeny population was merged together. Seven QTL markers for *Corynespora* disease resistance (one each mapped in LG 6, 11, 13 and two each in LG 8 and 16) were identified at an adjusted P value cut off 0.001 and a LOD threshold score of three. The identified potential QTL markers need to be validated.

1.3. Powdery mildew disease

In a disease control experiment with poly bag plants, efficiency of two bacterial biocontrol agents along with wettable sulphur recommended fungicide and a new fungicide trifloxystrobin + tebuconazole (Nativo) was tested. Among the treatments trifloxystrobin + tebuconazole (nativo) was found to be the most effective. About 50-70 per cent control of the disease was obtained with a bacterial isolate RH 34.

2. Tapping Panel Dryness (TPD)

Recent investigations on TPD-affected trees showed typical bark necrosis spreading downwards from tapping cut to root stock. DAPI (nuclear stain) stained TPD and healthy bark when examined under laser scanning microscope showed presence of living organism in phloem vessels and their absence in healthy trees. Phytoplasma-enriched DNA was isolated from TPD and few healthy bark samples. Nested-PCR assays using two universal primer pairs, R16mF2/ R1 and R16F2n/R2 yielded an amplification of ~1300 bp in all TPD-affected plants. Cloning and sequencing of amplified PCR product were carried out and the sequence on optional blasting showed similarity to other reported Phytoplasmas. The healthy plants which were positive in PCR reaction later changed into TPD. Scanning Electron Microscopic (SEM) studies of Phytoplasma-positive bark samples showed round pleomorphic bodies similar to Phytoplasma in the sieve tubes. The size of the Phytoplasma-like bodies ranged between 400-1800 nm.

3. Growth improvement of young rubber plants through microbial inoculants

3.1. Effect of PGPR inoculation at different fertiliser levels

Four selected PGPR isolates and two consortia were evaluated in root trainer plants at 25 and 50 per cent recommended levels of fertilizer. Uninoculated plants supplied with 25, 50 and full dose of fertilizer were kept as controls. Inoculated plants showed better girth and height of plants than fertilizer applied plants. The highest girth of plants was recorded by those applied with RH 104 at 25 per cent fertilizer application. Inoculated plants at 25 and 50 per cent fertilizer

application did not show much difference in growth and was higher than uninoculated plants at the same levels and with full level of fertiliser applied plants. Root growth was more for inoculated plants. Population of the inoculated cultures and total microbial population were more in potting mix than the controls in all the treatments.

PLANT PHYSIOLOGY DIVISION

The major areas of research in the Crop Physiology Division are studies on physiology of growth and yield, environment and stress physiology, tapping panel dryness, secondary metabolites, gene expression analysis in relation to abiotic stress responses and rubber biosynthesis and ecological impact on rubber cultivation.

A few common adaptive physiological traits were identified in drought and cold tolerant clones total of 14 wild accessions were identified with low canopy temperature during peak summer season revealing prospective drought tolerance germplasm lines. Lack of sync between photosynthetic CO_2 assimilation (A) and transpiration (E) in response to light was observed in leaves of young as well as mature rubber plants. A few phenotypic and molecular markers were shortlisted for drought tolerance and rubber biosynthesis. Growth regulator, paclobutrazol (PBZ) was tested in young plants of rubber for better root growth and drought responses.

Effect of ethephon stimulation on latex regeneration mechanism was studied in many high and low yielding clones. The latex biochemistry of a few clones which are not responding to yield stimulation was studied. A couple of crucial genes associated with rubber biosynthetic pathway which are over-expressing under stimulation were characterized.

1. Environmental and stress physiology

1.1. Developing early screening tools for drought tolerance in *Hevea*

Physico-chemical properties of a consistently over-expressing chloroplast stress protein under soil moisture deficit stress was analysed by ExPASy tool ProtParam. It had a molecular weight of 23.8 kDa and 214 aminoacids. In order to ascertain the association of this stress protein (23.8 kDa) with drought tolerance traits of *Hevea* germplasm accessions, western blot analysis was carried out with chloroplast protein profiles of irrigated control and drought imposed plants of four relatively drought tolerant (RO 3261, AC 612, RO 3157 and RO 3184) and two susceptible (RO 3242 and MT 1619) germplasm accessions along with two check clones (RRIM 600 and RRII 105). Chloroplast isolation, extraction of proteins and blotting exercises were carried out after imposing drought stress by withholding irrigation in polybags for 10 days. Expression of the protein was very prominent in relatively drought tolerant germplasm accessions, RO 3261, AC 612 and RO 3157 than susceptible accessions, RO 3242 and MT 1619 (Fig. Phy.1).

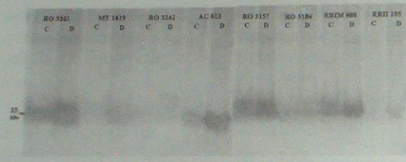


Fig. Phy.1. **Western blot profiles of 23.8 kDa chloroplast stress protein extracted from six *Hevea* germplasm accessions and check clones RRIM 600 and RRII 105**

Gene expression analyses were performed for sHSP23 (23.8 kDa chloroplast protein coding gene). Quantitative expression analysis (qPCR technique) indicated up-regulation of sHSP23 gene in drought exposed plants. Drought tolerant clones recorded more than two fold over-expression of this gene when compared to susceptible clones.

1.2. Molecular basis of drought tolerance in *Hevea*

1.2.1. Analysis of integrity of genomic DNA during water deficit/ high light stress

Excised leaf discs harvested from *Hevea* clones RRII 430 (relatively drought tolerant) and RRII 414 (relatively susceptible) were subjected to water deficit stress *in-vitro* using 40 per cent PEG under low and high light conditions (growth chamber) and under very high light (under sunlight in open field). The integrity of genomic DNA from the leaf discs was analyzed by agarose gel electrophoresis. A fair degree of DNA fragmentation was observed in the susceptible clone under high light alone as well as in the combination of high light and PEG stress (osmoticum) whereas, DNA was comparatively intact with no visible signs of fragmentation in RRII 430 at low light and at a low concentration of PEG. However, under very high light conditions in the open field (coupled with high temperature), DNA fragmentation was pragmatic in both the clones indicating that high light can inflict serious damages to DNA in all the clones.

1.2.2. Investigations on micro RNAs in Hevea brasiliensis: Role in gene regulation under abiotic stresses

Analyses were completed to find out miRNAs that show strong association with cold stress tolerance in *Hevea*. Next generation sequencing using Illumina HiSeq method revealed that strong expression of 29 and 21 conserved miRNA families in cold exposed and control samples, respectively. Around 42 novel miRNAs were identified. From the differential expression analyses, eight conserved miRNAs were found to be common to both the samples. Expression analyses were performed with six selected miRNAs by qPCR method in two clones (RRII 105 and RRIM 600). miR169, miR482 and miR159 showed strong association with cold tolerance and can be employed as markers for cold tolerance after validating in larger number of clones with varying degree of cold tolerance.

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

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

In an experiment at Central Experiment Station, Chethackal, RRII 400 series clones recorded better trunk girth (7th year) than the check clones RRII 105 and RRIM 600. Among the 400 series clones, the highest girth was observed in RRII 414 followed by RRII 422. Among the clones studied the number of trees which attained tappable girth was more in RRII 414 followed by RRII 430.

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

Polybag plants of two selected germplasm accessions (MT 4788 and MT 5100) were grown

along with other elite *Hevea* clones at RRII farm. Plants were subjected to drought stress by withholding irrigation for ten days to study the effect of drought in relation to oxidative stress and antioxidant responses. The selected germplasm lines showed better growth performance in comparison to RRII 105 and RRII 400 series clones. Leaf samples were collected after assessing the physiological performance of plants. Photosynthetic pigment content was on par with elite clones. Analysis of other biochemical parameters in relation to stress responses such as xanthophyll cycle pigments, lipids and antioxidants are in progress.

1.4. Identification of physiological, biochemical and molecular factors associated with drought tolerance in *Hevea* germplasm accessions

Leaf samples from six relatively drought tolerant and four susceptible wild *Hevea* germplasm accessions along with check clones were analysed for SOD activity and contents of antioxidants (thiols and ascorbic acid) in the leaf. Analyses after imposing stress showed increased activity of SOD and relatively higher levels of ascorbic acid in tolerant accessions and check clones compared to the susceptible accessions and clones. No definite trend could be observed for the contents of thiols.

1.5. CO_2 assimilation and transpiration under changing light conditions

The rate of CO_2 influx into and water vapour efflux out of the leaf was studied in six different species of plants under light and shaded conditions. The study was done in species with varying photosyntheisis rates *viz.Hevea, Pueraria, Hibiscus, Bauhinia,* cashew and papaya. When a leaf under steady-state photosynthesis was exposed suddenly to dark/ shade condition, CO_2 assimilation was reduced to almost zero within seconds, whereas stomatal conductance and transpiration rate declined slowly. The mismatch in these two processes causes considerable reduction in water use efficiency of plants, especially under partiall cloudy conditions. The situation is similar during early morning and late evening hours when light intensity changes fast. CO_2 assimilation rises fast in the morning as sunlight intensity goes up and it declines fast in the evening with fall in light. Transpiration rate is slow to respond (Fig. Phy. 2). Similar trend of lack of sync between CO_2 assimilation and transpiratory water loss in response to light was observed in other studies with canopy level CO_2 and water flux measurements using eddy covariance technique and whole tree level sap flow measurements in rubber plantation.

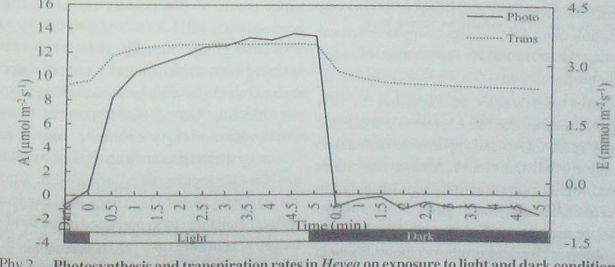


Fig. Phy.2. **Photosynthesis and transpiration rates in *Hevea* on exposure to light and dark conditions in the nursery**

1.5.1. Effect of high temperature stress on Hevea

Two clones (RRII 105 and RRII 430) were subjected to high temperature stress *in vitro* and photochemical efficiency in terms of PSI and PSII activities were measured using Dual PAM and Fluorescence Monitoring System (Hansatech, UK). Among the clones, RRII 430 showed better temperature tolerance capacity *in vitro* than RRII 105 in terms of chlorophyll fluorescence parameters at 2, 4 and 6 hours of high temperature treatment. Gas exchange parameters and biochemical components of the plants at high temperature stress are continuously monitored.

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

Adaptive mechanisms to low temperature was studied under controlled condition using polybag plants of nine *Hevea* clones. After 10 days under controlled condition (30^{0}C/22^{0}C day/night, 13h photoperiod), these plants were subjected to low temperature by gradually lowering the temperature to 15^{0}C/7^{0}C (day/night). Under field condition, another set of plants were subjected to drought stress for similar periods. Data on leaf water status and biomass partitioning were recorded. Clonal variation was observed in terms of cold adaptability in all the experiments. RRII 422 was found to be the most susceptible clone for cold stress. Significant clonal variation was observed for leaf area and biomass partitioning.

After two weeks of exposure to low temperature stress the rate of antioxidant enzyme activity (peroxidase and superoxide dismutase) was high in all the clones. Among the clones studied higher rate of activity was observed in SCATC 88/13 and low in RRII 105. All other clones were on par with each other. Similar trend was observed for leaf protein content. Pigment composition was varied and the reduction was more in accordance with extension of stress period. Lipid peroxidation as a function of membrane damage was found less in clones such as RRIM 600, RRII 208 and SCATC 88/13. On cold stress recovery also these clones showed the same trend. Analyses of samples collected from drought imposed plants for biochemical parameters (photosynthetic pigments, lipids, lipid peroxidation, antioxidant system and other adaptive mechanisms) are in progress to evaluate common behavior of selected clones under cold/drought conditions.

1.6.1. Physiological evaluation of root trainer plants

Two years after planting at RRS, Dapchari, Maharashtra, field evaluation of root trainer and polybag plants was carried out in two clones, RRII 105 and RRII 430. Casualty of young plants due to moisture stress was assessed. Comparatively more casualties were seen in polybag plants than root trainer plants in case of RRII 105 under dry conditions. The casualties due to severe moisture stress in root trainer plants of RRII 105 and RRII 430 were also high under Dapchari conditions.

1.6.2. Screening of wild germplasm accessions for mid-day canopy temperature

Mid- day canopy temperature, that indicates water status of plants, was monitored in 31 accessions of SBN 2004 and 14 accessions of SBN 2005 groups during peak summer (March) in field grown nursery plants. Canopies exposed to direct sun light were measured using an infra-red portable remote thermometer (Raytek Corporation, USA) on clear sunny days. Generally the most tolerant accessions maintained low canopy temperature during peak summer when a combination of high light, high temperature and soil moisture stress prevailed in the field. The canopy temperature varied from 35.7^{0}C (accession AC 2009) to 41.3^{0}C (accession RO

291) across genotypes. Accessions maintaining relatively low canopy temperature were identified.

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

Sixteen ortets and seven check clones were planted at CES, Chethackal in 2012 as part of a multi-location evaluation trial. Girth was recorded at 50 cm height from the bud union during February 2017. Mean stem girth of ortets ranged from 18.6 cm (ortet GH1) to 35.4 cm (ortet RRSA 98). Clone RRII 430 recorded the highest girth, whereas, ortet GH 1 had the lowest girth. Cumulative rubber yield of ten successive test tappings was recorded in September/October 2016. Among the check clones, the highest yield was recorded in RRII 417 followed by RRII 430 during peak yielding season. Ortets, RRSA 98 followed by RRSA 585 and DAP1 recorded better yield, whereas, ortets, NGK 69, NGK 47 and DAP 34 recorded the lowest yield.

1.6.4. Experimental cultivation of high yielding varieties of rubber plants for establishment in high elevation

A high elevation trial was initiated during the years 2006 and 2007 at Haileyburia Tea Estate, Elappara with 10 clones. Growth of rubber trees was not promising in the experimental trial mainly ascribed to the prevailing environmental factors and lack of proper farm management. The growth of polyclonal seedlings (45.8 cm) planted in between the tea plant rows was found better than clonal trees. Overall the performance of this plantation at high altitude was not satisfactory.

1.7. Proteomic studies of *Hevea brasiliensis* under abiotic stresses

1.7.1. Proteomic studies of Hevea brasiliensis under cold stress

Cold stress was imposed in five clones (RRIM 600, RRII 208, RRIC 100, RRII 414 and RRII 429) inside the growth chamber. More cold injury was noticed in clones RRIC 100 and RRII 414. Rate of net photosynthesis and ϕ_{PSII} was better in clones *viz*. RRIM 600, RRII 208 and RRII 429. Fifteen stress proteins were found more abundant in clones which showed less cold injury. Further, identification of cold responsive proteins and their role in stress tolerance are progressing.

1.7.2. Proteomic studies of Hevea brasiliensis under drought stress

Drought tolerant clones RRII 430 and RRIM 600 maintained higher rate of net photosynthesis and stomatal conductance than susceptible clones. After 10 days of withdrawal of irrigation, differential expression of chloroplast proteins was observed between susceptible (Tjir 1 & RRII 414) and tolerant clones (RRII 208, RRII 430 & RRIM 600). Eighteen stress responsive proteins were more abundant in drought tolerant clones *viz*. RRII 430 and RRIM 600. Crucial proteins responsible for drought tolerance traits will be shortlisted.

1.8. Growth regulation and high density planting in natural rubber for productivity enhancement

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

An experiment on high density planting (HDP) and plant growth regulation was initiated with field planting of clone RRII 430 at RRII, Kottayam in 2015. Planting was carried out in three densities and two types of planting materials were used (polybag and root-trainer plants). Soil application of paclobutrazol (PBZ), a growth regulator was given to one year old plants. Data on girth and plant height was recorded for two consecutive years. Initial growth was found to be better in plants raised from polybag plants.

The PBZ treated plants showed slightly shortened and compressed branches as expected.

In another experiment at CES, Chethackal, girth data was recorded after third round of PBZ application in five-year-old plants belonging to six clones. Shortening of internodes resulted in rosette appearance of side branches in PBZ treated plants in all the clones. No significant differences were noticed in girth among the treatments.

1.8.2. Application of PBZ in young plants for development of better root system towards improving drought tolerance

When five *Hevea* clones were exposed to drought, clone RRII 430, RRIM 600 and RRII 208 recorded higher net photosynthesis rate (P_N) than clones Tjir 1 and RRII 414. P_N improved further in all the clones pre-treated with PBZ. Similar trends were noticed for ROS scavenging enzyme activities such as SOD, peroxidase, proline and leaf sugar contents. In PBZ pre-treated plants, number of stomata was 23 per cent more with 33 per cent lesser stomatal aperture size and more stomatal striations. These anatomical changes might help in imparting drought tolerance that was evident from gas exchange parameters. In a field experiment at RRS, Padiyoor more casualties were noticed in drought susceptible clone, RRII 105 than relatively tolerant clone RRII 430 due to soil moisture deficit stress. However, plants pre-treated with PBZ showed comparatively less casualty than untreated plants.

2. Production Physiology (growth and yield)

2.1. Inter-cropping with tree crops in rubber

Growth of rubber trees after 14 years did not exhibit any significant variation between the treatments *viz.* with or without inter-cropping with tree species. Mean girth of rubber trees varied as 68.1 cm for control and 67.7 cm, 67.3 cm, respectively for treatment plots *viz.* rubber with three rows of mahogany and rubber with one row of mahogany and one row of pathimugom trees in between. The trees were continuously tapped for six years under S/2 d3 system of tapping and yield was recorded fortnightly. Mean rubber yield in rubber alone and rubber intercropped with tree species exhibited significant variation. Yield in rubber alone and with mahogany intercrops were 71.4, 50.5 and 57.7 g t^{-1} t^{-1}, for control, with three rows of mahogany and two rows of mahogany trees, respectively. Mahogany trees (53.4 cm) were much bigger than pathimugom (26.1 cm).

2.2. Wintering nature in double budded *Hevea* clones

Early wintering (RRIM 600 and PR 107) and late wintering (RRII 105 and GT 1) clones were budded (one early and another late wintering scions in one stock) on to a single stock and planted along with their single budded plants to study the scion-to-scion communication in these plants. Girth data indicated significantly higher trunk girth in single budded plants of four clones compared to double budded counterparts. The scions of double budded plants did not differ from their single budded counterparts with respect to wintering phenomenon, indicating lesser communication of wintering trait between the two scions in a double budded plant. Latex yield from single and double budded plants was compared. Initial yield (20 months yield) of two early wintering clones (RRIM 600 and PR 107) was higher in their respective scion of double budded plants than single budded normal plants, while, single budded plants of RRII 105 and GT 1 had higher yield compared to their respective scions of double budded plants. However, this type of combination of clones cannot be recommended for planting due to varying girth of scions in one stock, difficulty in tapping process *etc.*

2.3. Clonal variation and effect of stimulation on latex regeneration mechanism in *Hevea brasiliensis*

A new experiment was initiated at CES Chethackal in newly opened trees of clones with different rate of latex metabolism (RRII 105, PB 217, PB 260, RRIM 600, RRII 33 and RRII 38) to study the effect of tapping and stimulation induced latex regeneration mechanism and stress effects. The trees were under d3 system of tapping. Latex samples were collected from control and stimulated trees (with 2.5% ethephon) after each stimulation. Biochemical parameters related to rubber biosynthesis (ATP and sucrose contents), oxidative stress indicators (peroxidase, glutathione reductase, thiols and proline) and protein content were analyzed. Yield and dry rubber content (drc) were also recorded. Out of the seven clones studied, the highest sucrose content was observed in clone PB 217 (above 25mM) and the lowest in clone PB 260 (2.6mM). Medium yielding clone RRIM 600 and two low yielding clones RRII 33 and RRII 38 showed same level of sucrose. The highest protein content was observed in clone PB 260. Glutathione reductase and thiols were high in clone RRII 105 and PB 260. After stimulation with 2.5 per cent ethephon, more sucrose utilization was observed in clone PB 217. Lower levels of stress components (peroxidase and proline) were observed in clone RRII 105 and PB 260 than clone PB 217. Low yielding clones did not respond to stimulation in terms of biochemical parameters like sucrose, ATP and enzymes. Yield increase was also not significant after ethephon application in low yielding clones ascribed to weak response to stimulants.

2.4. Studies on rubber biosynthesis: Gene expression studies

qPCR analyses of 14 genes associated with rubber biosynthetic pathway were performed in the latex of high (RRII 105 and PB 217) and low yielding (RRII 38 and Tjir 1) clones before and three days after stimulation. Significantly higher expression level of genes corresponding to sucrose transporter, HMG-CoA reductase, and mevalonate diphosphate decarboxylase and rubber elongation factor 2 was observed in stimulated than control trees irrespective of yield potential of clones. In general, these genes were over-expressed in high yielding clones than low yielding clones.

2.5. Relationship of ATP status of latex with rubber yield

Five polyclonal selections with high latex ATP and three control clones (RRII 105, RRII 417 and RRII 430) were planted at RRII farm during July 2014 (24 plants each at 2.5x2.5m spacing) to evaluate the yield and stress tolerance potential of these selections compared to control clones. Biochemical parameters related to antioxidant defense mechanism were studied in these clones during stress and stress free seasons. Clone RRII 430 and selection 63 showed better antioxidant capacity than other clones/selections.

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

To study the molecular mechanism of ethylene induced latex production in different clones, an experiment was initiated at CES, Chethackal with six clones (RRII 105, PB 217, RRIM 600, Tjir 1, RRII 33 and RRII 38). Six trees were selected from each clone (three control and three trees stimulated with 2.5% ethephon). The trees were under d3 system of tapping. Latex samples were collected from control and stimulated trees and cDNA was synthesized from total RNA. Quantitative gene

expression (qPCR) analyses of ethylene receptors (*ETR1* and *ETR2*) and other genes involved in the signaling pathway are in progress.

3. Tapping panel dryness

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

Transcriptome sequencing of bark RNA from normal and tress with different levels of TPD (10-20%, 40-60% and 80-100%) incidence trees was carried out. Further annotations and bioinformatic works are progressing.

4. Secondary metabolites

4.1. Water relations of latex with reference to the content of inositols and sugars in the latex during drought periods

To understand the water relations of latex in terms of yield stability and drought responses of *Hevea,* an experiment was initiated during 2016. Field planting of three new clones (RRII 417, RRII 422 and RRII 430) along with one check clone (RRII 105) was done so as to evaluate osmotic variation from the initial stage onwards. Early growth performance of all the clones was very good and on par with each other.

5. Ecological impact of natural rubber cultivation

5.1. Estimating carbon footprint of rubber production

A new project was initiated to estimate the carbon footprint of rubber production. The objective is to assess the CO_2 eq. of all inputs like fertilizers, weedicides, fungicides, farm mechanization *etc.* in rubber plantation starting from nursery to yielding mature trees till life period and work out the footprint of a unit rubber production (for *eg.* 1 metric ton). Accounting various sources of CO_2 emission in rubber plantation activities was initiated. Quantities of recommended dose of inputs like fertilizers, pesticides, weedicides *etc.* are accounted in order to calculate the emissions from the field. CO_2 emission equivalents (CO_2 eq.) of various inputs were worked out. Emissions due to tillage and chemical fertilizers application alone were accounted to be around 20 MT CO_2/ha. Further accounting of emission from tapping process, transportation and primary processing units *etc.* are progressing.

LATEX HARVEST TECHNOLOGY DIVISION

The Division continued applied research on crop harvesting aspects of rubber. The collaborative programme on popularizing weekly tapping among small and medium holdings initiated with the active participation of Regional Offices of the Rubber Board was continued and progressed well during the period under report. Other activities of the Division included testing and evaluation of various products, advisory and training on all aspects of crop harvesting of rubber.

1. Low Frequency Tapping

1.1. Collaborative programme on popularizing weekly tapping

The growers who have participated in the programme continued weekly tapping with

promising results (Table LHT. 1). Based on the results, many growers are adopting weekly tapping with stimulation in other holdings as well.

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

Location (Regional Office)	Yield (kg tree^{-1})	Tapping days
Manjeri	5.0	34
Palakkad	3.6	40
Mannarkkad	3.4	34
Marthandam	5.5	46
Kozhikkode	4.7	42
Thrissur	4.5	50
Thodupuzha	6.4	51
Muvattupuzha	6.6	51
Pala	4.9	23
Thalaserry	5.9	43
Ernakulam	4.0	52
Kottayam	4.4	37
Kothamangalam	5.7	44
Changanaserry	4.9	52
Kottarakkara	3.4	37
Thiruvananthapuram	3.4	44
Adoor	3.8	44
Mean	4.7	43

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

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

In this trial, trees were tapped under d7 frequency of tapping with monthly stimulation in BO-2 panel from 2002-2004. From April 2004 onwards, the tapping frequency was changed to d10 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 during 2016-17. Yield of 1798 kg 400 trees^{-1} was obtained in the sixth year of BI-1 panel. 34 tapping days was realized out of 36 possible tapping days during the period.

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

The commercial evaluation trial on d10 frequency tapping initiated at Kanthimathy Estate, Kulasekharam, Tamil Nadu with once in 20 day's stimulation was continued. Mean dry rubber yield of 1943 kg ha^{-1} could be obtained under d10 frequency of tapping during 2016-17.

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

During 2016, two more blocks of demonstration plot on weekly tapping in clone RRII 105 were initiated at CES, Chethackal. Earlier, both blocks were tapped under S/2 d3 6d/7 in BO-2 panel. Both blocks were tapped under weekly tapping from July 2016 onwards. Nine rounds of yield stimulation were imposed at monthly interval with 2.5 per cent ethephon. Nine months yield of both blocks gave sustainable yield of 1656 (block 1) and 1480 kg 400 trees^{-1} (block 2) respectively during July 2016 -17 (Table LHT.2). It was 4.1 and 3.7 kg tree^{-1} respectively during nine months of tapping. Higher yield was obtained in block 1 when compared to block 2. Yield was declined in both the blocks during the month of October due to leakage of rain guard rubber trees. Mini rain guarding was not practiced during the period. Dry rubber content was higher in block 2 (42.1%) than block 1 (39.9%). In both blocks 38 tapping days was realized during the period (nine months).

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

Months	Yield kg 400 trees^{-1} (block 1)	Yield kg 400 trees^{-1} (block 2)	DRC (%) (block 1)	DRC (%) (block 2)	No. of taps
July'16	59.1	237.7	39.5	41.3	5
Aug	256.9	248.4	37.3	37.8	4
Sep	282.1	268.7	38.0	42.8	5
Oct	133.2	123.7	41.6	42.9	4
Nov	198.2	156.9	40.7	42.0	4
Dec	216.1	173.7	40.4	41.7	5
Jan'17	133.7	104.5	43.2	44.6	4
Feb	76.5	73.0	38.8	44.8	4
Mar	99.7	93.6	39.8	41.3	3
Total	1655.5 (4.1)*	1480.2 (3.7)*	39.9	42.1	38

*mean yield - kg tree^{-1}

In another demonstration trial in field 93C under weekly tapping at CES, Chethackal during the period the yield was 1400 kg 400 trees^{-1}.

2. Controlled Upward Tapping (CUT)

The share of old and senile trees has increased alarmingly for the last few years in India and is one of the major reasons for productivity decline in many plantations. An all out effort of proper implementation of Controlled Upward Tapping (CUT) can address this decline to a great extent.

2.1. Large scale on farm trial on low frequency controlled upward tapping (LFCUT) under weekly tapping

To evaluate low frequency controlled upward tapping (LFCUT) under weekly tapping, large scale on farm trial on LFCUT under weekly tapping was initiated at Kanthimathy estate in 12 tapping blocks (8 blocks mixed clone and 4 blocks seedling population) during November 2016. Initial results are promising. Per tap yield of 36 kg could be obtained under CUT in mixed clone block and 59.7 kg in seedling population.

3. Yield response to stimulation

3.1. Response of RRII 400 series clones to stimulation

At CES, Chethackal, RRII 400 series clones (RRII 414, RRII 422 and RRII 429) were identified for the experiment. The statistical design was completely randomized single tree single plot consisting of more than 100 trees of each clone. Tapping system adopted in this trial was S/2 d3 6d/7. Two rounds of stimulation (ET 2.5% pa) was given during 2016-17 in comparison with the unstimulated control trees (50% trees for stimulation and 50% trees unstimulated, for each clone).

No significant yield increase was noticed in stimulated clones of RRII 414, RRII 422 and RRII 429 (Fig. LHT. 1). Panel change effect was more in clone RRII 429 than other two clones (RRII 414 and RRII 422). Significantly higher yield was recorded in clone RRII 429 than RRII 414 and RRII 422.

There is no difference in annual mean drc of clone RRII 422 and RRII 429 both stimulated and unstimulated trees. But in clone RRII 414,

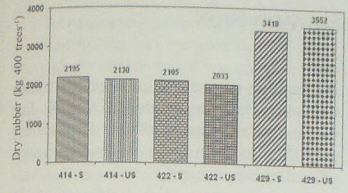


Fig. LHT.1. **Yield performance of RRII 400 series under S/2 d3 6d/7**

annual mean drc was 2 per cent higher in unstimulated trees than stimulated trees.

3.2. Response of RRII 430 clone to 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. The statistical design was RBD with four treatments and five replications. Each replication consist of more than 300 trees. The four treatments comprising d3 (without stimulation as control), and d3, d4 and d7 frequencies of tapping with one level of stimulation. Yield was recorded from July 2016 to March 2017.

Yield was significantly higher under d3 frequency of tapping with one round of stimulation than unstimulated d3 tapping system (Fig. LHT.2). Only 9 per cent of yield increase was observed in stimulated d3 tapping system when compared to control (d3 without stim) during nine months of tapping. Breakeven yield of 78 per cent was obtained when compared to d3 frequency of tapping with stimulation. Low yield under d4 and d7 frequencies of tapping was due to failure of proper depth and thickness of bark shaving for several months. Moreover more leakage of rain guard of rubber trees was reported under d7 frequency of tapping. The drc values of trees tapped under low frequency tapping system (d7) are higher than the d3 frequency of tapping.

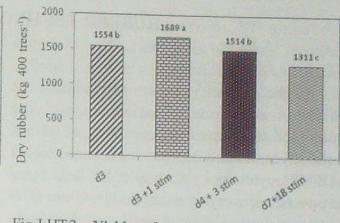


Fig. LHT.2. **Yield performance of clone RRII 430 under LFT systems**

3.3. Long term evaluation of various yield stimulants in clone RRII 105

The experiment is being conducted at CES, Chethackal with six blocks of clone RRII 105. The experimental design was RBD with three replications comprising 60 trees per replication. Tapping system followed was S/2 d3.6d/7 with panel application of 2.5 per cent ethephon (3/y and 6/y) and groove application of Vitex (3/y, 6/y and 9/y). During the period Agrowin gel was not imposed as per the schedule (T6, T7 and T8) and therefore low yield was obtained. Application of ethephon (3/y & 6/y) and Vitex (3/y, 6/y & 9/y) application showed comparable yield (Table LHT.3).

Table LHT.3. **Yield performance (kg 400 trees^{-1}) of clone RRII 105 with two yield stimulants at CES, Chethackal**

Treatments	Year 2016-17
T1- S/2 d3 6d/7 ET. Pa 2.5% 3/y	1916 ab
T2- S/2 d3 6d/7 ET. Pa 2.5% 6/y	2215 a
T3- S/2 d3 6d/7 VT. Ga 3/y	2068 a
T4- S/2 d3 6d/7 VT Ga 6/y	2315 a
T5- S/2 d3 6d/7 VT Ga 9/y	2259 a
T6- S/2 d3 6d/7 AG. Ga 0/y	1587 b
T7- S/2 d3 6d/7 AG Ga 0/y	1576 b
T8- S/2 d3 6d/7 AG Ga 0/y	1604 b

Values followed by a common alphabets are not significantly different

RUBBER TECHNOLOGY DIVISION

In the current year, the activities of the Division were focused mainly on evolving improved techniques in processing of rubber, production of solid deproteinisednatural rubber (DPNR), cytotoxicity analysis of NR latex based products, recovery of quality rubber from skim latex, radiation vulcanisation of NR latex (RVNRL), latex stage incorporation of fillers such as carbon black and silica in NR, reinforcement of NR using polymeric filler and devulcanization of used rubber products.

A novel method for the production of solid deprotenised natural rubber from field latex was developed. The cytotoxicity due to residual accelerators in a few commercial glove brands in Indian market was evaluated. The studies on NR latex - carbon black and NR latex - silica master-batch showed that high levels of filler incorporation in NR is possible through the master-batch route and properties comparable to that of the dry mix could be achieved through this method. A simple and cost effective method for complete recovery of good quality rubber from skim latex was developed. A stable free radical was identified as an efficient devulcanizing aid (DVA) for the mechanical devulcanization of used rubber products.

1. Processing

1.1. Solid deproteinized natural rubber (S-DPNR)

A novel method for the production of solid DPNR from NR latex, without employing processes such as centrifugation or creaming was developed. The method involves the treatment of field latex with a de-protenisation mixture developed at RRII, followed by coagulation of the latex with acid. This new protocol is simple, cost effective and suitable for high volume production of DPNR. Plasticity retention index (PRI) of the raw DPNR is quiet high compared to other commercially available DPNR. Properties of the DPNR produced under different treatment conditions by the new method are given in Table Chem.1.

Table Chem.1. **Raw rubber properties of solid DPNR**

Sample designation	Deprotenisation time (h)	Nitrogen content (%)	P_0	PR I	Mooney Viscosity (M_l(1+4), 100°C)
A1	24	0.1	54	61	87
A2	72	0.093	55	64	94
B1	2	0.157	43	67	82
B2	4	0.091	41	41	78
B3	24	0.07	39	36	78
E1	4	0.108	45	67	86
F1	4	0.092	46	65	86
Control	Nil	0.44	38	82	69
Commercial sample	-	0.07		18	

Technological properties of the DPNR prepared by the four methods A, B, E and F (through the new protocol) was compared with that of the corresponding control rubber without deprotenization. DPNR prepared by the new protocol (B1, B2, B3, A1, A2, E1, F1) and the control rubber sample was evaluated for its cure characteristics and technological properties using the formulation *viz*. rubber 100, stearic acid- 1 phr, zinc oxide – 4 phr, HAF – 50 phr, naphthenic oil-5phr, sulphur -1.5 phr and CBS -1.5 phr. Two levels of antioxidant 0.5 and 3 phr of Mernox-6c(N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) was also used in the study for evaluating the ageing behaviour of the DPNR vulcanizates.

At high antioxidant level, the ageing behaviour of DPNR is comparable to that of the non-deproteinised control. This indicates that the removal of proteins from NR does not significantly affect the ageing behaviour, provided sufficient amount of antioxidant is added to the formulation. The dynamic property (tan delta at M_H), compression set and abrasion resistance of the DPNR vulcanizates were found to vary significantly with the method of preparation employed. Tan delta at M_H varied from 0.05 to 0.12 and was found to increase with increase in deprotenization time.

2. Latex technology

2.1. New process for recovery of rubber from skim latex

The new process developed at RRII (Fig. Chem. 1.) is simple, efficient and cost effective. The skim latex after treatment with a chemical for 24-48 h is acidified. Skim rubber gets coagulated as a lump with complete recovery achieved in a single step.

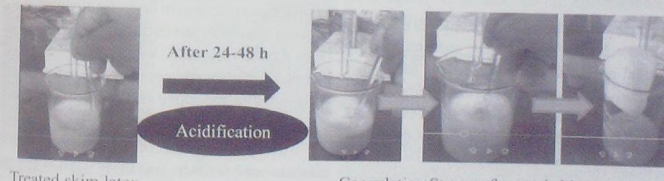


Fig. Chem.1. **Rubber recovery from skim latex using new protocol**

Rubber recovered from skim latex by different methods was evaluated for its vulcanizate properties, *viz*. rubber from skim latex through the new method (skim modified at RRII, skim modified at Cenex factory) and that from conventional skim recovery process (skim control). Formulation used for the study is skim rubber 100, stearic acid- 2 phr, zinc oxide – 4phr, ISAF – 50 phr, naphthenic oil-5phr, sulphur -1.5 phr and CBS -1.5 phr. Vulcanizates of these samples were also compared with the rubber coagulated from fresh field latex using the same formulation. Compared to rubber recovered from skim control, significant improvement in tensile strength (a), elongation at break (b) and reinforcement Index (c) was shown by the rubber recovered from skim latex through new process (both at RRII and at Cenex factory). The percentage improvement is given as (d) in Fig. Chem. 2.

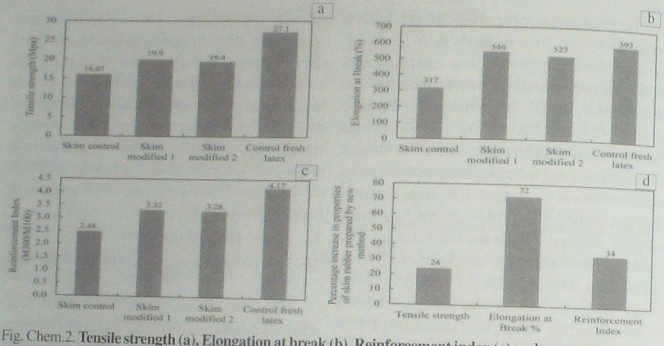


Fig. Chem.2. **Tensile strength (a), Elongation at break (b), Reinforcement index (c) and percentage improvemeant in various properties for the vulcanizates of rubber recovered from skim latex through different process(d)**

2. 2. Residual chemicals in rubber products and their cytotoxicity

A few samples of surgical gloves collected from the Indian market were analysed for the residual accelerator content. It was found that the main accelerator used in these samples was the dithiocarbamate, zinc diethyl dithio carbamate (ZDEC). The quantification of the total residual accelerators was done by UV (UV-Visible spectroscopy) analysis. The residual accelerator content varied from 926 to 2725 µg g^{-1} of the glove sample. The qualitative and quantitative analysis of the ZDEC accelerator in the glove sample was carried out using HPLC and revealed that ZDEC content varied from 873 to 2616 µg g^{-1} among the pre-powdered surgical gloves.

The quantification of bioavailable accelerator (aqueous leachable) from different glove brands (F15, B and F14) using UV spectroscopy showed that more than 14 per cent of the total residual accelerator in the glove is available in 'sweat equivalent' aqueous extracts.

The aqueous extract of the glove brand F14 was analysed for its cytotoxicity against L-929 cell line using MTT assay (indirect assay). Severe toxicity with less than 20 percentage cell viability (or more than 80% cell death) at various dilutions was noticed in the study (Table Chem. 2 and Fig. Chem.3).

Table Chem.2. **Bioavailability of residual accelerator by leaching in aqueous buffers**

Glove brand	Total residual accelerator by UV (µg g^{-1})	Bioavailable residual accelerator by UV (µg g^{-1})	Percentage of bioavailable accelerator (%)
F15	2276	318.5	14.0
B	2203	313.3	14.2
F14	2725	395.1	14.5

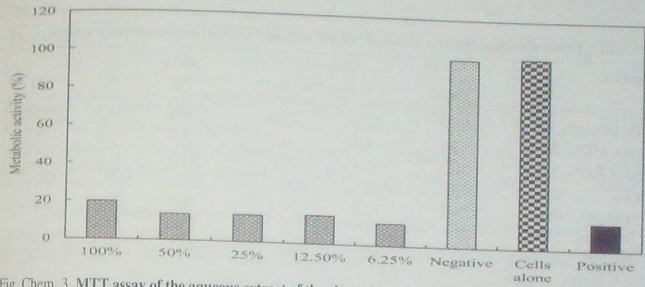


Fig. Chem. 3. **MTT assay of the aqueous extract of the glove sample at different dilutions against cell line L929**

The direct contact assay of the glove sample with the same cell line showed severe cytotoxicity of grade 4. This is evident from the loss of cell morphology in B, whereas it was intact for cell lines in A (negative control) as shown in Figure Chem 4

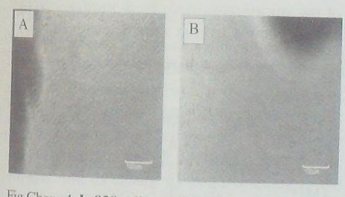


Fig.Chem.4. **L-929 cell lines after direct contact with polythene as negative control (A) and L-929 cells after direct contact with the glove brand 'F14' (B)**

A few leaching methods to minimise the residual chemicals in gloves is under development. Initial studies showed that 85 per cent of the residual accelerator residue in the glove samples could be removed by the new leaching methods developed at RRII.

2.3. Radiation vulcanized natural rubber latex (RVNRL)

Revalidated the license (from BARC) for operating the Gamma chamber and was made functional. Personal radiation monitoring system was made operational. Calibration of some of the important radiation measuring equipments was get done through authorized agencies. Regulatory inspection of RVNRL unit was done by Atomic Energy Regulatory Board (AERB). Samples from the institute and outside agencies were irradiated using Gamma chamber.

3. Rubber Technology

3. 1. Rubber reinforcement

3.1.1. NR/ Polymeric filler system

To improve the abrasion resistance and flex resistance of the tyre tread compounds based on NR and polymeric filler system, studies were conducted. Tread compounds were prepared with 70 parts NR, 30 parts BR and 12 parts polymeric filler. To this matrix 10, 20, 30 and 40 phr HAF carbon black was added along with other conventional rubber compounding ingredients. Cure characteristics and its technological properties were studied. Among these samples no significant change in the cure time and scorch time was observed. However, maximum and minimum torque increased proportionally with increase in the carbon black loading.

Table Chem.3. **Effect of carbon black in blends of NR/PB with and without polymeric filler system**

Properties	Control	HAF 0	10	20	30	40
Tensile strength, MPa	26.4	23.2	23.8	24.2	24.7	25.3
Modulus 100%, MPa	2.1	4.4	4.7	4.9	5.1	5.3
Modulus 300%, MPa	10.2	10.4	10.6	11.2	11.6	12.1
EB, (%)	570	638	620	598	568	560
Tear strength, N/mm	74.8	72.0	74.2	76.0	78.0	78
Heat build up, ΔT^0 C	20	9	10	12	14	16
DIN abrasion loss, mm^3	58	99	86	82	76	72
Dematttia flexing, Crack Initiation, kCys	54	38	38	39	42	44

Technological properties of these vulcanizates are given in Table Chem.3. Incorporation of carbon black to the NR/BR/ Polymeric filler system improved its technological properties such as modulus, tensile strength and tear strength. On increasing the loading of carbon black, these properties improved proportionally. With carbon black incorporation, elongation was found decreased and the heat build-up got adversely affected. Abrasion resistance increased with increasing loading of carbon black. Nevertheless, this improvement was not up to the level that is obtained for the control compound. Flex resistance (crack initiation) also showed improvement with carbon black loading.

3.1.2. NR Latex - carbon black masterbatch

Studies on the preparation of NR latex-carbon black master-batch was continued as a collaborative project with a tyre industry. Master-batch samples were prepared and sent to the collaborator for evaluation of its technological properties. Based on the feedback from the collaborator, new samples of NR latex-carbon black master-batches were prepared using three different surfactants S1,S2 and S3. These master-batches prepared were compounded as per the formulation given in Table Chem.4. Cure characteristics of the control (dry mix) and the master-batches with surfactants S1,S2 and S3 at 150^{0}C were determined. Cure characteristics for all the master-batches were closer to that of the dry mix (control). Technological properties of the vulcanizates made from master- batches and the control dry mix were determined.

Table Chem.4. **Formulation of mixes**

Ingredients	Phr (g)
Master- batch	150 (Effective NR 100g)
ZnO	4.5
Stearic acid	3
TMQ	1
6 PPD	2.75
TBBS	1.6
Sulphur	1.2

Tensile strengths of all samples are more or less on par with the control. Samples prepared with surfactant S3 showed highest elongation at break. Compared to the control, modulus and tear strength are higher for the master-batches. Cut growth and flex resistances of the vulcanizates are given in Table Chem.5. Cut growth and flex resistances are better for the master-batches when compard with the control.

Table Chem. 5. **Cut growth and flex resistance of the vulcanizates**

Properties	Samples			
	Control dry mix	S1	S2	S3
Cut growth development up to 12.5mm (No. of counts in kilocycles)	20139	231588	151539	257193
Initial crack observation (No. of counts)	49007	126476	126476	126476
Final failure observation (No. of counts)	143978	554960	557613	668130

Filler dispersion properties of the vulcanizates was tested using dispergrader (M/s. Alpha Technologies) and are given in Table Chem.6. Carbon black dispersion of master-batches was found to be closer to that of the corresponding dry mix.

Table Chem. 6. **Carbon black dispersion**

Sample name	Filler dispersion (X) dispersion (Y)	Agglomerate dispersion	% of carbon black
S2	8.48	9.68	97.24
S3	8.04	9.89	99.48
Control	8.23	9.9	99.18

3.1.3. Silica reinforcement of NR

Compared to carbon black, silica has poor interaction and dispersion in non-polar rubbers.In contrast to carbon black, the surface of precipitated silica is highly polar and hydrophilic due to the presence of silanol groups. In order to improve the compatibility of silica with non-polar rubber and to ensure its good coupling to the polymer matrix, silane coupling agents are to be used.

Silica with silane coupling agents have enabled tyre manufacturers to satisfy the 'magic triangle of tyre technology' where low rolling resistance of a tyre and improved wet grip for easy handling has been achieved while maintaining the abrasion resistance of the tyre. Mixing of silica in hydrocarbon rubbers in the dry state is difficult and is an energy intensive process. Dry mixing also causes pollution problems in the factory floor. Hence as an alternative to dry mixing, NR latex - silica master-batch was attempted. Six latex based master-batches of NR - silica were prepared from preserved fieldlatex. NR –Latex silica master-batches are denoted as M1 to M6 and dry mixes are denoted as D1 and D2. Formulation of the compounds prepared from the master-batches and dry mix are given in Table Chem.7. Silica filled latex based master-batches and the dry master-batches were treated with 6 per cent silane (TESPT), based on the silica weight and the composites were heat treated at 130 °C for five minutes in the Rheocord. Further compounding of the composites as per Table Chem.7 was done in the two-roll mixing mill. Cure characteristics of these compounds were determined using RPA -2000. Tensile and technological properties were determind as per the corresponding ASTM / DIN standards. Cut growth resistance (using Dematttia flex tester) of various composites are given in Table Chem.8.

Table Chem.7. **NR latex–silica masterbatch - formulation of mixes**

Ingredients	Compound number							
	M_1	M_2	M_3	M_4	M_5	M_6	D_1	D_2
NR	100	100	100	100	100	100	100	100
Silica	33	38	50	55	66	72	50	55
ZnO	4	4	4	4	4	4	4	4
Stearic acid	2	2	2	2	2	2	2	2
Mernox 6C	2	2	2	2	2	2	2	2
Naphthenic oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Plasticizer (Vegetable oil based)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
CBS	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DPG	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sulphur	2	2	2	2	2	2	2	2

Table Chem.8. **Comparison of cut growth resistance**

Property	Compound number							
	M_1	M_2	M_3	M_4	M_5	M_6	D_1	D_2
No. of kilo Cycles to crack up to 12mm	36	35	30	29	28	25	27	24

3.2 Recycling of rubber: Devulcanisation of used rubber products

3.2.1 Stable free radical assisted mechanical devulcanisation of carbon back filled natural rubber

The effect of type of carbon black filler and the amount of the same in the NR vulcanisates to be de-vulcanised was studied using the formulation given in Chem.Table 9. The vulcanisates prepared in the lab was mechanically devulcanized in a two roll mill with and without the presence of optimum amount of a stable free radical; 4-hydroxy TEMPO (4HT).

Table Chem.9. **Formulations of the original samples with varying filler type and filler quantity**

Ingredients	Original compound (phr)		Revulcanisation (phr)
	Filler type	Filler quantity	
Natural Rubber	100	100	10
Devulcanised rubber	-	-	100*
Carbon black	40@	20@@	-
Naphthenic oil	4	2#	-
ZnO	5	5	3
Stearic acid	2	2	2
TDQ	1.5	1.5	1.5
CBS	0.6	0.6	1.5
Insoluble sulphur	2.5	2.5	0.8

*Rubber hydrocarbon (RH) =50
@ Filler type was varied as 40phr of ISAF, HAF, FEF and GPF blacks
@@ HAF amount was varied as 20phr, 30phr, 40phr, 50phr and 60phr
#Oil was varied as 2phr,3phr, 4phr, 5phr and 6phr respectively for increasing filler content from 20phr to 60phr

The devulcanised rubbers were revulcanised as per the formulation given in Table Chem.9. The revulcanisate properties of the samples mechanically devulcanised with stable free radical (ISAF 4HT DV *etc.*) are significantly higher, when compared with the control samples devulcanised without 4HT (ISAF DV *etc.*). The revulcanisate tensile strength showed a marginal decrease with increase in particle size of the carbon black used to reinforce the original sample. A profound impact on the tear strength of the corresponding revulcanisate was noticed for the 4HT assisted devulcanisation. It was noted that, irrespective of the particle size of the filler, the tear strength of the revulcanisates are comparable and is comparable to the tear strength of the original GPF filled sample in stable free radical assisted devulcanised samples.

Studied the effect of amount of HAF black in original sample on revulcanisate properties of the corresponding devulcanised rubber. The amount of carbon black ranging from 20 phr to 60 phr with an increment of 10phr was studied. The general observations that can be made from the revulcanisate properties are:

(i). The tensile strength and elongation at break of the original samples decreased and the modulus values and tear strength increased with increasing HAF loading.

(ii). The revulcanisate properties of the 4HT assisted devulcanised samples were significantly higher than that of the mechanically devulcanised samples.

3.2.2 Cure charecteristics of devulcanised rubber: the issue of low scorch

The issue of low scorch associated with the revulcanization of devulcanized rubbers was also addressed. Based on the earlier reports that solvent extraction of devulcanized rubber does not improve the scorch time during revulcanization, the role of activators forming zinc bound non-extractable moieties was investigated using activator-free vulcanizates, which disproved the role of such moieties. Investigations on the role of revulcanizing agents showed that curing of devulcanized rubbers is associated with low scorch, irrespective of revulcanization formulation. Cure characteristics of devulcanised rubber originally vulcanised using peroxide is similar to typical sulphur/sulphenamide cure behaviour. This confirmed that low scorch of devulcanised rubber is related to the initial sulphur cure system used for the preparation of original sample.

The ability of PVI to induce an induction time before revulcanization of devulcanized samples proved that the MBT generated from initial crosslink formation from the unreacted crosslink precursors in the original sample is the cause of low scorch time during revulcanization of devulcanized rubbers. This led to the conclusion that the issue of low scorch during revulcanization of devulcanized rubbers can be tackled with the aid of PVI, if the devulcanized rubber is revulcanized as such without adding any virgin rubber. In the presence of incorporated virgin rubber, scorch time can be maintained only if the concentration of PVI is in enough excesses to maintain adequate or required concentration of PVI in the vicinity of devulcanized rubber which is not a practical proposition. This means that addition of devulcanised rubber or crumb rubber powder in rubber compounds can lead to reduction in scorch time depending on the amount of the devulcanised rubber in the blend.

3.2.3. Patent application

An Indian patent application titled "Stable free radical assisted mechanical devulcanisation of rubbers" was filed.

4. Collaborative project

Collaborative study with regard to the projects NR latex -carbon black masterbatch and DPNR are in progress.

5. Development / advisory work / project work

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

b. Latex /dry rubber testing – 480 Nos.

TECHNICAL CONSULTANCY DIVISION

The major activities of the Technical Consultancy Division are testing/certification of rubber products as per relevant standards *i.e.*, ISO, BIS, ASTM, EN, ASRTU *etc.* in addition to R&D activities. The Division also conducts research projects for the benefits of industries in the micro, small and medium (MSME) category. Research projects were undertaken in industrially important areas. Major research projects undertaken are summarised below. As part of the academic collaboration between Tripura university and RRII, the division has conducted two weeks training cum practical classes for the BVoc. Students of the former and provided course materials.The laboratory has NABL accreditation since November 2016.

1. Research Projects

1.1. Studies on preparation, characterization and evaluation of micron to nanosized dispersions in latex

In latex industry, the chemical dispersions required for latex product manufacturing are usually prepared by ball-milling. The performance of the products is highly dependent on particle size as well as the stability of added dispersions. The objective of the present research work was optimization of process parameters for the preparation of aqueous dispersions of latex compounding ingredients. Accordingly the process parameters such as ball size, milling time and concentration of the surfactant were optimized. The degradation behaviour of silica and titania nanoparticles incorporated natural rubber latex (NR) nanocomposites against various degrading agents *viz.* thermal, γ-radiation, UV radiation and chlorination were studied. The addition of nano silica and nano titania in latex formulations improved the degradation resistance of latex films. Starch nanocrystals (SNC) were isolated from modified corn starch and its efficacy as a potential bio-filler for natural rubber latex was explored. The barrier properties of latex films were improved with the addition of SNC suspension. Graphene derivatives such as exfoliated graphene oxide (XGO) and reduced graphene oxide (RGO) were prepared and characterized by various analytical and spectroscopic tools. The addition of RGO in RVNRL matrix yielded significant improvement in electrical conductivity and theoretical predictions showed random distribution of filler in the polymer matrix.

1.2. Studies on peroxide vulcanization of natural rubber

Organic peroxides are commonly used as alternatives to sulphur in the vulcanization of elastomers. In this study the kinetics of peroxide vulcanization has been studied at various temperatures. Thermal decomposition of peroxide followed a first order free radical decomposition

reaction. The role of various coagents in the peroxide vulcanization of naturely rubber was studied by Fourier transform infrared (FTIR) spectroscopy. Crosslinking mechanism of peroxide in NR and the predominance of hydrogen abstraction over radical addition have been established. An attempt has been made to identify a suitable coagent for NR so as to minimize the present problems associated with peroxide cured NR-vulcanizates.

A statistical method, response surface methodology, was used for assessing the role of various compounding ingredients used for the peroxide vulcanization of NR. A face centred central composite design (FCCD) was used to obtain the relationship between vulcanizate properties and the level of ingredients. Regression equations were generated to model the properties of interest and response surfaces and finally contour diagrams were plotted.

The thermal stability and thermal degradation kinetics of natural rubber layered silicate nanocomposites have been analysed by thermo gravimetric analysis. Among the different organoclays selected, the highest thermal stability was exhibited by MMT-M2HT.

1.3. Technically specified rubber from preserved field latex

Studies were conducted to develop technology for the processing of preserved field latex into technically specified rubber. Experimental trials were carried out and the latex was processed in to high quality block rubber like Indian Standard Natural Rubber-3L (ISNR 3L), ISNR 3CV and ISNR 5 grades. There is a demand in the manufacturing sector for these higher grades of ISNR for critical products and it fetches a premium price over standard RSS sheet grades. There is a niche market for high quality TSR in the country. This technology can be transferred to processing industries for production of higher grades of ISNR.

1.4. Development of viscosity modified natural rubber using chemical peptisers

Indian Standard Natural Rubber (ISNR 3CV) with a lower Mooney viscosity below 60 units is one of the most value added forms of NR marketed in the country. This was processed from latex by proper collection and processing within a stipulated period. Chemical peptisers are used in the mastication process to lower the viscosity of NR (dry rubber form) in product manufacturing units. In this study, latest chemical peptisers like Struktol A 89 and Struktol HT-107 were used to obtain low viscosity rubber suitable and ready to use for industries. Field latex was collected from plantations and optimum quantity of peptisers (0.15phr) was added as dispersion and the contents were coagulated using formic acid. The coagulum was allowed to mature for about three hours and then processed into technically specified rubber. Mooney viscosity of the rubber can be reduced to the required level which is beneficial to the consuming industry.

1.5. Analysis of hydrocarbon content in synthetic rubber products

Thermogravimetric analysis of elastomers containing a heteroatom such as chlorine produces a char or carbonaceous residue which is stable at 600°C in inert atmosphere and gets oxidised in air or oxygen atmosphere. While calculating the rubber hydrocarbon content, the carbonaceous residue should be accounted. The following conclusions are also drawn from the study. 1) Theoretical rubber hydrocarbon content (RHC) as per formulation and RHC obtained after experiment are to be matched. 2) As the unsaturated terpolymers of ethylene propylene rubber (EPDM) content in the blends increases, the heat resistance also increases. 3) As the

EPDM content increases the carbonaceous residue in the blend decreases. This is clear from Figure TC.1

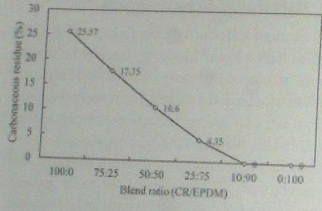


Fig. TC. 1. **Variation in carbonaceous residue with CR content in CR: EPDM blends**

1.6. Comparative study of the latex products from prevulcanized field creamed latex and cream latex compound

Effect of creaming on prevulcanised field latex (PFL) and unvulcanized field latex (UFL) was studied. The effect of total solid content (TSC) during creaming was monitored at regular intervals in both the latices. A comparison was made between prevulcanised field creamed latex (PFCL) and creamed latex compound (CLC). Here CLC was the conventional latex compound or the control compound.

In the case of PFCL and CLC the tensile strength and modulus (stress) decreased after ageing. However before ageing, modulus was higher in PFCL than in CLC. The maximum modulus values in PFCL indicated higher level of cross linking. All these are shown in the stress–strain graph (Fig. TC. 2). After a particular period due to over-vulcanization and the consequent restricted coalescence of rubber particles a decrease in modulus was observed.

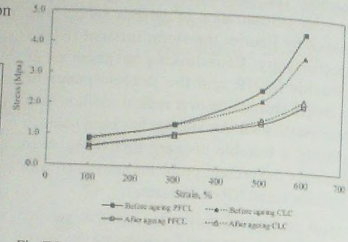


Fig. TC.2. **Stress- Strain graph of prevulcanised field creamed latex (PFCL) and creamed latex compound (CLC)**

1.7. Effect of latex processing techniques on protein content and particle size

Field latex had high content of protein than all concentrated forms of latices. In cast and coagulated films the trend observed was field latex>creamed latex > HA latex> LA latex.

In continuation with the above study, storage study of preserved field latex (PFL) at different dosages of ammonia also was conducted. Preserved field latex had higher particle size than fresh field latex. One per cent ammoniated PFL had higher extractable protein content, particle size and lower viscosity than 0.5 per cent ammoniated PFL.

1.8. Finger-printing of natural rubber latex

Adulterations made in minute quantities are difficult to detect in natural rubber latex. Such adulterations very often cause high rejection rates in products like gloves, condoms, elastic threads *etc*. Quality control of latex using particle size analysis is an ideal means of overcoming such difficulties. It was observed that adulteration is more detectable if the adulterated latex was subjected to mechanical destabilization followed by particle size analysis. The combined

techniques of DSC, TGA and FTIR with particle size analysis would be highly beneficial to find out adulteration.

1.9. Natural rubber field latex based adhesives

The role of dry rubber content and the size of rubber particles in the latex on adhesive properties of ammonia preserved natural rubber (NR) field latex were studied. The effect of particle size on the adhesive properties of NR latex was confirmed by comparing the adhesive peel strength of centrifuged latex and its combinations with skim latex, field latex and water. The peel strength of centrifuged latex was improved by the addition of both field latex and skim latex. Dilution with water could not improve the peel strength of centrifuged latex. Blending of centrifuged latex with both skim latex and field latex increased the share of small sized particles in centrifuged latex (Table TC.1). It is concluded that both dry rubber content and small sized particles in the latex play significant role on the adhesive properties of NR latex-based adhesives.

Table. TC.1. **Comparison of particle size distribution and peel strengths of centrifuged latex and its various combinations**

Types of latex	Particle size (Volume density, %)			Peel strength
	<500 nm	500-1000nm	>1000 nm	(N/mm)
Centrifuged latex (60% DRC)	46.2	39.0	14.8	2.1
Centrifuged latex- field latex blend (45% DRC)	51.1	38.4	10.6	3.1
Centrifuged latex- skim latex blend (45% DRC)	50.0	38.8	11.2	2.8
Centrifuged latex diluted with water (45% DRC)	47.5	39.1	13.5	2.2

2. Testing and certification

For the testing of rubber compounds/ products and raw materials, consistent support and a large number of small and medium level entrepreneurs availed this service. Total number of samples tested and the revenue collected during the reporting period are given in Table TC. 2.

Table TC. 2. **Total number of samples tested and the revenue collected during the reporting period (2016-17)**

No. of clients	511
No. of samples tested	1051
No. of parameters analysed	5104
Consultancy letters/e-mails	1255
Trouble shooting of factory process	17
Component analysis	206
Total revenue collected (Rs.)	22,91,134.00

3. Product development

Services to new entrepreneurs as well as existing rubber based industries were provided in the development of rubber products based on both synthetic and natural rubber. In this area, the services given are categorised as follows (Table TC.3).

Table TC.3. **Know-how transfers to industries**

Type of rubber products developed	Number
Adhesives	3
Automobile components	22
Fire resistant sealings and mats	2
Latex based dipped and foamed goods	3
Pharmaceutical closures and agitator bush as per US FDA spec	2
Pre-cured tread, bonding gum and tube valve (ASRTU specification)	5
Rubber based agro-machinery components	2
Rubber based engineering components for railway, defence, BSF, Kochi Metro *etc.*	1

List of major clients who availed the services of the division are listed below.

- Apollo tyres, Kochi
- BPCL, Kochi
- Fluid Control Research Institute, Palaghat
- Good Way Rubber Industries, Malaysia
- Indian Railways, Ranchi
- Kochi Metro
- MRF tyres, Chennai
- Naval Dockyard, Mumbai
- Over 500 numbers of small, medium and large scale industries in India
- Rubber Seals Limited, Saudi Arabia
- VSSC, Thiruvananthapuram

4. Project profiles/Technical bulletins

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

5. Advisory services

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

6. NABL Accreditation

Technical consultancy laboratory received NABL accreditation as per ISO/IEC 17025: 2005 on 25/11/2016.

ECONOMICS DIVISION

The Division conducts research in the broad areas of farm management, primary processing and marketing, rubber products manufacturing industry and foreign trade and intercrops and by-products. Inter-divisional collaborate projects were also undertaken for comprehensive understanding of the rubber sector.

1. India's tariff policies on rubber and rubber products under regional trade agreements

The study was an attempt to provide thorough understanding about the duty concessions offered by India to different types of rubber and rubber products under various regional trade agreements to the member countries. All the 174 tariff lines (8 digit level of HS) of rubber and rubber products were classified and tabulated based on the tariff policy pursued by India under different regional trade agreements. A compendium on product-wise tariff policies of India on rubber and rubber products under various RTAs was prepared and published.

2. Impact of Regional Comprehensive Economic Partnership (RCEP) on India's Rubber and Rubber Products: An ex-ante analysis

Regional Comprehensive Economic Partnership (RCEP) Agreement is an agreement on the anvil between the ten member countries of ASEAN and its six Free Trade Agreement partners namely, Australia, China, India, Japan, Korea and New Zealand. An analysis of export potential of India's rubber and rubber products to RCEP member countries was made. The study assumes importance in the context of membership of most of the major NR producing and consuming countries in the world under this agreement.

The benefit due to trade diversion for India's rubber and rubber products in the RCEP region is limited to the product groups HS 4002, HS 4004 and HS 4005. During the year 2016 these three product groups together constituted only less than five per cent of the total export of rubber and rubber products to the RCEP region. Moreover, the items under these product groups are raw materials (HS 4002) and raw materials with minor value addition (HS 4004 and HS 4005) and are used as intermediate products. Conversely, the three major product groups of rubber and rubber products exported from India to the RCEP region during the year 2016 were HS 4011 (58.0 %), HS 4016 (12.0 %) and HS 4003 (6.1 %). While India has comparative advantage in the RCEP region in the case of HS 4011, HS 4016 exhibited comparative disadvantage. Among the three product groups HS 4011 and HS 4016 did not show any regional orientation but HS 4003 exhibited regional orientation towards the RCEP. These three product groups together constituted more than 76 per cent of India's export of rubber and rubber products to the RCEP region.

3. Strategies for rejuvenation of Rubber Producers' Societies in Kerala

A study was conducted to examine the reasons for poor/non-performance of Rubber Producers Societies (RPSs) and device strategies to address the same.The study was conducted in Kottayam district. The criteria for classification of RPSs as followed by the Rubber Production (RP) Department were used in the study. RPSs under Category A and B were only considered for the study. The study addressed ways to

improve the performance of category B RPSs to become category A and the low performers in category A to become better performers. Existence of a group processing facility was the prime criterion which differentiated Category A and B RPSs in the study area. For enhanced resource use efficiency and to improve the income of farmers under Category B the possibility of sharing non-functional GPCs among RPSs was proposed. The importance of group processing of sheets in RPSs for producing good quality sheets for realising better farm gate price and the need to sensitize the farmers/RPSs on the same were highlighted. While majority of the RPSs limited their role as stockists and distributors of farm inputs, RPSs under category A having labour banks went a step ahead by organising input application in member farmers' fields. Only 20 per cent of RPSs studied had labour banks. Though SHGs existed in 67 per cent of the RPSs visited, only 20 per cent were functioning properly. The study also revealed a decline in the share of members actively engaged with the RPSs. Since success of RPSs to a large extent depends on active participation of members and commitment/ leadership qualities of the executive committee members, the study suggests identification and encouragement of dedicated farmers and youth having leadership qualities and commitment to serve the community, to participate in management of RPSs. To keep pace with the changed socio-economic scenario, the study advocates diversification of activities of the RPSs to qualify as complete farm service providers.

4. Adoption of Low Frequency Tapping (LFT) by the smallholder sector in Kerala: A Socio-Economic Analysis

Though *prima facie* LFT is packed with advantages, its adoption by the smallholder sector of the state is negligible in the past two decades, despite efforts by the Board to popularize it. The tappers census conducted earlier had revealed that only 5.1 per cent of the tappers employed in the smallholder sector of the state practiced LFT. In this backdrop, during the reporting period, the Division initiated a research project to identify the reasons for low adoption of LFT in the smallholders sector in Kerala and the project is underway.

5. Shortage of rubber tappers in the small holders sector of Kerala: An explorative analysis

During the reporting period the Division initiated a project to identify the reasons for the scarcity of rubber tappers in the smallholders sector of Kerala. The first tappers census conducted by the Division revealed existence of huge demand supply gap of tappers in the sector. The study would be helpful to understand various aspects of the shortage of rubber tappers confronted by the rubber smallholdings in Kerala and suggest policy options to overcome the shortage. The study is progressing.

6. Commercial yield performance of *Hevea* clones in Tripura

The commercial yield performance of *Hevea* clones in the NE state of Tripura was evaluated to assess the performance of *Hevea* clones in the smallholding sector in the state. It was observed that growers of South Tripura were getting higher yield as compared to the growers of West Tripura. The yield data from 1st to 22nd year of tapping showed a maximum yield of 1987 kg ha^{-1} during 6th year of tapping in South Tripura and the average yield during the 1-22 year period was 1418 kg ha^{-1} in South Tripura. The average yield during the 1-22 year period realized by the growers in West Tripura was 1268 kg ha^{-1}. It was also found that except during the yield

increasing phase the yield in growers' farm was lower than the experimental yield.

Consultancy services /other works

1. Prepared a detailed note for the submission to the Ministry of Commerce and Industry on the article titled "Asean-India free trade agreement and India's balance of trade in rubber and rubber products: a preliminary assessment" that appeared in the Business Line on 18-05-16.
2. As directed by the Chairman a brief note on Natural Rubber Exports from India was prepared and submitted.
3. A detailed note on ASEAN India Free Trade Agreement (AIFTA) for the Parliament Committee was prepared and communicated to the Planning Division, Rubber Board.
4. Estimated the timber volume and upset values of 42422 rubber trees in 14 blocks of the State Farming Corporation of Kerala Ltd., Punalur, 832 trees in three plots at CES Chethackal, Rubber plantations of Devaswom Board, Mundakkayam and 863 trees in RRII farm.
5. Associated with Rubber Production Department, Rubber Board in the preparation and submission of "Project proposal for registration of rubber nurseries/certification of planting materials".
6. Received an external funded project for Rs. 4.9 lakhs for the project "Evaluation of Pollination Support through Beekeeping" from State Horticulture Mission, Kerala.
7. As directed by the Chairman, Rubber Board, finalised the following projects for submission to the Government of Tripura.
   i. Project proposal for setting up knowledge resource centre in NR sector of Tripura
   ii. Project proposal for productivity enhancement of rubber plantations in Tripura through rain-water harvesting and rain-guarding
   iii. Project proposal for promoting multi-cropping in NR plantations in Tripura
   iv. Special project for skill development on harvesting of natural rubber and processing of latex under PMKVY in Tripura
   v. Special project for productivity enhancement in NR sector through up-skilling latex harvest technicians under recognition of prior learning (RPL) programme under PMKVY in Tripura

QUALITY CONTROL DIVISION

The Quality Control Division which was functioning under the P&PD Department was brought under the Research Department with effect from December 2016 for the effective functioning of the Central Quality Control Laboratory and provide synergy for technical consultancy work.

The Central Quality Control Laboratory, established in 1980, 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. The laboratory is ISO:17025 certified and accredited by NABL for performing various tests in natural rubber and water. 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 of the following items on payment basis.

(i) Preserved Field Latex

(ii) Concentrated latex (Cenex and Creamed)

(iii) Dry rubber (ISNR,RSS, EBC, Skim and Crepe)

(iv) Chemicals used in rubber processing, rubber product manufacturing and plant protection.

(v) Fertilizers and organic manures

(vi) Effluent water from rubber processing units

(vii) Drinking water and water for civil construction

Table QC 1. **Details of samples tested during the period 2016-17**

Sl. No.	Type of samples	Number of samples	Number of parameters
1	Field latex	7063	7150
2	Dry rubber	2913	10336
3	Concentrated latex	253	1801
4	Water	1009	8318
5	Effluent	179	1186
6	Chemicals	304	552
	Total	11721	29343

An amount of Rs. 25,12,006/- was collected as testing fee during the year.

2. Specification section

2.1. BIS scheme of testing and Inspection

The Bureau of Indian Standards (BIS), in association with the Rubber Board, is operating a Scheme of Testing and Inspection (STI) for block rubber and centrifuged latex. A processing unit who becomes a member of this scheme is licensed to use the BIS standard mark (ISI) on his produce which conform to standards prescribed in the relevant IS Specifications.

The officers of BIS and Rubber Board (Specifications officers) conduct periodic inspection to ensure proper maintenance of quality in the processing units. So the BIS Certification mark stands as a token of assured quality. The product with the certification mark will be welcomed in the market. The consumers of raw rubber covered by BIS certification can get protection from exploitation and free replacement in case the product is found to be of substandard quality.

For implementation of the Scheme of Testing and Inspection (STI), BIS has appointed Rubber Board as their agent and 66.7 per cent of the marketing fee collected is paid to the Rubber Board. The scheme covers the natural rubber processing units in the four states, *viz*. Kerala, Tamil Nadu, Karnataka and Tripura.

Inspections were conducted during the period under BIS scheme and an amount of Rs. 6,28,607/- was received as share of marking fee from BIS.

2.2. Preliminary inspection to issue BIS certification License

A processing unit who intends to become a member of STI has to apply to BIS to get license to use the ISI standard mark on his produce. A preliminary joint inspection by the officers of BIS and the Rubber Board is conducted prior to issue of License by the BIS. One such inspection was conducted during the year.

2.3. Quality control inspections at rubber processing units (not covered by BIS's STI)

Random surprise inspections are 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 :38

Dry rubber	:27
Latex	:11

2.4. Quality enforcement for TSR

Under the rule 48 of the Rubber Rules 1955, every processor shall grade and market his products in conformity with suchstandards 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.

For the enforcement of the above rule in the TSR sector, the Rubber Board decided to introduce compounding of offences for violation of quality norms by the processors with effect from 1st January 2013. The compounding amount is Rs. 15,000/-. For the first offence and will increase in arithmetic progression for repetition of offences till the 6th offence before revocation of the license of the processor.

Eight latex centrifuging units were compounded for violation of quality norms during the financial year and an amount of Rs.1,65,000/- was collected from these units.

2.5. Import of natural rubber

It is mandatory that natural rubber imported to India shall conform to Indian Standard Specifications or any other National or International Standard Specifications. NOC for import of natural rubber for own consumption by manufacturers having valid Manufacturers' License (M license) is issued by Rubber Board, provided all the documents *viz.* Bill of Entry, Invoice, Test Certificate and the request from the importer are in order. Inspections are carried out at random to ensure conformity with these standards. However, in the case of first time import, inspection of the consignment/collection of samples for quality check is always carried out by an authorized Officer of the Board prior to the issue of NOC. A total of 65 inspections were conducted during the year and this includes inspections by officers from other offices also.

Table QC. 2. **Details of NOC issued for import of NR during 2016-17**

Sl. No	Type of NR	Quantity (MT)
1	Block Rubber	362301.385
2	Sheet Rubber	70902.542
3	Crepe Rubber	115.2
4	Latex	1112.05
5	Others	25.6
	Total	434456.777

No. of consignments of import : 4401

2.6. Export of natural rubber

Rubber Board conducts quality checking to ensure the quality of rubber exported from the country. NR can be exported either under the brand name "Indian Natural Rubber" with logo or as an individual export without logo. Consignments for export are inspected and samples drawn by the officers of Market Promotion Department. All samples are analyzed at the Central Testing Laboratory for conformity with BIS Specifications. Quality certificates are issued based on test results. This procedure is applicable to both block rubber and concentrated latex.

Details of samples tested at the Central Testing Laboratory are given below.

Latex samples - 120
TSR samples - 106

3. Other activities

3.1. Training programmes of Rubber Training Institute

Officers of the Division functioned as resource personnel in various training programs organized by the Rubber Training Institute.

3.2. Training programmes to University students/ Industry personnel

(i) Imparted training to M.Tech (Polymer Technology) students of Cochin University of Science and Technology.

(II) Conducted practical examination for B.Tech (Rubber and Plastic Technology) students of Madras Institute of Technology, Chennai.

3.3. Advisory work

Natural rubber processors approach the Division frequently for advice on quality improvement, reducing wastage, reducing cost of production and better environment management systems *etc.* Individual cases are studied and appropriate measures are suggested.

CENTRAL EXPERIMENT STATION, CHETHACKAL

The Central Experiment Station (CES), Chethackal is situated at a distance of about 50 km from Kottayam. The Station was established to cater the research needs of the different Divisions of RRII. The Station has a total land area of 254.76 ha which is planted for different research projects. The total tapping area is 174.8 ha and immature area is 24.58 ha. Bud wood nursery and close planting was established in 20.35 ha during the current year.

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, intercropping systems, reduction in cost of cultivation, low frequency tapping systems *etc*. Total number of trials is 85.

During the reporting period, the total crop realized was 1,14,687 kg. Total of 298 tapping days were possible in the year and 70 tappers were engaged for tapping. The total man-days engaged were 47882 days. The Station having 193 permanent workers is managed by Officer-in-Charge with 37 staff for office administration, farm management, dispensary, security and canteen. The dispensary with full-time doctor provides medical care to the workers. The total number of patients attended to during the period under report was 4142.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

1. Crop improvement

1.1. Performance of clone RRII 429

Girth of RRII 429 and RRIM 600 were on par. Yield over four years showed that it was significantly higher in RRII 429 by around 27 per cent than RRIM 600 under the agroclimate of Goalpara, Assam (Table Ghy. 1). Yield during 2016 was poorer than that of other years due to frequent rain, lack of rainguarding and more number of late tapping.

Table Ghy. 1. **Yield of RRII 429 compared to RRIM 600**

Clone	Mean yield (kg ha^{-1})			
	2013	2014	2015	2016
RRII 429	1339	1731	1863	1292
RRIM 600	1074	1355	1453	1020
T-stat	3.5	5.6	4.7	5.5

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

The best three selected ortets from Assam were cultivated under the agroclimate of Morigaon, Assam to evaluate the performance of these three primary ortets *viz*. RRSG 9, RRSG 3 and RRSG 1 (Table Ghy. 1). RRIM 600 and RRII 429 were the check clones. Girth of ortets at 30 cm height was at par with RRIM 600 after 20 months of planting. Girth of RRSG 3 ranked first followed by RRSG 1 but it was low in RRSG 9 due to severe casuality after 12 months from planting.

Table Ghy. 2. **Growth of ortets in growers' field after 20 months of field planting**

Clones/Ortets	Mean girth (cm)
RRSG 9	7.5
RRII 429	9.2
RRSG 1	10.4
RRSG 3	10.6
RRIM 600	8.8
CD (P=0.05)	NS

1.3. On-farm evaluation of potential clones / ortets under the agroclimate of Arunachal Pradesh

In trial I initiated during 2015-16, planting of 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 was completed. In trial II, also initiated during 2015-16, budding 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 further planted in polybags.

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The activities of the Station were focused mainly on evaluation of clones, crop management, latex harvesting and ecosystem studies. Advisory services on discriminatory fertilization to growers and analysis of dry rubber content of latex samples for industries were also continued.

1. Crop improvement

The development of location specific clones, evaluation of promising clones and the standardization of DUS norms projects 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 location specific clones to identify promising clones for the region. To develop location specific clones for this region, 131 locally recruited clones *viz*. 20 hybrids, 21 OP progenies and 90 half-sib progenies are under evaluation in six clonal and seedling nurseries.

1.2. Evaluation of clones for their adaptability and yield performance

Two On Farm Trials (OFT) and two Large Scale Trials (LST) initiated for the purpose are in progress. Based on evaluation, 15 clones *viz*. DD/6/16, DD/6/5, RRSA 114, RRSA 121, RRSA 585, RRSG 248, RRST 37, 98/38, 98/46, 99/1/24, 99/5/9, RRII 208, RRIM 600, RRSA 98 and RRSA 315 were planted during 2015 in LST. In another LST (GxE) trial, clone RRII 429 (66.7 g t^{-1} t^{-1}) and RRII 417 (59.1 g t^{-1} t^{-1}) were observed to have higher yield than RRIM 600 (54.7 g t^{-1} t^{-1}) (Table 1) during 2016-2017.

High yielding clones are under evaluation in one mature and one immature OFTs, in which RRII 429 recorded the highest mean yield (35.4 g t^{-1} t^{-1}) that was above RRIM 600 (33.3 g t^{-1} t^{-1}) in the second year of tapping in OFT, Pathalia (2005). Clone RRII 429 had the highest girth (54.1 cm) in another OFT, Hirapur (2009).

Field trial involving 57 clones for the identification of reliable juvenile and mature characteristics for clone identification with regard to standardisation of DUS testing norms is also in progress. A germplasm garden having 213 wild *Hevea* accessions, source bush nurseries and a breeding orchard is being maintained in the Station.

Table Agar. 1. **Yield performance of 13 clones in LST (1996)**

Clone	Mean yield (g t^{-1} t^{-1}) 2016-17	Mean yield (g t^{-1} t^{-1}) over 14 years
RRIM 600	54.7	47.2
RRII 429	66.7	53.8
RRII 203	45.1	36.6
PB 217	41.9	35.0
RRII 51	39.2	31.0
RRII 414	47.2	37.2
RRII 430	46.9	45.2
RRIC 100	46.7	42.2
RRII 422	49.0	53.2
RRII 105	45.4	42.4
RRII 417	59.1	48.8
RRII 176	55.1	40.1
PB 235	40.1	39.9
Mean	49.0	42.5
CD (P=0.05)	11.2	4.5

2. Crop management

2.1. Soil fertility mapping

Studies on soil fertility status of rubber plantation in Tripura was continued to develop fertility maps for macro and micro nutrients. During the reporting period, 178 geo-referenced soil samples were collected and 73.9 per cent of these soils were medium in OC status and 82.3 per cent were low in available phosphorous.

A demonstration plot on influence of silt pits on soil conservation and latex yield of mature rubber was initiated at Taranagar farm. The gradient of the field was 5-7 per cent and 200 pits ha^{-1} with a pit size of 120 x 45 x 60 cm^3 were dug for soil and water conservation. Upon extrapolation of preliminary data, it was estimated that about 5.5-7.1 t ha^{-1} of soil could be conserved annually under rubber plantation in Tripura.

2.2. Development of cropping system and management practices

In the experiment on evaluation of cropping system model, the girth of rubber after nine years were 53.9 and 53.5 cm for the intercropped plots and 54.6 and 55.2 cm for monoculture in Model I and II, respectively. Intercropping was carried out during the entire immaturity period in Model I in paired row system and for five years in rectangular planting system in Model II. Among all the crops cultivated under immature rubber, BCR for colocasia (3.3) was found to be the highest followed by banana (2.5) and cowpea (2.0) indicating the economic feasiblility for growing these crops as intercrops during the immature phase of rubber. For the intercropping trial with fodder, the herbage yield was the highest for guinea grass (437 q ha^{-1} $annum^{-1}$), followed by stylo with signal grass (405 q ha^{-1} $annum^{-1}$). These three crops are recommended for growing under immature rubber in North East region.

For the zero tillage experiment, the girth data indicated that pits of larger dimensions had no advantage over smaller pits on growth of plants. The girth was 34.2, 34.1 and 32.8 cm for conventional tillage, zero pit (polybag) and zero pit (root trainer), respectively in the fifth year. The comparative labour cost analysis of pitting normal pits (size 75x75x75 cm) and small pits [60 cm (depth) with 25 cm (diameter)] for accommodating polybag plants showed that

around 66 per cent saving can be made by planting in small pits and 80 per cent saving can be made by planting in still smaller pits [55 cm (depth) with 15 cm (diameter)] for root trainer plants.

In the experiment on development of specific package of practices, the mean girth of rubber in vertical mulching (40 cm) and conventional mulching (39.3 cm) were on par with control (38.8 cm).

3. Latex harvesting

The Station continued field experiments on low frequency tapping at Taranagar farm. In this experiment clone PB 235 was tapped under three tapping systems *viz*. d3, d4 and d6. Higher yield was observed in d3 system compared to d4 and d7 systems.

Response of different RRII 400 series clones to yield stimulation in two systems of tapping *viz*. d2 and d3 are being continued. Yield of d3 system of tapping with stimulation and d2 system with rest was comparable. Clone RRII 429 continued to be the highest yielder among the clones.

The experiment to evaluate the effect of planting density on two tapping systems (d2 and d3) was continued with clone RRII 429. It was observed that yield of d3 system was comparable with d2 system of tapping in all the densities. Higher yield was observed in high density planting compared to lower density.

4. Ecosystem studies

Initial modelling of potential distribution of guayule rubber was attempted for Mexico region using the species occurrence points of Arizona region of South America. Maxent model was simulated with modified species occurrence points shape files of guayule rubber in its native range in the background of global model simulation. Preliminary results indicated that the most suitable regions in India appear to be the foothills of the Himalayas in Jammu and Kashmir, Uttaranchal, parts of Sikkim and Arunachal Pradesh. Parts of Rajasthan and Gujarat states were found to be moderately suitable. More number of occurrence points of guayule rubber have been collected from GBIF database and other published literature.

Initial model simulation using Maxent interface was done with additional new variables such as annual mean radiation (Wm-2), highest weekly radiation, lowest weekly radiation, radiation seasonality, annual mean moisture index, moisture index seasonality derived by CSIRO and MIROC-H global climate models. Data downloading and pre-processing using ARC-GIS was carried out. Maxent model was successfully simulated with 35 variables with 10 min spatial resolution. Among the new variables, annual mean radiation (bio20) and mean moisture index (bio33) showed 5.9 per cent and 14.8 per cent contribution in *Hevea* model building in case of NE region. High resolution bioclimatic spatial data (bio1- bio19) was merged with coarse resolution data (bio20 – bio35) in the same model. However, effect of resolution merging was not realized in the pattern recognition process. Maxent modelling with 35 variables for NE region revealed that around 61600 hectares of land is best suited for *Hevea* plantation having the suitability index of 0.64 to 1.0. Around 95800 hectares of land showed good suitability with the similar index of 0.48 to 0.64. These results show that the response of climatic variables to distribution probability may vary with scale of the input data.

5. Advisory work

Discriminatory fertilizer recommendation based on soil analysis was offered to 82 rubber growers in the state of Tripura. A total of 270

latex samples were analyzed for drc and other latex parameters. Conducted six training classes for the rubber growers on evaluation of soil fertility, nutrient management and fertilizer application and method of soil/leaf sampling were demonstrated.

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 has been started at the Rubber Board campus, Dakopgre, Tura in two designs. Due to the acute fund shortage, the trial could not be managed properly and some plants are broken because of the dense bushes and weeds.

In another set of 2011 population, a total of 34 top yielders were selected on the basis of growth performance and juvenile yield and maintained in the field for further evaluation.

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

A set of 350 seedlings has been raised at Rubber Board campus, Tura for bud-grafting during August, 2017 with the selected bud-woods in 2008 trial.

1.3. Clonal nursery evaluation trial-(2010)

The experiment was completed during the year and research paper was presented during the 22nd PLACROSYM at CPCRI, Kasargod during December, 2016.

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. Tapping was started during August, 2016 in both the trials. Highest mean annual yield was recorded in PB 235 (36.5 g t^{-1} t^{-1}) closely followed by RRIM 600 (35.45 g t^{-1} t^{-1}) while minimum yield was recorded in RRII 203 (18.7 g t^{-1} t^{-1}). Maximum girth was recorded in PB 235 (53.7 cm) and RRII 429 (52.7 cm) while minimum girth was recorded in RRII 422 (48.0 cm) (Table Tura 1). In the West Garo Hills, highest mean plant girth was recorded in RRII 417 (42.6 cm) followed by RRII 422 (37.0 cm). Similarly, the highest girth increment was recorded in RRII 417 followed by RRII 422 (Table Tura 2).

Table Tura 1. **Growth of different clones (girth in cm) in the On-Farm Trial at Mendipathar, North Garo Hills**

Clones	Locations		
	Rabha	Momin	Mean
PB 235	53.2	54.2	53.7
RRII 417	47.5	54.6	51.1
RRII 429	49.2	56.1	52.7
RRII 422	46.3	49.6	48.0
RRII 203	51.5	53.6	52.6
RRIM 600	48.3	52.2	50.3
SD	2.6	2.3	1.9
CV (%)	3.66		

Table Tura 2. **Growth of different clones at Bolchugre, West Garo Hills**

Clones	Girth (cm)	Girth increment (cm)
RRII 417	42.6	7.6
RRII 422	37.0	6.9
RRII 429	32.7	6.5
RRIM 600	33.1	6.6
Mean	36.4	6.9
SD	4.60	0.50
CV (%)	12.6	7.20

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, and RRSs of Agartala, Guwahati and Tura and the selected bud woods are maintained in the nursery for further study.

1.6. Fundamental studies on the nature of wintering, flowering and seed germination in *Hevea* clones in Garo Hills of Meghalaya

This trial has been completed after the detailed study of nature of wintering and flowering pattern in the Garo Hills region of Meghalaya. This experiment was continued for five years in the 1985 clone evaluation trial. Data on visual observation were recorded.

1.7. Nursery evaluation of poly-clonal seedlings trial (2013 and 2014)

The poly clonal seeds collected from Poly Clonal Seed Garden, Mizoram were planted in the field during 2013 at two locations of the RRS *viz*. Ganolgre farm and Rubber Board campus, Dakopgre, Tura. Two years after planting, the plant had a height of 6.2 m and girth of 19.5 cm and the mean test tap yield of the population was 11.5 g t^{-1} 10 t^{-1} (with a range of 1.0 - 46.8 g t^{-1} 10 t^{-1}). On the basis of test tap yield, top 25 best performing progenies were selected and maintained in the bud-wood nursery at Rubber Board campus, Tura and five progenies were selected from RRS, Ganolgre farm for further evaluation.

1.8. Seedling evaluation for screening potential ortets under the agro-climatic conditions of Garo Hills of Meghalaya

The new set of evaluation of 200 seedlings was started at RRS, Ganolgre farm by selecting healthy seeds.

1.9. Germplasm Arboretum at Teksragre Farm

To maintain the 1st, 2nd, 3rd and 4th sets of Germplasm Arboretum under the agro-climatic condition of Garo Hills of Meghalaya, 534 poly bag plants (belonging to 178 Accessions) were planted in the field at Teksragre farm near Anogre, during September, 2016 and the plants were maintained. Six hundred and ninety two budded stumps belonging to 82 accessions and 10 control clones were planted in the polybag nursery in December, 2016. Sprouting success was very less (24.3%) probably due to the planting of budded stumps during winter period.

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 reasons for depression of yield and percent dry rubber content (DRC) in *Hevea* under the agro-climatic condition of Garo Hills. The annual mean yield (46.2 g t^{-1} t^{-1}) and DRC (33.1%) for the current year was recorded. Low temperature adversely affected the yield and DRC. Early defoliation and refoliation was observed and during winter DRC ranged from 28.4-29.8 per cent. Lowest soil moisture content was recorded in the months of February and March.

2.2. Study on controlled upward tapping system

Treatment wise monthly yield was recorded and six years mean data were processed and the result showed that under CUT, yield was higher (79.3 g t^{-1} t^{-1} or 2527 kg ha^{-1} $year^{-1}$) than the normal tapping system (S/2 d2 system) (55.9 g t^{-1} t^{-1} or 1780 kg ha^{-1} $year^{-1}$) of downward tapping in BI-1 panel. Difference in DRC was not seen/observed. An increase of 26.5-52.3 per cent was observed in yields under the CUT system compared to the normal tapping system under the agro climatic condition of Garo Hills of Meghalaya.

2.3. Shallow tapping – an option for stress alleviation in *Hevea* plantations during winter

There was no significant difference between treatments. Highest yield was recorded in normal tapping system (33.2 g t^{-1} t^{-1}) followed by normal continuous tapping (33.1 g t^{-1} t^{-1}) and LFT + normal tapping (31.7 g t^{-1} t^{-1}) and lowest was in shallow + normal tapping system (28.5 g t^{-1} t^{-1}). DRC was low in normal continuous tapping and was high in shallow + normal tapping system.

Normal continuous tapping system showed higher TPD (8.2%) followed by the shallow + normal tapping system (7.0%) and LFT + normal tapping (5.8%) and lowest was in normal tapping system (5.3%).

2.4. Location specific stimulant application

Ethylene induced stress response in the tapping panel of the *Hevea* trees was initiated with the aim to reduce the stress in tissues in the tapping panel. In RRIM 600 six treatments were adopted with bark applications of five per cent ethaphon. Results showed that maximum yield (44.6 g t^{-1} t^{-1}) and low DRC (33.3%) were recorded in T3 (Bark application of 5 per cent ethaphon at 150 cm above from bud union and near bud union and minimum yield (30.8 g t^{-1} t^{-1}) and high DRC (34.3%) was recorded in T6 (unstimulated trees). The difference in DRC between treatments was not significant.

3. Crop management

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

The results of the experiment on nutrient management for 11 years showed that the highest girth and yield were obtained in the treatment combination, $N_{60}P_{30}K_{45}$ kg ha^{-1} compared to other treatments.

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

Soil samples were collected each month at the depth of 0-15, 15-30 and 30-60 cm for soil moisture study. Soil moisture content increased with increasing depth. The highest soil moisture content was observed in September and lowest in January/February. Annual mean was 23.6, 24.4 and 25.5 per cent, respectively for 0-15, 15-30 and 30-60 cm depth.

3.3. Analytical/Advisory work for fertilizer recommendation

During the year, twenty surface (0-30 cm) soil samples from the rubber growing areas were analysed. Organic carbon ranged from 0.96 - 1.31 per cent, available phosphorus from 2.3-6.9 mg kg^{-1} and available potassium from 76.0-95.0 mg kg^{-1}. The soil was acidic in nature and pH ranged from 4.4-5.6. Discriminatory fertilizer recommendation was given accordingly.

3.4. Evaluation of soil fertility status and mapping of soil fertility in Meghalaya- A collaborative research project with Crop Management group of RRII, Kottayam

Initiated the project and collected 83 soil samples from the rubber growing areas of Garo Hills of Meghalaya (West, North, East and South-west district) using GPS system.

3.5. Generation of advance planting materials by in-situ budding on stocks raised in root trainers in the Garo Hills conditions of Meghalaya

The experiment on different potting media for root trainers was repeated with all the treatments for further confirmation of the best performing potting media/treatments.

3.6. Weed flora studies

Due to the extreme fund shortage, much progress was not made during the reporting year.

REGIONAL EXPERIMENT STATION, NAGRAKATA, BENGAL

1. Crop improvement

1.1 Evaluation of clones

Studies on evaluation of promising clones under the agroclimate of Sub-Himalayan Bengal were initiated in 1990, 1991 and 1993 with an aim of screening clones better adapted under the region in terms of growth, yield and other attributes. In Trial I and II at the 26th year of planting, girth was superior in RRIM 612 followed by RRII 118 and SCATC 93/114 when compared to check clone RRII 105. In Clone Trial III, girth of all the clones was at par with RRIM 600 (check clone). However, SCATC 93/114 was the superior in terms of girth among the clones. However, in terms of girth five the clones were found superior to the check clone RRIM 600 in Clone Trial IV (Table Nag. 1).

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

Trial I and II	Girth (cm)	Trial III	Girth (cm)	Trial IV	Girth (cm)
Gl 1	71.2	HK 1	69.3	HK 1	68.3
GT 1	77.5	PB 235	71.4	PB 235	68.4
HK 1	85.9**	PB 260	75.0	PB 280	69.7
PB 235	82.7**	PB 310	75.4	PR 261	70.8
PB 311	76.7	PB 86	76.8	RRIC 104	73.2
PB 5/51	75.4	PR 107	78.6	RRII 105	74.2
PB 86	81.2**	RRIC 102	78.8	RRII 208	74.6
PR 107	81.7**	RRII 208	78.9	RRII 300	75.0
RRII 118	89.8**	RRIM 612	79.6	RRII 308	75.4
RRII 203	74.7	SCATC 93/114	86.5	SCATC 93/114	76.1
RRII 208	78.9*	RRIM 600	78.6	RRIM 600	74.1
RRII 300	82.5**	CD (P=0.05)	NS	CD (P=0.05)	NS
RRIM 605	83.2**				
RRIM 612	97.1**				
RRIM 703	86.9**				
SCATC 88/13	78.5*				
SCATC 93/114	86.9**				
RRII 105	70.0				
Collective mean	81.2				
CD (P=0.05)	8.0				

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

Yield in PB 235, SCATC 88/13, RRII 300, PB 311 and RRII 208 was significantly higher than that of the check clone RRII 105 in Clone trial I and II. Rest of the clones were at par with the check clone. In Trial III and IV, none of the clones was superior to the check clone in terms of yield (Table Nag. 2).

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

Trial I and II	Yield (g t^{-1} t^{-1})	Trial III	Yield (g t^{-1} t^{-1})	Trial IV	Yield (g t^{-1} t^{-1})
GI 1	30.9	HK 1	33.3	HK 1	38.1
GT 1	40.1	PB 235	39.3	PB 235	35.6
HK 1	40.0	PB 260	32.3	PB 280	43.0
PB 235	49.1**	PB 310	44.7	PR 261	39.9
PB 311	44.7*	PB 86	27.3	RRIC 104	31.0
PB 5/51	33.6	PR 107	33.1	RRII 105	38.5
PB 86	41.5	RRIC 102	31.9	RRII 208	42.1
PR 107	43.4	RRII 208	40.4	RRII 300	31.1
RRII 118	40.8	RRIM 612	26.3	RRII 308	34.4
RRII 203	36.9	SCATC 93/114	34.9	SCATC 93/114	34.9
RRII 208	44.6*	RRIM 600	42.3	RRIM 600	36.0
RRII 300	46.7*	CD (P=0.05)	NS	CD (P=0.05)	NS
RRIM 605	31.7				
RRIM 612	23.2				
RRIM 703	44.2				
SCATC 88/13	48.0*				
SCATC 93/114	40.4				
RRII 105	32.0				
CD (P=0.05)	12.2				

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

1.2. Evaluation of germplasm

To evaluate the performance of germplasm accessions under the agro-climate of Nagrakata, West Bengal in 1998, twenty one germplasm accessions were evaluated with RRII 105 as check clone. Maximum girth was recorded in RO 3172 followed by RO 2890 and RO 5348. Six accessions *viz.* RO 3172, RO 2890, RO 5348, RO 2635, AC 619 and RO 5557 showed significant higher girth than RRII 105. Yield of the germplasm accession RO 5363 and MT 196 was significantly higher than the check clone. In general, the performance of Rondonia was better when compared to the Acre and Mato Grosso accessions.

1.3. Performance of polyclonal seedlings

A polyclonal seedling plantation was maintained in Nagrakata since 1990 with 240 trees in CRD (single tree single plot) at normal distance (5x5m). After 26 years of planting, mean girth of the population was 76.2 cm. The average block yield of the population was 30.4 g t^{-1} t^{-1} where 43.1 per cent plants showed above average yield. Selected Ortets were maintained in the nursery for further evaluation.

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

Half sib progenies raised from seven different clones showed that seedlings raised from seeds of RRII 429 attained maximum average girth followed RRII 208 and RRII 422. The percentage of coefficient of variance for yield indicated that variation in the half-sib progeny of RO 5363 and RRII 422 was more than the other clones.

1.5. On - farm evaluation of promising clones

Study was initiated to observe the performance of selected promising clones under the agro climate of Sarugaon, Ethalbari, Jalpaiguri, Bengal. At the third year highest girth was attained by RRII 417 (46.5 cm) followed by RRII 429 (41.0 cm) and RRIM 600 (38.6 cm).

1.6. Performance of polycross progeny in high pH soil

Evaluation of polycross progeny raised from the seeds of three different geographical locations under high pH soil of cold prone Dooars area of Sub-Himalayan West Bengal was initiated in 2014. Open pollinated seeds were collected from Kanyakumari, Kolasib (Mizoram) and Nagrakata. Seedlings were raised in polybags and field planting was done in June-July, 2015 at Sarugaon Tea Estate, Ethalbari, Jalpaiguri, Bengal. Vacancy filling was done in June-July 2016. The experiment is under progress.

1.7. GxE Trial: Performance of new generation clones under the agro-climate of Sub Himalayan Bengal

To study the performance of five new generation clones in different agro-climate, trial was initiated in 1996 along with check clone RRIM 600 in Orissa, Kerala, Bengal and Tripura. In Nagrakata, Bengal, RRII 429 (85.6 g t^{-1} t^{-1}) showed significantly higher yield and RRII 422 (67.9 g t^{-1} t^{-1}) and RRII 417 (63.7 g t^{-1} t^{-1}) showed superior yield in comparison to check clone RRIM 600 (58.4 g t^{-1} t^{-1}). However, in terms of growth, only RRII 429 (79.8 cm) attained significantly higher girth than check clone RRIM 600 (74.40 cm).

2. Crop management

2.1. Inter-planting trial

Inter-planting of rubber under tea in the Dooars area of Bengal showed that green tea leaf yield in inter-planted plots was significantly lower than that of the pure plot due to heavy shade imposed by the mature rubber trees and also due to severe pest attack in inter-planted plots. Rubber yield in pure plot was significantly higher than the inter-planted plots due to more crop stand.

3. Crop physiology

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

Study of the performance of seedlings, raised from seeds developed in varied agroclimate, under the climatic condition of Sub-Himalayan Bengal, showed that mean girth and first year yield of seedling plants from different seed source were similar.

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

Six clones were planted in the high pH soil (pH 7.9) of Sarugaon Tea Estate, Birpapar under Dooars area of Sub-Himalayan West Bengal in 1.25 ha area in blocks of 77 plants of each clone with a control block having soil pH at 5.5 at the experiment station of RES, Nagrakata. Girth at 150 cm height (Table Nag. 3) was taken at 6th year of planting.

Table Nag.3. **Clonal variation in girth (cm) of plants during 6th year under different soil pH**

Clone	Soil pH	
	7.9	5.5
RRIM 600	32.8	36.9
RRII 208	37.1	42.7
RRIM 605	32.0	34.2
RRII 417	32.6	40.2
RRII 422	25.1	28.8
RRII 429	25.5	40.8
CD (P=0.05)	7.6	8.4

In both the soil types, there was significant difference in girth between the clones studied. Growth of plants in high pH soil was lower than the soil with pH 5.5. In high pH (7.9) soil, mean girth of RRII 422 was the lowest and RRII 208 was the highest. In pH 5.5 soil also, girth was low in RRII 422 and RRII 208. RRII 429 and RRII 417 recorded significantly higher girth than RRII 422.

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

Promising ortets selected from the cold and drought prone (non-traditional) areas were evaluated in nursery trials under the agro-climate of Sub-Himalayan Bengal (Nagrakata), Konkan region of Maharashtra (Dapchari) and Traditional region (Kottayam). The girth data of plants at sub-Himalayan Bengal showed that the clone RRII 429 attained significantly higher girth than the check clone RRIM 600. Girth of RRSG 1, RRST 37 and RRSG 9 was comparable among the clone/ortets followed by RRSG 3 and RRII 414. In terms of juvenile yield, significant difference was observed among the clones / ortets studied. Ortets RRSG 1 was found superior to the clones / Ortets (Table Nag. 4).

Table Nag. 4. **Girth and yield of ortets**

Ortet/Clone	Girth (cm)	Juvenile winter yield ($g\ t^{-1}\ 10t^{-1}$)	Ortet/Clone	Girth (cm)	Juvenile winter yield($g\ t^{-1}\ 10t^{-1}$)
RRSD 1	33.6	127.0	RRII 417	35.3	165.1
RRSD 34	31.4	64.4	RRII 422	36.2	143.2
RRSD 35	33.9	107.9	RRII 429	43.5	147.1
RRSD 36	28.1	76.2	RRII 430	32.5	110.7
RRSG 1	39.9	223.3	RRSA 98	27.1	67.4
RRSG 3	37.9	119.9	RRST 24	33.3	103.0
RRSG 9	38.1	179.4	RRST 315	32.9	88.5
RRSN 1	34.1	59.6	RRST 37	39.2	203.3
RRSN 47	34.6	124.8	RRST 39	30.5	66.0
RRSN 69	35.2	170.5	RRST 585	34.1	90.6
RRII 105	35.7	147.6	RRIM 600	38.7	177.6
RRII 414	37.1	155.1	CD (P=0.05)	2.9	53.6

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

REGIONAL REESEARCH STATION, DAPCHARI, MAHARSHTRA

The mandates of this Station are to develop suitable clones and location specific agro technology for prevailing drought condition. The experiments on crop improvement and environmental physiology are being carried out.

1. Environmental physiology

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

The trial started in 2011 to evaluate the physiological and biochemical adaptation potential and common mechanisms involved in cold and drought tolerance using molecular physiology/ biochemical tools in ortet selections from cold and drought by interchanging the clones to different agro-climatic regions and to study the G x E interaction for growth and yield under varying agro-climates. A significant difference in girth was recorded among the ortets. The RRSA 98 continued to record highest girth (18.3) while lowest girth was recorded in GH 9 (10.9 cm). RRII 105 recorded lowest girth (14.6 cm) while highest girth noticed in RRII 414 and RRII 429 (19.6, 19.5 cm). In general RRII 400 series showed higher girth than RRII 105 and RRIM 600 (14.0, 14.3 cm).

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 twelve experiments are being conducted to evaluate the growth and yield performance of clones, pipeline clones, hybrid clones, ortets, wild *Hevea* accessions, half sib progeny and polycross seedlings, different root stock plants and root trainer plants with objective to identify drought tolerant clones for their adaptability and stability to this agro-climatic condition of Maharashtra.

2.1. Large scale trials

2.1.1. Evaluation of selected Ortets

Trial started during 2008 to evaluate the growth and yield performance of ortets selected from polycross seedlings planted at this station with control clones. Significant difference in girth was noticed. Girth among the ortets and check clones ranged from 26.5 cm in OS 8 to 38.4 cm in OS 236. Among the check clones, RRII 430 recorded higher girth (34.7 cm) while least girth was noticed in clone RRII 105 (28.4 cm). All ortets were at par with OS 236 except OS 42, RRII 105. All ortets were found superior in girth to clone RRII 105 except ortet OS 42.

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

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

2.2.1. Field evaluation of selected Hevea *clones for drought tolerance at RRS, Dapchari (2007)*

The field experiment was laid out using 25 potential drought tolerant wild *Hevea* accession for drought under Dapchari condition along with five HP clones and RRII 105, RRIM 600, Tjir 1, RRII 430, RRII 208 as a check clone in augmented RBD. In order to confirm the drought tolerance potential of selected wild accessions from various preliminary field screenings and five

HP clones were grown at normal spacing at drought prone region and subjecting to detailed studies along with recording mature yield. The accessions showed wide variability for all characters studied, after experimenting nine summer periods 2008 to 2016, 4 wild accessions recorded girth higher than the proven drought tolerant clone RRIM 600. MT 40 recorded highest girth at 9th years under drought. RRII 430, RRII 414 registered significantly better growth than RRIM 600 under prevailing drought. Hybrid 93/270 recorded highest girth.

2.2.2. Small scale further field evaluation trial of selected wild accession 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) with the objective to confirming the drought tolerance potential of selected seven wild accessions from preliminary field screening- 2003 by growing them at normal spacing and subjecting to detailed studies along with recording mature yield.

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

In order to evaluate juvenile and mature performance under drought condition a field experiment was laid out in rectangular lattice design with 11 wild *Hevea* accessions along with two check clones *viz*. RRII 105 and RRIM 600.

2.3. Clonal nursery evaluation

A total of five clonal nursery evaluations are in the initial stage for evaluation of half sib progeny, polycross, half sib progeny of prepotent clones and pipeline clones. Experiment on identification of reliable juvenile and mature characteristics for clone identification (50 divergent clones) in *Hevea* and standardization of distinct, uniform and stable (DUS) testing norms for evolving specific guidelines for varietal registration.

2.3.1 Germplasm screening

Screening for wild *Hevea* accession (130) for drought tolerance under Dapchari condition was laid out in 2003 with RRII 105, RRIM 600 and Tjir 1 in augumented block design. In general, Mato Grosso accessions were superior in all growth characters studied than those from Randonia and Acre provenances. 25 potential drought tolerant accessions were identified based on 3-4 years of field performance and further 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

Clonal nursery evaluation of promising *Hevea* clones (half sib progeny of prepotent clones) for drought tolerance (2010) with the objective to evaluate the clones in a clonal nursery and advance the potential ones showing drought tolerance along with rubber yield to LST and PCE to shorten the breeding cycle is in progress.

2.3.3. Clonal nursery evaluation of pipeline clones (2011)

Trial laid out in rectangular lattice design with pipeline clones (50 number with 2 check clone RRII 105, RRIM 600) with the objective to identify drought tolerant clones. Clone responses for field establishment were assessed. Adaptation of standard cultural practice for establishment and early growth will be carried out. 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)

A half sib progeny of 15 clones was planted for selection of primary ortets with objective 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. 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 a few superior locally adapted clones. The test tapping yield in peak winter and peak summer season were analysed.

2.3.5. Evaluation and selection from progenies of polyclonal seed gardens and multi-clone populations (2015)

Trial aimed at evaluation of progenies for promising individuals, selection and multiplication of superior individuals, study of yield, growth and other secondary characters of selections in clonal nurseries and evolving locally adaptable clones. Seeds collected from multiclonal trial, polycross seeds, polyclonal seed garden of Mizoram, Karnataka and Tamil Nadu.

2.3.6. Development of drought tolerant root stock for the non-traditional areas (2015)

Trial aimed at developing drought tolerant root stocks for the non-traditional area by evaluating the drought tolerance capacity of the seedlings from seeds produced from drought tolerant clones and seeds from tree grown in drought prone non-traditional areas as against the seedlings for traditional areas.

REGIONAL RESEARCH STATION, DHENKANAL, ORISSA

The Station located in a dry sub humid climate region continued its research activities with the particular objective of identifying clones suited to the drought prone conditions.

1. Crop improvement

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

1.1. Clone evaluation

In trial 1 (1987), the elite clone RRIM 600 exhibited a suerior mean yield of 41.1 g $t^{-1}t^{-1}$ while GT 1 recorded the least yield (30.5 g t^{-1} t^{-1}). Further, GT 1 recorded significantly higher mean girth (82.0 cm) over RRII 105. In terms of growth all three clones performed well in this region.

In clone trial (1990), RRII 208 (70.8 g t^{-1} t^{-1}) and SCATC 88-13 (67.4 g t^{-1} t^{-1}) were found the most high yielding clones. Another popular clone RRIM 600 (64.2 g t^{-1} t^{-1}) also performed well in the region. SCATC 93-14 recorded comparatively lesser yield (41.9 g t^{-1} t^{-1}). However, SCATC 93-14 recorded comparatively better growth in terms of girth, followed by SCATC 88-13 and RRII 208 (Table Odi. 1).

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

Clones	Yield (g t^{-1} t^{-1})	Girth (cm)
Haiken 1	59.5	83.4
RRIM 600	64.2	84.4
RRIM 701	50.0	86.8
RRII 5	54.6	87.7
SCATC 88-13	67.4	91.7
SCATC 93-14	41.9	92.8
PB 310	61.2	87.0
RRII 208	70.8	87.6
PCK 1	54.5	87.0
RRII 300	54.9	87.0
CD (P=0.05)	12.7	—

In the 1991 experiment, clones varied significantly in terms of mean yield. RRII 208 (76.5 g t^{-1} t^{-1}), PR 255 (72.4 g t^{-1} t^{-1}) and RRIC 102 (83.6 g t^{-1} t^{-1}) recorded highest yield among the clones. Polyclonal seedling population (54.7 g t^{-1} t^{-1}) yielded lesser though exhibited better growth and adaptability under the prevailing stress conditions (Table Odi. 2).

Table Odi. 2. **Performance of different clones in the region**

Clones	Yield (g t^{-1} t^{-1})	Girth (cm)
GT 1	55.7	98.4
RRII 105	70.5	85.5
RRII 208	83.6	94.5
RRII 5	71.3	88.1
RRII 300	58.0	92.2
PR 261	55.2	84.9
PR 255	72.4	92.9
RRIC 102	76.5	88.6
RRIM 600	69.9	85.9
Polyclonal	54.7	110.9
CD (P= 0.05)	---	8.02

In another clone trial (2000), superior mean yield was observed in RRIM 600 (66.0 g t^{-1} t^{-1}) and RRII 208 (61.2 g t^{-1} t^{-1}) and least mean yield was recorded in RRII 51 (34.0 g t^{-1} t^{-1}). Maximum girth was observed in RRII 300 (66.7 cm) (Table Odi. 3).

Table Odi. 3. **Yield and growth performance of clones**

Clones	Yield (g t^{-1} t^{-1})	Girth (cm)
RRII 300	47.1	66.7
RRII 208	61.2	58.5
RRII 357	48.1	58.2
RRII 352	59.9	57.5
PB 28/59	58.4	60.6
RRIM 600	66.0	62.3
RRII 351	59.9	56.8
IRCA 109	58.3	48.4
RRII 105	66.9	54.7
RRII 51I	34.0	57.4
RCA 111	46.0	62.5
CD (P=0.05)	17.00	---

1.2. Polyclonal ortet evaluation

Ortet clones OR 3 and OR 4 recorded comparatively higher yield and was almost at par with high yielding clone RRII 208 in the region under the test tapping. The ortets OR 8 (40.9 cm) and OR 10 (40.2 cm) exhibited comparatively better girth attainment.

REGIONAL RESEARCH STATION, PADIYOOR

Identification of locally adaptable clones suited to the region, evaluation of clonal tolerance to drought/disease incidence and development of suitable agro-management techniques for reduction of gestation period in rubber are the major thrust areas of research activity in the station.

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 fertilizers *viz.* recommended dose (D1), twice (D2) and thrice (D3) the recommended dose and a zero fertilizer control. Differences in yield were not significant (Table Pad.1).

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

Treatment	Yield ($g\ t^{-1}\ t^{-1}$)		
	RRII 105	RRII 414	RRII 429
D1	118.6	99.5	98.5
D2	110.5	109.9	89.9
D3	93.7	98.9	70.6
Control	113.3	79.2	75.4
CD (P=0.05)		NS	

2. Crop improvement

2.1. Large scale evaluation of clones

Mean annual yield in the renewed bark panel of PB 255 (Table Pad.2) was the highest at 54.2 $g\ t^{-1}\ t^{-1}$ and was on par with IRCA 130 (46.6 $g\ t^{-1}\ t^{-1}$), IRCA 18, PB 314 and RRII 105. Summer yield of PB 255 was the highest (36.6 $g\ t^{-1}\ t^{-1}$) and was on par with IRCA 130 (35.4 $g\ t^{-1}t^{-1}$) while being significantly superior to RRII 105 at 25.9 $g\ t^{-1}\ t^{-1}$ and other clones.

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

Clones	Mean annual yield ($g\ t^{-1}\ t^{-1}$)	Summer yield ($g\ t^{-1}t^{-1}$)
PB 255	54.2	36.6
PB 314	39.5	24.5
PB 330	36.6	21.1
PB 28/59	29.1	17.2
RRIM 703	32.5	18.5
IRCA 18	39.0	20.6
IRCA 109	30.3	21.4
IRCA 111	30.5	21.0
IRCA 130	46.6	35.4
IRCA 230	33.8	18.8
RRII 105	47.0	25.9
CD	15.9	10.4

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

The average annual yield of PB 86 was the highest with 65.3 $g\ t^{-1}t^{-1}$ followed by RRIC 100 (63.7 $g\ t^{-1}\ t^{-1}$) (Table Pad.3). Yield of clone RRII 203 and ortets Iritty, P 270 and P 213 were on par with PB 86 and RRIC 100 and were significantly superior to that of RRII 105 and other clones/ortets tested. RRIC 100 recorded the highest summer yield of 55.6 $g\ t^{-1}\ t^{-1}$ and was significantly superior to RRII 105 (15.2 $g\ t^{-1}t^{-1}$) and on par with the summer yield of PB 86, RRII 203, P213, P270 and Iritty.

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

Clones	Mean yield (g t^{-1} t^{-1})		Clones	Mean yield (g t^{-1} t^{-1})	
	Annual yield	Summer yield		Annual yield	Summer yield
RRII 105	21.7	15.2	P 121	27.9	21.9
RRII 203	47.6	33.1	P 155	23.5	16.3
RRIC 100	63.7	55.6	P 213	51.3	41.4
RRIC 102	13.5	10.6	P 270	56.5	49.1
PB 86	65.3	49.1	P 280	24.3	21.0
P 1	26.3	22.7	P 296	20.5	14.3
P 2	21.6	17.4	Iritty	56.5	39.5
P 90	25.7	20.2	CD (P=0.05)	36.1	29.4

HEVEA BREEDING SUB-STATION, KADABA, KARNATAKA

Rubber research began in south coastal Karnataka in 1986 after the establishment of the Hevea Breeding Sub-station with a research farm at Nettana. The major challenges for commercial rubber cultivation in the region are the prevalence of drought during summer months and occurrence of pink disease, *Phytophthora* and *Corynespora* leaf fall diseases. The research programmes in the station are envisaged to evaluate planting materials evolved through breeding and selection. Prevalence of biotic and abiotic stress factors is utilized for screening tolerant genotypes suited for non-traditional regions for future planting. The research farm has a source bush nursery for generating nucleus planting material and a well-established Class B Agro-meteorological Observatory.

1. Large scale clone trial (1990)

In the 1990 A large scale clone trial, HP 372 recorded the highest mean yield in 2016 (91.1 g t^{-1} t^{-1}) followed by HP 223 (83.2 g t^{-1} t^{-1}) while check clone RRII 105 recorded 60.5 g t^{-1} t^{-1} under S/2 d6 6d/7 system of tapping with ethephon stimulation. Mean yield over 13 years of tapping was highest for HP 372 (67.1 g t^{-1} t^{-1}) followed by PB 235. Clones HP 372, PB 235, PB 260 and HP 223 recorded significantly superior yield compared to RRII 105 (52.5 g t^{-1} t^{-1}). At 26 years after planting, HP 372 (107 cm) recorded the highest girth among the clones and was significantly superior to check clone RRII 105 (77.5 cm).

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

In 1991, three Small Scale Trials were planted *viz.* 1991 A (36 clones), 1991 B (13 clones) and 1991 C (13 clones). In trial 1991A with 36 clones, PB 280 (108.8 g t^{-1} t^{-1}) recorded the highest yield in 2016, followed by PB 235 (78.3 g t^{-1} t^{-1}) under S/2 d6 6d/7 system of tapping with ethephon stimulation. Clones PB 314, PB 312, PB 235, RRII 203, RRII 300 and PB 280 recorded

significantly higher mean yield over 13 years than check clone RRII 105 (50.3 g t^{-1} t^{-1}). In 2016, among the 13 clones evaluated in the trial 1991 B, RRII 5 (89.1 g t^{-1} t^{-1}) recorded highest mean yield and there was no significant difference between the clones tested (Table Kad. 1). Clones RRII 3 (59.3 g t^{-1} t^{-1}) and RRII 5 (72.4 g t^{-1} t^{-1}) had significantly higher mean yield over 13 years compared to RRII 105 (42.6 g t^{-1} t^{-1}). Clones GT 1, NAB 17, RRII 3, RRII 5, RRII 118 and RRII 308 recorded significantly higher girth than RRII 105 at 25 years after planting. Among the clones tested in 1991 C, PB 28/59 (88.1 g t^{-1} t^{-1}) recorded the highest mean yield in 2016 followed by HP 83/225 (71.7 g t^{-1} t^{-1}).

Table Kad.1. **Performance of clones in Small Scale Trial (1991B)**

Sl. No.	Clone	Mean yield 2016 (g t^{-1} t^{-1})	Mean yield over 13 years (g t^{-1} t^{-1})	Girth 2016 (cm)
1	AVROS 255	43.7	32.1	71.2
2	CH 2	25.3	26.8#	71.0
3	CH 3	54.2	26.8#	73.0
4	GT 1	54.3	36.0	80.9*
5	NAB 17	55.1	46.8	88.8*
6	PB 5/139	34.6	25.3#	64.2
7	RRIC 102	41.6	33.5	62.8
8	RRII 105	46.9	42.6	63.9
9	RRII 118	61.9	39.6	87.2*
10	RRII 208	50.1	36.8	73.2
11	RRII 3	71.6	59.3*	86.3*
12	RRII 308	42.5	40.4	86.0*
13	RRII 5	89.1	72.4*	88.7*
	CD (P=0.05)	NS	13.99	11.4

* Significantly higher than RRII 105
\# Significantly lower than RRII 105

3. Large scale trial 2000

The large scale trial (LST) for evaluation planted in the year 2000 consisted of six RRII 400 series hybrids *viz.* RRII 403, RRII 407, RRII 414, RRII 422, RRII 429 and RRII 430 and their parents *viz.* RRIC 100 and RRII 105. Mean yield recorded in 2016 did not reveal any significant difference between the clones (Table Kad. 2). Clone RRII 430 (108.9 g t^{-1} t^{-1}) recorded the highest yield in 2016 followed by RRII 422 (101.1 g t^{-1} t^{-1}). Mean yield over eight years was highest for RRII 430 (75.3 g t^{-1} t^{-1}) and was significantly superior to RRII 105 (51.2 g t^{-1} t^{-1}). Clones RRII 414 and RRII 422 also recorded significantly superior mean yield than RRII 105. Clones RRII 414 (84.5 cm) and RRII 430 (78.5 cm) had significantly superior girth compared to check clone RRII 105 (68.3 cm).

Table Kad. 2. **Performance of clones in LST 2000**

Sl. No.	Clones	Mean yield 2016 (g t^{-1} t^{-1})	Mean yield over 8 years (g t^{-1} t^{-1})	Girth 2016 (cm)
1	RRIC 100	65.5	49.7	75.2
2	RRII 105	66.5	51.2	68.3
3	RRII 403	72.7	53.1	64.1
4	RRII 407	56.0	47.2	70.5
5	RRII 414	81.7	70.6*	84.5*
6	RRII 422	101.1	71.5*	66.0
7	RRII 429	84.9	67.3	70.9
8	RRII 430	108.9	75.3*	78.5*
	CD (P=0.05)	NS	17.7	8.3

* Significantly higher than RRII 105
\# Significantly lower than RRII 105

4. Polycross garden 1995

A polycross seed garden consisting of nine pre-potent clones (RRII 105, PB 215, PB 217, PB 242, RRII 203, PB 5/51, PB 28/83, AVT 73 and Ch 26) planted as per Simmonds (1986) design is being maintained for collection of open pollinated seeds for evaluation and selection.

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 2016-17.

1.1.1. Clone evaluation

Evaluation of field performance of modern high yielding clones in the block trials initiated at Palazhi and Bethany estates was done during the reporting year. The clones were in their third year of tapping. Comparison of the girth of the trial clones with that of RRII 105 after 10 years of growth indicated that the clones RRII 429, RRII 414 and RRII 422 were relatively more vigorous.

The commercial yield data over three years indicated clones RRII 422 and RRII 430 to be top yielders with 57.0 g t^{-1} t^{-1} and 54.9 g t^{-1} t^{-1}, respectively in the block trial at Bethany estate (Table Par. 1).

Table Par.1. **Growth and yield performance of 400 series clones in the block trial at Bethany estate**

Clone	Girth (cm)	Mean yield (g t^{-1} t^{-1})	
		2016-17	Over 3 years of tapping
RRII 414	60.7	47.1	41.1
RRII 417	60.3	40.4	41.6
RRII 422	61.5	57.1	56.9
RRII 429	62.4	48.8	42.4
RRII 430	60.1	61.2	54.9
RRII 105	55.1	53.8	51.6
Mean	60.0	51.4	41.1
CD (P=0.05)	1.0	3.1	2.9

* at 10th year

The yield data recorded at the Palazhi estate indicated that the clone RRII 430 was the highest yielder with 92.1 g t^{-1} t^{-1} (Table Par. 2). During the same period, the yield of RRII 105 was 51.6 g t^{-1} t^{-1} and 79.9 g t^{-1} t^{-1} respectively at Bethany and Palazhi estates.

Table Par. 2. **Growth and yield performance of 400 series clones in the block trial at Palazhi estate**

Clone	Girth (cm) at 10 years	Mean yield (g t^{-1} t^{-1})	
		2016-17	Over 3 years of tapping
RRII 414	62.5	73.2	71.3
RRII 417	62.2	79.5	67.6
RRII 429	64.3	61.0	60.6
RRII 430	62.1	93.8	92.1
RRII 105	57.1	74.4	79.9
Mean	61.6	76.4	74.3
CD (P=0.05)	1.2	5.3	5.4

Based on the results of the concluded large scale experiments conducted in the region, a block trial was envisaged for evaluating the commercial yield performance of the clones PB 255, PB 314, IRCA 109, IRCA 111, RRII 203, RRII 414, RRII 417, RRII 422, RRII 430 and RRII 105. The Arasu Rubber Corporation had agreed to allocate land for the purpose at the Chithar Division. The bud grafting of the clones is proposed to be undertaken in their nursery at Kuttiyar during the ensuing season.

1.1.2. Hybridization and clonal selection

Two breeding orchards at the research farm at Paraliar comprising of 51 parental clones were properly maintained. During the current year, the flowering was very sparse and hence around 1321 hand pollinations alone could be attempted with six different parental combinations. The

hybrids obtained during 2016 were raised in seedling nursery for preliminary evaluation. The hybrids of 2015 were test tapped to evaluate the performance and secondary characters.

1.1.3. New generation polyclonal seed garden

The seed garden at New Ambady Estate was maintained well. The polyconal seedlings raised out of polycross seeds collected during 2015 were test tapped and the promising ones were pollarded for multiplication. Around 2000 healthy polyclonal seeds were collected and a seedling nursery was raised for evaluation.

1.1.4. Participatory clone evaluation experiments

The annual growth of 11 pipeline clones and the three check clones planted at Thiruvaiyar estate were recorded and the data tabulated. The clones P 10 (52.9 cm) and P 21 (52.8 cm) were found more vigorous after eight years. However, the average tappability was only around 65 per cent.

In the OFT at Bethany estate planted during 2010, of the 14 trial clones assessed for growth, clones P 44 (53.5 cm) and P 27 (52.4 cm) were relatively more. The tappability assessment indicated that the trial had crossed 65 per cent in majority of the plots. Hence, the management had decided to put the trial into tapping during the current season.

The assessment of the annual girth of 11 clones in the OFT planted at Bethany estate in 2012 indicated that the trial clones P 102, P 104 and P 49 were better than the rest of the clones in relative growth.

During the period under report, Phase V of Participatory Clone Evaluation was commenced and the constituent trial for the region was laid out at the Chithar Division of Arasu Rubber Corporation Ltd. The trial was planted during July 2016 with 16 trial clones and three check clones.

LIBRARY AND DOCUMENTATION CENTRE

The Library and Documentation Centre attached to Rubber Research Institute of India was well maintained with a collection of 22934 books, 23801 bound volumes of periodicals, 6003 standards, 1563 reprints, 180 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. Fourteen books and one standard were added to the stock. Received and registered 422 issues of journals as subscription/exchange.

Compiled Information bulletins *viz*., Documentation List (1-4) 2016, New Additions List 2016, Staff Publications list 2010-2016 and Cumulative index to *Rubber Science* Vol. 1-29. Databases were updated with the details of 14 books and 311 articles. Rearranged 1298 bound journals, circulated 900 books, filed 2303 press clippings of relevant articles and provided 9399 photocopies. Library membership issued to 26 users and extended library services to 711 outsiders.

As a part of sales promotion of RRII publications, library organized the sale and distribution of 571 copies of the journal *Rubber Science* and 264 other publications including RRII Annual Reports.

SCIENTIFIC ADVISORY COMMITTEE RECOMMENDATIONS

- Three best growing fodder crops *viz.* guinea grass, signal grass and *Stylosanthes* were recommended for NE region as fodder intercrops during the immature period of rubber.
- Recommended adoption of reduced spiral tapping system S/4 d3 6d/7 ET 2.5 per cent (6/y) @ 45 days interval during May to January, in clone RRII 105 with trunk girth of 45 cm or above. The system ensures comparable yield to S/2 d3, longer economic life and compact blocks. Adoption of this system for regular tapping ensures more years of virgin panel tapping (7 x 4 = 28 yrs) and possibility of enhancement of task size at least by 25 per cent more than conventional system.
- Use of Wood Pecker HTP power sprayers *viz.* WPH 21K and WPH 30K were recommended for high volume spray to control leaf diseases in rubber plantations having height about 21 and 30 feet, respectively.
- In mature rubber plantations with high or very high status of soil organic carbon (OC) and deep soils (more than 1m), fertilizers may be skipped for an initial period of three years. After this period, soil may be tested and if required recommended level of fertilizers may be applied *(Ad hoc recommendation)*
- Following secondary and micronutrient applications in rubber plantations (ad hoc recommendations) were recommended:

  For areas low in available Zn status, zinc sulphate at the rate of 50 g plant^{-1} may be applied once. In sloppy areas (slope >20%), a second dose with 25 g plant^{-1} also may be given after two years. For areas low in available B, borax at the rate of 20 g plant^{-1} may be given. These nutrients may be given once in immature (except in sloppy areas, where a second dose of Zn also may be given) and once in mature phases and subsequent application of Zn and B should be considered only after testing the soil.

  In areas with soil pH less than 5.0 (very strongly or extremely acidic soil) and with low available calcium status, it is advantageous to apply powdered shell lime (neettukakka) at the rate of 250 g plant^{-1} or dolomite at the rate of 500 g plant^{-1} two to three weeks before planting and this should be thoroughly mixed with soil. Planting should be done after the receipt of a few rains.

  In mature rubber plantations with soil pH <5.0 (very strongly or extremely acidic soil) and low in available calcium, application of powdered shell lime at the rate of 1.0 to 1.5 kg tree^{-1} at an interval of three years (one application only in the particular year), two to three weeks before the application of chemical fertilizers will be advantageous.
- Recommended web based system of fertilizer recommendations to enable rubber grower to access fertilizer recommendation online.
- Recommended use of oxo-degradable polythene manufactured by M/s. Everest polymers for rainguarding rubber trees. Rainguarding with this material served the purpose of protecting the tapping panel effectively without degradation during both the monsoon seasons (SW and NE monsoons, a total of seven months) and degraded completely thereafter in the next 5-6 months.

ANNUAL EXPENDITURE

2016-17

Head of Account	Expenditure (Rs. in lakhs)
Non-Plan	
RRII HQ	646.73
CES, Chethackal	519.30
Total	1,166.03
Plan	
North East	367.33
Other than North East	1,889.55
Total	2,256.88
Grand Total	3,422.91

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GEN BANK SEQUENCE SUBMISSION

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Awards/ achievements

Jacob, J. (2016). **Distinguished Scientist Award** by Dr. C.S. Venkataram Memorial Trust, *National Symposium on Plantation Crops, PLACROSYM XXII*, 15-17 December 2016, Central Plantation Crops Research Institute, Kasargod, India.

Mercy, M.A., Nair, D.B., Meenakumari, T., Singh, M. and Mydin, K.K. (2017). **Best Research Paper Award.** Performance of wild Amazonian accessions and hybrid *Hevea* clones of Wickham origin in a drought stressed environment. *National Seminar on Natural Resources Management for Horticultural Crops under Changing Climatic Conditions NRMHCCC-2017* held at CWRDM, Calicut during 16-17 March, 2017 under the theme "Drought management of plantation crops".

Pradeep, B (2016). **Young Scientist Award (YSA)**, International Rubber Research & Development Board (IRRDB) 2016, Malaysia.

Predeep, B. (2016). **Young Scientist Award** by International Rubber Research and Development Board. The award was given for his contributions on application of satellite based imaging and geospatial technology towards mapping rubber plantations in India.

Roy, C.B. (2016). **R.L. Narasimha Swami Memorial Award** by Indian Society of Plantation Crops.Construction of a high density genetic linkage map using SNP and silicoDArT markers and QTL mapping for disease resistance in rubber.*NationalSymposium on Plantation crops* held at Central Planation Crops Research Institute, Kasaragod, India.

Roy, C.B. (2016). **Guman Devi Memorial Best Woman Scientist Award.** Targeting disease resistance loci conferring tolerance to major leaf diseases of rubber tree through QTL mapping using high density genetic linkage map with DArT markers. *Thirty Eighth Annual Conference and National Symposium on Challenges Towards Plant Health Under Changing Climate Scenario for Sustainable Agriculture,* Indian Society of Mycology and Plant Pathology, 24-26 November 2016, Indian Society of Mycology and Plant Pathology, Bidhan Chandra KrishiViswavidhyalaya, Kolkatha, India

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Continued from inside front cover

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 anther culture systems for propagation and crop improvement of *Hevea* are the important areas in which the Biotechnology Division is engaged. The important fields of research of the Botany Division are breeding, evaluation and selection of new clones, propagation techniques, planting methods, anatomical studies and cytogenetic investigations. The Germplasm Division is concentrating on the introduction, conservation and evaluation of *Hevea* germplasm. The Plant Pathology Division is engaged in investigations on the diseases and pests of rubber and associated cover crops and their control. The Plant Physiology Division conducts studies on both fundamental and applied aspects of *Hevea* tree physiology. The Latex Harvest Technology Division is concentrating on all applied aspects of crop harvesting in rubber. The Rubber Technology Division concentrates on improvement in primary processing of rubber, its chemical modification, rubber product manufacture and quality control of processed rubber. The Technical Consultancy Division provides consultancy services for the promotion of the rubber industry. The Economics Division undertakes studies on economic aspects related to rubber plantations.

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

Central Experiment Station

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

Regional Research Stations

RRII has established a North-Eastern Research Complex with headquarters at Agartala having regional research stations at Agartala in Tripura, Guwahati in Assam and Tura in Meghalaya. The RRII has also set up regional research establishements at Dapchari (Maharashtra), Dhenkanal (Orissa), Nagrakatta (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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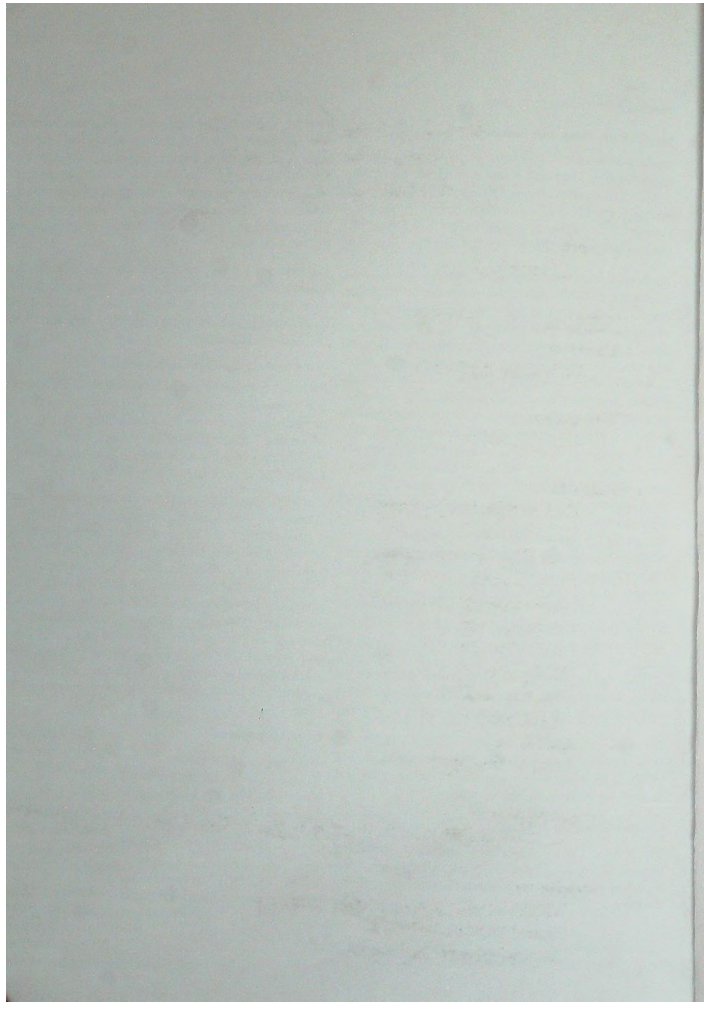

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