RUBBER RESEARCH INSTITUTE OF INDIA

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Rubber Research Institute of India

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

Location

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

Organization

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

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ANNUAL REPORT
2014-2015

RUBBER RESEARCH INSTITUTE OF INDIA
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THE RUBBER BOARD

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

Organization

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

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PREFACE

I am happy to note that substantial progress has been made in several projects during the reporting year. Long and painstaking efforts in developing clones through traditional breeding and field trials lasting for almost a quarter of century or more have paid off well. The high yielding clones developed by RRII are some of the best in any rubber growing countries. It is important that this tempo is maintained to produce more number of high yielding and climate-resilient clones suited for different agro-climatic regions of India. To meet this end, the best science and technology should be tapped, including genetic engineering, functional genomics, marker assisted selection etc. I am glad that RRII has a strong team of scientists working in these areas.

Good agricultural practices for sustaining high yield without compromising soil and environmental health, reducing cost of production etc. should continue to be research priorities at RRII. Developing viable models of rubber-based cropping systems for small growers in their homesteads is a felt need of the hour. I am happy that RRII has been successfully adopting geospatial technology, mathematical modeling etc. to monitor and understand the impact of climate change on rubber plantations. GIS based soil fertility mapping is being taken up for the entire rubber growing soils of South India. Compared to the impact RRII has made on the agricultural side, its impact on rubber products manufacturing side has been rather modest so far and I am sure there must be good historical reasons for this. But it is now time that RRII started focusing on this important area also, particularly on the SME sector. Indian rubber products manufacturing and exports are pegging high rate of growth which will certainly benefit from appropriate R&D support from RRII.

Scientists have a tremendous responsibility to the society. Scientists of RRII should not stop with generating results, but they should also research how far the results reach the stakeholders and what are the barriers that prevent the growers from implementing useful results. A typical case in point is the weekly tapping recommended by RRII which is not getting the expected popularity even at a time of low rubber price. Similarly, it is perplexing why growers still keep very old, senile and uneconomic trees without replanting them. However, RRII will always strive to hand hold the growers in the trying times and keep him abreast of the latest farming techniques.

A.Ajith Kumar, I.A.S.
Chairman, Rubber Board

Kottayam
29 November 2016

DIRECTOR'S REVIEW

Despite shortage of manpower and funds, all the major research disciplines made substantial contribution during the reporting year. After consolidating 25 years of data pertaining to growth and yield performance of RRII 208 from extensive field trials conducted in the North East, it was decided to upgrade this clone to Category I for exclusive cultivation in North East India. This was the first time a region-specific clone was recommended in the country. Long term field data has shown the superiority of this clone in terms of tolerance to cold stress and Powdery Mildew and Abnormal Leaf Fall diseases. The former two are major factors affecting growth and productivity of rubber in North East India. Efforts to popularize low cost tapping techniques, particularly weekly tapping have been another significant area of focus. During a given year almost 50 to 60% of the production cost (operating cost) is tapping expenses. Reducing tapping cost by adopting weekly tapping is an immediate and viable option before the growers to reduce cost of production which is particularly relevant at a time when natural rubber price is very low.

RRII remains focused on developing new high yielding clones. A large number of high yielding clones are in the pipeline and many of them are at advanced stages of field evaluation at multiple locations under participatory clone evaluation program. Breeding for drought and disease tolerance has been taken onboard as an important objective. The present breeding cycle is at least 23 years (and one should be quite lucky too), which is the major limitation in developing new clones. In order to shorten the breeding cycle, it's important that more focused attention is given in genome sequencing and functional genomics. There are several agronomically important traits seen in the large germplasm collections which otherwise have very poor yield. Incorporating these lines as parental materials in classical breeding has obvious disadvantages. Developing and validating genomic markers will help in faster and effective exploitation of these genomic resources.

The Advanced center for Molecular Biology and Biotechnology under RRII continued its active programs in functional genomics and genetic engineering towards genetic improvement of this crop. Gene expression analyses in several rubber clones indicated that high rubber yielding potential is associated with over-expression of specific genes in the mevalonate pathway. We could identify several SNP markers having putative association with rubber yield. We identified SNPs from the complete genomic sequence of eight major genes involved in the rubber biosynthesis pathway. Different types of DNA markers (RAPD, SSR, STS, SNPs, ITS based, SCAR) were developed for differentiating rubber clones and fungal pathogen.

Identification of genes involved in disease resistance and mapping of disease tolerance QTL are pre-requisites for identifying DNA markers that will directly help in molecular breeding for disease resistance and efforts in this direction are progressing well. We Identified QTL markers for Phytophthora and Corynespora disease

resistance using high density linkage map generated with DArT markers. Virulent isolates of Corynespora cassiicola from Karnataka were identified with the presence of a DNA marker (~0.6 kb) discriminating avirulent isolates. Whole genome sequencing and de novo assembly of the genome of RRII 105 are continuing. So far more than 300 GB sequence data was generated giving more than 85X genome coverage.

Efforts were continued in making more number of transgenic rubber plants using different target genes, as well as studies on polyembryony, haploids, polyploids etc. Studies on development of antibiotic marker-free GM rubber have been initiated. No state government has given NoC for taking up field trials with GM rubber so far.

There has been some evidence suggesting the association of a Phytoplasma with tapping panel dryness syndrome, although further confirmation is required. A toxin-based bioassay for Corynespora tolerance has been developed and successfully used in large number of clones. An integrated waste water treatment system was designed and installed at a couple of group processing centres.

About 12000 soil samples were collected from the rubber growing regions of South India and the chemical analyses have been completed under a collaborative programme with the National Bureau of Soil Survey & Land Use Planning (NBSS&LUP) (ICAR), Regional Centre, Bangalore. Soil fertility data will be mapped using GIS, after interpolating the fertility data which is based on the soil samples collected at a frequency of 50 ha.

More evidence in support of intercropping with a number of different crop species in immature rubber holdings has emerged. Scientific intercropping does not adversely affect growth of young rubber plants. For prevention of soil erosion, erecting biological bunds, especially planting Vetiver, across the slope was effective. A biodegradable plastic for rain-guarding and soil mulching is under evaluation and the initial results have been highly promising. An association was observed between potassium content of leaf and roots and drought tolerance in young rubber plants which needs further consolidation.

Geo-spatial techniques were used to map existing areas of rubber plantations in the state of Odisha as well as assess the age profile of rubber plantations in Kanyakumari district. The latter proved to be expensive. Using geo-spatial techniques we could identify patches of rubber plantations encircled by forests in Wayanad and Idukki districts. This doesn't mean that the forest lands were encroached. But the advantage with geo-spatial techniques is that any changes in rubber area, including intrusion into forests can be monitored in future and the technology can be applied regardless of geographic borders.

Weather-disease modeling has been attempted for the past many years and we could come up with some weather indices to define the best congenial set of conditions for the onset of some fungal diseases. However, we have not yet been successful in validating the use of these indices. For example, during the reporting year, a humidity-minimum temperature index has been developed to define the humidity and minimum temperature conditions congenial for Corynespora leaf disease. But there will be several instances of adjacent plots with or without this disease even as the index would be the same in both plots.

The two Divisions in the Advanced Center for Rubber Technology of RRII, namely Rubber Technology and Technical Consultancy Divisions took up rubber technology research and consultancy work for the rubber products manufacturing industry. A new and efficient mechano-chemical devulcanisation process was developed that could achieve significantly high percent of devulcanisation. This process offers obvious advantages if it can be scaled up to industrial level. Production of de-proteinised natural rubber (DPNR) by enzyme treatment of preserved latex followed by creaming and coagulation was upgraded from laboratory scale to pilot plant scale. The DPNR produced by RRII and commercial DPNR samples had similar nitrogen contents and comparable properties. Comparison of vulcanizate properties of compounds prepared from NR latex-carbon black masterbatch and corresponding dry mixes having equivalent amounts of carbon back indicated the need to improve the master batch preparation process for industrial use. In the case of NR latex-silica master batch the results obtained showed that master batch process can achieve comparable vulcanisate properties with NR-silica dry mix. More work is needed to close the gap in properties of NR-silica vulcanisates with that of NR - carbon black vulcanisate.

The Technical Consultancy services cater to the needs of new entrepreneurs as well as existing ones by providing supporting services like testing/certification of raw materials, rubber chemicals, rubber products etc. as per relevant standards and help in the development of rubber products required by clients. During the reporting year, a total of about 6800 different parameters were analysed for the purpose of product certification and technology for making 24 rubber products was transferred to the SME sector.

The technology team of ACRT at RRII has made remarkable progress in developing various protocols such as preparation of fine dispersions for latex industry, selection of the right co-agent for peroxide vulcanization, cost effective tread formulations complying with specifications of Association of State Road Transport Undertaking, rubber compounds for improved flex-crack resistance, selection of blowing agents etc. Effect of EPDM reclaim in EPDM products and checking of adulteration of NR latex with synthetic analogues and effect of non-rubber constituents on adhesive properties of field latex were also studied. For the NABL accreditation of the Technical Consultancy Division, quality manual, quality system procedures and system operating procedures have been prepared and internal audit as per ISO/IEC 17025: 2005 was conducted.

While the consultancy services were provided to the entrepreneur based on his specific needs and requirements, technology research is largely taken up in broad generic areas (and not necessarily specific to any particular product). Findings from such research seldom find full acceptance with industry partners. The issues that emerge during industry level (factory floor) scaling up are very different from what scientists face in the laboratory. This could be addressed effectively only if research in Rubber Technology that we do have an industry/vendor partner from the beginning. Strengthening the manpower and infra-structure capabilities, obtaining NABL accreditation for the laboratories and increasing the visibility of the ACRT team before the products manufacturing industry are required for

making more tangible progress in industry related research and consultancy work.

Decline in 10 year average yield of selected clones planted during different decades in the estate sector was reported by Economics Division. Assessment of seasonality in NR production in India during 1991 to 2012 showed no significant change in the seasonality of NR production in the country at the aggregate level, but at the disaggregate level (intra month), significant trend breaks in production were observed. A revival strategy for NR Planting Subsidy Scheme based on the trends in development cost and planting subsidy and the strategies followed elsewhere were proposed. Study on female tappers in the smallholder sector revealed very low share of participation. Intercropping activity in the immature phase in the traditional region revealed a shift from subsistence farming to a highly commercial activity. Study on rubber cess countervailing duty imposed on imported NR justified its imposition as it is consistent with the existing rules.

High per cent of vacancies in technical posts and poor funding are beginning to adversely affect progress of various research programs at RRII. However, substantial progress has been made in several projects during the reporting year.

Dr. James Jacob

AGRONOMY AND SOILS DIVISION

Generating additional income from the plantation through crop diversification, maintaining high yield, sustaining soil health and reducing cost of cultivation through refinement of agro-management techniques were important thrust areas of research. Reducing the gestation period through good agricultural practices, management of biotic and abiotic stresses and ground cover management were also priority areas of research. Chemical analysis of soil samples for soil fertility mapping of rubber growing areas in South India was completed and fertility mapping is in progress. More than 100 permanent soil health monitoring sites were identified and analyses of the profile samples are in progress. Experiments on nutrient management in nurseries, young and mature rubber were continued. The Division also functioned as a centre for dissemination of knowledge on various soil and crop management techniques, undertook feasibility studies on cultivation of rubber and intercrops in various agroclimatic regions and investigated specific field problems.

1. Nutrient management

A nursery trial was initiated at Central Nursery, Karikattoor for the evaluation of an organic manure, 'Geo-green'. The treatments comprised different doses of 'Geo-green' as basal application and subsequently as top dressing in addition to the standard practice as control. Preliminary observations on growth of seedlings indicated that basal application of 'Geo-green' @500kg ha^{-1} enhanced the growth of seedlings compared to the standard practice.

Based on the results of experiments conducted at different locations, the fertilizer recommendation for rubber seedling nursery was revised as N: P_2O_5: K_2O @ 250: 125: 50 kg ha^{-1} respectively, with N applied in two equal split doses. This can be applied as urea, rock phosphate and muriate of potash, or as factamfos and potash. Application of MgO @ 37.5 kg ha^{-1} is recommended from Thiruvananthapuram to Eranakulam region, where available Mg status is low.

Field experiments on secondary and micronutrients were started during 2011, in four low status areas *viz.*, New Ambady estate in the southern region, Cheruvally estate in the central region, Palappilly estate in the north central region and Thamarassery estate in the northern region are in progress. There was no significant difference between treatments on growth of plants in any of the four locations. Secondary and micronutrients were applied during the first year of planting and the soil status was maintained in the sufficiency level during the fourth year also.

In an attempt to establish the critical level of nutrients, soil and leaf samples were collected from five year old plants of RRII 105, 430 and 414. Girth of plants (100 plants) was measured from each field and analysis of soil samples is in progress.

The field experiment at CES, Chethackal to study the effect of long term use of inorganic fertilizers and organic manures on the growth and yield of rubber and soil physico-chemical properties was continued. The treatments with 25 per cent recommended dose of chemical fertilizer with 75 per cent FYM and 50 per cent recommended dose of chemical fertilizer with 50 per cent FYM were comparable in yield and significantly superior to the standard practice.

The study on leaf nutrient status of trees with low and high yield is in progress.

The field experiment on use of coir pith organic manure (CPOM) as soil amendment in marginal soils, at Thanneermukkom, Cherthala is in progress. CPOM and FYM were comparable in promoting initial establishment and growth of rubber plants in sandy soil. Microbial analysis of soil during the seventh year showed that bacterial population was significantly higher in treatments with CPOM and FYM with chemical fertilizer, compared to the treatment with chemical fertilizer alone.

The study on rhizosphere chemistry and growth of natural rubber under varying pH and base status is in progress. The total and available nutrients and organic P varied between rhizosphere and bulk soil samples depending on the pH variations.

The poly bag experiment to study the effect of different soil pH on growth of rubber plants is also in progress. Higher contents of N, P, K, Ca, Mg and micronutrients in the shoot and root were observed at pH 7.3. Contents of nutrients at soil pH 4.3 and 5.5 were comparable.

2. Soil and water conservation

The experiment on evaluation of vegetative hedges *viz.* vetiver, guinea grass, pineapple and *Strobilanthes* sp. for soil conservation in rubber plantation was continued. Five years after planting of rubber and the vegetative hedges, there was no significant difference between treatments in the quantity of soil deposited in trenches. No difference in growth of rubber was observed in plots with and without vegetative hedges over a period of five years.

3. Intercropping and cropping systems

The experiment initiated to develop a multi species rubber based cropping system for Tamil Nadu region is in progress. The establishment of intercrops did not influence the growth of rubber. The experiment to evaluate the feasibility of growing perennial intercrops with rubber is also in progress and the growth and yield of rubber continued to be not influenced by cultivating perennial intercrops.

Feasibility study on interplanting ornamental plants in mature rubber plantations at CES, Chethackal showed that *Dracaena massangeana* can be cultivated in mature plantation. The leaves can be harvested once in 45-60 days, and can be sold in the local market @ Rs. 2.50 per leaf. The different shade tolerant medicinal plants also established well in rubber plantations.

The experiment to evaluate the performance of timber trees inter-planted with rubber is in progress at CES, Chethackal. Growth of rubber under normal system of planting was adversely affected by the presence of wild jack.

4. Ground cover management

The field experiment on the effect of legume covers and natural flora on growth of rubber, soil physico-chemical and biological properties, biomass and nutrient turnover laid out during 2010 at CES Chethakkal was continued. The girth as well as girth increment of the rubber plants under natural cover with life-saving weeding was significantly less compared to the treatments, rubber + *Pueraria*, rubber + *Mucuna* and rubber + natural cover with $1m^2$ weeding around the plant basin.

Evaluation of biodegradable plastic for weed control and soil moisture conservation was in progress at CES, Chethackal. Biodegradable plastic was durable for nine months and reduced the cost of weed control. Biodegradable plastic conserved soil moisture during summer season.

The observational trial initiated at Malankara estate, Thodupuzha during 2009 to explore the feasibility of establishing *Mucuna* under partial shade is in progress and soil moisture status during dry season continued to be higher in *Mucuna* established fields. The field trial initiated at CES, Chethackal for the evaluation of *Calopogonium caeruleum* was continued. Growth of C. *caeruleum* was less than that of *Mucuna*. However, it survived during summer season. Flowering and seed set were also observed.

5. Planting techniques

The field experiment initiated to assess the effect of mechanized land preparation on soil erosion and physical and chemical properties of rubber growing soils was continued. The land preparation methods evaluated were (a) pitting, terracing and tilling inter rows by earth mover (b) pitting and terracing by earth mover (c) pitting by tractor mounded hole digger and manual terracing and (d) standard practice - manual pitting and terracing (control). Different types of land preparation methods had significant effect on soil erosion (Table Ag. 1). The rate of soil erosion was significantly higher in plots where pitting, terracing and tilling inter rows were done by earth mover compared to all other treatments.

Table Ag. 1. **Quantity of soil eroded (2014)**

Treatments	Quantity (t ha^{-1})
Manual pitting and terracing (control)	1.8
Manual terracing & pitting by tractor mounded hole digger	2.0
Pitting & terracing by earth mover	2.2
Ploughing, pitting & terracing by earth mover	2.9
SE	0.17
CD (P=0.05)	0.55

The experiment to evaluate different planting designs is in progress at Cheruvally estate and the canopy of rubber has an asymmetrical growth pattern in the modified planting designs.

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

The field experiment at Malankara Estate, Thodupuzha initiated during 2005 to evolve an agronomic package to reduce the immaturity period was continued. The growth of rubber under the integrated management was significantly superior followed by irrigation, enhanced nutrient application, irrigation and conservation oriented tillage which were comparable. Early yield did not indicate any significant difference among treatments.

The field experiment initiated at CES, Chethackal during 2008 to evolve an agronomic package to reduce the immaturity period of rubber starting from planting material onwards in which the treatments included combinations of two types of planting material and two management options was in progress. The superiority of direct-seeded green budded plants under integrated management continued.

The field experiment investigating the comparative field performance of one-whorl, two-whorl and three-whorl polybag and root trainer rubber plants initiated at the CES, Chethackal during 2008 indicated that advanced three-whorl polybag plants had significantly more growth compared to all other planting materials studied *viz*., polybag: (one-whorl, two-whorl) and root trainer: (one-whorl, two-whorl and three-whorl) plants.

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

7. Rubber growing soils

The study on management of active and microbial carbon pools at Pottamkulam estate, Yendayar was concluded. Integrated nutrient management with chemical fertilizers (50% of the recommended dose), biofertilizers and green manure improved soil quality in terms of microbial activity and nutrient status.

The project on soil CO_2 flux measurements in mature rubber plantations in different agro-climatic zones was continued. The mean soil CO_2 flux, temperature and moisture during summer and winter period from Dapchari are shown in Table Ag. 2. The soil CO_2 flux and soil moisture content were more during summer than the winter period. The lower soil moisture content coupled with lower temperature might have contributed towards the lower soil respiration rate during the winter period in Dapchari. It appeared that the stress for microbes was more during winter than in summer.

Table Ag. 2. **Average soil CO_2 flux, temperature and moisture during summer and winter periods at RRS, Dapchari**

Parameter	May 2014	Feb. 2014
Soil CO_2 flux (μ moles m^{-2} s^{-1})	0.9	0.7
Soil temperature (°C)	30.7	22.8
Soil moisture (%)	13.4	11.3

A study was started during 2014 to assess the contribution of root respiration towards soil respiration. Immature rubber fields with banana and pineapple and a mature rubber field at Areeparambu village near Kottayam were selected and trenches were made and refilled with root-free soil in each experimental area and polythene sheets inserted on all sides to avoid root penetration. Soil CO_2 flux and temperature measurements were taken from 10 sites each, trenched and non-trenched from all the experimental areas since October 2014. The soil CO_2 flux was more in trenched sites with no roots during the initial month. In subsequent months, soil respiration rate was found to be less in trenched sites with no roots, especially in rubber-pineapple and rubber-*Mucuna* fields.

The study on characterization of soil organic matter in rubber plantations was concluded. It was found that carbon quality in mature rubber system allows the carbon to remain in soil for longer periods. Cover crop *Pueraria* generated more carbon in the labile pool and improved soil quality than *Mucuna*. Among the intercrops banana was found to be better in improving soil quality than pineapple.

8. Stress management

8.1. Drought management

Two field experiments, one with RRII 105 (Experiment I), a drought susceptible clone and the other with RRII 430 (Experiment II), a drought tolerant clone initiated at Chimoni Estate, Puthukkadu during 2012 to study the effect of different types of agricultural mulch materials *viz*. coco tree mat, poly propylene woven fabric, coir pith and dry leaf mulch on growth of rubber, soil moisture conservation and weed control were continued. Polypropylene woven fabric improved soil moisture retention, suppressed weed growth and was found durable. But there was no significant impact on plant growth.

In the experiment on drought mitigation, water stress was imposed by withholding irrigation for a week in polybag plants of clone RRII 417. Leaf potassium content was higher in Kaolin and anti-transparent treated (2.5%) plants than in control. In another poly bag experiment, on treatment of anti-gibberalin (to

enhance drought tolerance by inhibiting auxin production), leaf K content was enhanced both in drought tolerant and susceptible clones.

A polybag experiment on disease management was initiated to study the effect of supplementing Ca, Mg and B on disease incidence. The plants were inoculated with *Phytophthora* strains and leision size was recorded. Lesion size was significantly lower in treatments with Ca, B and K.

9. Environmental aspects of rubber cultivation

A polybag experiment was conducted with seven levels of copper, *viz.*, 0, 10, 20, 30, 40, 50 and 100 ppm Cu (supplied as $CuSO_4$), incorporated in soil used for polybag filling. Copper @10 mg kg^{-1} soil showed a positive effect on growth of polybag plants. Copper levels up to 50 mg kg^{-1} soil did not adversely affect the growth of polybag plants of *Hevea*, while Cu @ 100 mg kg^{-1} soil resulted in significant reduction in growth compared to control.

Initiated a study on assessment of water quality in a watershed dominated by rubber plantations. Delineated watershed and identified locations for sampling and surface 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 NBSS & LUP, ICAR, Regional Center, Bangalore)

Analyses of soil samples for soil fertility mapping of rubber growing soils was completed. Soil fertility mapping of Kottayam district was initiated using geostatistical technique. For soil health monitoring, profile samples were collected from 100 monitoring sites in the different agroclimatic regions and analysis is in progress.

FERTILIZER ADVISORY GROUP

Advisory services were given to smallholders and large estates on optimization of fertilizer use based on soil testing and leaf analysis. The service is provided through the laboratory at RRII, Kottayam and the eight regional laboratories. Clarifications for the queries by smallholders and large estates on fertilizer use were provided. Dry rubber content estimation of latex samples were also provided at the regional laboratories.

- Offered site-specific fertilizer recommendations to 1400 individual fields from 29 large estates on the basis of analysis of 750 leaf samples and 1545 soil samples.
- 315 leaf samples and 2649 soil samples from small holdings were analyzed and offered site-specific fertilizer recommendations to smallholders.
- 58904 latex samples were tested for dry rubber content.
- 114 advises to smallholders were provided through telephone and visit of the farmers to RRII.

CLIMATE CHANGE AND ECOSYSTEM STUDIES

The thrust areas of research in this multi-disciplinary area include development of information system on rubber cultivation using remote sensing (RS) and geographic information system (GIS) to identify area under rubber cultivation and identification of suitable new areas where rubber can be newly cultivated in the country. Agro-climatic data management system in traditional and non-traditional rubber growing regions is updated and being examined to assess climate change issues. Studies were also undertaken on carbon sequestration, bio-diversity conservation and impact of extreme weather events in rubber cultivation.

1. Applications of geospatial techniques in rubber plantation sector

1.1. Area estimation in Odisha

Information system on rubber cultivation developed using RS and GIS could estimate the natural rubber (NR) growing areas of Odisha using Landsat satellite data (2014). Satellite based NR area in Odisha was 561 ha while based on the traditional survey statistics it was 752 ha indicating a difference of 191 ha. Majority of the NR holdings was distributed in Mayurbhanj district of Odisha. The difference in statistics is attributed to the area under young plantations of less than three years old having poor spectral signature. Spatial distribution of rubber areas in different districts of Odisha is given in Fig. CCES. 1.

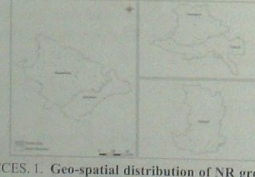


Fig. CCES. 1. **Geo-spatial distribution of NR growing areas in different districts of Odisha**

1.2. Age profile of NR in Kanyakumari district of Tamil Nadu

Investigations were carried out to segregate rubber holdings according to age in Kanyakumari district of Tamil Nadu using multi-temporal satellite data (2014). Satellite data was digitally classified and critically interpreted using 283 GPS points collected from rubber plantations of different ages for validation. Table CCES. 1 shows age-wise statistics of rubber holdings in Kanyakumari district as studied using this technique. It was noticed that 59 per cent of the total rubber area (14111 ha) fell under the age group of more than eight years.

Table CCES. 1. **Satellite data on age-wise area of NR plantations in Kanyakumari district for the year 2014**

Age class (years)	NR area (ha)
1-3	1095
4-7	8670
>8	14111

1.3. Estimation of NR area surrounded by forests in Wayanad and Idukki districts

Reserve forest boundaries of Wayanad and Idukki districts were vectorized using Survey of India toposheets of scale 1:50,000 for estimating (likely) rubber cultivation inside and outside the forests. About 313 and 4783 ha of rubber was present inside forests in Wayanad and Idukki districts, respectively (Table CCES. 2)

Table CCES. 2. **Satellite based estimation of rubber area (ha) surrounded by forests in Wayanad and Idukki district (2012-2013)**

District	*NR area (ha) surrounded by forests	NR area (ha) outside forests	Total area (ha)
Wayanad	313	7254	7567
Idukki	4783	35491	40274

* This does not imply mean forest encrodachment.

1.4. Identification of suitable lands for NR cultivation in Assam

Existing rubber holdings and suitable lands for rubber cultivation were identified from Cachar district of Assam using high resolution PAN sharpened CARTOSAT satellite data (2012). Ground truth verification was also carried out. About 899 ha of existing NR holdings and another 1139 ha of suitable lands (outside forests, cultivated and flood-prone areas) were identified from this district.

1.5. Soil fertility mapping using GIS

Soil fertility mapping of traditional and Konkan regions was carried out using GIS. Kriging analysis was carried out to interpolate soil fertility parameters of Kasaragod district determined at a frequency of 50 ha. Interpolation maps were prepared for soil quality parameters such as organic carbon, soil pH, Fe, Zn, Mn, Mg, B, Al, K, Cu and gravel contents. Soil fertility maps were integrated with place names and Taluk boundary of Kasaragod district and a GIS database was developed.

2. Agrometeorology

2.1. Weather modelling for disease prediction

A field trial was initiated to study the Corynespora Leaf Disease (CLD) occurrence in a three year old rubber plantation at Shiradi, Karnataka. A field unit of meteorological observation was installed in this field. Twice daily (morning and afternoon) meteorological observations on maximum temperature, minimum temperature and RH were collected from this station. Leaf fall data was collected from the field at quarterly interval using the standard quadrant method. Daily weather data was collected from January to May, 2014. The daily Humidity Minimum Temperature index (HT_mI) was computed and it was related with the daily leaf fall. Moderate level of disease intensity was observed and the HT_mI index values of 5.5 were below 7 days, which did not cause any severe leaf fall (Fig. CCES. 2). The experiment will be continued further for assessing and validating the HT_mI index for the start of leaf fall both for occurrence and severity of the disease.

Fig. CCES. 2. **Daily variations in Humidity Minimum Temperature Index (HT_mI) for the occurrence of Corynespora leaf disease in Shiradi, Karnataka**

2.2. Environmental protection and climate change studies

The change-point analysis was adopted for identifying climate change points within periods of datasets of 30 years and daily temperature data were subjected to extreme event analysis. Since temperature is considered to be normally distributed (IPCC, 2001), Gaussian curves were fitted to the daily series of T_{max} and T_{min} for both the periods of before (Period 1) and after (Period 2) a change point. The two periods were analysed for variability *i.e.*, for changes in probability of occurrences of particular extreme events at temperature thresholds above/below 95% significance level. The cumulative probability (also called as probability of non-exceedance) was calculated for each of the series and the return periods or the probability of exceedance was calculated which indicates the expected waiting time (seasons in this case) until the exceedance occurs again. Analysis was completed for five rubber growing stations in the NE region. Decrease in occurrence of seasonal minimum temperature were prominent in Nagrakatta (Winter), Padiyoor (Pre-monsoon), Tura (Monsoon) and Agartala (Post-monsoon). No significant decrease in number of cold days per year was observed in the hot and dry sub-humid climate of Dhenkanal. In Dhenkanal, it was found that there was more than 25-fold increase in the chances of warming in Period 2 of the data set.

2.3. Agrometeorological database management

Agrometeorological data from10 stations including the RRII Regional Research Stations in the traditional and non-traditional regions were consolidated. Weather data were recorded by trained field staff in Excel format (prepared data sets for 2015) for archival of meteorological records on a daily basis.

2.3.1. Salient features of weather in traditional and non-traditional NR growing regions in India during 2014

a. Traditional region

In Kottayam the mean annual temperature fluctuated from 32.5 to 23.3 ^{0}C for the year. The highest temperature was 38.5^{0}C during the pre-monsoon season. Unlike earlier years there were no days with minimum temperature below 19.0 ^{0}C. The annual rainfall recorded during the year was 3370 mm (8% increase above normal). Lowest relative humidity (RH) was below 25% for a few days during the winter season with mean maximum bright sunshine duration of 7.8 hours/day. In Chethackal the mean maximum seasonal temperatures were above 35.0^{0}C for the year. Daily temperatures fluctuated from 39.5 to 16.0^{0}C. The rainfall recorded during the winter season (62.4 mm) in Chethackal was much higher than that at Kottayam which received only 6.2 mm. In Chethackal the total annual rainfall received was 3980 mm with 53% contribution during the monsoon season. Annual rainfall recorded in Paraliar was 2302 mm. Warm weather was observed in Paraliar with a maximum monthly temperature anomaly of +1.3^{0}C from the normal during the month of March. The daily maximum temperature fluctuated from 38.5 to 24.5^{0}C.

b. Non-traditional region

The annual rainfall of 1505 mm for Agartala in 2014 was 24% lower than the long term average with 50% contribution from the monsoon period. Monthly maximum temperature fluctuated from 36.1 (January) to 25.3^{0}C (April). In Guwahati

the mean seasonal temperature fluctuated from 12.0°C during winter to 32.9°C during summer. Guwahati received 1563 mm of rainfall with 70% being contributed during the monsoon season. In Tura, seasonal minimum temperature recorded a low of 8.6°C during winter. The total rainfall received was 2195.1 mm which was only 87% of the long-term average. The rainfall distribution was skewed with more than 70% of the annual rainfall received during the monsoon season. The monsoon season rainfall recorded a 26% increase than that of the normal rainfall. Average seasonal temperature fluctuations for Nagrakata ranged from 9.3°C during winter to 32.2°C in summer. This station received copious rainfall during the southwest monsoon period with 82% of the annual rainfall of 2893 mm.

2.3.2. Climate resource characterisation of the rubber growing areas

The percentile thresholds of mean maximum and minimum temperature in the rubber growing areas of Northeast were standardized and were compared with traditional areas. The temperature regime for optimum yield (as per reported literature) was kept as the standard. Temperature profiling using percentile thresholds categorically proved that thermal regime prevailing in NE during Southwest monsoon is remarkably high compared to that of traditional area. Hence high minimum temperature prevailing in NE during the SW monsoon could also be taken into account for charactering the substantially low yield against the pre conceived notion.

3. Rubber ecosystem and water use efficiency

Water availability is a major factor limiting plant growth and latex yield in the traditional and non-traditional rubber growing regions of India. Thus exploring the ecosystem water use efficiency (WUE) is critical in revealing the response of ecological and hydrological processes to global climatic changes and optimizing the water and carbon managements in practice. Annual, monthly and seasonal WUE of rubber plantations was estimated from the carbon flux (FC) and evapotranspiration (ET) data obtained from the eddy covariance measurement facility installed at CES, Chethackal. Annual water use efficiency of rubber plantations was estimated as 4.31 g CO_2 / kg H_2O. Monthly average of WUE varied from 3.22 g kg^{-1} during February to 6.32 g kg^{-1} during May. Seasonal variation in WUE was estimated based on weekly average during pre monsoon (February, March, April, May), monsoon (June, July, August, September) and post monsoon seasons (October, November, December, January). WUE was the least during post monsoon season and highest during pre monsoon season. The monthly and seasonal variations in WUE clearly indicated that water availability and climatic factors influenced the water use and evapotranspiration rate in the ecosystem. WUE ecosystem is a useful tool for monitoring the ecosystem health, efficiency of water management practices, carbon sequestration capacity and climate change impacts on rubber plantations.

BOTANY DIVISION

Introduction of domesticated *Hevea* germplasm *via* bilateral and multilateral clone exchange programmes with other rubber growing countries was a major thrust of crop improvement efforts during the year. A total of 18 promising clones developed in Vietnam, China and Thailand were introduced and efforts are underway for multilateral clone exchanges with other IRRDB member countries. These clones are expected to provide a further boost to breeding programmes with the much needed diversity of parental genes. Programmes for the development of clones possessing high rubber yield and dual purpose latex-timber clones via conventional methods of hybridization, polycross breeding and ortet selection were continued. The fourth phase of the farmer participatory clone evaluation programme with 23 pipeline clones was launched with the establishment of field experiments in seven new locations. Studies on response to stimulation in some promising pipeline clones gave encouraging results. In the area of plant propagation, the use of elephant dung as a viable alternative potting medium for root trainer plants was established.

1. Evolving high yielding clones for the traditional area

1.1. Hybridization and clonal selection

Out of 34 hybrid clones under evaluation in SST 1995 A, 20 clones showed significantly superior girth. Girth in the 11th year of tapping ranged from 50.6 to 93.8 cm with check clone RRII 105 having 63.7 cm. Yield in the 11th year ranged from 16.9 to 101.4 g t^{-1} t^{-1} with check clone RRII 105 having 41.9 g t^{-1} t^{-1}. Sixteen clones showed significantly higher yield than the check clone RRII 105. Hybrid clone 89/27 (PB 260 x RRIM 600) continued to yield more than 100 g t^{-1} t^{-1} superior girth.

The top selections of Wickham x Amazonian germplasm hybrids *viz.*, 90/10 (83.3 g t^{-1} t^{-1}) and 90/271 (92.9 g t^{-1} t^{-1}) along with other high-yielding hybrids, continued to exhibit superior yield performance over the check clone RRII 105 during the 11th year of tapping. These selections *viz.*, 90/10 (70.1 g t^{-1} t^{-1}) and 90/271 (55.6 g t^{-1} t^{-1}) also showed superior performance based on mean long-term yield over 11 years of tapping compared to control clone RRII 105 (36.7 g t^{-1} t^{-1}). In another small scale evaluation of W x A hybrids (11th year of tapping) maximum girth was recorded in the hybrid clone 90/55 (94.4 cm) followed by 90/109 (87.0 cm) and 90/129 (85.8 cm), while most promising yield was obtained in hybrids 90/274 (70.7 g t^{-1} t^{-1}), 90/55 (68.8 g t^{-1} t^{-1}) and 90/109 (64.0 g t^{-1} t^{-1}).

Yield of small scale trial (SST) 1998 A (11th year) ranged from 28.1 to 78.2 g t^{-1} t^{-1} with the check clone RRII 105 having 57.3 g t^{-1} t^{-1}. Eight clones showed comparable yield to the check clone RRII 105 with hybrid clone 93/48 being the highest yielder (78.2 g t^{-1} t^{-1}). Seven clones were significantly superior in girth to any of the parent clones while 13 clones showed significantly superior girth to the check clone RRII 105.

Table Bot. 1. **Family means of important traits among hybrids in the 1999 small scale trial**

Trait	RRII 105 x RRIM 703	RRII 105 x RRII 118	RRII 105 x PB 86	Check clone RRII 105
Dry rubber yield over six years (g t^{-1} t^{-1})	35.5 (2)	48.3 (14)	35.8 (4)	39.8
Girth at opening (cm)	45.2 (4)	51.9 (9)	47.8 (4)	47.8
Girth increment –immaturity (cm yr^{-1})	7.4 (1)	8.7 (14)	8.1 (9)	8.2
Girth increment on tapping (cm yr^{-1})	2.6 (16)	3.5 (18)	3.1 (17)	1.6
Total bark thickness (mm)	7.1 (1)	6.9 (nil)	6.7 (nil)	7.8
Total number of latex vessel rows	7.7 (nil)	9.0 (nil)	9.1 (nil)	13.7
Annual mean dry rubber content (%)	37.5 (9)	39.8 (14)	39.7 (14)	37.1
Annual mean volume of latex (ml t^{-1} t^{-1})	100.8 (8)	126.9 (10)	87.3 (3)	102.0

Number of hybrids better than check clone is given in parentheses

When hybrids evolved from three bi-parental crosses involving RRII 105 as female and RRII 118, RRIM 703 and PB 86 as male parents were evaluated for their performance with respect to yield and yield components over six years of tapping in panel BO-1 (SST 1999), the family of RRII 105 x RRII 118 was found superior (Table Bot. 1).

Hybrids of RRII 105 and RRII 118 showed superior annual mean yield as well as summer yield performance compared to check clone RRII 105, in another (1999) SST. Hybrids *viz*., 95/346 (72.4 g t^{-1} t^{-1}) and 95/306 (70.8 g t^{-1} t^{-1}) showed superior yield performance compared to check clone RRII 105 (59.8 g t^{-1} t^{-1}).

In a SST of 17 hybrid clones from seven cross-combinations along with seven parental clones (1995), maximum girth (15th year) was noticed in hybrid clone 95/62 (85.2 cm) followed by 95/124 (83.3 cm) and 95/575 (81.2 cm). Maximum yield (8th year of tapping) was recorded in 95/7 (64.6 g t^{-1} t^{-1}), 95/9104 (58.7 g t^{-1} t^{-1}) and 95/95 (56.1 g t^{-1} t^{-1}). Of the 24 hybrid clones evaluated in another SST, four hybrids *viz*., 94/24 (RRII 105 x RRII 208); 95/92 (RRII 105 x Mil 3/2); 95/296 (RRII 105 x RRII 118) and 95/567 (RRIM 600 x RRII 203) maintained superior growth and yield in the 3rd year of tapping. Hybrids 94/24 and 95/296 registered significantly higher yield (62.0 g t^{-1} t^{-1} and 64.0 g t^{-1} t^{-1} respectively). These were pollarded to generate budwood for establishing source bushes towards further evaluation.

Among the small scale trials laid out in 2001 for evaluation of hybrids from 1995 HP, in Trial I, hybrid clones 95/413 (RRIM 600 x RRII 203) and 95/425 (RRIM 600 x RRII 203) registered superior yield while clone 95/63 (PB 242 x RRII 105) registered maximum growth (74.5 cm) than done RRII 105 (52 cm). In Trial II, clones 95/196 (81.2 cm) and 95/355 (73 cm) continued to show vigorous growth than RRII 105 (55.7 cm), while clones 95/519, 95/535 and 95/129 registered significantly higher yield than RRII 105. Clones 95/304 (82.9 cm), 95/514 (74.0 cm) and 95/562 (70.6 cm) registered exceptional growth traits than control (RRII 105, 56.6 cm) while clone 95/410 (RRIM 600 x RRII 203) and 95/304 (RRIM 600 x RRII 118) maintained higher yield continuously for last five years in the Trial III.

In the clonal nursery evaluation of hybrids evolved from the 2002 HP, clones 02/683, 02/638, 02/688 and 02/335 emerged as fast growing clones when compared to RRII 414, RRII 429 and RRII 105. Test tap yield of recombinants varied from 3.9 to 28.0 g t^{-1} t^{-1} when compared to that of the check clones (4.0 to 15.3 g t^{-1} t^{-1}).

In order to widen the genetic base of breeding populations and to develop improved clones by crossing promising Wickham x Amazonian hybrids (WxA) with RRII 400 series clones and RRII 105, progenies were developed every year from 2011 to 2014 and planted in seedling nurseries along with half sibs which were collected from clones used as 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. The 91 hybrid and 216 half sib seedlings obtained in 2014 were maintained in the nursery. Among the 275 test tapped HP as well as half sib seedlings, 30 seedlings including 6 hybrid seedlings yielded more than 10 g 10 tap^{-1}. A breeding orchard was maintained at RRII consisting of 48 clones among which 13 clones flowered.

1.2. Ortet selection

In the clonal nursery of ortets/hybrids at RRII (2007B), the ortets and hybrids from Paraliar (Kanyakumari) showed better test tap yield than those from Guwahati and Padiyur over five consecutive seasons. The ortet Par O 18 showed a maximum test tap yield of 16.8 g t^{-1} t^{-1} followed by Guw 4 (12.3 g t^{-1} t^{-1}), Guw 9 (11.0 g t^{-1} t^{-1}), Par O 10 and Pad 270 (10-11 g t^{-1} t^{-1}). Par O 18 also recorded highest girth (38 cm). Guw 4 and Guw 9 recorded 30-31 cm girth which was comparable to RRII 414. These selections also exhibited desirable secondary traits with respect to bark thickness and number of latex vessel rows.

In the SST of ortets (1995) selected from smallholdings, two ortets O 72 (81.5 g t^{-1} t^{-1}) and O 73 (87.6 g t^{-1} t^{-1}) recorded significantly superior yield over RRII 105 (50.4 g t^{-1} t^{-1}) during the 10th year of tapping. Mean yield of these two clones over 10 years of tapping was significantly superior (72-78 g t^{-1} t^{-1}) to the check clone (48.0 g t^{-1} t^{-1}). In the clonal nursery trial (2012) consisting of 23 Andaman ortets, no significant variation in girth could be observed.

2. Evaluation of clones

2.1. Large scale trial (LST)

Among the RRII 400 series under large scale evaluation at CES, (11th year of tapping; panel BI-1), clones RRII 430 (62.6 g t^{-1} t^{-1}) and RRII 417 (57.0 g t^{-1} t^{-1}) were found superior to RRII 105 (47.0 g t^{-1} t^{-1}). In trial 1, RRII 417 had the highest yield in summer (volume yield of 110 ml t^{-1} t^{-1} and dry rubber yield of 46.8 g t^{-1} t^{-1}). In trial 2, RRII 430 showed a summer yield of 47.8 g t^{-1} t^{-1} and highest volume yield of 98.3 ml t^{-1} t^{-1} during summer season. The summer yield of RRII 105 was 41.3 and 35.5 g t^{-1} t^{-1} in trials 1 and 2 respectively. In the LST of PB clones at CES, Chethackal (panel BO-1), clones PB 312 and 314 were found superior while in panels BO-2 and BI-1 clone PB 280 was found superior (Table Bot. 2). Clone PB 280 was found superior in terms of yield per unit girth also.

In an LST of nine clones at CES (1999), highest girth (15 cm) was recorded in clone 4 (71.5 cm) followed by clone 12 (66.8 cm) and RRII 105 (61.9 cm). In the fifth year, maximum yield was recorded in RRII 105 (65.0 g t^{-1} t^{-1}) followed by clone 12 (62.5 g t^{-1} t^{-1}) and clone 4 (55.3 g t^{-1} t^{-1}).

Tapping was initiated in two large scale trials of ortets selected from large estates. While tappability was 48% in ortet CyO 48, it was 55% in RRII 105. Ortet CyO 32 (42.8 g t^{-1} t^{-1}) was found the highest yielder while seven Cheruvally ortet clones were comparable to clone RRII 105 (30.0 g t^{-1} t^{-1}). Based on first year yield data in another LST of ortets from Koney and

Table Bot. 2. **Yield (g t^{-1} t^{-1}) performance of the clones in the virgin and renewed panels**

Clone	Yield over 6 yrs in BO-1 panel	Yield over 4 yrs in BO-2 panel	Mean yield over 10 yrs in BO panel	Yield over 4 yrs in BI-1 panel	Yield per unit girth over 14 yrs (gm cm^{-1})
PB 235	45.6 cd	73.6 cde	56.8 cd	61.9 de	0.6 d
PB 311	50.0 bc	88.7 ab	65.5 ab	87.8 bc	0.7 c
PB 280	54.2 ab	95.4 a	70.7 a	123.3 a	0.9 a
PB 314	58.3 a	84. 6 abcd	68.8 a	100.1 b	0.8 ab
PB 312	59.8 a	87.9 ab	70.0 a	76.5 cde	0.8 bc
PB 217	38.4 d	79.2 bcd	54.7 d	100.1 b	0.7 bc
PB 260	48.3 bc	86.2 abc	63.5 abc	74.5 cde	0.7 bc
PB 255	43.3 cd	66.4 e	52.5 d	81.4 bcd	0.7 c
RRII 105	49.7 bc	72.6 de	58.8 bcd	56.6 e	0.7 cd
CD	7.2	11.8	7.7	19.8	0.09

Means followed by a common alphabet are not significantly different by DMRT.

Mundakkayam estates, ortets KO 27 (50.2 g t^{-1} t^{-1}) and KO 25 (41.5 g t^{-1} t^{-1}) had yield on par with RRII 430 (54.8 g t^{-1} t^{-1}) which was found superior in yield, girth and tappability. The yield of clone RRII 105 was 32.5 g t^{-1} t^{-1}.

In the LST (1996) of a set of 13 hybrid clones evaluated in RRS, Padiyur, seventh year yield was maximum in clone 86/597 (77.0 g t^{-1} t^{-1}). Over 7 years of tapping, three clones performed (56-60 g t^{-1} t^{-1}) better than RRII 105 (53.0 g t^{-1} t^{-1}). The bark thickness and LVR of clones 86/597 and 86/468 were comparable to RRII 105. Two clones *viz.*, 86/400 (70.0 g t^{-1} t^{-1}) and 86/61 (61.0 g t^{-1} t^{-1}) were identified as superior from another LST planted in 1996 at RRS, Padiyoor.

2.2. On-farm Trial (OFT)

Post release observations on RRII 400 series clones in small holdings across North, Central and South Kerala were continued. Regional performance of RRII 400 series clones was evaluated in farmers' fields (eight locations) at Central Kerala. Location wise yield variation was evident for clones. Mean yield across the locations showed higher yield for RRII 400 series clones over control clone RRII 105. Highest yield (g t^{-1} t^{-1}) in central Kerala was recorded for RRII 430 (63.9) followed by RRII 414 (62.7). In North Kerala, (OFT at Ottappalam), RRII 417 performed better than RRII 105 in terms of growth and yield (41.6 g t^{-1} t^{-1} and 35.7 g t^{-1} t^{-1} respectively). In the fourth year of tapping, RRII 417 gained higher mean girth (55.2 cm) when compared to RRII 105 (51.1 cm). The RRII 400 series clones were found relatively tolerant to ALF disease.

To evaluate the performance of promising pipeline clones in South Karnataka region, an on-farm trial was laid out at KFDC plantations, Karnataka with 14 top yielding clones selected from those included in the first three phases of the participatory clone evaluation programme along with seven check clones. On-farm evaluations that were ongoing in large estates were continued. In the 1992 OFT, four imported clones and two indigenously developed clones were evaluated in block trial in Shaliackary

Estate, Punalur. Clone PB 280 with more than 2000 kg ha^{-1} yr^{-1} was found the highest yielder based on mean yield over six years. Clone PR 261 with an overall mean yield of about 1560 kg ha^{-1} yr^{-1} was the lowest yielder. Based on mean girth in the 14th year of tapping, PB 260 (85.0 cm) and PB 280 (78.0 cm) was found superior when compared to RRII 105 (75.0 cm). Remaining clones showed almost similar mean girth. The experimental clones exhibited more than 30% overall mean TPD incidence. Among the clones, PB 260 exhibited maximum TPD incidence (43%) while the other clones had 27-34% TPD incidences.

In the 1993 OFT at Shaliackary Estate, Punalur, (A panel), PR 255 exhibited a mean yield of 1301 kg ha^{-1} yr^{-1} while PB 255 yielded 1136 kg as compared to check clone RRII 105 (1578 kg). Under controlled upward tapping system with stimulation, there was remarkable increase in rubber yield. While mean yield of the clones in the lower virgin panel was 1248 kg ha^{-1} yr^{-1}, mean yield in the higher panel (under CUT system) was 2644 kg ha^{-1}. Maximum yield was recorded in clone RRII 176 (3782 kg ha^{-1}) followed by RRII 50 (3501 kg ha^{-1}) while clone RRII 105 yielded 3105 kg ha^{-1}.

2.3. Genetic studies and investigations on G x E interactions

In the G x E interaction studies (1996) across five locations, two large scale trials in Kanyakumari and Odisha were closed. Sufficient long term yield data was generated. Mean yield from four locations was consolidated for site-wise analysis and GEI for long term yield over 36 environs.

3. Participatory evaluation of rubber clones in the pipeline

Field planting of 23 pipeline clones under Phase 4 of the project was completed in seven locations *viz.*, the central large scale trial at RRII farm, Kottayam, and on-farm trials in six locations (Thrissur, Mundakayam, Kulathupuzha and Kozhikode of Kerala; Nettana in Karnataka and Bethany Estate in Kanyakumari). The first batch of 13 promising W x A hybrids evolved in India has thus been planted for the final multilocational evaluation. Growth of pipeline clones planted in various locations in 2012 under phase 3 and in 2010 under phase 2 of the project was monitored. In Calicut where incidence of diseases was comparatively high, all the clones in the phase 3 trial (except P 112 -susceptible to pink and P 066 – susceptible to CLD) were found better than RRII 105 in terms of disease tolerance. In the 2010 OFT at Pudukkad Estate under Phase 2 of the project, clone P 044 exhibited maximum girth (39.9 cm) when compared to RRII 430 (38.7 cm), while RRII 105 had 33.1 cm. In the 2010 OFT at Calicut Estate, Kozhikode, the girth of clone P 064 was comparable to RRII 414 and RRII 430.

Among the multi-locational on-farm trials established in 2008 under phase 1 of the project, the check clones RRII 414 and RRII 430 exhibited maximum girth and tappability across the locations observed. In Be Be estate, Punalur, pipeline clones P 063 and P 026 exhibited maximum girth and tappability and were comparable to the check clones RRII 414 and RRII 430 while the performance of RRII 105 was not up to the mark. In terms of bark thickness, RRII 414 and RRII 430 were found better than RRII 105. While in Gokul estate, Perinthalmanna P 063 and P 087 were found superior in terms of tappability and bark thickness, P 072 and P 063 were found better in Koottikkal estate, Mundakkayam. At Athirapilly estate, RRII 414 recorded superior girth (52.2 cm) followed by the experimental clones- P 021 (48.0 cm), P 074 (45.0 cm).

In the LST under Phase 1 (CES in 2008), there was significant clonal variation for girth and tappability at 6 ½ years after planting. Among

the check clones, RRII 414 was found superior in terms of girth and tappability with 57-73% of trees attaining tappable girth in the two trials. While pipeline clones P 021, P 061, P 063, P 044 and P 026 were comparable to RRII 414, clone RRII 105 was found inferior.

4. Breeding for other specific objectives

4.1. Breeding for drought tolerance

In the two small scale evaluation trials (1998), one hybrid clone 93/58 (70.0 g t^{-1} t^{-1}) and two ortets Dap 111 and Dap 236 (80-83 g t^{-1} t^{-1}) continued their superior performance in the 9th year of tapping too. Summer yield of hybrid clones 93/58 and 93/272 were comparable to RRII 105. Wide range of variability for the major xylem vessel characteristics was found among 47 clones. Vessel density ranged between 2.7 (93/105) and 5 (AVT 73) while diameter was found minimum in 93/109 and maximum in 93/214. Clones 93/105 and 93/270 short listed for other intrinsic drought tolerance traits showed relatively low vessel density and least incidence of tyloses.

In an SST (1998; CES) with 15 hybrids derived from hybridization between high-yielding female parents and drought tolerant male parents, hybrid 93/214, exhibited superior yield (70.9 g t^{-1} t^{-1}) over nine years of tapping. Hybrids 93/80, 93/88, 93/216 and 93/145 were also found superior with more than 45.0 g t^{-1} t^{-1}. Yield in BO-1 panel was superior in hybrids 93/214 (64.4 g t^{-1} t^{-1}), 93/80 (4.8 g t^{-1} t^{-1}) and 93/88 (41.9 g t^{-1} t^{-1}). In BO-2 panel, 93/214 continued to maintain high yielding trend with more than 80.0 g t^{-1} t^{-1}. The hybrids 93/216, 93/88 and 93/145, yielded more than 60.0 g t^{-1} t^{-1} in BO-2 panel.

Among the 14 hybrids and their parents evaluated in an SST (1999), maximum girth (15th year) was found in hybrid 94/23 (80.4 cm) followed by 94/90 (80.0 cm) and 94/44 (74.5 cm). Maximum yield (8th year of tapping) was found in hybrid 94/44 (87.8 g t^{-1} t^{-1}) followed by 94/50 (76.3 g t^{-1} t^{-1}), 94/101 (75.3 g t^{-1} t^{-1}) and 94/23 (70.2 g t^{-1} t^{-1}).

A Large Scale Trial (LST) with 17 pipeline clones (RRII-105 x RRII 118; 3 half-sib selections and one ortet) was established at RRS Dapchari to select pipeline clones for yield under drought prone situation. In order to develop drought tolerant clones for the non-traditional regions, the drought tolerance capacity of 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 which was given only life-saving irrigation during the intense summer. Among the forty experimental clones (with nine check clones), seven experimental clones showed superior post summer girth than the top most check clone RRIM 600.

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 *viz.* Kerala (CES Chethackal) and Tamil Nadu (HBSS Paraliar). Assorted seeds as well as seeds from polyclonal seed gardens and also from drought tolerant clone RRIM 600 and drought susceptible clone RRII 105 were collected from each location. Seeds of drought tolerant clone RRII 203 from Orissa were also collected. When the growth of the assorted seedlings maintained under rain fed conditions were assessed, the plants from Kanyakumari were found superior (Table Bot. 3). Source location significantly influenced pre and post-summer growth. Post-summer data on growth showed that seedlings from Kanyakumari,

Table Bot. 3. **Growth of seedlings from various sources under drought**

Type of seedling	Seedling height (cm)	
	Pre-summer	Post-summer
Assorted Dapchari	48.6 de	70.6 ab
Assorted Orissa	44.9 ef	66.5 bc
Assorted Nettana	40.8 f	69.4 bc
Assorted Chethackal	56.2 ab	73.9 ab
Assorted Kanyakumari	61.8 a	82.8 a
RRIM 600 Dapchari	48.9 cde	68.2 bc
RRIM 600 Orissa	53.4 bcd	76.6 ab
RRIM 600 Nettana	43.1 ef	70.1 ab
RRIM 600 Kanyakumari	55.9 abc	74.7 ab
RRII 203 Orissa	46.0 ef	62.6 c
RRII 105 Dapchari	45.1 ef	62.5 c
RRII 105 Orissa	43.5 ef	62.2 c
RRII 105 Nettana	41.0 f	63.8 bc
RRII 105 Chethackal	54.2 bcd	74.3 ab
RRII 105 Kanyakumari	53.4 bcd	73.2 ab
CV	8.7	9.6

Means followed by a common alphabet are not significantly different by DMRT.

CES and Orissa were significantly superior. It was observed that seedlings from Orissa showed better growth during summer.

Physiological evaluation for drought tolerance in 45 pipeline clones was attempted at RRS Dapchari. Tolerant and susceptible plants were selected based on parameters such as membrane permeability, chlorophyll index and general growth characters including leaf yellowing/drying. Physiological parameters such as gas exchange parameters and photosynthetic efficiency were recorded in selected clones *viz.*, P 001, P 020, P 021, P 057, P 064, P 073 and P 101 and in check clones *viz.*, RRII 429, RRII 430, RRII 105, RRII 208 and RRIM 600. Under drought conditions, clones P 055, 64, 62, 59, 8, 84, 66, RRII 429 showed better chlorophyll index. Clones P 075, P 001, P 057, P 064, P 067, P 084 had higher membrane permeability. Under drought situation, clones P 001 and P 073 maintained better photosynthesis, Fv/Fm ratio and Phi PS II ratio while P 021, RRII 105, P 020, P 069, P 067 and RRII 429 were found moderate and RRII 208 and RRIM 600 were found poor. Similar parameters were recorded in selected clones (P 078, P 083, P 101, P 001, P 076, P 092, P 072, P 071, P 069, P 027, RRII 429 and RRII 208) during winter of 2014 at RRS, Agartala. CO_2 assimilation in clones P 078, 083, 101 and 001 was better under cold conditions.

4.2. Breeding for disease tolerance

Hybridizations between RRII 400 series clones (female parents) and Fx 516 (male parent tolerant to ALF disease) for improving disease tolerance in *Hevea*, and for developing a mapping population were continued. Observation on fruit setting showed about 10% initial success. For evaluating disease tolerance in open pollinated progenies, progenies from the disease tolerant clone Fx 516 were collected and grown in a seedling nursery established at RRII, Kottayam.

4.3. Polycross progeny evaluation

The clones under evaluation in the 1993 trial of polycross progenies were in the 14th year of tapping. Among the 33 selections, 15 selections had yield between 47.0 and 84.0 g t^{-1} t^{-1} in the renewed panel B.I.1 superior to RRII 105 (44.6 g t^{-1} t^{-1}). Response to stimulation in the selections was studied by applying three rounds of stimulation as per recommended practice. In Trial 1, clones 80 (77.0 g t^{-1} t^{-1}) and 27 (97.0 g t^{-1} t^{-1}) exhibited better response to stimulation (58 and 59% increase, respectively) when compared to RRII 105 (64.0 g t^{-1} t^{-1}) which had 36% increase.

Twenty rubber clones from half-sib progenies of diverse female parents were subjected to accelerated field evaluation through a clonal nursery trial laid out in close spacing of 2.5 x 2.5 m. Four high yielding precocious control clones (RRII 430, RRII 414, RRII 105 and PB 235) were included as check clones. Superior clones were identified from the population for agronomic traits *viz.*, growth, juvenile yield and bark thickness within a short span of six years. Half-sib progeny of mother clone PB 86 (HS PB 86/57) registered significantly (p=0.05) superior juvenile yield of 18.0 g t^{-1} t^{-1}, with a girth of 43 cm in the 6th year after planting when compared to RRII 105 (11.9 g t^{-1} t^{-1}; 34.1 cm girth) and on par with RRII 430 (14.0 g t^{-1} t^{-1}; 31 cm girth) and RRII 414 (14.0 g t^{-1} t^{-1}; 38 cm girth). The second best clone HS LCB 1320/30 also recorded comparable yield and growth with RRII 430 and was better than RRII 414.

In the polycross progeny trial (1995), half-sibs of all the eight clones showed better yield performance ranging from 31.3 to 43.9 g t^{-1} t^{-1} when compared to check clone RRII 105 (29.9 g t^{-1} t^{-1}) in the second year of tapping. Similarly, half-sib families of polyclonal origin showed better girth than check clone.

4.4. Biodiversity museum of rubber and miscellaneous forest trees

A model repository of 62 local / indigenous / rare forest trees, species, was planted in a biodiversity museum in the RRII main campus, Kottayam, with assistance from the Kerala Forest Research Institute, Peechi, Kerala.

5. Anatomical investigations

Various histochemical methodologies were employed to identify the moving objects earlier found in the sieve tubes of rubber. In older sieve tubes, the moving objects were identified as starch granules. Comparatively small particles with similar movement were noticed in sieve tubes of petiole.

Anatomical and histochemical aspects of TPD affected trees of *Hevea* were studied. TPD affected bark showed high degree of lignin biosynthesis which involved enzyme peroxidase. A positive indication of peroxidase activity in the cell walls of sieve tubes, and cytoplasm of phloic rays was noted. Phloic rays exhibited seasonal variation in the levels of peroxidase enzyme whereas in the sieve tubes the activity was found constant throughout the year. Deactivation of both these type of tissues were evident in TPD affected trees.

In studies on yield contributing bark anatomical parameters (bark thickness and latex vessel rows) of experimental clones from on farm trial at Nagrakatta comprising of 11 clones,

PB 280 was found superior (8.26 mm bark thickness, 19 rows of latex vessels) to clone RRII 105 (7.3 mm bark thickness, 13 rows of latex vessels).

6. Investigations on rubber wood

Among the eight Prang Besar clones (Table Bot. 4), clear bole volume was found maximum in PB 235 followed by PB 312 in the 23rd year. There was no significant variation in wood specific gravity.

Table Bot. 4. **Rubber wood parameters in PB clones**

Clone	Clear bole volume (m^3 t^{-1})	Specific gravity
PB 235	0.31	0.649
PB 311	0.25	0.659
PB 280	0.19	0.664
PB 314	0.18	0.652
PB 312	0.28	0.664
PB 217	0.18	0.634
PB 260	0.24	0.631
PB 255	0.22	0.618
RRII 105	0.14	0.668
CD	0.049	ns

7. Studies on propagation

Nursery technology in rubber has been revolutionalised with the recent introduction of root trainer plants grown in containers with soil free media. Partially dried elephant dung was tried as an alternative to coir pith alone or in combination with coir pith or soil. Among the treatments, plants grown in a medium comprising elephant dung and soil had early bud sprouting; better shoot length (44.3), diameter (6.8 m) and number of leaf whorls (1.6) when compared to other potting medium. This was followed by elephant dung+soil+coir pith (38.1, 6.4 and 1.5) and the least values were recorded for elephant dung alone (32.2, 5.8 and 1.2). The Board have recently recommended elephant dung as potting medium for root trainer plants. Two private rubber nurseries could raise 20, 000 root trainer plants successfully.

Five types of dormant buds of one year old scion of the most popular clone RRII 105 *viz.* brown leaf scar buds, greenish brown leaf scar buds, greenish brown scale leaf buds, clustered leaf storey buds and tender leaf buds were grafted onto one year old stock seedlings. Differences in budding success, survival rate and growth parameters in young stage were not found significant (P=0.05), except the height of the plants originated from brown leaf scar buds. However, differences became apparent with respect to percentage of trees attaining tappable girth in the seventh year. Trees raised from greenish brown leaf scar buds showed the highest percentage of tappable trees (63.9%) followed by greenish brown scale leaf buds (54.2%) which indicated relatively better performance of these buds in terms of uniform growth and tappability of the trees. Trees originated from brown leaf scar buds attained moderate (45.5%) tappability. Clustered leaf storey buds and tender leaf buds attained significantly low tappability of 37.5 and 36.7%, respectively with higher intraclonal variation in growth indicating graft incompatibility of these buds. Planting materials with optimum qualities such as uniform growth and tappability and shorter gestation period could be achieved by grafting greenish brown leaf scar buds collected from one year old scion. Quality of buds is one of the major factors which determine the quality of the scion in terms of growth and tappability. Selection of good quality buds while bud-grafting is important in producing viable planting materials.

GERMPLASM DIVISION

The genetic resources of *Hevea* comprise three gene pools - the 1981 IRRDB wild *Hevea brasiliensis* germplasm collection, domesticated clones derived from the original Wickham origin and five other *Hevea* species. Their conservation, evaluation and utilization in the genetic improvement of *Hevea* form the focus of the activities of the Division. Alternative natural rubber yielding plant species suitable for marginal lands are also being explored.

1. Introduction, conservation and documentation

1.1. Domesticated genepool (Wickham collection) from secondary centers

This genepool comprises 183 Wickham clones being conserved in a clone museum at RRII, Kottayam, and three germplasm gardens at CES, Chethackal. While the clone museum is in the form of a budwood nursery, the germplasm gardens are in the form of arboreta and serve the primary purpose of conservation, scientific data collection as and when necessary, and a source of flowers for breeding when required.

51 introduced clones are being conserved in Germplasm Garden 77. This arboretum was planted in 1977 and regenerated in 2000 by ratooning at a height of 20 cm and gapfilling vacancies with the corresponding polybag clones. Among the five IRCA clones in the Germplasm Garden 92, IRCA 130 and IRCA 111 continued to be better than RRII 105 for vigour, while the remaining 3 clones were on par with it. In the 13th year of tapping, dry rubber yield of IRCA 130 was far superior to all other clones, which were on par with RRII 105. A budwood nursery of IRCA clones was established at CES for generating required budwood for supply. In the Germplasm Garden 94, statistically significant clonal differences were recorded for girth and yield. In the 11th year of tapping, among the 20 clones, RRIC 100, RRIC 148 and RRIM 609 were superior to the remaining clones (72.2-70.9 g $t^{-1}t^{-1}$) for yield; RRII 23, PB 255 and RRIC 100 were superior in terms of girth (102-100.7 cm). Control RRII 105 had an average girth of 73.8 cm and yield of 38.0 g $t^{-1}t^{-1}$. Bark samples collected from these trees at the age of 15 years were analysed for anatomical traits. RRIM 609, RRII 23, RRII 105 and RRIC 100 had the highest bark thickness (9.0-10.3 mm) while RRII 105 and RRII 27 had the least bark thickness of 6 mm. RRII 23 also had the highest number of latex vessel rows (44.5) followed by RRIC 36 (34.0), compared to RRII 105 (27.0).

1.1.1. Formulation of DUS testing norms in Hevea

Data on trunk and branch traits was initiated in a set of 51 mature clones in the Germplasm Garden 77 at CES, and wintering pattern was recorded. Quantitative data on pre and post winter and summer data in all three locations were recorded to identify GxE effects.

1.2. IRRDB 1981 wild gene pool

1.2.1. Conservation nurseries

3576 wild accessions are being maintained in the original field conservation-cum-source bush nurseries (SBNs), as well as in reestablished nurseries. Preliminary evaluation of the wild accessions in the first four years of growth before cutting back to form BWNs, is being carried out in the reestablished nurseries from 2003 onwards. Establishment of a separate working collection comprising all the wild accessions showing potential for yield, yield related traits, or any biotic or abiotic stress tolerance, was initiated this year.

1.2.2. Arboretum

The first set of accessions for the arboretum being established at Tura, was planted comprising 69 wild and 6 Wickham clones. The second set

of accessions was multiplied and established in the polybag nursery at Tura. Another arboretum established earlier and comprising of 120 accessions, is being maintained at CES, Chethackal.

1.3. Other *Hevea* species

This genepool, comprising 6 accessions of five other species available at RRII *(H. benthamiana, H. spruceana, H. nitida, H. camargoana* and two accessions of *H. pauciflora)*, along with five natural putative interspecific hybrids, two *H. brasiliensis* clones, and F x 516 (an interspecific cross between *H. brasiliensis* and *H. benthamiana*), are being conserved in an arboretum planted at CES in 2006. *Phytophthora* tolerance of these accessions was recorded. *H. benthamiana* and FX 516 showed minimum ALF.

2. Characterization and preliminary evaluation

At RRS, Padiyoor, 171 wild accessions were evaluated for growth and yield in PET 2000A. High yielding accessions such as AC 3131, AC 552 and RO 2136, and vigourous accessions MT 4219, AC 4140 and MT 387 for timber traits were selected in PET 2000A. Among 166 wild accessions in PET 2000B, accessions AC 341, MT 4351 and RO 210 were identified as potential yielding accessions, while AC 647 and RO 2883were identified as potential timber clones. These accessions are conserved as male parents for future W x A hybridization programmes at RRS, Padiyoor. RO 1313, AC 567 and AC 1964 were selected from PET 2002. Selections from these two trials were maintained for further W x A hybridization programmes at Padiyoor.

3. Further evaluation and selection

3.1. Clonal nursery evaluation

First round of test tapping of 15 wild accessions at CES could not identify any potential wild accession. The highest yielder was MT 5078 and among the check clones, RRII 414 recorded the highest yield. Though the second round of test tapping was due to be done last year, it was not carried out due to shortage of laborers and funds. Recorded girth of the clones and the highest girth was recorded by MT 5078. Among the check clones RRII 414 had the highest girth.

3.2. Further evaluation trials (FET)

From the further evaluation trial FET 1995 at RRS, Padiyoor, eight relatively high yielding wild accessions and 10 potential timber yielding accessions could be identified and were selected as male parents for Wickham X Amazonian (W X A) hybridization programmes. Last year the trees were marked as male parents and are preserved in the trial area as hybridization programme could not be carried out due to shortage of fund.

Out of 22 wild accessions in the FET 2003, RO 287, RO 2629, MT 999 and AC 163 recorded the highest annual girth while RO 2629, AC 4149, RO 3804 and AC 716 recorded the highest yield. Analysis of girth of 22 wild accessions and three controls in FET 2005 showed highly significant clonal differences. AC 2004 followed by MT 43 and MT 4788 had the highest girth, on par with the best check PB 260. RO 1241 showed the highest tappability of 69.2%, while seven other wild accessions showed more than 50% tappability, compared to 69.2% and 53.3% of PB 260 and RRII 105 respectively. The trial will be opened for regular tapping next year. In FET 2008, AC 176, RO 2846 and RO 4149 recorded the highest girth in the sixth year of growth.

Among the 13 accessions in FET 2010, the highest girth was recorded by RO 1769 followed by AC 3146. Another set of 22 selected wild accessions along with 3 control clones are in the first year of growth in FET 2013 at CES, Chethackal A set of 12 wild accessions selected on the basis of preliminary evaluation in the nursery, were planted along with 3 control clones in a further evaluation trial (FET 2014),

employing randomized block design (RBD) at RRS, Dapchari, as part of the multilocation detailed evaluation of selections from preliminary evaluations.

3.3. On-farm trials

On-farm trials have been established at five locations *viz.*, B.C. Cheruvally estate in Erumely, Malankara estate at Thodupuzha, Mooply estate at Trissur, Calicut estate at Kozhikode and Bethany estate at Kanyakumari for evaluating the performance of the three selected IRCA clones (IRCA 130, IRCA 111, IRCA 109) and one wild accession (AC 166) at multi locations. 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 and the lowest girth was in the clone IRCA 109. Among the check clones RRII 430 recorded the highest girth followed by RRII 414. At Malankara estate, among the test clones IRCA 111 recorded the highest girth and the lowest girth was in the clone IRCA 109. Among the check clones RRII 414 recorded the highest girth. At Calicut estate, the clone IRCA 130 showed better growth performance followed by IRCA 111, IRCA 109 and the least in AC 166. At BC Cheruvally estate, highest girth was recorded by IRCA 130, followed by IRCA 111 and AC 166.

4. Screening for stress tolerance

4.1. Screening for biotic stress tolerance

A hotspot evaluation trial was established in 2013 at Ulickal nursery, Iritty. A set of 41 short listed wild *Hevea* accessions along with 2 control clones were monitored for its field tolerance to *Corynespora*.

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 in the fourth year of growth was recorded by the family of PB 5/51. Among the four check clones, the highest girth was recorded by RRII 105. There was significant difference in clonal response towards drought stress. The clones were subjected to test tapping for comparing their yield potential. The highest average yield was recorded by the family of PB 242.

In the clonal nursery at RRS, Padiyoor with 29 potential half-sibs of 8 clones and 2 hybrid progenies, during second year also the highest average girth was recorded by hybrid 93/10 followed by the family of PB 5/51. Among the four check clones, the highest girth was recorded by RRIM 600 followed by RRII 430. The highest test tap yield was recorded by the family of PB 5/51.

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 seven summer periods from 2008-2014, nine wild accessions and 4 HP clones recorded girth higher than the proven drought tolerant clone RRIM 600. Accession MT 40 recorded highest girth at seventh year under unirrigated condition at Dapchari. The modern clones RRII 430 and RRII 414 showed significantly better growth than the proven drought tolerant clone RRIM 600 under Dapchari conditions. Among the five hybrid clones, 93/270 recorded the highest girth followed by 93/105.

4.2.2. Cold tolerance

Two cold evaluation trials comprising of sixty four wild *Hevea* accessions are under evaluation for growth and yield at Regional Experiment Station, Nagrakatta, West Bengal.

Higher annual 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 MT 915, RO 2727 and RO 3169 recorded the highest girth compared to that of the controls Haiken 1 and RRIM 600.

5. Screening for timber characteristics

5.1. Field screening

Annual girth and monthly yield were recorded in 25 accessions at RRS, Padiyoor. MT 941 and MT 1032 recorded the highest girth, while RO 685 and AC 707 the highest yield.

6. Utilisation of *Hevea* germplasm

6.1. Hand pollination programmes

Growth was monitored in the 75 progenies of the 2009 hand pollination programme at CES, Chethackal, involving three wild accessions, six cultivated Wickham clones, along with the OP seedlings of the Wickham parents. Five cross combinations with testtap yield higher than the population average, were identified for further evaluation.

At RRS, Padiyoor 29 seedling progenies derived from two crosses in 2009, along with 25 OP seedlings of RRII 105, are under evaluation in a seedling nursery. Mean test tap yield was higher in the combination of RRII 105 x AC 675 than the progenies of RRII 105 x RO 368. Steps were initiated to multiply high yielding hybrid seedlings at Ulickal nursery in order to raise a budwood nursery of these high yielding hybrids.

43 seedling progenies of the 2014 HP involving four potential wild accessions and RRII 105 were planted in the nursery last year, of which 29 have survived. The 46 hybrids of 2013 HP are under evaluation in the seedling nursery. Growth in terms of girth and height was monitored and four vigourous hybrids identified. The seedlings were also screened in the nursery for *Phytophthora* tolerance. The four vigourous seedlings did not show symptoms of shoot rot, but *Colletotrichum* was observed in two of them.

6.2. Generation of mapping population

An additional 110 seedlings were generated in the mapping population cross between *H. brasiliensis* (RRII 105) and *H. benthamiana* (F 4542) and planted in the seedling nursery this year, of which 80 survived. Together with the previous years' plantings, the total population now stands at 261. Growth of the 2013 HP set was monitored: the 72 seedlings had an average girth of 6.7 cm in the first year of growth, and ranged from 2-16 cm. Control plants (budded RRII 105) plants had an average girth of 6.1 cm and a range of 4-10 cm. Three hybrids were very vigourous with a girth of more than 10 cm. Incidence of *Phytophthora* shoot rot was observed in the hybrids and in the controls.

7. Other studies

7.1. Feasibility of ratooning in *Hevea*

This exploratory study had been superimposed on an existing germplasm garden planted in 1977 comprising 51 introduced clones, by felling the original trees at a height of 20 cm when they were 23 years old, to see the effect of ratooning in a tree crop like *Hevea*. At the age of 15 years, the ratoons were superior to the conventionally raised polybag plants for girth and reached tappability about 2-3 years ahead of their conventionally raised counterparts of the same age. The 51 clones showed variations for yield which was largely due to the small sample size (2-6) for each clone. The effect of ratooning on the yield potential needs to be confirmed in a larger sample size with a single clone. Wind damage and disease incidence were the same for both sets of plants. Morphologically, the ratoons had a 'shoe' on one side of the base of the tree corresponding to the old stump of the original planting. This area, roughly the bottom 20 cm, cannot be tapped. The other side was perfectly normal up to the base. The trunk of these trees also had a vertical groove on the side of the stump. Data collection from this area has

been completed. The technique has been successful in rejuvenating the trees, which continue to serve the purpose of conservation as Germplasm Garden.

7.2. Assessment of the performance of rubber plantations of ITDA, Andhra Pradesh

Growth of rubber plantations was assessed in 12 selected farmers' fields (2008 plantings) at RC Varam, A.P., a collaborative project with the Integrated Tribal Development Agency (ITDA), Govt. of Andhra Pradesh. In the seventh year of growth, Farm 7 recorded the highest growth (47cm) while the lowest was recorded in the Farm 4 (23.5cm).

7.3. Studies on alternative sources of natural rubber yielding plants

One accession of guayule rubber (*Parthenium argentatum*) and two accessions of Ceara rubber (*Manihot glaziovii*) are being conserved at RRII. Another accession of Ceara rubber from Palakkad was multiplied for inclusion in the germplasm collection.

BIOTECHNOLOGY DIVISION

Biotechnology research at RRII is mainly focused on the genetic improvement of *Hevea brasiliensis* using modern tools. The major ongoing research programmes in Biotechnology Division are: i) development of efficient protocols for *in vitro* propagation of elite *Hevea* clones; ii) transgenic plant regeneration for better adaptation to environmental stresses and tapping panel dryness, latex yield and disease tolerance; iii) i*n vitro* approaches to complement conventional breeding programmes; iv) ploidy variation through *in vitro* techniques; and v) study of molecular mechanisms and characterization of genes controlling tolerance to abiotic stresses.

1. Somatic embryogenesis and plant regeneration

Somatic embryogenesis from leaf cultures was attempted from *Hevea* clones RRII 105, RRII 414, RRII 417 and RRII 430. Viable cultures were initiated for callus induction with leaf explants collected from glass house grown bud grafted plants maintained in poly bags. The medium standardized earlier for somatic embryogenesis from leaf explants of clone RRII 105 was used for callus and embryo induction in 400 series clones. The callus obtained from clones RRII 105 and RRII 414 were proliferated by two repeated subcultures in proliferation medium fortified with a higher cytokinin/auxin ratio and increased levels of sucrose and phytagel. The earlier standardized embryo induction medium devoid of charcoal was used for the third subculture. Embryogenic callus obtained in the embryo induction medium was proliferated and somatic embryo induction was obtained. It was observed that the proliferation rate of the callus obtained from clones RRII 430 and RRII 417 was low in the earlier standardized medium.

Simultaneously work was carried out for direct embryo induction in leaf cultures. *In vitro* cultures were raised with shoot tips and axillary buds and the sterile leaves developed were used for culture initiation. Pretreatment experiments such as liquid culture and auxin shock prior to culture initiation were also carried out. Auxin shock was provided by exposure of the explants to high levels of 2, 4-D and NAA for different time intervals in both solid and liquid medium. Modified MS and $ChuN_6$ medium were experimented with different concentrations (0-10 mg L^{-1}) of phytohormones such as BA, TDZ, picloram, triacontanol and TIBA individually as well as in combination with lower concentration of auxins. Callus induction, leaf swelling and globular structures were observed in cultured explants after 2-3 weeks in different media when

kept in dark. Leaf explants at the early maturation stage, cultured after auxin shock, in medium supplemented with higher concentration of picloram and triacontanol, induced pro-embryos.

A study was initiated to examine the feasibility of *in vitro* screening for drought tolerance of *Hevea* clones. Few clones relatively tolerant and susceptible to drought were short listed based on previous observations made at whole plant/ field levels and their callus induction ability was assessed before *in vitro* screening. Immature anthers of clones RRII 430, RRIM 600, GT 1, RRII 105, RRII 414 and Tjir 1 were inoculated on standardized callus induction medium and dark incubated. It was observed that all the clones except GT 1 induced callus. To test the effect of agar on callus induction efficiency and genotypes, callus induction medium was solidified with various concentrations of agar ranging from 0.2 - 0.35%. Immature anthers of clones RRII 430, RRIM 600 and RRII 105 and Tjir 1 were inoculated in four agar concentrations and the experiment was repeated five times. All the four clones induced callus after two months of culture in all agar concentrations tried.

Paclobutrazol, an antigibberellin was reported to promote profuse rooting in budgrafted poly bag plants of *Hevea*. The response of this chemical on root enhancement in *in vitro* cultures was studied. Viable cultures were initiated with clonal materials and zygotic embryos. Seedlings raised *in vitro* from zygotic embryos and *in vitro* developed somatic plants were subcultured in medium supplemented with paclobutrazol. Clonal and seedling shoots after *in vitro* adventitious root induction was also exposed to medium containing paclobutrazol. These cultures exhibited morphological symptoms typical to the effect of antigibberellins such as reduced shoot growth and root thickening. Experiments are being continued to assess the effect of paclobutrazol on root enhancement.

1.1. Cloning and characterization of *SERK* gene

Somatic embryogenesis receptor kinase *(SERK)* gene has been reported to be differentially expressed during somatic embryogenesis in numerous plant species. An attempt was made to isolate and characterize *SERK* gene from *Hevea* and related its association in somatic embryogenesis. Somatic callus induced from immature anther of clone RRII 105 were separated after 20 and 40 days of culture. A portion of the callus was subcultured for embryogenic callus formation. RNA was isolated from 20 and 40 days old callus, embryogenic callus and mature leaf. First strand cDNA was synthesized from all the four samples by reverse transcription reaction with oligo-(d) primers using Improm-II reverse transcription system. Amplification of *SERK* gene from embryogenic callus was performed with primers designed from the consensus sequences of other reported crops. PCR with cDNA of embryogenic callus yielded a specific band of 0.4 kb. The PCR product was cloned and sequenced. The cloned fragment showed 78 per cent sequence homology with cDNA sequence of *AtSERK*1 and the sequence was 408 bp in length. The amino acid sequence of *HbSERK* gene when compared with *AtSERK*1 at the protein level showed 99 per cent identity and the sequence contained 135 amino acids. RT-PCR analysis revealed good amplification with embryogenic callus, while no amplification was detected either in primary callus or with leaf, suggesting its role in somatic embryogenesis. Since *SERK* gene expression was detected only in embryogenic callus, this could be a potential marker for *Hevea* somatic embryogenesis.

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

2.1. *In vitro* fertilization and plant recovery

2.1.1. Embryo rescue and plant regeneration

A system for the rescue of immature

embryos with 42 per cent success was developed earlier. In the reporting period the feasibility of utilizing the system for developing haploids and interspecific hybrids is being tested. For developing haploids, hand pollinations were carried out in RRII 105 plants with irradiated pollen grains (2500GY) following standard procedure. The developed fruits were collected after six weeks of pollination and cultured employing *half ovulo* embryo culture technique. The developing embryos were dissected after one month and cultured in the maturation medium. After maturation, embryos were transferred to germination medium. The regenerated plants were hardened and transferred to big poly bags.

2.1.2. Induction of polyembryony

For developing new embryogenic lines from the zygotic embryo and for improving the already developed pathway, fruits were inoculated in different combinations of culture media. The ovules with multiple embryo induction were identified and further cultured on proliferation medium. The uniform seedlings already developed were used to study the root stock induced variation in scion. The uniform seedlings and their bud-grafted counter parts were subjected to genetic and epigenetic analysis using RAPD and Methylation sensitive AFLP respectively. It was observed that all the plants tested were genetically uniform. However polymor- phism in the DNA methylation pattern was observed among bud grafted plants and it was absent in uniform seedlings of single zygotic origin. Two polymorphic bands obtained were eluted, cloned and sequenced. The sequence data compared with NCBI data base showed similarity with some important genes in the *Hevea* whole genome shotgun sequences reported earlier.

2.2. Ploidy variation through *in vitro* techniques

2.2.1. Androgenic haploids

Pre culture of the mature anthers of clone RRII 105 and 430 at the uni-nucleate stage of the pollen in colchicine containing medium with simultaneous application of temperature shock for 20 days resulted in the division of microspores producing micro callus in liquid callus induction medium. The micro callus obtained from the cultured pollen grains of clone RRII 105 when plated on solid callus induction medium resulted the formation of callus. Small group of callus obtained earlier from the pollen grains of clone RRII 430 was transferred to callus proliferation medium. Simultaneously mature anthers of clone RRII 430 were pre-cultured in mannitol medium and incubated at 35°C for 15 days. The anthers dried up and callusing was achieved from the pollen grains on the surface of the anthers. This callus was separated and proliferated. The proliferated callus was cultured in different media combinations for embryogenic callus formation.

The purification and culture of pollen protoplasts being highly cumbersome and the induction and proliferation of microcallus was at low frequency, another experiment was initiated for culturing intact dehisced anthers. Anthers from mature male flowers, prior to anthesis, were separated and cultured over different media combinations for callus induction and kept at different ranges of temperatures at lower (10-12°C) and higher (38-42°C) regimes. In some cultures heat shock was imparted by exposing the explants to lower temperatures for some time and shifting to higher temperatures. After specific time intervals the cultures were returned to normal temperature. It was observed that pollen grains remained intact within the anther lobes in some media combinations at temperatures up to 40° C. Division of pollen grains giving rise to multiple cells was observed, within two months, in a few cultures subjected to heat shock. These microcalli were subcultured for multiplication and further proliferation. Haploid nature of the callus has been proved through cytology (n=18) and flow cytometry. Haploid callus has been obtained from dehisced anther for the first time in *Hevea.*

2.2.2. Gynogenic haploids

The unfertilized ovules exposed to temperature shock (38°C) resulted in the enlargement of the ovules. The cultured embryo sac produced callus and the proliferated callus showed embryogenic potential in high sucrose medium (90 g L^{-1}) supplemented with calcium nitrate (500 mg L^{-1}). Pro-embryogenic masses appeared in the medium supplemented with IAA, GA_3 and BA. Repeated subculture of the pro-embryogenic masses resulted in the formation of globular embryos at a low frequency (Fig. Biotech.1).

Fig. Biotech. 1. **Embryogenesis from the embryo sac cells of mature unfertilized ovules**

Different methods were tried for the isolation of a good number of intact embryo sacs from the ovules. Mechanical isolation of embryo sacs from ovules subjected to pre-treatment in liquid MS medium enriched with 10% sucrose was found to be the most efficient technique, enabling easy separation of intact embryo sacs in more numbers compared to the other techniques. Isolated embryo sacs were cultured in the earlier standardised callus induction medium. Some of the cultures were exposed to high temperatures (38-40°C) whereas the rest of the cultures were kept under the dark at normal temperature. Callus induction at a frequency of 5-10% was observed in the cultures kept at room temperature. Exposure to high temperatures did not favour callus induction, on the contrary those cultures turned brown and finally dried up. Calli emerging from the embryo sacs were proliferated and later transferred to embryo induction media. Embryogenic callus induction followed by the emergence of globular embryos has been observed in clones RRII 105 and 414. These embryos have been sub cultured for further development. A few mature embryos were developed from the clone RRII 414 and are in the germination medium (Fig. Biotech. 2). Meanwhile, ploidy determination of the embryo sac derived callus has been carried out both through cytological method and flow cytometry.

Fig. Biotech. 2. **Germinating haploid embryos**

In both methods these calli were found to be haploid in nature (n=18).

2. 3. *In vitro* development of polyploids through colchicine treatment

Experiments on *in vitro* induction of polyploidy in diploid callus of *Hevea* through colchicine treatment were continued. Parameters like colchicine concentration and exposure time were optimized. Embryogenic calli as well as

Fig. Biotech. 3a. **Embryos from colchicine treated callus**

Fig. Biotech.3b. **Plant regeneration from colchicine treated callus**

embryos (Fig. Biotech. 3a) could be induced from the colchicine treated callus. Mature embryos are at different stages of germination and plant regeneration (Fig. Biotech. 3b). Meanwhile, ploidy determin- ation of the regenerants has been carried out, both through cytological analysis and flow cytometry. Proliferating callus as well as actively growing root tips of the regenerating plants were used for cytological analysis. Chromosome count of 72 could be obtained in some samples indicating those cells are tetraploid. Flow cytometry analysis also confirmed higher ploidy levels including tetraploidy and mixoploidy in the callus samples analyzed.

3. Development of transgenic plants

3. 1. Genetic transformation of *Hevea brasiliensis* for stress tolerance

With the objective of improving the stress tolerance in *Hevea* clone RRII 105, *MnSOD* gene was integrated in the callus derived from immature anther and leaf explants to develop more transgenic lines. Embryo induction was also attempted from transgenic embryogenic callus maintained from earlier experiments. Transgenic plants could be regenerated *in vitro* from the embryos obtained. Plantlets obtained from previous experiments were multiplied by bud gratting and the grafted plants were maintained in glass house for stress tolerance studies. New *Agrobacterium* infections were carried out with MnSOD gene construct using leaf callus of clone RRII 105 and anther callus derived from *Hevea* clones RRII 105, 414 and 430. Media modification experiments were conducted for the induction of embryogenic callus from the proliferated MnSOD transgenic callus lines obtained. The callus lines derived from clone RRII 105 turned friable and embryogenic callus induction is awaited. Transformation experiments were also carried out with Sorbitol 6-phosphate dehydrogenase gene for enhanced drought tolerance. Embryos as well as plantlets were regenerated from transformed cell lines; however, the plantlets could not be hardened.

Proliferated friable callus obtained from leaf cultures initiated during the year as well as embryogenic callus were used as target tissues for new *Agrobacterium* infections carried out for incorporating *ipt* gene. Infected tissues were recovered free of bacterial over growth and transgenic callus obtained were cultured for proliferation. Experiments were also carried out using pre-cultured leaves as target tissue for *Agrobacterium* infection. Embryo induction was also obtained from transgenic embryogenic callus maintained from earlier experiments by routine subculture and *in vitro* transgenic plant regeneration was obtained.

Transgenic plants integrated with osmotin gene, developed earlier were evaluated for their drought tolerance capacity. Initially, for the reassurance of the gene integration, PCR and RT-PCR were carried out using the gene specific primers with the DNA /RNA isolated from the six transgenic plants maintained in the shade house. Positive amplification of the 0.75 kb gene insert could be obtained in the PCR and RT-PCR reactions indicating the integration and expression of the transgene. For the evaluation of drought tolerance, free proline content was estimated in transgenic and non-transgenic leaf discs following standard procedures under stressed and unstressed condition (PEG 6% 100mM). When the leaf discs were subjected to PEG stress for different durations, the transgenics showed a higher value for proline compared to control. With increase in time interval the proline content increased in both transgenic and control plants. However, the increase was much higher for transgenics compared to control. Under salt stress also there was a steady increase in the proline content with increase in salt concentration up to 18 hours for fransgenics and control up to 18hours then the proline value decreased in control, but the high value for free proline was maintained in the transgenics.

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

Genetic transformation was carried out for the development of transgenic plants integrated with *hmgr*1 gene for yield enhancement. Primary callus was generated from the immature anthers of clone RRII 430 and made friable by culturing in the medium with sucrose (70 g L^{-1}) and calcium nitrate (500 mg L^{-1}). Genetic transformation was performed using this friable callus, co-cultured for 3 days and transferred to selection medium containing hygromycin and cefotaxime. Transgenic cell lines emerged from the infected callus within 60 days of culture. In order to transform a low yielding phytophthora tolerant clone F x 516, callus was induced from immature anthers, proliferated and subcultured for embryogenic callus initiation. Expression studies were also initiated in the transgenic plants integrated with *hmgr*1 gene.

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

3.3.1. Functional validation of the pNS14 binary vector in tobacco plants

Agrobacterium mediated genetic transformation was carried out with tobacco leaf disc as well as callus. Transgenic shoots were regenerated from the transformed leaf disc and from the callus lines. The transgenic shoots regenerated were rooted in hormone free ½MS medium fortified with 50 mg L^{-1} kanamycin. Transgene integration was ascertained by performing PCR with the genomic DNA isolated from transgenic plants as test (T), one untransformed control plant (NC) and plasmid DNA of pNS14 as the positive control (PC), using *npt*II and *cre* gene specific primer-pairs. When PCR was performed with *nptII* gene-specific primer, amplification was obtained in all the test samples and also in the positive control, whereas this band was absent in the untransformed negative control plant. This band corresponds to

the *npt*II fragment of the T-DNA portion of the pNS14 vector which was integrated in tobacco plants by *Agrobacterium* mediated transformation. Similarly with *cre* gene specific primer, amplification was observed in all the tested plants and positive control but it was absent in the untransformed control plant, indicating the presence of *cre* gene in the transgenic tobacco plants transformed with the pNS14 binary vector.

3.3.2. Marker gene excision and molecular confirmation of transgene deletion

In order to excise the antibiotic (*npt*II) and *cre* genes from the transgenic tobacco plants, they were subjected to heat shock at 42°C for 3 h in a temperature controlled air oven. When PCR was performed using the genomic DNA isolated from all the transgenic plants subjected to heat shock, no amplifications were detected with *npt*II or *cre* gene specific primers in the test and untransformed control plant, whereas, amplification was observed in the transgenic tobacco plant which was not given any heat shock. This is an indication of the deletion of *npt*II and *cre* genes when the transgenic plants were subjected to heat shock.

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

Agrobacterium mediated genetic transformation experiments were carried out with anther callus derived from *Hevea* clones RRII 105, 414 and 430 as well as with polyembryony derived embryogenic callus. The kanamycin resistant callus lines emerged were selected and cultured individually over the proliferation medium optimized earlier for other gene constructs.

3.4. Multiple gene integration in *Hevea brasiliensis*

The MnSOD and *hmgr*1 gene integrated embryogenic callus lines obtained earlier were cultured for embryo induction and plant regeneration. Multiple gene integration was also attempted by co-transformation with MnSOD and osmotin gene constructs using *Hevea* zygotic embryo derived embryogenic callus. Transgenic embryogenic callus incorporated with MnSOD and *ipt* genes maintained from earlier experiments were also routinely subcultured for somatic embryo induction. Proliferated friable callus obtained from leaf cultures initiated during the year as well as embryogenic callus were used as target tissues for new *Agrobacterium* infections carried out for incorporating genes coding for *MnSOD* and *ipt*. Infected callus tissues were recovered free of bacterial over growth and transgenic callus obtained were tried for proliferation.

4. Characterization of genes controlling tolerance to abiotic stresses

4.1. Characterization of calcium-dependent protein kinase (*cdpk*) genes

Characterization of stress signal transducer gene family calcium-dependent protein kinase genes are important for genetic engineering imparting stress tolerance in *Hevea* clones. Novel genes in this cdpk family were isolated and characterized to find out their functions in response to abiotic stress. A 1.6 and a 1.5 kb *cdpk* gene from cDNA of *Hevea* clone RRII 105 was PCR amplified and cloned. The recombinant plasmids were sequenced and analyzed with bioinformatic tools ensuring the isolated sequences are *cdpk* genes itself. The sequences were submitted in the NCBI database as cdpk1, cdpk3 and cdpk4 under the accessions KJ939362.1, KP742352.1 and KR028482. The temporal expression of the three *cdpk* genes was analyzed in callus during cold stress through real time PCR. The expression trend of cdpk1 and cdpk3 was similar with varying patterns of up regulation and down regulation at the same time intervals while that of cdpk4 showed consistent down regulation. The results indicate that all the three genes are responsive to cold stress. In

addition to their response in callus, the gene expressions were compared in stress susceptible clone RRII 105 and stress tolerant clone RRIM 600. The expression of cdpk1 increased in stressed samples of RRIM 600 as compared to controls; while it remained the same in both control and stressed samples in RRII 105. The expression trend of cdpk3 and cdpk4 were found to be down regulating in both the clones. The study paves the way for further investigations on the role of these genes, especially cdpk1, in abiotic stress tolerance.

GENOME ANALYSIS LABORATORY

Major research activities of the Genome Analysis laboratory are grouped under the following heads: (1) development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome 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) Genome sequencing and *de-novo* assembly of rubber (*Hevea brasiliensis*) genome.

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

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

1.1.1. Transcriptome derived micro-satellites SSR detection in NB-LRR domain containing transcripts

Transcriptome data generated from control and Corynespora challenged leaf samples of two rubber clones RRII 105 and GT1 was analysed for developing genic-SSR markers containing dinucleotide (>6 repeats), trinucleotide (>5 repeats) and tetranucleotide (>5 repeats) repeat motifs, associated with resistance genes containing characteristic NB-LRR motifs. Multi-step computational pipeline was setup for detecting SSRs in NB-LRR domain containing transcripts using clustering methods, Pfam database search using hmmscan, in-house Perl script, BLAST search and MISA tools. From the NB-LRR domain containing transcritps 17, 25, 36 and 24 SSRs were identified in C1, C2, T1 and T2 respectively.

In silico prediction of polymorphic SSR loci

Prediction of polymorphic SSR loci was performed *in silico* with control and pathogen challenged transcriptome data of two rubber clones RRII 105 (C1 & T1) and GT1 (C2 & T2). Polymorphic SSRs were distinguished from monomorphic SSRs by the representation of varying motif lengths within an alignment of sequence reads from RRII 105 and GT1 *i.e.*, C1C2 and T1T2. In control transcriptomes (C1C2) 42 di-, 37 tri- and 3 tetra-nucleotide polymorphic SSR loci were detected and in pathogen challenged samples (T1T2) 67 di-, 38 tri- and 4 tetra-nucleotide SSR loci were identified. Among these polymorphic SSR loci, 9 di-, 6 tri- and 1 tetra-nucleotide loci were common between two clones. Altogether 191 SSR loci (109 di-, 75 tri- and 7 tetranucleotide) showing polymorphism between RRII 105 and GT1 were identified which could be used to develop informative markers.

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

1.2.1. SNPs in gene/ gene families involved in root functions

Sequence data was generated for root transcriptome of *Hevea* at Xcelris lab. In assembled root transcriptome, 64,893 transcripts were identified. Clustering of these transcripts with 90 per cent identity reduced the transcript count to 43085 unigenes. Variant calling was performed for identification of base substitutions in root transcriptome. Mixing of root samples facilitated SNP identification. In total 275918 single nucleotide variations were predicted with minimum mapping quality of 25 and read depth of 10. SNPs identified through bioinformatics tools in various gene families need to be validated through Sanger sequencing using four rubber clones RRII 105, RRII 430, RRIM 600 and GT1 for marker generation.

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

Full length genomic sequence of hydroxymethylglutaryl CoA synthase (*HMGS-2*) and mevalonate kinase (*MVK*) genes from rubber was reported for the first time and submitted to NCBI Genbank (Accession No: KM272629 and KM272630)

In total, 20 SNPs constituting 8 haplotypes were confirmed in HMG-CoA synthase (*HMGS*) gene through re-sequencing. Twenty-six SNPs were identified in *MVK* gene containing 5 exons and 4 introns. Eight haplotypes were identified in *MVK* gene from five genotypes/ clones: RRII 105, RRII 118, RRIM 600, GT1 and RRIC 52 using DnaSP program.

Full-length phosphomevalonate kinase (*PMVK*) gene was constructed using RRII 105 genome sequence data. The entire *PMVK* gene was found to be approximately 9kb in size with 10 exons. Partial sequencing of the *PMVK* gene from five clones revealed the presence of 18 SNPs.

SNP genotyping

SNP genotyping of 46 progenies of RRII 105 x RRII 118 using high-resolution melting (HRM) analysis was performed with the SNPs *HMGS* (3059 G/A) and *FDPS* (1380 C/T). As expected, a segregation ratio of ~1:1 was obtained.

HRM genotyping of SNP (C/T) at position 1438 in *cis-prenyl* transferase gene was also performed with 46 progenies of RRII 105 (C/T) X RRII 118 (T/T) for linkage mapping of the gene.

Expression analysis of rubber biosynthesis genes

Relative quantification of gene expression of eight genes (*HMGR, HMGS, FDPS, MVK, PMVK, GGDPS, CPT* and *REF*) in the latex of five clones was carried out to understand clonal variation in latex production and to estimate the possible association between srip haplotypes and gene expression. Preliminary analysis indicated that RRII 105 and RRII 118 showed comparatively better expression of the above genes among the five clones.

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

Parental genotypes RRII 105 (*H. brasiliensis*) and F4542 (*H. benthamiana*) of the mapping population were screened with a new set of SSR primer pairs, derived from the transcriptome sequencing project for developing a new set of informative SSR markers and 60 new markers were generated. Genotyping of the mapping population was carried out with dinucleotide and

trinucleotide informative SSR markers. All together 37 SSR markers were used to analyse segregation pattern of the progeny population. Selected 21 polymorphic RAPD primers were also used in segregation analysis and the profiles were documented. Genotyping of segregating progeny population with both marker types is in progress.

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*

Genes involved in host tolerance to Corynespora leaf disease of rubber

Gene expression analysis of RRII 105 (susceptible clone) and GT 1 (tolerant clone) in response to *Corynespora* infection revealed that they responded differently upon infection. Results of the study showed that the genes encoding disease resistance proteins, leucine-rich repeat proteins and proteins involved in carbohydrate metabolic processes were significantly over-expressed in GT 1, whereas these transcripts were either completely suppressed or down regulated in RRII 105 upon pathogen infection. Gene ontology term enrichment identified that majority of the transcripts was enriched for defence response, response to stimulus and stresses.

Microarray gene signatures for* Corynespora *disease tolerance in rubber

DNA microarray experiments were designed to validate differentially expressed transcripts derived from *Corynespora* challenged and control transcriptomes of both susceptible and resistant clones of rubber. Triggering of genes involved in hormone signalling, cell wall synthesis, proteolysis, transcription factors and defence genes were identified. Further data analysis is in progress.

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

2.2.1. NAC domain containing sequence analysis in leaf transcri- ptome

NAC domain containing transcripts were extracted from unstressed control, drought and cold-stressed leaf transcriptomes. Non-redundant sets of 52, 50 and 64 NAC transcripts were identified in control, drought and cold leaf transcriptomes respectively. Common and unique NAC sequences were extracted from control, drought and cold transcriptomes (A, B and C), which resulted in 49 & 3, 50 & 0 and 57 & 7 common and unique sequences in A, B, C transcriptomes respectively.

2.2.2. Transcriptome sequencing

Transcriptome sequencing was performed with 15 samples derived from leaf, root, bark and latex of rubber plant subjected to biotic and abiotic stresses.

De novo *assembly of transcriptome data*

De novo assembly of filtered PE RNA-Seq reads, derived from 15 *Hevea brasiliensis* transcriptomes, was performed using Velvet-Oases at four different k-mers (45, 47, 49 and 51) for optimization of the assembly. Quality assessment of the Oases and Trinity assemblies was performed by the mappability of sequence reads against the assembled transcriptome of YB1, PHY_600_CO, PHY_600_TP and PHY_FX_TP using both the tools. Trinity assembly was found better than the Oasis assembly. Therefore, Trinity assembly for the rest of RNA-seq data was performed.

Root transcriptome analysis

In assembled root transcriptome, 64,893 transcripts were identified and clustering of these

transcripts using CD-HIT tool with 90% identity reduced the transcript count to 43085. Finally 6490 full-length open reading frames were detected after sequence clustering, 1 kb length filter, ambiguity filter and single ORF filter. Transcript abundance estimation was performed with root transcriptome using the pipeline based on RSEM tool.

2.2.3. Identification of genes responsible for de-novo DNA methylation and de-methylation in rubber

Full-length sequences of DRM methyltransferase and DNA N-glycosylase genes were identified from the whole genome sequence data using the partial sequences of the genes reported last year. DRM transferase was found to be 2.31 kb in size comprising of 3 exons with an ORF of 1.218 kb. DNA N-glycosylase was 11.935 kb in length with 20 exons with an ORF of 5.859 kb. Overlapping primers spanning the entire cDNA sequence of DNA N-glycosylase and genomic sequence of DRM methyltransferase gene was designed. Real time PCR primers from the 3' end were also designed for both the genes to study the expression pattern under various stress conditions.

2.2.4. Methylation AFLP for identification of somaclonal variants in Hevea

Sequencing of two more MS-AFLP bands, amplified using EC8-Met-TAG primer combination, yielded nucleotide information of 273 bp and 289 bp fragments. Blastx analysis revealed that 273 bp fragment encoded a phospholipase-like protein (PEARLI 4) family conserved domain. Blastn with draft genome of *Hevea* yielded the contig AJJZ011043409.1 which harboured an exon of the same family protein. The 289 bp fragment showed high similarity to the contig AJJZ011043409.1, which had no similarity to any known protein sequence. Confirmation of the results by bisulfite sequencing is essential to locate the exact methylated loci.

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

Genes encoding CAD, CCR and COMT involved in cell wall lignification through phenylpropanoid pathway were cloned and characterized in previous years. Efforts are being made to clone these genes in binary vector for *Agrobacterium* mediated genetic transformation of rubber for timber quality improvement.

3.2. Cloning and characterization of NAC gene

cDNA encoding NAC gene and NAC genomic sequences were amplified to their full-length from three rubber clones RRII 105, RRIM 600 and GT1 and cloned. NAC cDNA was found to be 849 bp long encoding 282 amino acids. Genomic sequence of NAC contained two introns of 105 bp and 95 bp in length.

3.2.1. Prokaryotic expression of NAC gene

NAC cDNAs from RRII 105 and RRIM 600 were cloned in pRSET vector and transformed to *E.coli* strain BL21 (DE3) pLysS for expression of recombinant protein. Expression of recombinant fusion protein was detected on SDS PAGE within one hour of induction with IPTG and confirmed through western blotting using antibody for N-terminal Xpress epitope tag (Invitrogen).

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

Sequence d1ata generation of RRII 105 genome was completed with three sequencing platforms. Altogether 342 Gb sequence data was generated providing 97.7X genome coverage. Extensive quality control analysis carried out on these data at RRII to obtain high quality

sequencing data for whole genome assembly. Sequence reads generated from different long-insert mate-pair libraries (2 kb, 4 kb, 6 kb, 8 kb & 20 kb) were filtered. In total 38.8 Gb filtered sequence data segregating in true mate pair, paired end and tandem reads was achieved.

Initial assembly of Illumina sequence data alone (paired-end and mate-pair data) using k-mer 43 with SOAPdenovo2 assembler could reveal 1.69 Gb genome length with gaps. Optimization of k-mer is being continued. Assembly was also performed with the sequence data generated on Roche GS-FLX platform alone (4.1 Gb shotgun & 0.98 Gb 20 kb PE data) using Newbler v2.9 resulting in ~434 Mb genome with scaffold N50 length of 1.4 kb.

PacBio SMRT sequencing of rubber genome was also performed using long insert library (20 kb) of the *Hevea* genomic DNA, as the PacBio reads are very useful in achieving better assembly by reducing the gaps in the scaffolds generated by short read data. The first set of data comprised of 414901 reads was obtained from 7 SMRT cells. PacBio data generation is in progress.

PLANT PATHOLOGY DIVISION

The main foci of research in Plant Pathology division were improving disease and pest management strategies by chemical and biological agents, assessing yield loss and other impacts due to diseases, improving the growth of rubber using efficient microorganisms and in designing and developing efficient treatment technologies for waste water.

1. Leaf diseases

1.1. Abnormal leaf fall disease

Multilocational trial was carried out to evaluate the efficiency of half- dose (20 kg ha^{-1}) application of oil- based COC against abnormal leaf fall (ALF) disease in the clone RRII 105. The treatments were 4 kg COC: 20 L spray oil, 8 kg COC: 40 L spray oil and untreated control. Spraying was undertaken during the second fortnight of May and leaf retention was recorded periodically. The results showed that leaf retention in the half- dose applied plot was comparable to full dose application of COC.

Evaluation of the crop loss due to the combined effect of ALF disease and powdery mildew diseases was continued. Powdery mildew disease was mild and abnormal leaf fall disease was very severe. The leaf retention was only 20% in the RRII 105 unsprayed area and it was < 5%, 30 % and 40% 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 34%, 48%, 42% and 39% respectively. Reduction in girth increment was also noticed in the unsprayed plots of all the clones.

The studies to assess the impact of ALF in four modern clones *viz.*, RRII 414, RRII 422, RRII 429 and PB 260 indicated yield loss of 14, 8, 14 and 7 per cent respectively. The leaf fall ranged from 50-60 per cent in unsprayed blocks, whereas it was only up to 30 per cent in sprayed except in RRII 422. The girth increment and bark thickness were higher in sprayed blocks.

The clone PB 260 recorded higher TPD (up to 28%) irrespective of sprayed or unsprayed, whereas RRII 429 registered lower (11%) incidence.

The crown budding experiment at CES, Chethackal on clone PB 260 with crown Fx 516 recorded significantly higher leaf retention, girth and yield in crown-budded trees than control. In another experiment at Malankara Estate, Thodupuzha on clone PB 311 with crowns RRII 33 and Fx 516, the leaf retention was only 10 per cent in control, whereas crown-budded trees recorded 70 per cent leaf retention. The estimated timber volume in crown-budded trees with clone Fx 516 was double that of control. The properties of latex of crown-budded trees in both experiments did not show any adverse effects. Attempts to raise plants in root trainer cups of bigger size (1000 cc) for crown budding purpose did not succeed as the maximum height obtained was 6 feet when the cups were placed on the stands from the beginning without touching the soil, necessitating still bigger cups to attain the desired height of 10 feet.

Studies for the identification of genes involved in the disease resistance of ALF disease were attempted. *Phytophthora* susceptible (RRIM 600) and tolerant (Fx 516) clones were challenge-inoculated with zoospores of *Phytophthora* and transcriptome sequencing of control and pooled challenged leaf samples was performed on NextSeq. *De novo* assembly of RNASeq data was carried out across a range of kmers (kmer 39 to 51). The control transcriptome sequence of RRIM 600 assembled 44831 transcripts with a maximum transcript size of 8483 bases and a read participation of 62.61%. In the treated samples of RRIM 600, a total of 36522 transcripts were assembled with a maximum transcript size of 13678 bases and a read participation of 60.91%. In the control transcriptome of Fx 516, a total of 64151 transcripts were assembled. The maximum transcript size was 7392 at kmer 51 and the read participation was 76.57%. While in the treated samples of Fx 516, a total of 41377 transcripts were assembled and the maximum transcript size was 14968 bases with a read participation of 50.74%.

1.2. Powdery mildew disease

Trial was conducted to evaluate the efficacy of two bacterial biocontrol agents along with recommended fungicide wettable sulphur, a new fungicide trifloxystrobin + tebuconazole and untreated control. Treatments were imposed in 8- day intervals and disease intensity was assessed on a 0-5 scale before each treatment. Among the treatments trifloxystrobin + tebuconazole was the most effective one. About 50% control of the disease was obtained with the bacterial isolate RH 34 when compared to control.

1.3. Colletotrichum leaf disease

The level of disease tolerance in 185 Wickham clones to *Colletotrichum* spp. was assessed by detached leaf technique. Consolidation of the results on inherent tolerance of these clones revealed that 10 clones possessed over 70 per cent tolerance and 36 clones were in the highly susceptible group (> 80 %).

1.4. Corynespora leaf disease

Nursery evaluation of new generation fungicides *viz*. pyraclostrobin+ metriam and thiophanate methyl against Corynespora leaf fall disease on budwood plants of RRII 105 was continued. The results showed that the fungicides were effective and on par with recommended fungicides.

Evaluation of three fungicides *viz*. tebuconazole, thiophanate methyl and pyraclostrobin + metiram at different concentrations along with recommended

fungicides and untreated control was continued at two locations in Karnataka. Experiment was laid out in Randomized Block Design with eight treatments and three replications. Among the treatments, thiophanate methyl 0.07% was found significantly superior to all other treatments in controlling the disease (Table Path. 1).

Table Path. 1. **Efficacy of fungicides on *Corynespora* disease intensity**

Treatments	Disease intensity	
	Location 1	Location 2
Tebuconazole (0.25%)	1.4	1.7
Thiophanate methyl (0.07%)	0.5	0.5
Thiophanate methyl (0.035%)	1.1	0.9
Carbendazim (0.05%)	1.4	1.2
Pyraclostrobin+ metiram (0.06%)	1.4	1.0
Mancozeb (0.75%)	1.4	1.4
Pyraclostrobin + metiram (0.03%)	1.5	1.6
Control	2.8	3.1
CD (P = 0.05)	0.4	0.4

Antagonistic microbes were evaluated against Corynespora leaf disease in budwood plants of RRII 105 at Ulickal nursery. Experiment was laid out in Randomized Block Design with five treatments including the recommended chemical control. Among the biological agents tested antagonistic endophyte RB 66 showed reduction in disease.

In order to study the diversity in cassiicolin gene in relation with pathogenicity of *Corynespora cassiicola*, the pathogen was isolated from different rubber growing regions of Kerala and Karnataka during January and February 2015. Aggressiveness of isolates was studied by leaf wilt bioassay using crude toxin and virulence of the isolates by spore inoculation method. Among different isolates, one isolate from Bathiyadukka was avirulent/nonaggressive. Cas1, the cassiicolin encoding gene was amplified from the genomic DNA of all aggressive isolates but not from the non-aggressive isolate of Bathiyadukka.

Fx 516 showed tolerance against *Corynespora* in the toxin bioassay/spore inoculation method. Root trainer plants of Fx 516 and RRII 105 kept at Ulickal nursery for natural disease incidence in the disease season also indicated the tolerance of Fx 516 compared to RRII 105.

Studies on identification of genes involved in the disease resistance of *Corynespora* disease by transcriptome sequencing were continued. Microarray-based gene expression technique was adopted to get an understanding on the expression profiles of genes based on functional classification and gene ontology. 10063 transcripts identified from the transcriptome sequencing and 459 additional genes / cDNAs identified earlier in response to Corynespora disease through differential gene expression studies were used as probes on an array in 8 x 60 K format. Triggering of genes involved in hormone signalling, cell wall synthesis, proteolysis, transcription factors, defence genes *etc*. was identified, which is being validated through real time PCR assay.

2. Stem diseases

2.1. Pink disease

Two new generation fungicides were evaluated for its prophylactic and curative effect against pink disease at Mundakayam estate on 3- year-old plants. Fungicides were applied by high volume spraying using a single man operated power sprayer. Observation on the recovery of plants showed that both the fungicide trifloxystrobin + tebuconazole (Nativo) and tebuconazole (Folicur) were better in preventing the pink incidence .

Trial was conducted to evaluate the efficacy of bacterial antagonists against pink disease as prophylactic and curative methods. In the prophylactic treatment, 19% of the plants were infected in the biocontrol plots as against 24% in the Bordeaux paste- treated control plots. In the curative treatment, among the different bio-agents the bacterial isolate RB 88 was found to be better. However, the treatment was effective only in plants treated in the initial period of infection.

3. Root disease

To identify potential alternate fungicides for the recommended fungicides *viz.* tridemorph and propiconazole for the control of brown root disease caused by *Phellinus noxius*, sixteen fungicides were tested under *in vitro* condition. The fungicides tebuconazole + trifloxystrobin, metiram + pyraclostrobin, provalicarb + propineb and epoxyconazole + pyraclostrobin were found to be effective by inhibiting the growth of pathogen at concentrations less than 20 ppm.

4. Tapping panel dryness

In order to characterize the presence of any organism present in the TPD- affected phloem sieve tube, DNA was isolated from the bark samples of seventeen TPD- affected trees by modified MLO enrichment procedure. The isolated DNA amplified through nested PCR yielded ~1200 bp band in all the seventeen TPD plants. The PCR product was cloned and sequenced . All the sequence data from the amplified product gave homology to "Phytoplsama-like organism" on optional Phytoplasma blast analysis. The sequence data was submitted in NCBI(KPO 25850).

5. Pests of rubber

The efficacy of a 100 per cent plant protectant (tag folder) produced by Tropical Agrosystem (India) Pvt. Ltd. was tested against Mooply beetles under *in vitro* and *in vivo* conditions. Spraying of the protectant at a dose of 5ml L^{-1} on the cluster of beetles showed 100 per cent mortality after four days of application under *in vitro* condition. In the field studies, the efficacy was compared with other insecticides like imidacloprid, deltamethrin and untreated control in Completely Randomized Design with three replications. Imidacloprid and deltamethrin gave 98 and 87 % mortality of beetles by a single application, while tag folder gave 73 % mortality. Tag foder required two rounds of application at two- day interval for the maximum mortality.

Conducted survey on the incidence of bark feeding caterpillar, *Aetherastis circulata* (Lepidoptera :Yponomeutidae) in rubber trees and observed Kodumon estate in Pathanamthitta district as the highly infested area. Collected pre-pupal and pupal stages of caterpillar and out of 146 larvae 77.6% were found dead due to the attack of entomopathogenic fungus *Aspergillus flavus*. The pathogen was multiplied in the laboratory and evaluated its pathogenicity against fresh larvae and pupae by spraying fungal spore suspension of *A. flavus* at 50 spores ml^{-1}. The result showed 100 per cent mortality of larvae and pupae within seven days of application.

Occurrence of a new beetle eating the leaves of rubber seedlings from Kumbazha estate and a kind of bark eating caterpillar from Thaliparamba region was reported.

6. Improvement of rubber and cover crops through microbial inoculants

Evaluated the growth promoting efficiency of four phosphofungal isolates, four PGPR isolates and four consortium of selected PGPR in root trainer plants at 50% recommended levels of fertilizers .The cultures were applied at the rate of 10 ml to the plants at fortnightly intervals.

Plant growth, root volume and fresh weight were higher for the inoculated plants than full fertilizer applied plants. Among the treatments, plants inoculated with the isolate RH 104 and consortium 3 showed better growth. Among the phosphofungi, Pf 11- inoculated plants showed higher girth and height. The different bacteria inoculated in root trainer plants were found to survive in the potting mix but their population varied with the isolates.

In biofarming trial, plants in the integrated treatment showed higher girth (42.1 cm) than chemical (38.7 cm) and biological (38.4 cm) treatments, which were on par. The total bacterial population in the three treatments did not vary, total fungal, phosphobacterial and *Pseudomonas* populations were more in biological and integrated plots. Inoculated bacterial population was also more in these plots.

The ability of 49 isolates to use ACC as nitrogen source was confirmed by checking the turbidity in different broth media. The growth of these isolates in DF media alone and DF amended with ACC or $(NH_4)_2SO_4$ was compared to distinguish between ACC deaminase activity and nitrogen fixing activity. Out of 49 isolates tested 34 showed good growth in ACC amended media. The isolates were also checked for the production of IAA, the growth hormone and siderophores. The IAA production of the isolates ranged from 1.3-40.4 µg ml^{-1} and most of the isolates were siderophore positive. These isolates were evaluated for their effect on drought tolerance of rubber seedlings in polybags. The plants were maintained without watering during summer from the end of January till the heavy rain in mid- March. Weekly observations were made for drought effect including yellowing, shedding of leaves and drying of plants. Plants inoculated with an isolate from Uduppi, Karnataka showed 90% survival of plants. Ten isolates showed more than 50% survival of plants after the drought season and were selected for further screening using drought susceptible rubber clones.

7. Waste management in rubber processing

7.1. Design and development of thorough- put treatment technologies for the efficient treatment of waste water from NR processing.

Integrated waste water treatment system was designed for the installation at three group processing centres as per their specific requirements. The performance evaluation of the system installed at Poothrika showed removal efficiency in BOD (99%), COD(99%), TS(57%) and DS(50%). The reed bed system was redesigned and installed. Rooted vetiver plants were planted in the three compartments of the reed bed system and evaluated . The system showed removal efficiency in BOD(98.5%), COD(99%), TS(80%) and DS(79%).

7.2. Development of biogas/producer gas cum solar thermal energy- based integrated drying system for efficient drying of Ribbed Smoked Sheets (RSS) - (Collaborative project between RRII and Dept. of Bioenergy, TNAU)

Preliminary data collection from the conventional smoke house such as fuel consumption, heat transfer in the furnace to smoke house, heat utilization and heat loss were carried out. Temperature at various points in the smoke house was monitored at 15 points for each drying period for 74 h with a capacity of 400 kg/ batch. Convective heat loss is occurred at the top region due to flue gas exit in chimneys (2 nos) which is higher than the average temperature (60°C) of the smoke house. The estimated useful energy was about 24 % of input heat supplied. Due to heat losses at the top and base region of the smoke house, heat gained in

middle portion of smoke house was more than the upper and lower portions.

7.3. Design and development of economically viable urban solid waste management system

The specially-designed electrically-operated mechanical aerobic prototype device for the rapid decomposition of solid waste was modified by fitting with energy meter, temperature gauge (maintaining temperature- 28- 36°C) and an automatic timer. The modified prototype device decomposed the cooked food waste by 18 h, raw food waste by 13 h and combination of cooked and raw food waste by 15 hours. The macronutrients estimated in the decomposed material were as follows, nitrogen - 2.0%, phosphorus- 0.3%, potassium - 0.6%, calcium- 0.5% and magnesium - 0.1% and the micronutrients in the digested materials were iron - 771.6 ppm, copper - 10.1 ppm, manganese- 15.0 ppm and zinc – 37.3 ppm. The cellulose content in the waste material before and after digestion was 0.1 mg ml^{-1} and 0.1 mg ml^{-1} respectively.

8. Farm mechanization

As a part of standardization of various mini tractors available in the market for the attachment of the ATB/RRII High-tree Mist blower, a special attachment system was designed for mounting Mist blower on the KAMCO Tera TRAC 4W manufactured by M/S Kerala agro-machinery Co-Operation. The tractor- mounted sprayer delivered the spray fluid to a height above 80 ft. in the rubber plantation. This new technology was recommended for adoption.

In view of up-grading the present mist blower, the Honda GK300 engine was replaced with GX 200. The impeller was made out of heavy duty aluminium fins and backside plate by replacing the double layer stainless steel fins. The boss flange was also redesigned in such a way to reduce the vibration and for dynamic balancing. The total weight of the equipment became 45 kg with reduction of 10 kg than the existing Turblow mist blower with Honda GK 300 engine. The performance of the modified mist blower system was evaluated in the field. The equipment was very comfortable and convenient for handling by four persons. The discharge of the machine was 1 L/min and the delivery height was 70 ft.

9. Web-based 'Distance Diagnostic Identification System (Rubber Clinic) for rapid diagnosis of pests and diseases of NR

Cases related to any aspect of rubber cultivation and processing were attended daily and timely interventions made to address the complaints through online and offline depending on the merit of the cases. Need-based updation of the clinic was attended and the performance of the clinic was also done periodically. The clinic diagnosed 857 cases through 'Assisted Diagnosis' and 1499 cases through 'Self Diagnosis' accounting for a total of 2356.

PLANT PHYSIOLOGY DIVISION

The major areas of research in Plant Physiology Division are studies on environmental and stress physiology, physiology of growth and yield, ecosystem level flux analysis, tapping panel dryness, secondary metabolites and gene expression analysis in relation to abiotic stress responses and rubber biosynthesis.

1. Environmental physiology

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

The relative abundance of a chloroplast small heat shock protein (hsp) was tested under drought stress in many elite clones and six germplasm accessions using western blotting technique. The result indicated that expression level of the stress protein was relatively higher in stress tolerant clones and accessions than susceptible ones. On the basis of the stress protein abundance and drought responsive physiological parameters in terms of relative stable level of activities clones such as RRIM 600, RRII 208, RRII 429 and RRII 430 were classified as relatively drought tolerant.

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

Six months old polybag plants of four *Hevea* clones *viz*., RRIM 600 and RRII 208 (relatively drought tolerant), RRII 105 and RRII 414 (relatively drought susceptible) were subjected to drought stress for 10 days. Quantitative gene expression analyses were performed for seven drought associated transcripts. Among the genes studied, Mitogen Activated Protein (MAP) kinase, Myeloblastosis (Myb) transcription factor, C-repeat responsive element/Dehydration Responsive Element (CRT/DRE) binding factor and Nuclear Factor Y subunit A (NFYA) showed positive association with drought tolerance in *Hevea*. The expression analyses of 20 more drought responsive transcripts are in progress.

1.2.1. *Investigations on micro RNAs in* Hevea brasiliensis: *Role in gene regulation during abiotic stresses*

Expression analyses of six conserved miRNAs *viz*., miR184, miR164, miR167, miR398, miR482, miR169 and one novel miRNA (HbmiRn_42) were carried out in four *Hevea* clones with varying levels of drought tolerance. The miR482 was found down regulated in RRIM 600 and RRII 208 (relatively tolerant clones) while there was no much change in RRII 105 and RRII 414 (relatively susceptible clones) under drought stress. The miR164 and miR167 showed down regulation in susceptible clones while there was no much change in tolerant clones. The novel miRNA, HbmiRn_42 was found over expressed in tolerant clones while it got down regulated in RRII 105 and RRII 414.

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

1.3.1. *Physiological evaluation of RRII 400 series clones for drought tolerance: Field trial at CES Chethackal*

The RRII 400 series clones recorded better girth (5th year) than RRII 105 and RRIM 600. Among the RRII 400 series clones, the highest girth was observed in RRII 422 followed by RRII 414.

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

Quantification of xanthophyll cycle pigments was continued in different *Hevea* clones. Leaf samples from control and drought imposed plants of RRII 105, RRIM 600, RRII 414 and RRII 430 were collected for analysis of xanthophyll cycle activity. HPLC analysis was performed for quantification of pigments from controls and drought exposed plants. Higher level of xanthophyll pigment pool size was found in

drought imposed plants than control plants. Level of neoxanthin, lutein and zeaxanthin was found enhanced in stressed plants compared to control plants. A significant reduction was observed in the case of total chlorophyll content under stress. The relative level of carotenes under stress was found higher in RRII 430 and RRIM 600 than RRII 414 and RRII 105. Significant enhancement in the overall xanthophyll cycle activity was observed in RRII 430 followed by RRIM 600.

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

Relatively drought tolerant (6 nos.) and susceptible (4 nos.) wild *Hevea* germplasm accessions along with check clones were raised in polybags. Six month old plants were subjected to drought stress and analysed for drought tolerant traits. Relative water content (RWC%) was found to be higher in tolerant than susceptible accessions under drought stress. Anthocyanin content was higher in tolerant accessions compared to susceptible while no definite trend could be observed for peroxidase activity.

1.5. Ecosystem flux measurements

1.5.1. Sap-flow measurements in mature rubber plants

The water use of mature rubber plants was estimated using TDP-sap flow system in a dry sub-humid climatic region, at RRS, Dapchari, Maharastra. The sap flow system was installed in clone RRIM 600 with two different treatments. Around 0.5 ETc level of irrigation was provided during January 2014 on wards in the 'irrigation' treatment till the onset of monsoon rain. The rainfedtrees were left as unirrigated throught the year. Irrigated trees consumed significantly higher amount of water than rainfed trees during summer season. Generally the water mining rate declined during wintering period and subsequent drought periods even in irrigated trees (Fig. Phy.1).

The water mining rate of mature rubber trees of two clones was estimated in a cold stress prone region, Nagrakatta. West Bengal. Average water using rates of 21 and 24 L/tree/day was recorded for clones RRII 429 and RRII 417, respectively.

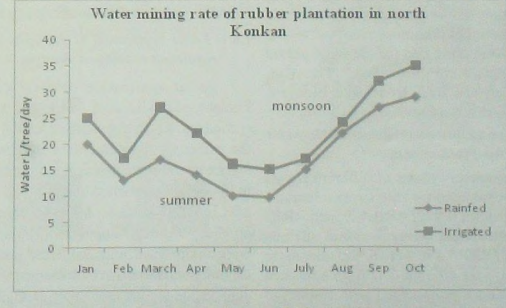


Fig. Phy. 1. **Sap flow rate of mature trees of clone RRIM 600 at RRS, Dapchari, Maharashtra**

Table Phy. 1. **Monthly mean net carbon dioxide (NEE g $m^{-2}day^{-1}$) and water vapour (ET mm day^{-1}) flux in a mature rubber plantation at CES, Chethackal**

Month	Net ecosystem exchange of w_2 (NEE)	Water vapour Flux (ET)	Ecosystem level WUE_{NEP} (g CO_2/kg water)
May 2014	11.3±0.5	3.8±0.13	2.9
June	10.4±0.5	4.0±0.10	2.6
July	10.9±0.8	3.7±0.14	3.0
November	7.8 ±0.9	3.3±0.10	2.4
December	10.6±0.5	2.8±0.10	3.8
January 2015	7.9±0.6	2.4±0.12	3.3
March	9.7±0.7	2.3±0.13	4.2

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

The ecosystem level carbon dioxide and water vapour fluxes and canopy level net ecosystem exchange (NEE) of CO_2 in rubber plantation were measured during the period (May 2014 to March 2015) using an eddy covariance system. The average NEE was 9.8g CO_2 m^{-2} day^{-1}, which is equivalent to 35 MT CO_2 $ha^{-1}year^{-1}$. The evapo-transpiration (ET) rate of this plantation was 3.2mm per day (Table Phy. 1). The ecosystem level water use efficiency (WUE_{NEP}) was worked out to be 3.2g CO_2/kg water in mature rubber plantation in traditional area. The amount of carbon sequestered by the rubber trees was estimated for the same period using biometric method also. The CO_2 sequestration realized from tree biomass increment alone was around 20 MT CO_2 $ha^{-1}yr^{-1}$.

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

Polybag nursery plants of different *Hevea* clones were evaluated for cold stress tolerance. Clones such as RRII 422, RRII 429, and RRIC 100 were susceptible whereas clones such as RRII 208 and RRIM 600 were found tolerant to cold stress. Under drought condition a marked clonal variation was observed with respect to the response of plants to water deficit stress. In general the chlorophyll content was relatively high in RRII 400 series clones, among this, RRII 422 and RRII 429 recorded higher chlorophyll *b* content. Anthocyanin content was higher in clone RRII 429 than other clones. The Photosynthetic measurements showed that RRII 400 series clones were on par with each other for *A* and *gs* and have a marginal increase of these parameters under early stress period. The clone RRII 422 has better adaptation features under high light intensity. Diurnal alterations and clonal variation of xanthophyll pigments under sun and shade conditions indicated the role of accessory pigments in high light adaptations in young plants.

1.6.1. *Foliar application of nutrients and growth regulators to mitigate drought*

Foliar application of MOP (Murate of Potash), salicylic acid (SA), and kaolin was carried out in polybag plants of RRII 417 to study the effect of supplementary nutrient, growth regulators and antitranspirant on drought mitigation in *Hevea*. Drought stress was imposed for 10 days after foliar application. A booster dose was given on 5th day of drought imposition. The control plants were maintained with saturated soil moisture conditions till the end of the experiment. The kaolin applied plants were found morphologically better than other treatments. Among the treatments T1

(1% MOP + drought) was found to have higher total plant biomass followed by T2 (2.5% Kaolin + drought). The whole plant water status and relative water content were better in treatments T1, T2, and T3 (150 ppm SA+ drought) than the treatment T4 (drought alone). The photosynthetic measure- ments showed that the percent reduction in assimilation rate (A) and stomatal conductance (g_s) was relatively low in treatment T1 followed by T2. The biochemical analysis showed that high level of chlorophyll and anthocyanin pigments in T2 followed by T1. Anthocyanin content was higher in foliar treatments than control plants (T5). Other analyses are progressing towards unraveling the mode of action imparting tolerance by these components.

1.7. Physiological evaluation of root trainer plants

Plant growth and biomass indicators such as height, leaf area, leaf number, leaf weight, shoot and root biomass were significantly less in root trainer plants compared to polybag plants. When a single whorled root trainer and polybag plants compared, the root trainer plant had 55 per cent less biomass (on dry weight basis) than polybag plants, irrespective of the clones (Fig. Phy. 2). The root: shoot ratio of plants grown in root trainer and polybags ranged between 1.2 and 1.5, respectively, indicating very little variation between the planting materials, irrespective of the type of containers. Most of the biochemical parameters indicated relatively lower rate of activity in the case of root trainer plants than polybag plants.

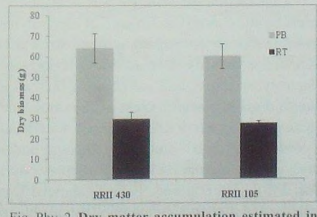


Fig. Phy. 2. **Dry matter accumulation estimated in single -whorled plants of root trainer and polybag. (PB: polybag, RT: root trainer; n=6)**

1.7.1. Photosynthetic rate under water stress and recovery in glass house

Photosynthetic rate (A) was measured in young plants of two clones, RRII 105 and RRIM 600 during a cycle of water stress (withholding irrigation) followed by recovery (irrigation) periods in glass house at 5 days interval for a total period of 40 days. Complete recovery of A to the initial level could not be achieved after re-watering in water stress treatment. The initial decline in photosynthetic rate was steep when water was withdrawn for 10 days, subsequent reduction in A was not as steep as the initial step, indicating possible priming/acclimation under stress. On first re-watering, A recovered to almost 66% (RRIM 600) and 53% (RRII 105) of control level and on second re-watering it was only 50% and 46%, respectively (Fig. Phy. 3).

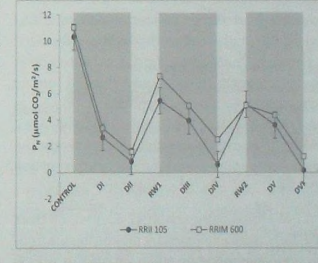


Fig. Phy. 3. **Photosynthesis rate of RRII 105 and RRIM 600 on exposing to cycles of drought and recovery periods for 40 days at 5 days interval (shaded portion in the figure indicates drought period of 10 days each; D: drought; RW: Re-watering)**

1.8. Drought survey in young rubber plantations

A survey on drought impact on young rubber plantations during summer season was done in Nedumangad, Punalur, Kottarakkara, Pala, Kanjirapally, Erattupetta and Thrissur regions. Among the surveyed fields, very few planters resorted to irrigation practice in South (3%) whereas around 30 and 50 per cent planters irrigate in Central and Thrissur regions, respectively. Recommended management practices in one year old rubber plantations, like mulching, shading and cover-cropping were not followed by majority of farmers surveyed in the Southern Kerala.

1.9. Screening of wild germplasm accessions for mid-day canopy temperature stress tolerance in summer

Top ranking 162 accessions were evaluated for mid-day canopy temperature under field condition during summer when peak drought and high light prevailed in the field. Increase in canopy temperature under drought stress is an indicator of susceptibility of plants to drought during the hot summer. A simple and rapid technique was used for this study. The method was standardized for *Hevea* by monitoring the diurnal variation in canopy temperature using an infra-red remote thermometer (Raytek Corporation, USA) at peak summer during the months of March, April and May. The accessions exhibiting relatively low canopy temperature were selected as promising ones.

For clonal comparison 42 accessions from SBN 2003 were evaluated by measuring the mid-day canopy temperature and it variably increased from 35.6 ^{0}C for accession AC 3964 to 40.3 ^{0}C for MT 4225. All accessions were ranked for tolerance to canopy temperature stress and found that accessions AC 3964, RO 2432, AC 4046, MT 67, MT 69, RO 2889, RO 4415, RO 142, RO 1323 and RO 2846 were showing low canopy temperature at peak mid-day hours and characterized as most intrinsic tolerant ones for the drought and high temperature stresses in field. Contrastingly, accessions MT 4225, RO 2753, RO 850, AC 4095, AC 3874 and MT 41 ranked bottom showing higher canopy temperature and characterized as the most susceptible lines.

1.10. Multi-location physiological evaluation of ortets for abiotic stresses

In Ortets planted at CES, as a part of multi-location clone evaluation trial, the growth was monitored during the months of November 2014 and May 2015 by measuring the tree girth at 150 cm height from the bud union. Clone RRII 430 and ortet RRSA 98 acquired maximum girth and ortets RRST 39 and GH 1 recorded the lowest girth at CES, Chethackal. Girth increment during stress period was the highest in ortets RRSA 98, GH 9, NGK 47, Dap 34 and the lowest in NGK 69 and GH 3. Among the elite clones RRII 414, RRII 429 and RRII 430 showed higher girth increment and clones such as RRIM 600, RRII 105 and RRII 417 showed the lowest girth increment in summer period.

Cell membrane stability was analysed in ortets by measuring the leakage of electrolytes from leaf tissues under *in-vitro* drought. Membrane stability decreased and leakage of electrolytes across cell membranes increased due to drought stress in most of the ortets and clones. The ortets RRSA 315 and RRST 37 recorded increased rate of cell leakage under stress and low membrane stability whereas, ortets Dap 34, RRIM 600 and NGK 47 showed less leakage and high membrane stability attributed to intrinsic tolerance to drought stress (Table Phy. 2).

Table Phy. 2. **Variation in cell membrane injury in ortets under *in-vitro* desiccation stress**

Clone/ Ortet	Cell membrane injury (%)	Clone/ ortet	Cell membrane injury (%)
RRII 105	38.6	RRSA 315	43.8
RRIM 600	29.6	RRSA 585	31.5
RRII 414	38.8	NGK 1	40.0
RRII 417	35.9	NGK 47	30.2
RRII 422	31.7	NGK 69	36.8
RRII 429	41.2	GH 1	38.4
RRII 430	30.2	GH 3	32.2
DAP 1	38.2	GH 9	33.6
DAP 34	28.8	RRST 24	39.9
DAP 35	30.4	RRST 37	43.0
DAP 36	37.0	RRST 39	32.5
RRSA 98	36.2	—	—

1.10.1. *Biochemical studies: Antioxidant defense mechanism in ortets/clones*

Data on biochemical factors (sugars, thiols, protein, proline, malondialdehyde) and antioxidant enzymes (SOD, Catalase, Peroxidase and glutathione reductase) were measured in leaf samples of 16 ortets and 7 clones (samples collected from Nagrakatta and Dapchari and CES during stress and stress free season) were compiled and statistical analysis is in progress.

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

Among five clones planted at Haileyburia Tea Estate, Elappara, Idukki, a high altitude location, clone PB 260 followed by RRIM 600 continued to perform better than all other clones. Clone PR 261 was the least performer showing 20 cm girth at ninth year of planting. Overall the growth of rubber plants at cold stress prone high altitude was affected by multiple environmental factors and lack of proper cultural practices compared to the traditional belt. However, the polyclonal seedlings planted in tea garden seem to be promising than the hybrids. Polyclonal seedlings planted between tea rows as a substitute for the traditional shade trees (Silver oak) had attained 33 cm (mean) girth.

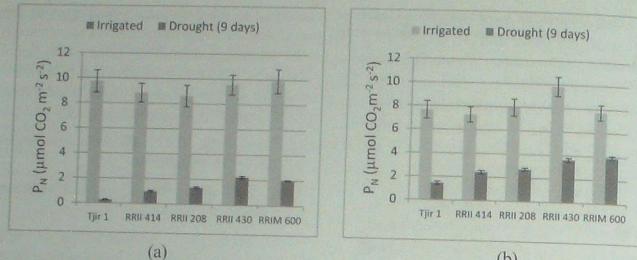
1.12. Proteomic studies of *Hevea brasiliensis* under abiotic stresses

1.12.1. *Proteomic studies of Hevea brasiliensis under cold stress*

Differential expression of several chloroplast proteins in SDS-PAGE were noticed among top five (RRII 429, RRST 24, RRST 39, DAP 1, GH 9) and bottom five (NGK 47, RRSA 585, NGK 1, NGK 69, GH 3) ranked ortets during winter season at RES, Nagrakata. Cold treatment of polybag plants of five *Hevea* clones *viz.,* RRIC 100, RRII 414, 208, 429 and RRIM 600 was initiated in growth chamber. Preliminary physiological observations indicated that severe cold injury in RRIC 100 and RRII 422, relatively less injury in RRII 429 and no injury marks in the case of RRII 208 and RRIM 600 at 6°C temperature (T min) for 10 days.

1.12.2. Proteomic studies of Hevea brasiliensis under drought stress

Drought stress was imposed by withholding irrigation in five *Hevea* clones inside glasshouse. Among the five clones net photosynthesis rate and stomatal conductance were higher in RRII 430 and RRIM 600 followed by RRII 208, RRII 414 and Tjir 1. RWC was higher in RRIM 600 followed by RRII 208, 430, 414 and Tjir 1 after nine days of drought exposure. Differential expression of about fifty chloroplast proteins was noticed among susceptible (Tjir 1 & RRII 414) and tolerant clones (RRII 208, RRII 430 & RRIM 600) after drought imposition for nine days in comparison to respective control samples. Around fifteen stress proteins have been specifically more abundant in drought tolerant clones.



(a) (b)

Fig. Phy. 4. **Net Photosynthesis rate (P_N) of drought imposed polybag plants of *Hevea***
(a) without PBZ
(b) pre-treated with PBZ

1.13. Growth regulation and high density planting in natural rubber for productivity enhancement: Application of PBZ in young plants for development of better root system towards improving drought tolerance

Polybag plants pre-treated with paclobutrazol (PBZ) exhibited better net photosynthesis (P_N) on 9th day of drought, in all clones (1.6-3.9 µmol.m^{-2}.s^{-1}) than untreated plants (0.2-2.1 µmol.m^{-2}.s^{-1}) (Fig. Phy.4). Stomatal conductance (g_s) was higher in susceptible clones, RRII 414 and Tjir 1 (40-45 mmol.m^{-2}.s^{-1}) than untreated plants (15-20 mmol.m^{-2}.s^{-1}). Similar trends were found for RWC values. Epicuticular wax content was better in drought imposed plants of RRIM 600 (5 mg.dm^{-2}) followed by RRII 208, RRII 414, Tjir 1 and RRII 430. Plants treated with PBZ-50mg were found to withstand soil moisture deficit conditions including susceptible ones. PBZ treated leaves showed higher stomatal density, small stomatal pores, thicker leaf lamina, more palisade tissue with densely packed chloroplasts; spongy layer consists of two zones: upper zone consists of 2-3 layered cells with more intercellular space; lower layer is formed of compactly arranged cells; thick cuticle with more striations on the lower epidermis that partially covers the stomatal pore, upper epidermis was en-sheathed with thick cuticle intruded into the epidermal cells.

The level of antioxidants and enzymes was assayed in PBZ-50 mg treated, drought imposed and control plants in five clones of *Hevea*. The results indicated significant alteration in the contents of thiol, sugar and enzyme activities in treated plants. These might conferred certain degree of drought tolerance to plants.

2. Production physiology

2.1. Intercropping with tree crops in rubber

The impact of tree intercrops with rubber trees was periodically monitored by measuring the annual girth and fortnight cup lump yield at CES, Chethackal. Rubber was planted along with three rows of mahogany trees and single row of pathimugom trees in between rubber rows. The girth of rubber trees did not show any significant variation between treatments. Similarly, the rubber yield ($gt^{-1}t^{-1}$) in rubber alone and intercropped area did not exhibit any significant difference. Among the intercrops the growth was largely affected in pathimugom trees due to

shading by mature rubber trees. Mahogany trees (47 cm girth) found growing as better intercrop than the pathimugom (24 cm girth) trees in rubber-intercrop trial.

2.2. Wintering nature in double budded *Hevea* clones

Trunk girth and wintering observations were taken in double and single budded plants which were planted in the field during 2000. After 14 years of growth, 32% double budded scions in the combination of RRII 105 x RRIM 600 attained tappable girth, while it was 56% in the combination GT 1 x PR 107. The single budded plants of the four clones are under tapping for two years. Among the four clones, RRIM 600 and PR 107 are early wintering whereas RRII 105 and GT 1 are late wintering types. No marked variation was observed in double budded plants in terms of wintering and wintering pattern of their single budded counterparts.

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

Studies on latex regeneration mechanism in different clones indicated that clones RRII 105 and PB 260 showed efficient latex regeneration capacity and clone PB 217 was characterized by very high sucrose (above 20 mM), low ATP and ATPase activity indicating slow metabolism. Based on these findings, a new experiment was initiated at CES, Chethackal during November 2014 in newly opened trees of clones RRII 105, PB 260 and PB 217 to study the tapping activated latex regeneration mechanism and effect of stimulation on latex regeneration. Eighteen trees from each clone (nine control and nine stimulated with 2.5% ethephon) were selected and latex physiological parameters were measured. After first stimulation, yield, drc and biochemical parameters and enzymes activities were measured in whole latex and C-serum. Immediate effect of stimulation was noticed in clone PB 217 with high rate of energy metabolism and sucrose utilisation.

2.4. Studies on rubber biosynthesis: Gene expression studies

Quantitative gene expression analysis of seven genes associated with rubber biosynthetic pathway was carried out in high yielding (RRII 105 and PB 217) and low yielding (Tjir 1 and RRII 38) clones. Analysis of the data for picking up crucial genes associated with yield parameters is in progress.

3. Tapping Panel Dryness

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

Statistical analysis of three years yield data on biochemical parameters in latex and bark after stimulating the trees with ethephon (Clone RRII 105 with eight treatments, application of stimulant at different locations and different concentrations) was carried out. A significant reduction in sucrose content but better energy availability (ATP) in latex was observed in trees stimulated with 5 per cent ethephon above and below the tapping panel. However, the stress effects were more in trees with 5 per cent ethephon application above and below the panel than 2.5 per cent ethephon treatment.

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

Transcriptome sequencing of bark RNA from normal and trees with different levels of TPD (10-20%, 40-60% and 80-100%) incidence is continuing.

4. Secondary Metabolites

4.1. Quantification and identification of inositol in *Hevea*

In order to licensethepatented protocol related to isolation of L-quebrachitol from *Hevea* latex, permission was obtained from the

technology transfer committee and the Chairman of Rubber Board. By following the procedures of technology transfer committee arrangements were made for website advertisement.

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

Analysis of data on osmolyte concentration with respect to the water relation of latex showed significant clonal variation. Clones such as GI 1, GT 1 and RRII 308 had high sucrose concentration than other clones during stress season. Total amino acids were found higher during stress season in all the *Hevea* clones. Significant increase in total inositol content was observed in all the clones except RRII 43 during stress season. RRII 105 and GT 1 showed high level of inositol content followed by RRIM 600 under stress season. The contribution of total sugars (sucrose, reducing and inositol content) to the measured osmotic potential was found at a range of -0.35 to -0.64 MPa and -0.48 to -0.72 MPa in peak yielding and stress seasons, respectively. Inositols alone accounted for 28 per cent to 41 per cent of the latex osmotic components during stress season. Sugars and inositols together contributed more than 50 per cent of the total latex osmolality during stress in some of the clones studied.

LATEX HARVEST TECHNOLOGY DIVISION

The research and advisory services on different aspects of Latex Harvest Technology were actively continued by the division. The very low and uncertain rubber price, and acute shortage of skilled tapper availability warrant development, popularization and adoption of measures to reduce cost of production of each kilogram of rubber produced. The division has conclusively proved that weekly tapping from year one of harvesting is the best tool to achieve this target. In India, the share of old and senile trees has crossed 50 per cent mark which is one of the major reasons for productivity decline year after year. An all-out effort of implementing proper Controlled Upward Tapping (CUT) can very easily stop this decline; in fact can lead to upward movement of the productivity graph. Popularizing these two techniques are our major thrust.

1. Low frequency tapping

The division successfully continued applied research on various aspects of Low Frequency Tapping. The comprehensive study on frequencies ranging from d2 to d7 at HML Koney estate continued successfully in the fourth year under modified stimulation schedules. Four years yield data was subjected to statistical and economic analysis. Dry rubber yield was at par under all treatments except S/2 d3 6d/7 without yield stimulation, where it was lower (Table LHT. 1). The benefit cost ratio (BCR) under d2 frequency was 5.8 whereas it was 12.4, more than double under weekly tapping (d7). Annual average dry rubber content was in the range of 29.9 (d2) to 41.6 (d7) per cent. Four years cumulative incidence TPD was over 21 per cent under d2 frequency (without yield stimulation), whereas it was only six per cent only under d7 (72 rounds ethepon application in 4 years). No sign of degradation of the laticiferous system was observed under d6 and d7 with frequent stimulant applications, indicated by high thiol, non accumulation of stress indicators like proline and phenol. These systems showed high utilization of sucrose after stimulation (indicated by very low sucrose and high invertase activity), normal

Table LHT. 1. **Yield (kg/400 trees), dry rubber content (%), Benefit Cost Ratio, TPD and panel status under various tapping systems at Koney estate (April 2011 to March 2015)**

Tapping system	Mean over 4 years	Mean drc %	Benefit cost ratio	Cum. TPD %	Tapping panel & year
S/2 (RG) d2 6d/7	2109 a	29.9	5.8	21	BO-2 (2)
S/2 (RG) d3 6d/7	1727 b	36.0	6.9	13	BO-2 (1)
S/2 (RG) d3 7d/7 ET 2.5% Pa 2/y	2132 a	33.8	7.0	11	BO-2 (1)
S/2 (RG) d3 6d/7 ET 2.5% Pa 3/y	2052 a	36.5	7.8	6	BO-2 (1)
S/2 (RG) d4 7d/7 ET 2.5% Pa 4/y	2098 a	36.7	9.1	9	BO-2 (1)
S/2 (RG) d4 6d/7 ET 2.5% Pa 6/y	2113 a	38.6	10.0	10	BO -1 (5)
S/2 (RG) d6 7d/7 ET 2.5% Pa 10/y*	2004 a	39.5	11.1	17	BO -1 (5)
S/2 (RG) d6 6d/7 ET 2.5% Pa 12/y*	2082 a	41.6	12.4	46	BO-1 (5)

** Initial two years of opening, number of stimulation rounds are double*

thiols and sufficient energy availability. Though low, the level of sucrose is maintained throughout the experimental period. High frequency (d2) tapping trees had the highest bark consumption compared to lowest in trees under d7.

1.1. Collaborative programme of RRII and Regional Offices of Rubber Board in popularizing weekly tapping with stimulation in smallholdings

More growers have joined the programme during the year and the total participants are more than 40 now (under various Regional Offices), and are continuing weekly tapping with good results. As anticipated, many neighbouring growers of demonstration plot owners started showing interest, and have voluntarily started weekly tapping with yield stimulation. Under regional office at Thalassery, there are more than 15 growers practicing weekly tapping.

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

The exploratory trial started with the objective of further reduction in cost of production of NR. at CES, Chethackal on d10 frequency continued to give similar performance as that previous years. A commercial evaluation trial of S2 d10 system of tapping was initiated in 10 tapping blocks of clone RRII 105 at Kanthimathy Estate, Kulasekharam, Tamil Nadu, during 2014-15. Mean dry rubber yield of 1596 kg/block could be obtained under d10 as against estate average of 1679 kg/block. Initial year result (10 months period) is encouraging.

1.3. Demonstration trial on weekly tapping in clone RRII 105

The demonstration plot at Central Experiment Station (CES) under weekly tapping with monthly application of 2.5% ethephon gave yield of 1854 kg/400 trees during 2014-15 and incidence of tapping panel dryness was only 3.3%. Since the tapping panel reached bud union, yield was less compared to previous years.

2. Controlled Upward Tapping

The RBD experiment on Low Frequency Controlled Upward Tapping (LFCUT) under d10 frequency initiated in 2013-14 at EFU, RIT, Pampady was continued. Similar to previous year, significant yield variation was observed

among the treatments. Dry rubber yield was significantly higher under d3 (2560 kg) system as against 2120 kg under d10 frequency.

All India coordinated project on CUT continued successfully in various locations.

3. Other experiments

3.1. Onfarm evaluation of reduced spiral tapping

The onfarm trial of reduced spiral tapping at Piravom road and Ottappalam continued successfully. The commercial yield (kg tree^{-1}) performance of S/4 d3 at HML, Mundakkayam estate was 2.7 (RRII 430), 3.2 (RRII 414) and 2.9 (RRII 105) during the first year of tapping. All the relevant information so far were compiled.

3.2. Response of RRII 400 series clones to yield stimulation

Three RRII 400 series clones (RRII 414, RRII 422 and RRII 429) under tapping in the second year from opening were identified at CES, Chethackal, 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 is S/2 d3 6d/7. Two rounds of stimulation (ET 2.5% Pa) was given during 2014-15 in comparison with the unstimulated control trees (50% trees for stimulated and 50% trees unstimulated, for each clone).

Though not significant, RRII 429 showed higher yield than RRII 422 and RRII 414. The duration of stimulatory effect in these clones were monitored. The trees were stimulated on 16.09.2014. Yield increase under stimulation was minimal and lasted only for the next three tapping in clone RRII 414. Whereas it was better under RRII 429 and RRII 422 and lasted longer, *i.e.*, for next nine tappings (Fig. LHT. 1).

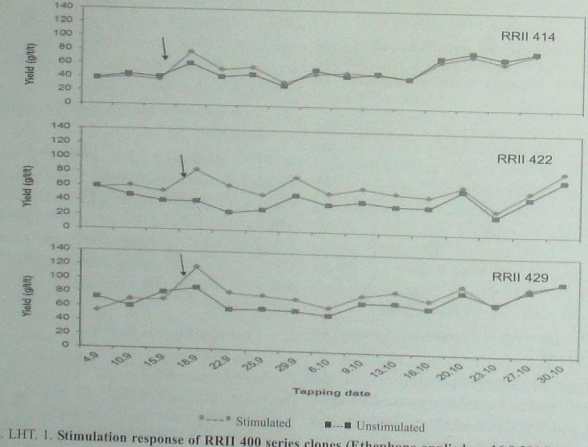


Fig. LHT. 1. **Stimulation response of RRII 400 series clones (Ethephone applied on 16.9.2014)**

3.3. Evaluation of non-conventional tapping methods

3.3.1. Comparison of needle tapping (d3) with conventional tapping system (S/2 d3)

Needle tapping was compared with conventional half spiral tapping, both under d3 frequency in clone RRII 105 at EFU, RIT, Pampady. About 48% yield reduction was observed in needle tapping. The average yield under conventional and needle tapping was 41.1 g $t^{-1}t^{-1}$ and 21.9 g $t^{-1}t^{-1}$, respectively.

3.3.2. Evaluation of vertical tapping

The RBD experiment on vertical tapping under d3 frequency of tapping laid out at EFU, RIT, Pampady was continued. Yield comparable to that of S/2 d3 frequency of tapping could be obtained with vertical tapping cut of 22 or 10 cm with higher levels of stimulation. (Fig. LHT. 2)

3.4. Testing and evaluation of products

3.4.1. Testing and evaluation of ethephon (New brands)

The RBD experiment with four treatments and six replications laid out at CES, Chethackal to test and evaluate two makes of ethephon 10 per cent in comparison with already tested and evaluated ethephon and control (unstimulated) under d3 frequency of tapping was concluded. Test reports were issued to the firms after approval by Scientific Advisory Committee (SAC).

3.4.2. Farm mechanisation – Testing and evaluation of tapping knives

As part of Farm Mechanisation programme, one new trial comprising of five experimental plots for evaluation of three models of tapping knives (one mechanised and two mechanical) in comparison with "Mitchie" and "Jebong" knives was laid out at CES, Chethackel. Pre-treatment yield recording completed.

3.5. Long term response of yield stimulation in clone RRII 105

Two products from the market, Vitex (2.8% CEPA) and Agrowin gel (9.0% CEPA) were evaluated at CES, Chethackal under S/2 d3 6d/7 system of tapping. Application of 2.5% ethephon (3/y) showed comparable yield of three rounds of Vitex and Agrowin gel application. The stimulant cost per tree per application for these two are Rs. 2 where as it is only 40 paise for ethephon.

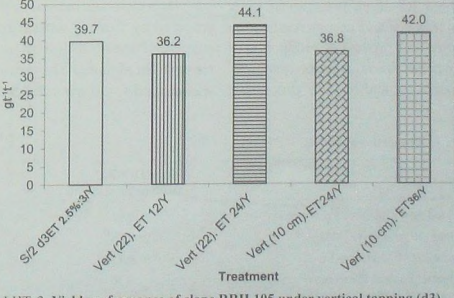


Fig. LHT. 2. **Yield performance of clone RRII 105 under vertical tapping (d3)**

3.6. Development and evaluation of biodegradable polythene

Seven different grades of polythene having different levels of additives were prepared in collaboration with M/s Everest Polymers, Mangalore and tested in the field. One sample has given the desired performance during the monsoon and degraded within few months thereafter. The identified sample will be evaluated commercially during 2015 before recommending.

RUBBER TECHNOLOGY DIVISION

The activities of the Division were focused mainly on evolving improved techniques in processing of rubber, deproteinisation, radiation vulcanisation of latex (RVNRL), latex stage incorporation of fillers, reinforcement of NR using polymeric filler and silica, devulcanization of used rubber products and rubber nanocomposites based on RVNRL.

1. Primary processing

1.1. Deproteinised natural rubber (DPNR)

Development of technology for the processing of deprotenised natural rubber (DPNR) under the Transfer of Technology (TOT) project was carried out. A Memorandum of Understanding (MOU) was signed with a party and Rubber Research Institute of India (RRII) for the transfer and commercialization of the technology. The process of production of DPNR involves preservation, enzyme treatment, creaming, coagulation and further processing of rubber under controlled conditions into technically specified rubber. Pilot plant scale trials were carried out at RRII crumb rubber factory and the DPNR produced was found to meet the standards with regard to the nitrogen content. DPNR produced were given to the industry for evaluation. The properties of the DPNR developed were comparable to that of the commercial sample (Table Chem. 1). However some more improvement in process and properties are required to match with the industrial requirements of the clients. These include high production cost, tedious method of preparation, low quality of DPNR, etc.

1.2. Carbon black filled DPNR

Deproteinized natural rubber (DPNR) was prepared by treating natural rubber latex with enzyme (Papain) followed by creaming with ammonium alginate. The creamed DPNR latex was diluted to 20 per cent dry rubber content

Table Chem. 1. **Properties of DPNR**

Property	DPNR (Commercial sample)	Sample developed
1. Dirt retained on 44 im sieve (% wt max)	0.01	0.02
2. Ash content (% wt. max)	0.15	0.15
3. Nitrogen content (% wt. max)	0.12	0.11
4. Volatile matter content (% wt max)	0.30	0.30
5. Wallace plasticity, P_o (min)	35.00	33.00

Table Chem. 2. **Comparison of technological properties of Carbon Black filled NR and DPNR**

Particulars	DPNR	DPNR MB	Latex MB	ISNR 5 (Control)
Tear strength, N/mm	94	53.3	73.0	83.4
Tensile strength, MPa	27.9	16.3	20.3	25.5
EB (%)	682	272.8	398.1	652.6
Modulus at 100 %, MPa	2.5	4.7	3.20	1.7
Modulus at 300 %, MPa	9.3	-	13.9	7.7
Heat build up, ^{D}T ^{0}C	16	20	16	13
DIN abrasion loss, mm^3	138	103	111	100
Hardness, Shore A	62	76	70	60

(DRC). HAF black was added to it as a 20 per cent dispersion (so as to get 40 phr black) in presence of a surfactant (0.5 phr). The latex-HAF black mix was coagulated using formic acid. Coagulam was washed and dried at 70°C thus getting a masterbatch (MB) of DPNR with carbon black referred to as DPNR MB. Another MB of natural rubber latex with HAF black (40 phr) was also prepared as control and is referred to as Latex MB. Similarly a control DPNR was prepared as described earlier. DPNR and ISNR 5 were mixed with carbon black (40 phr) through dry mixing. These samples were compounded and compared its technological properties and the results are given in Table Chem. 2.

1.3. Modified coagulant in sheet making

The variation of colloidal properties of NR latex on incorporation of varying concentration of a surface active agent was studied by measuring the variations in isoelectric point (Fig. Chem. 1), viscosity and particle size distribution. The isoelectric point shifts from pH 4.00 (fresh field latex) to 4.9 at 10 iM and then to 5.16 at

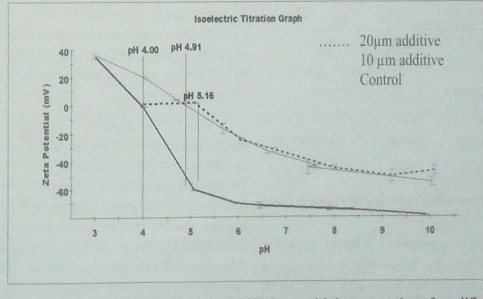


Fig. Chem. 1. **Variation of iso electric point of NR latex with incorporation of modifier**

20 iM concentration of additive indicating the variation in the surface charge of latex particles.

With increase in additive concentration, an increase in viscosity of NR latex especially at higher shear rate and a shift in the particle size distribution towards the higher size range was noted. These property changes of latex with additive content indicate a higher flocculation of latex particles.

2. Latex Technology

2.1. Radiation vulcanised latex (RVNRL)

Attempt was made to prepare nancomposites based on RVNRL using modified clay. An *in-situ* modification of bentonite clay was done. A dispersion of bentonite clay (50 %) in water was exposed to gamma radiation (5 kGy) in presence of n-butyl acrylate. Finally this modified bentonite clay (2.5 phr modified clay) was mixed with centrifuged latex and used for preparing RVNRL. The incorporation of modified bentonite clay improved mechanical properties of RVNRL. Results are given in Table Chem. 3.

Table Chem. 3. **Mechanical properties of RVNRL with and without nanoclay**

Properties	RVNRL Control	RVNRL with nanoclay
Modulus at 100%, MPa	0.58	0.77
Modulus at 300%, MPa	1.02	1.25
Modulus at 500%, MPa	1.55	2.04
Modulus at 700%, MPa	3.21	4.56
Tensile strength, MPa	26.69	30.20
Elongation at break,%	1360	1258

2.2. Superior processing rubber (SP rubber) from RVNRL

An attempt was made to prepare superior processing rubber by blending g-irradiated vulcanized natural rubber latex (RVNRL) with styrene butadiene rubber (SBR) latex to get a final dry mix containing 60 parts of SBR and 40 parts of NR by weight which is referred to as SBR 60/NRXL 40. Similarly another mix of 80 parts of SBR and 20 parts of RVNRL was also prepared and is referred to as SBR 80/NRXL 20.

Carbon black filled (40 phr HAF) RVNRL-SBR blend and ISNR 5 based compounds were prepared and tested. Properties which showed improvement are given in Table Chem. 4.

R 5 baSed comP°unds were

Table Chem. 4. **Mechanical properties of the SBR-RVNRL blend**

Particulars	SBR 60/NRXL 40	SBR 80/NRXL 2	ISNR 5 (Control)
Modulus at 100 %,MPa	3.0	3.2	1.9
Modulus at 300 %,MPa	10.2	11.1	6.1
Compression set, %	27.0	25.0	40.0
Hardness, Shore A	69.0	71.0	60.0

2.3. Recovery of skim rubber from skim latex

Skim latex obtained as a by-product from centrifugal concentration of NR latex is having higher protein content and has very low rubber content. Rubber particles are smaller in size. Skim rubber recovery from skim latex was attempted by two methods.

a) Skim latex was acidified to optimum pH followed by coagulation by the addition of limited quantity of field latex. b) Skim rubber recovery by creaming process followed by acidification for coagulation.

Deproteinization of skim latex was attempted by two methods - a) by urea treatment and b) by enzyme treatment. Skim latex treated for protein removal was coagulated by the above two methods. Rubber thus recovered was tested for raw rubber properties and nitrogen content. Both urea treatment and enzyme treatment gave rubber with low nitrogen content. Also the above two methods gave smooth techniques for recovery of rubber from skim latex.

2.4. Cytotoxicity studies of NR latex and latex products

NR latex products face the challenges of protein allergy and residual chemical toxicity. For the analysis of residual chemicals in the latex rubber products, an HPLC based method has been developed, to distinguish between three classes of accelerators namely thiurams, dithiocar- bamates and benzothiazoles by chromato- graphic run using a UV-Visible detector at wavelengths of 290, 430 and 320 nm respectively. This is possible due to the variation in retention time of these accelerators. Retention time of various accelerators studied are given in Fig. Chem.2.

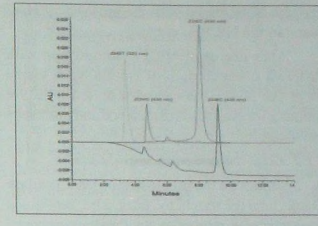


Fig. Chem. 2. **HPLC profile of various accelerators**

3. Rubber Technology

3. 1. Reinforcement

3.1.1. NR/ Polymeric filler system

The effect of carbon black on the properties of polymeric filler natural rubber composites was studied. Carbon black used in this study was HAF black. The formulation used in this study is given in Table Chem. 4.

A control compound with blends of natural rubber polybutadiene was included in this study for a comparison. The compounds CB0, CB10, CB20 and CB30 contain 0, 10, 20 and 30 parts HAF carbon black respectively. The technological properties have been investigated in detail among these samples. With the addition of HAF black to the NR/PF system improved its tensile strength, modulus, hardness, abrasion residence, tear strength etc. and these values are comparable to that of the control compound (Table Chem. 5). However, the heat build-up

Table Chem. 4. **Formulation of carbon black filled NR- Polymeric system**

Ingredients	Control	CB_8	CB_{10}	CB_{20}	CB_{30}
NR	60	100	100	100	100
Polybutadiene	40	0	0	0	0
Polymeric filler	0	12	10	8	6
HAF (N330)	50	0	10	20	30

ZnO - 5, Stearic acid - 2, Naphthenic oil - 6, 6PPD - 1.5, TMQ - 1, MC Wax - 1, TBBS - 1.2, Sulphur - 1.3

Table Chem. 5. **Technological properties of carbon black filled NR-PF system**

Properties	Control	CB_8	CB_{10}	CB_{20}	CB_{30}
Tensile strength, MPa	22.3	25.7	26.2	27.0	28.6
Modulus at 100%, MPa	2.6	4.3	4.5	4.5	4.7
Modulus at 300%,MPa	10.8	10.8	11.3	12.1	13.4
Elongation at break, %	558	628	613	602	586
Tear strength, N/mm	72	78	82	86	92
Hardness, Shore A	62	62	64	64	66
DIN Abrasion loss, mm^3	58	102	94	83	80
Heat build-up, $^{D}T^{o}C$	22	7	9	12	14

properties increased with the presence of HAF black in the NR/PF system.

Also prepared NR/PF composites with different concentration of carbon black (same as in the above trials) and supplied to a tyre industry for evaluation at their end and the results are awaiting.

3.1.2. Latex- carbon black masterbatch

Studies on the processing of latex - carbon black master batch by fatty acid sensitized method was continued as a collaborative project with M/s. Apollo Tyes Ltd., Perambra, Trichur District. Based on our laboratory trials, protocols were standardized for pilot plant studies. The machinery for the continuous coagulation and processing of master batch was designed, fabricated and installed in the factory at RRII. Pilot scale production of master batch was carried out. About 60 kgs. of masterbatch was supplied to the collaborating tyre manufacturing unit for further evaluation of the technical properties at their end. Evaluation report from the industry revealed that the master batch giving satisfactory results in comparison with the corresponding dry mixes conventionally produced at the factory. However some more improvement is required with regard to batch to batch consistency, moisture retained in the product and some of the critical properties such as compound viscosity, modulus, heat build-up, tanä, reinforcing index and abrasion loss. Comparison of the results is given in Table Chem. 6. The process has to be fine tuned to suit the requirement of the industry.

3.1.3. Silica reinforcement of NR - Latex- silica masterbatch

a. Effect of plasticizer

Silica filled natural rubber was prepared through Latex-filler masterbatch route. Natural rubber with varying proportions of silica *viz.*, 41 to 58 parts of silica per hundred rubber were prepared with two different plasticizers *viz.*, hydrocarbon based and vegetable oil based. Sulphur vulcanizates prepared from these composites were evaluated for its mechanical properties. Composites with vegetable oil plasticizer exhibited superiority over to that of

Table Chem. 6. **Comparison of properties - Dry mix from industry vs masterbatch from RRII**

Property	Carbon black, phr		Carbon black, phr	
	45 Dry mix Industry	45 Masterbatch RRII	50 Dry mix Industry	50 Masterbatch RRII
Mooney viscosity, ML (1+5),135 °C	48.6	26	53	36
Modulus at 300%, MPa	12.3	9.9	13	10.8
Reinforcibility index	5.7	4.6	5.5	4.7
Tensile Strength, MPa	28	27	27.7	24.6
Tear strength, N/mm	107.5	105.8	113	109
Heat build up, T, °C	20	19.4	21	26.7
DIN abrasion loss, mm^3	108	118	107	122
Tan ä at 60 ° C	0.206	0.22	0.217	0.25

the hydrocarbon based (Table Chem. 7). Also the vegetable oil based plasticizer shows lower Payne effect compared to hydrocarbon oil (Fig. Chem. 3).

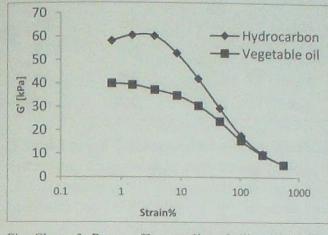


Fig. Chem. 3. **Payne effect studies of silica filled NR containing different plasticizers**

b. Latex-Silica masterbatch from latex with varying preservation systems

Incorporation of silica in rubber is an energy intensive process. NR-Silica masterbatches containing 48 parts silica per hundred rubber (phr) were prepared from NR latex preserved with four different preservation systems and are denoted as MP_1 to MP_4. Sulphur vulcanizates prepared from these composites were compared with a corresponding dry mixed system for its cure characteristics and technological properties. Composites made from masterbatches prepared through latex route exhibited properties closer to that of the dry mixed system and are given in Table Chem. 8 .

Table Chem. 7. **Effect of plasticizer on the properties of silica filled natural rubber**

Property	Silica loading, phr (Hydrocarbon oil plasticizer, 5 phr)			Silica loading, phr (Vegetable oil plasticizer, 5 phr)		
	41	46	58	41	46	58
Modulus 300%, MPa	11.6	13.1	14.3	10.5	11.7	14.5
Tensile strength, MPa	29.5	28.5	27.5	27.5	27.5	28
Tear strength, N/mm	99	96	107	98	85	103
Heat build up T(°c)	8	8	15	8	9	10.5
Abrasion loss, mm3	119	121.1	127	89.5	94.2	90.6

Table Chem. 8. **Technological properties of NR-Silica composites**

Property	Latex- silica (48 phr) masterbatch from NR-Silica				
	NR latex MP_1	NR latex MP_2	NR latex MP_3	NR latex MP_4	(48 phr) dry mix
Tensile Strength, MPa	28.0	23.1	24.3	24.9	26.8
Modulus at100%, MPa	2.2	2.2	2.4	2.5	2.7
Modulus at 300%, MPa	12.6	9.7	10.5	12.8	11.9
Elongation at break, (%)	518	565	537	486	563
Tear strength, N/mm	93	90	77	77	79
Abrasion loss, mm^3	107	107	111	114	109
Heat build up T, °C	6	5	6	6	6

Table Chem. 9. **Cure properties of the rubber –silica compounds with and without modifier**

Property	MS_0	MS_1	MS_2	MS_3	MS_4
Cure time ,T90, min	11.1	8.2	8.520	8.1	8.1
Ts2, min	2.1	3.01	3.210	2.6	2.4
Maximum Torque, M_H, dNm	25.6	24.7	25.2	25.1	23.9
Minimum Torque, M_L, dNm	5.9	4.4	4.5	4.0	3.5
Tan δ at M_H	0.38	0.33	0.29	0.27	0.25

3.1.4. Reinforcement of natural rubber using modified silica

Sulphur cured natural rubber-silica composites were prepared using silica and NR serum modified silica. Cure system used was S/CBS/DPG combination in the ratio 2.5:1.5:1.5 (phr) and filler loading was 50 phr. Precipitated Silica (Ultrasil VN3) was modified using varying concentration of serum contents at 0, 0.5, 1.0, 2.0 and 5.0 weight percent of silica weight and the corresponding compounds are namely MS_0, MS_1, MS_2, MS_3 and MS_4. The cure behaviour and physical properties of the composites were studied. Cure characteristics are given in Table Chem. 9. Modified silica composites showed better processability due to the reduced filler – filler network as is evident from the Payne effect studies of the compounds are given in Fig. Chem. 4. It was observed that technological properties (modulus, tensile strength, Elongation at Break, tear strength) of the unmodified and modified composites were comparable.

3.2. Recycling of rubber : Devulcanisation

A new devulcanising agent (DVA) has been identified for the mechano-chemical devulcanisation of vulcanized rubber products. The optimum amount of DVA required for devulcanisation depends on the type of cure system used (CV, semi-EV or EV) for the vulcanization of the original sample. Fig. Chem. 5 gives the comparison of percent devulcanisation for various cure systems with DVA at optimum concentration and with out DVA.

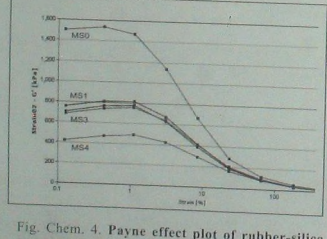


Fig. Chem. 4. **Payne effect plot of rubber-silica compounds (70 °C, 0.1 Hz, strain 0.1 to 500%)**

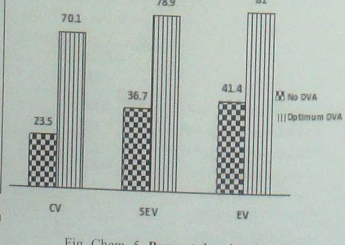


Fig. Chem. 5. **Percent devulcanization**

Table Chem. 10. **Comparison of the vulcanizate properties original and revulcanised samples**

Cure system of original sample	Vulcanizate	Tensile strength MPa	EB,%	M_{100}, MPa	$M3_{60}$, MPa	Tear strength N/mm
	Original	30	671	2.2	9.4	119
CV	0 DVA	19.7	533	1.9	7.8	37
	Optimum DVA	25.5	668	2.1	8.3	64
	Original	31.6	634	2.6	11	110
Semi-EV	0 DVA	22.5	579	2	7.7	39
	Optimum DVA	27	662	2	7.9	65
EV	Original	27	699	2	8	110
	0 DVA	16	457	2.2	8.5	48
	Optimum DVA	21.5	587	2	7.9	59

Chemical probe analysis of the samples revealed the nature of bond scission involved in the devulcanization process. The study revealed that the reduction in cross link density during devulcanization was due to the scission of the majority cross link type present in the original sample *ie.*, polysulphidic crosslinks in CV and Semi EV systems and disulphidic crosslinks in EV system. The presence of DVA considerably increased the percent devulcanization and the same may be due to the blocking of re-crosslinking of cleaved crosslinks.

The revulcanisate properties of the devulcanised rubbers (prepared with and without using DVA) were compared with the original sample. The results are given in Table Chem. 10. Devulcanisation of various synthetic rubber vulcanistes also showed better efficiency of the DVA system.

4. Collaborative projects

4.1. Development of rubber components for defense

A national seminar on 'Indigenous Warships: Avenues and opportunities for Indian Rubber Industries' was organized jointly by National Institute of Research and Development in Defence Ship building (NIRDESH), Calicut and Rubber Research Institute of India (RRII) in June 2014 at RRII. It was attended by more than hundred participants from Navy, DRDO, Coast guard, VSSC, PSU's and Industries. In continuation to this an MOU is being processed with Naval Ship Repairing Yard of Indian Navy for the joint quality monitoring of rubber products being procured for warships.

4.2. BEL Development work

4.2.1. Service life prediction of rubber based products in sea water

Compounds of NR, EPDM and HNBR were prepared, moulded and aged in sea water at different temperatures (70°C, 80°C and 90°C) for 0, 1, 3 and 5 days. Variations in tensile strengths with and without ageing were measured. The service life was predicted by applying Arrhenius equation. For predicting the service life of a particular rubber, minimum time required to attain a cut off tensile strength of 20 MPa for NR, 10 MPa for EPDM and HNBR was noted. Data was plotted as 'Log days' vs '1/K' (where K is

the temperature in Kelvin scale). The plot was extended to ambient temperature expressed as 1/K and can get the service life. From the study it was found that NR can withstand up to 79 days, EPDM up to 794 days and HNBR up to 1000 days in sea water.

5. Collaborative work with rubber industry

Technology transfer with industries with regard to the three projects *viz*., Polymeric filler, NR-carbon black masterbatch and DPNR are in progress.

6. Development / advisory work / project work

a. Tested and report given for the damage of 14 tyres referred from various Consumer Disputes Redressal Forums in the country.
b. Tested and report given for 20 polythene samples for the RP Department
c. General advisory-7, Latex/ Dry rubber testing - 250
d. Project students (PhD/ M.Tech / B.Tech / M.Sc.) - 8, Sastradarsan - 20

TECHNICAL CONSULTANCY DIVISION

The Division provided consultancy services to small and medium enterprises, individual entrepreneurs, central and state government departments and agencies. The Division caters to the needs of new entrepreneurs as well as existing rubber goods manufacturers. The services offered by the Division are: i) R&D of rubber products; ii) Reverse Engineering & Component analysis; iii) Protein analysis, iv) Trouble shooting; v) Customs, Dispute analysis; vi) Training for specialized products, vii) Testing of raw materials/rubber products, viii) Preparation of Project profiles, Technical bulletin and Project reports.

1. Research Projects

1.1. Nano-dispersions for latex technology

1.1.1. Preparation of ultra-fine dispersions of zinc oxide by simple ball-milling: Optimization of process parameters

Ultra-fine aqueous dispersions of zinc oxide were prepared by a combination of wet ball-milling and ultrasonication in the presence of various concentrations of surfactant. The particles in the dispersions were characterized by dynamic light scattering (DLS) and zeta potential measurements. Six hours of ball-milling followed by ultrasonication on the zinc oxide dispersion containing 3 weight per cent surfactant resulted in the formation of particles having size below 500 nm. (Fig. TC. 1).

1.2. Peroxide vulcanization of rubbers

1.2.1. Effect of coagents on the mechanical properties of peroxide cured natural rubber vulcanizates

The effect of different coagents like trimethylol propane trimethacrylate (TMPTMA), triallyl cyanurate (TAC) zinc diacrylate (ZDA) and n-butyl acrylate (NBA), on the mechanical properties of peroxide cured natural rubber vulcanizate has been studied. Dicumyl peroxide (DCP) was used as the crosslinking agent. Mechanical properties like tensile strength, elongation at break and modulus (100 and 200 per cent elongations) were measured. ZDA offers ionic bridges which behave like polysulphidic crosslinks and it can be used for applications where good mechanical properties

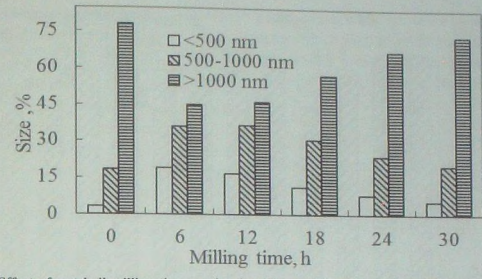


Fig. TC. 1. **Effect of wet ball-milling time on the size (%) of zinc oxide particles in aqueous dispersions containing 3 weight per cent surfactant**

are required. TMPTMA increases the modulus of the vulcanizates and is suitable for high modulus applications. The effects of temperature and concentration on the cure kinetics and physical properties of peroxide cured natural rubber have been investigated. Dicumyl peroxide (DCP) having forty per cent activity was used as the crosslinking agent. The Monsanto Rheometer was used to investigate the kinetics of the peroxide vulcanization. It was found that thermal decomposition of the peroxide followed a first order free radical decomposition reaction and therefore, half-lives at various temperatures were estimated from the kinetic data. The optimum mechanical properties can be achieved by completely decomposing the peroxide in the vulcanizates. Moreover as the concentration of peroxide increases the addition mechanism competes with abstraction mechanism, producing a stiffer vulcanizates with lower mechanical properties.

1.3. Design of cost effective tread formulations as per national specifications

The poly butadiene rubber (PB) content in the tread decides the mileage of the respective tread formulation. However a high amount of PB content will decrease the tear strength which is related to the cutting and chipping resistance of the tread. Accordingly the tear resistance of the blends having different PB content was estimated. The variation of PB in 60/40 (A series), 50/50 (B series) and 40/60 (C series) of NR/PB blend proportions are given in Fig. TC.2. In all blends it was found as the PB content increases the tear strength decreases. The trend is the same in 50/50 and 60/40 NR/PB combinations. However as the proportion of PB increases, the tear strength value also takes lower values. Similarly the specific gravity, heat build-up, compression set, hardness etc. increased as the PB content increases. All possible blend proportions of NR/PB were formulated along with essential rubber chemicals and testing was carried out as per national specifications.

1.4. Rubber compounds with improved flex crack resistance

Nitrile rubber vulcanizates filled with three different grades of carbon black fillers at varying loading were prepared and the effect of fillers on flex-crack resistance has been studied. Among the fillers used, the one with the largest particle size (GPF) produced the highest flex-crack resistance.

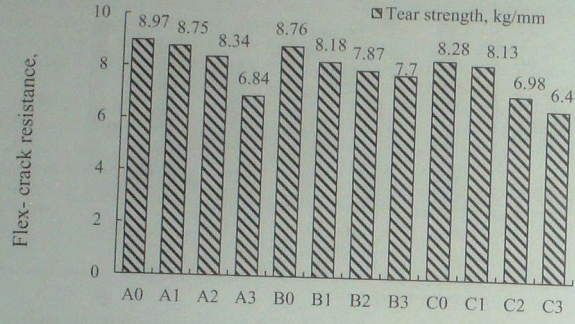


Fig. TC. 2. **Variation of tear strength with 60/40, 50/50, 60/40 NR/PB blends**

1.5. Cell structure and performance of expanded rubber

The technological properties of expanded rubber prepared using three type of blowing agents *viz.* N, N´- dinitrosopent- amethylentetramin (DNPT), azodicarbon- amide (ADC) and Oxydibenzenesulfonyl Hydrazide (OBSH) were studied. The dosage of all ingredients were kept constant and only the loading of blowing agent has been progressively increased. It has been observed that DNPT at moderate loadings produced expanded sheets conforming to IS 10702 for Hawai sole applications (Table TC. 1). ADC and OBSH produced hard sheets with most of the properties out of the specification limits.

Table TC. 1. **Physical properties of expanded sheet with DNPT**

Sl. No.	Parameters	Requirement	DNPT loading, phr		
		IS 10702	*4*	*6*	*8*
1	Relative density	0.4-0.5	0.54	0.41	0.36
2	Hardness, Shore A	45±5	49	46	43
3	Split tear strength, kgf	3.5 (Min)	6.2	5.0	4.5
4	Compression set, %	25 (Max)	23.5	24.8	30.5
5	Flexing resistance				
a.	Initial crack, number of cycles	60,000 (Min)	No crack	No crack	41,750
b.	Cut growth at the end of 1lakh cycles, %	600 (Max)	34	0	4

1.6. Electromagnetic interference shielding composites based on NR

Expanded graphite reinforced NR composites exhibited significant electromagnetic interference shielding effectiveness (-18dB). Effective dielectric constant and loss of the composite is derived over the frequency range and it can be seen that the increased absorption emerged from the increased conductivity of the composite. Expanded graphite, which is cheaper reinforcing filler than carbon nanotubes, can find application in EMI shielding materials.

1.7. Quantification and characterization of blooming in rubber compounds

Variation of cure-time and temperature on blooming of a typical V-strap compound has been studied. Effect of certain chemical ingredients which promote blooming has been studied. Accordingly, design of bloom free formulations for various products are in progress.

1.8. Effective utilization of EPDM reclaim in EPDM compounds

Ethylene-propylene rubbers (EPDM) continue to be one of the most widely used and fastest growing synthetic rubbers having both specialty and general-purpose applications. As EPDM is a costly rubber, incorporation of reclaimed EPDM to virgin EPDM compound has been attempted and evaluated the resulting vulcanizates. Mechanical properties such as tensile strength and modulus showed gradual deterioration while hardness increased to some extent which was in correlation with the modulus values. Tear strength showed a drastic reduction while the heat build-up increased to some extent. Overall results revealed that addition of 10 phr of reclaimed EPDM in to EPDM compounds registered an optimum balance of almost all properties. Further addition of reclaimed rubber is not recommended as far as various vulcanisate properties are concerned. Also, it may be noted that the presence of reclaimed EPDM rubber does not adversely affect the ageing properties of vulcanizates. Mixing large quantities of reclaimed rubber with virgin EPDM resulted in poor dispersion which eventually gives vulcanizates with inferior properties (Table TC. 2).

1.9. Variation of particle size/extractable protein in different processing/product development techniques used in natural rubber latices

Field latex showed high values of protein compared to other concentrated forms. The effects of sulphur pre-vulcanization (PVNRL) and radiation vulcanization (RVNRL) were compared with sulphur post vulcanized latex compound (POVLC) on extractable protein content, particle size and particle size distribution. In all cases extractable protein content was higher in the film prepared from post vulcanized latex compound. This trend is in agreement with the peaks associated with the FTIR spectra of the respective films.

Table TC. 2. **Dispersion rating of various EPDM vulcanizates using Dispergrader**

Mix	EP-EPRR-0	EP-EPRR-10	EP-EPRR-20	EP-EPRR-30
Dispersions	x=8.2, y= 10	x=7.8, y= 9.9	x=7.8, y= 9.9	x=6.9, y= 9.8
Rating	z=98.6	z=96.4	z=95.7	z=92.1

1.10. Particle size variation of natural rubber latex with the addition of styrene butadiene latex, skim latex and precipitated calcium carbonate dispersion

Adulteration of natural rubber latex concentrates cause difficulties in manufacturing process. It is proposed to use particle size analysis as quality control tool for various latex/ latex mixtures. The work has been initiated by incorporating different proportions of SBR latex and calcium carbonate dispersions (major adulterants) in natural rubber latex. Particle size measurements of the mixtures and colloidal stability were evaluated.

1.11. Natural rubber field latex based adhesives

The role of natural rubber field latex as an adhesive for bonding leather to leather specimens was investigated. Natural rubber field latex (preserved with ammonia) was used for bonding leather to leather specimens. Optimization of the dry rubber content of natural rubber latex on the adhesive strength and the effect of different ageing conditions like thermal ageing immersion in water ultraviolet rays on adhesive strength were carried out. It was found that the adhesive strength of the bonded specimens were increased with increase in the dry rubber content, and maximum registered strength values are at 45 DRC. Design of a cost effective formulation is in progress.

2. Testing support to industries

For the testing of raw materials and rubber compounds/products, consistent support was offered especially to small and medium level entrepreneurs. Total number of samples tested was 1295 and the revenue collected was Rs. 20,98,470/-.

The dry rubber products tested are pre-cured/conventional tread, bridge bearings, bonding gum, black vulcanizing cement, tyre flaps, inner tubes, rubber channels/ tubes, floor mats, hawai soles, sponge rubber, O-ring, bushes, engine mounts, automobile components etc. The latex products tested include examination and surgical gloves, latex adhesive, latex thread, balloons and condoms. Protein analysis of latex products and evaluation of chemicals/ latex based paints were also done.

3. Product development

As per the requests of the clients, 25 rubber products were developed as per the specifications and the know-how were transferred to the respective clients.

- Rubber mould
- Neoprene beading
- Surgical tubing
- Vulcanizable adhesive
- Synthetic rubber compound
- Rubber gasket (2 nos)
- Transparent rubber band
- Rubber bearing (2 nos)
- Fire retardant floor mat
- Rubber Compound (2 nos)
- Latex adhesive (2 nos)
- Rubber band
- House hold gloves (2 nos)
- Rubber flap
- Fluorescent rubber band
- Standard rubber compound
- EPDM flap (2 nos)
- Rubber mat
- Heat resistant rubber band

4. Evaluation of chemicals

In the reporting period, two chemicals from clients were analysed and evaluated their suitability in rubber and the detailed reports were communicated to the clients.

5. Project profiles/Technical bulletins

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

Project profiles

- Industrial gloves
- House hold gloves
- Hospital and industrial sheeting
- Rubber Band
- Latex thread
- Latex adhesive

Technical bulletins

- Hot water bottle
- Rubber bladder
- Surgical gloves

6. Advisory services

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

7. NABL accreditation

In connection with the filing of application for NABL accreditation, following documents were prepared: i) Quality Manual, ii) Quality System Procedures, iii) System Operating Procedures, iv) Formats and Work Instructions, v) Test Procedures. In addition to these, officers in the Division have attended various training programmes on laboratory management system and internal audit as per ISO/IEC 17025: 2005.

ECONOMICS DIVISION

Studies on farm management, primary processing and marketing of NR, rubber products manufacturing industry and foreign trade, intercrops and by-products, and inter-divisional collaborative projects are the five major areas of research of the Division. Seven projects were completed and reported during the period. A summary of the results are given in the following sections.

1. Comparative advantage and decomposition of export growth: The case of India's selected rubber products

The study on the comparative advantage and export growth of the four selected rubber products from India during the 25 year period (1988-2012) revealed varied trends with important policy implications. An improvement in the revealed comparative advantage (RCA) in all the selected export destinations during Phase II was observed only in the case of New pneumatic tyres - others (especially off the road tyres) (HS 401199) whereas RCA of tyres used in buses or lorries (HS 401120), Other articles of vulcanized rubber other than hard rubber- Other- Other (HS 401699) and tyres used on motor cars (including station wagons and racing cars) (HS 401110) exhibited market-specific trends. The observed export performance of all the selected products was mainly due to world trade effect supplemented by competitiveness effect. However, negative market distribution effect and varied trends in commodity composition effect pose important policy challenges. Hence, it is necessary to segment and streamline product and market-specific strategies to reap the potential advantages arising from the inherited strengths of India's rubber products manufacturing industry.

2. Intercropping in the immature phase of natural rubber cultivation: Emerging trends and policy challenges

The study found that the basis and objectives of intercropping have undergone a paradigm shift from subsistence farming employing family labour to a highly organised commercial activity employing hired labour and culminating in contract farming for short term monetary gains as observed in Central Kerala. As a corollary, the choice of intercrops grown and the management practices pursued were tuned to maximize the returns. The three important consequences of the observed changes have been indiscriminate mechanization in land preparation, excessive use of fertilizers and

adoption of very high density of intercrops. In Central Kerala, where the contract farming is more popular, even the rubber planting materials are provided by the contractors with the attendant issues related to the quality of planting materials. The potential consequences of these indiscriminate practices on soil fertility and rubber productivity deserve immediate attention.

These observations emerging from the study highlight the need for appropriate institutional interventions to sustain soil fertility and rubber productivity through efficient dissemination of scientific rubber production technologies. To this end, promotion of producer' consortiums based on the Rubber Producers Societies (RPS) shall be evolved as centres of scientific technology dissemination by encouraging them to take up contract planting of intercrops based on the recommended package of practices in order to ensure sustainability of NR cultivation in the region.

3. Cess on imported rubber

It was found that though the rate of the rubber cess collected under the Rubber Act has been revised eight times since its inception in 1947 (Table Eco. 1), real value of the cess has declined significantly during the past 33 year period. In real terms, it has declined significantly from Rs. 0.40/kg in 1980-81 to Rs.0.24 kg in 2012-13 at 1980-81 base rate.

Table Eco. 1. **Trends in the rate of rubber cess**

Period	Rate of cess (₹/kg)
1/10/1947 to 31/7/1955	0.01
1/8/1955 to 31/3/1958	0.14
1/4/1958 to 31/3/1961	0.14
1/4/1961 to 29/9/1975	0.30
30/7/1975 to 23/8/1984	0.40
24/8/1984 to 31/8/1994	0.50
1/9/1994 to 31/8/1998	1.00
1/9/1998 to 31/8/2011	1.50
1/9/2011 till date	2.00

The study justifies the imposition of excise duty collected as rubber cess for natural rubber produced in India and on rubber imported as rubber cess CVD. Otherwise it becomes disadvantageous for the domestically produced rubber, as domestically produced rubber becomes costlier to that extent than imported rubber. The study pointed out inconsistency in imposition and collection of the cess CVD on NR imported through different ports causing revenue loss to the Government.

4. Female labour participation in tapping

One of the major constraints faced by the rubber smallholder sector in Kerala is the growing shortage of rubber tappers. Increasing female labour participation is considered as one of the options to address this issue. The analysis has revealed that the share of female labour in the total workforce of rubber tappers in the state was 7.5 per cent (Table Eco. 2) with notable regional variations. The study revealed that the continuance of the lower female labour participation in the smallholder sector is more than a supply side issue with important socio-economic and region-specific aspects. Analysis of age and caste compositions of rubber tappers, tapping tasks and wage rates did not reveal any significant entry barrier for female rubber tappers across the regions. However, the unique socio-economic milieu encompassing tapping job in the smallholder sector, especially the smaller size of holdings, appear to be the critical barrier in

Table Eco. 2. **Region-wise composition of rubber tappers**

Region	Male (%)	Female (%)
South Kerala	84.0	16.0
Central Kerala	94.6	5.4
North-central Kerala	91.0	9.0
North Kerala	96.7	3.3
State average	92.5	7.5

ensuring adequate supply of female labour. Hence, any long-term policy approach has to consider the multi-dimensional aspects of the issue for promoting group approaches rather than implementing *ad hoc* measures for addressing the shortage of rubber tappers.

5. Trends in seasonality of natural rubber production in India: A disaggregate level analysis

The study examined the seasonality in NR production in India during the 21 year period from 1991 to 2012. Change Point Analysis (CPA) was used to identify trend breaks in the seasonality of NR. The analysis showed no significant change in the seasonality of NR production in the country at the aggregate level *ie*., during 1991-2012. At the disaggregate level (intra-month), significant trend breaks in production were observed in the months of February, March, April, May, September and November. Among the six months, production witnessed an increase after the trend break in the months of February and March, whereas it declined in the remaining four months. Though the divergent trends observed in the monthly shares are not reflected in the seasonal shares, the trend breaks observed in the cases of six months are indicative of impending changes in the seasonality of NR production in India. Hence, it is imperative to initiate a multidisciplinary investigation to explore the underlying factors in order to initiate detailed studies at the disaggregate level. Such R&D initiatives would be helpful in recasting the agro-management policy packages.

6. Decadal trends in the commercial yield of selected clones in the traditional regions of India

This study is a preliminary attempt to understand the decadal trends in the yield of selected *Hevea* clones in the traditional regions. The analysis was based on time series data on commercial yield of three popular clones *viz*., GT 1, RRIM 600 and RRII 105, from the organized large estates. The trends in average yield (kg/ha^{-1}) of the three clones for the first 10 years of tapping during different decades was assessed and inter- decadal growth rates in yield were estimated. Trends in other yield related variables *viz*., the planting density and tapping systems followed were also considered to understand their potential influence on yield performance. Comparison of the yield performance of clones planted during different decades revealed a steady decline in the ten year average yield except an aberration in the case of RRIM 600 during the 1970s (Fig. Eco.1). The decline in

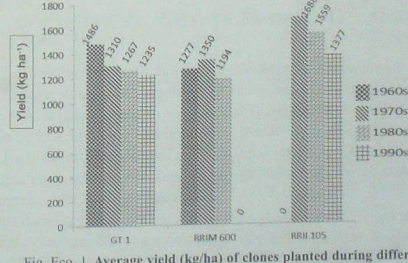


Fig. Eco. 1. **Average yield (kg/ha) of clones planted during different decades**

decadal average yield varied from 311 kg/ha^{-1} (-18.42%) in RRII 105 (in three decades), 251 kg^{-ha} (-16.89%) in GT 1 (in four decades) to 83 kg/ha^{-1} (-6.50%) in RRIM 600 (in three decades). The analysis of time series data on planting density found that the average decadal growth rates in the planting densities were not significant. Conversely, there had been remarkable shift to low frequency tapping systems (LFTS) in the case of all clones. The decadal trends in yield clearly indicated the prevalence of yield fatigue among the selected clones in the traditional rubber growing regions in India. Although the decadal trends in yield fatigue has been reversed to certain extent by the higher yield of HYV clones like RRII 105, the trends in yield of the same underline the inherent limitations. The observed changes in density of planting and shifts in tapping system are not expected to be yield depressing. Hence, the contributory factors for the observed yield fatigue over decades are construed to be the cumulative effect of depleting soil organic content and fertility as well as climate change.

7. Rubber planting subsidy in India: objectives, achievements and challenges in the era of market integration

The study discusses a revival strategy for NR Planting Subsidy Scheme based on the trends in development cost and planting subsidy and the strategies followed elsewhere. Planting subsidy which accounted for a significant share in Rubber Board's total expenditure since 1957 showed a declining trend during the past two decades. The subsidy schemes had been effective in popularizing high yielding variety (HYV) planting materials and extending the area under natural rubber (NR) in non-traditional regions. The trends in the estimated development cost and rates of planting subsidy in the traditional rubber growing regions found that there had been a decline in planting subsidy as a proportion of development cost from 25.5 per cent in 1981 to 8 per cent during 2014. This is in contrast to the trends in other major NR producing countries like Malaysia and Thailand where planting subsidy compensates a substantial portion of the development cost. Though rates of planting subsidy is subjected to periodical revisions the real value declined from 988 per/ha (1957) to 491 per/ha (2014) showing a negative growth rate of 2.3 per cent. The analysis observed that considering the strategic commercial importance of the crop there is a need to reconstruct the conceptual basis of planting subsidy by linking the life cycle average yield of the most popular clone and the rate of cess imposed on rubber produced in India.

CENTRAL EXPERIMENT STATION, CHETHACKAL

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

The station meets the field trial needs of the scientists of various disciplines of Crop Improvement, Crop Management, Crop Protection, Crop Physiology and Latex Harvest Technology. The station works under A and B Divisions of almost equal area. Apart from clone trials and bud wood nursery of pipeline clones, trials on low frequency tapping, CUT, Germplasm accessions, disease management and fertilizer dosages make up bulk of the experimental areas. Specialized trials like gas based tapping (G-Flex), intercropping and immaturity reduction etc. also make part of the experimental area. A three

part tree crown budded area with canopy from Fx 516 is laid to study disease resistance mechanisms. The Eddy covariance tower gives micro environmental data.

During the reporting period, the total crop realized was 222753 kg (PFL) and 73779 kg (scrap) and 13764 kg (coagulum). A total of 296 tapping days was possible in the year and 50 tappers (per day) were engaged for tapping. The CES Dispensary attends to the medical needs of the workers and the total number of visits of patients during the period under report was 4816.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

1. Crop improvement

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

In four different areas (Umsiang, Byrnihat, Bhakuagoog and Sonapur), *Hevea* clones *viz.* RRII 417, RRII 422, RRII 429 and RRII 430 along with two check clones RRIM 600 and SCATC 88/13 were planted in farmer's field. Trial in Umsiang showed better growth than the other three locations.

1.2. Evaluation of potential primary ortets in clonal nursery

Average girth at 5th year of planting and girth increment of RRSG 9 and RRSG 4 in nursery trial was significantly higher than that of the check clone RRIM 600 (Table Ghy. 1). Mean juvenile yield of RRSG 9 with or without 2.5% Ethephon was significantly higher than the check clone. Mean juvenile yield of RRSG 8 and RRSG 1 was appreciable and thus, RRSG 9 along with RRSG 8 and RRSG 1 were shortlisted for further evaluation.

1.3. Performance of the clone RRII 429 in grower's field in Assam

Girth and mean yield of RRII 429 was better than RRIM 600 in grower's field at Goalpara, Assam. The difference in yield was higher during the monsoon period (June-September).

Table Ghy. 1. **Growth of ortets in juvenile stage as compared to that of their respective mother trees**

Name of ortets / clones	Growth of ortet in SST		Juvenile yield efficiency (g cm^{-1}tap^{-1} tree^{-1})	
	Average girth at fifth year (cm)	Average girth increment from 3rd year to 5th year (cm month^{-1})	Without Ethephon	With 2.5% Ethephon
RRSG 1	24.7	0.6	0.6	0.6
RRSG 3	21.8	0.5	0.3	0.4
RRSG 4	29.2*	0.7*	0.3	0.5
RRSG 5	24.5	0.6	0.2	0.4
RRSG 6	28.2	0.6	0.3	0.4
RRSG 8	29.1	0.6	0.5	0.7
RRSG 9	33.5**	0.7**	0.7**	1.0**
RRIM 600	25.1	0.6	0.4	0.6
CD (Pe"0.05)	4.05	0.07	0.17	0.24

* = Significant at 0.05 % level ** = Significant at 0.01 % level

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

In order to evaluate the performance of three selected primary ortets along with two check clones, an OFT was initiated in Gopalkrishna Tea Estate, Morigaon, Assam. Field planting of polybag plants raised would be carried out next year.

1.5. Evaluation of germplasm accessions under the agroclimate of Assam

Tapping in mature plants was conducted at 30 cm height after 29 years of planting. In the existing plants, mean dry rubber yield in Acre accessions was 73.1 g $t^{-1}10\ tap^{-1}$ ranging from 1.6 to 959.2 g $t^{-1}10\ tap^{-1}$. In Mato Grosso, average dry rubber yield was 70.6 g $t^{-1}10\ tap^{-1}$ ranging from 0.75 to 409.7 g $t^{-1}10\ tap^{-1}$. The dry rubber yield was 60.9 g $t^{-1}10\ tap^{-1}$ in Rondonia accessions where yield ranged from 1.0 to 678.2 g $t^{-1}10\ tap^{-1}$.

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

The Namsai region of Arunachal Pradesh was selected for the study. The area has a per-humid to humid climate and receives copious rain. The soil is classified red sandy to laterite. Most of the land in Arunachal Pradesh is uncultivated and a large part (nearly 94%) is classified under forests. The maximum temperature recorded in the foothills is 40°C; however, average temperature during winter varies from 15° to 21°C. In order to encourage multiclonal plantation, screening of more clones for this region is necessary. In order to study the suitability of 20 potential primary clones / ortets selected from NE, necessary actions were taken.

2. Crop Management

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

The experiment was conducted at Sarutari experimental farm, RRS, Guwahati to develop locally adoptable and viable root trainer technique for rubber propagation in North-East India. For the study, there were three treatments *viz*, root trainers made of clay, bamboo and polypropylene material (600 cc). The clone used for the study was RRIM 600.

Bamboo root trainers recorded better sprouting percentage (91%) followed by earthen root trainers (89%) and plastic root containers (83%). The girth recorded was found to be non-significant among the three treatments (Table Ghy. 2).

Table Ghy. 2. **Sprouting percentage and growth**

Treatments	Sprouting percentage (%)	Girth (mm)
Control (Plastic root trainers)	83	5.5
Bamboo root trainers	91	4.5
Clay root trainers	89	6.0
SE		0.48
CD (P = 0.05)		NS

3. Crop protection

3.1. Dynamics of microbial status of rubber, tea, forest and barren soil in Assam and North Bengal

The microbial status of four different ecosystems was assessed consecutively for 2 years to study the population dynamics and impact of pesticides on the population on seasonal basis. The forest soil harboured the highest microbial population and the least population was observed in barren soil. In tea growing soils the total microbial population in the top soil (2- 5 cm depth) was found to be less. The populations reached its peak during the post-rainy season and decreased gradually towards winter and a gradual increase in the population was observed from summer season due to increase in temperature and moisture. The bacterial population was the highest in the forest habitat. The fungal species composition remained similar among all the habitats. An estimation of nutrient content of soil revealed that the forest soil

Table Ghy. 3. **Antagonistic activity of bacterial and fungal isolates against *Phellinus noxius***

Bacterial isolates	Antagonistic effect	Fungal isolates	Antagonistic effect
Pseudomonas sp.	+	*Aspergillus* sp.1	+
P.fluorescens	++	*Aspergillus* sp.2	+
Bacillus sp.1	++	*A.niger*	+
Bacillus sp.2	+	*Penicillium* sp.1	+
B. subtilis	++	*Penicillium* sp.2	-
Azotobacter sp.	+	*Trichoderma* viride	++
		Fusarium sp.	-
		Cladosporium sp.	-

+ = good; ++ = very good

contained highest N, P, and K status followed by rubber, tea and barren soil.

3.2. Exploitation of naturally occurring antagonists against diseases of rubber

Six bacterial and five fungal species were isolated from the rhizosphere of rubber and the antagonistic activity was tested by dual culture technique against the brown root pathogen, *Phellinus noxius*. The efficient isolates were selected for further evaluation in polybag plants (Table Ghy. 3).

3.3. Evaluation of PGPR and its influence on early growth of *Hevea brasiliensis*

Plant growth promoting rhizobacteria *viz. Bacillus subtilis, Pseudomonas* sp., *P. fluorescens* and *Azotobacter* sp. were isolated from the rhizosphere of rubber and mass multiplied in the NA broth. The pure culture of these PGPR was applied to the 15 days old seedling nursery at the root base singly as well as in dual mixture. The growth parameters like girth, height and leaf number were recorded in quarterly interval. The preliminary data revealed that rubber seedlings attained better growth in the treatment of *B. subtilis* + *P. fluorescens*. The growth of seedlings in single dose treatment of *B. subtilis* and *P. fluorescens* was at par.

4. Crop Physiology

4.1. Effect of application of stimulant away from tapping cut

The yield data over four years showed that bark application of 5.0% ET at 1 cm above bud union and 2.5% ET panel application was significantly higher than that of the unstimulated (control) trees. Incidence of TPD was high in 5.0% ET at 1 cm above bud union region, 5.0% ET at 125 cm above bud union and 2.5% ET panel application compared to the control (Table Ghy. 4).

4.2. Shallow tapping for stress alleviation in *Hevea* plantation during winter season in NE region

Different tapping systems like shallow tapping during winter tapping rest, S/2 d3 tapping during rest period etc. were adopted. Yield and TPD in RRIM 600 clone at Sarutari Research Farm in Assam was recorded. There was no significant difference between yields in different tapping systems. The incidence of TPD was more in shallow and S/2 d2 continuous tapping in comparison with the normal and S/2 d3 tapping (continuous) where TPD incidence was low.

Table Ghy. 4. **Effect of stimulation on yield of rubber**

Treatments	Yield (Kg ha^{-1} – 420 plants)	Percentage of plants showing 80-100 % TPD
5.0% ET above 125 cm height	2033	7
5.0% ET at 1 cm above the bud union	2206*	10
5.0% ET at 125 cm above bud union	1948	13
2.5% Panel application	2133*	12
Base material (Oil)	1865	5
Control (unstimulated)	1948	5
CD (P>0.05)	224	

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The Station is actively involved in various research programme under Crop improvement, Crop management, Latex harvesting, Ecosystem study and Socio-economic aspects of rubber cultivation. Advisory services for crop protection, discriminatory fertilization to growers, latex analysis for Industry are also taken up.

1. Crop improvement

The crop improvement programmes in the station include the development of location specific clones, evaluation of promising clones and the standardization of DUS norms.

1.1. Development and evaluation of clones

To develop location specific clones for this region, populations developed through different strategies *viz.*, hybridisation, ortet selection and polycross breeding were screened in eight seedling nursery evaluations involving 3026 genotypes. Based on growth performance, test tap yield and yield per girth in seedling nursery evaluation, 191 genotypes including 136 hybrids, five OP progenies and 50 half-sib progenies were selected for further evaluation. Based on girth and test tap yield in clonal nursery evaluations (2008 and 2009), hybrid clones 99/5/9, 99/3/61, 98/38, 99/1/24 and 98/46 were selected and multiplied for further evaluation. Locally recruited selected clones *viz.*, 29 hybrids, 21 OP progenies and 13 half-sib progenies are under evaluation in six clonal nurseries. In the clonal nursery evaluation of ortets and hybrids from traditional region (2009), RRII 403 (22.9 g $t^{-1}t^{-1}$) and RRII 407 (25.3 g $t^{-1}t^{-1}$) had significantly higher peak yield, while, ortet P 132 (16.8 g $t^{-1}t^{-1}$) was found on par with RRIM 600 (13.2 g $t^{-1}t^{-1}$) and was selected for further evaluation.

In poly cross progeny evaluation, eleven selected ortets were evaluated in SST with check clone RRIM 600 and ortet 114 (52.2 g $t^{-1}t^{-1}$) gave significantly higher yield than RRIM 600 (41.6 g $t^{-1}t^{-1}$). Among the 13 ortets from four regional stations in the North Eastern region RRSG 248 recorded highest mean test tap yield (23.7 g $t^{-1}t^{-1}$) followed by RRST 37 (19.6 g $t^{-1}t^{-1}$) while check clone RRIM 600 recorded 22.6 g $t^{-1}t^{-1}$.

Evaluation of clones for their adaptability and yield performance was undertaken in four On Farm Trials, two Large Scale Trials and four clonal nursery trials. PB 311 (58.6 g $t^{-1}t^{-1}$) and RRII 105 (51.9 g $t^{-1}t^{-1}$) had significantly superior yield compared to RRIM 600 (41.1 g $t^{-1}t^{-1}$) in the LST (1995) of 10 clones. Clone RRIM 600 (59.1 g $t^{-1}t^{-1}$) recorded the highest mean yield in LST 1996 (G x E trial), while RRII 422 (52.57 g $t^{-1}t^{-1}$) and RRII 429 (52.0 g $t^{-1}t^{-1}$) had significantly higher mean yield than RRIM 600 (45.5 g $t^{-1}t^{-1}$) over 12 years of tapping (Table Agar.1). Among the onfarm trials, at Killamura (1997) and Bagefa (2000), RRIM 600 recorded superior yield (1109 kg ha^{-1} and 41.8 g $t^{-1}t^{-1}$ respectively). RRII 429 recorded the highest mean yield (27.1 g $t^{-1}t^{-1}$) when compared to RRIM 600 (24.4 g $t^{-1}t^{-1}$) in the first year of tapping in OFT, Pathalia (2005). Clone RRII 429 had the highest girth (39. cm) as well as the highest plant stand (98.7%) in OFT, Hirapur (2009). In the clonal nursery evaluation of popular clones (2009), PB 255 (158.7 g t^{-1} t^{-10}) had the highest test tap yield. Among the pipeline clones from traditional area evaluated in three clonal nurseries (2009, 2010 and 2011), P 21 and P 107 recorded superior test tap yield in the peak season in 2014.

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

2. Crop management

2.1. Soil nutrient mapping

Evaluation of soil fertility status and nutrient mapping of soils under rubber plantation in Tripura was initiated during 2014-15. Composite soil samples are being collected from 56 administrated blocks of Tripura based on GPS points. During the reporting year, 181 soil samples, distributed in 12-blocks were collected and 112

Table Agar.1. **Growth and yield of thirteen clones of *Hevea* in LST (1996)**

Clone	Girth (cm)	Mean yield (g $t^{-1}t^{-1}$)	
	February 2015	2014-15	Over 12 years
RRIM 600	73.8	59.1	45.5
RRII 429	79.1*	56.4	52.0*
RRII 203	76.6	41.1	35.3
PB 217	75.1	39.8	34.0
RRII 51	70.5	33.3	30.3
RRII 414	68.1	39.7	36.2
RRII 430	65.7	48.0	44.5
RRIC 100	76.9	45.0	41.3
RRII 422	70.6	57.5	52.6*
RRII 105	70.8	53.0	41.4
RRII 417	73.7	57.0	47.5
RRII 176	**83.8***	55.0	36.9
PB 235	73.9	41.7	39.4
CD (P=0.05)	4.4	12.18	4.68

** Significantly higher than RRIM 600*

soil samples were analyzed. Rubber soils of Tripura were predominantly acidic in nature with soil pH ranging from 3.9 to 5.5 and were poor in NPK contents. Soils of most of the mature rubber plantations in Tripura were sandy and sandy loam in texure. A decline in clay content (13.2 to 18.7 %) in the upper surface of soil was observed.

2.2. Development of cropping system and management practices

In the experiment on evaluation of a cropping system model, after 7 years of intercropping, benefit cost ratio (BCR) for colocasia (3.3) was found to be the highest followed by banana (2.7) and cowpea (2.0) indicating the economic feasibility of growing these crops as intercrops during the immature period of rubber cultivation. At the end of seven years of intercropping, girth of rubber in the intercropped and in non-intercropped system was not statistically different.

For the fodder intercropping trial, it has been found that herbage yield was the highest for guinea grass, i.e. 43.7 t ha^{-1} $year^{-1}$, followed by stylo with signal grass (40.5 t ha^{-1} $year^{-1}$), Napier with Para grass (31.5 t ha^{-1} $year^{-1}$), fodder maize (22.7 t ha^{-1} $year^{-1}$) and fodder cowpea (15.8 t ha^{-1} $year^{-1}$).

In the experiment on identification and standardization of potting media, the performance of the treatment with top soil, cow-dung and rice husk, (7:2:1) was found to be better and used for mass propagation.

For the zero tillage experiment, the girth data after 3 years indicated that pits of larger dimensions had no advantage on growth of plants. Moreover, the comparative cost analysis of larger pits (size 75x75x75 cm) and smaller pits (accommodating polybag plants) showed that around 66% saving can be made by planting in small pits and further saving (80%) can be made by planting in still smaller pits for root trainer plants.

In the experiment on development of specific package of practices, the mean girth of rubber in vertical (20.7 cm) and conventional mulching (19.9 cm) was significantly higher than control (16.5 cm). The moisture content in all the three layers of soil, viz. 0-15, 15-30 and 30-60 cm was higher in vertically mulched pits (14.8 %) than in conventional mulching (13.7%) and control (10.9%).

3. Latex Harvest Technology

The results from experiment on comparison of different tapping systems in clone PB 235 continued to give highest yield under S/2 d3 system of tapping compared to S/2 d4 and S/2 d6 system of tapping. Yield stimulation for d4 and d6 need to be enhanced. In another experiment, yield under S/2 d3 system tapping is at par with S/2 d2 system and higher than S/2 d4 system in clone RRIM 600 and RRII 429.

Relationship of latex ATP with rubber yield was being continued for second year in polyclonal population. Latex ATP in polyclonal trees showed positive correlation with dry rubber yield.

4. Ecosystem Study

Present distribution of Natural Rubber (NR) trees in Western Ghats (WG) and Northeast region (NE) and predicting the changes in future expansion of the species influenced by climate change is one of the research priorities. Predicting the distribution of novel region based on the existing plantations is referred as transferability of Ecological Niche Modelling (ENM). Optimization of ENM transferability between the two introduced regions was achieved by including pooled occurrence data of the species from both the regions for model calibration. Several non-climate variables such as rooting condition, altitude, slope, village

population, labour availability and Human Influence Index (HII) etc. were successfully incorporated into the model based on the theoretical knowledge of interactions between the species and the environment. Climate variables such as precipitation in the driest month (bio14) in NE and winter months (bio19) in WG contributed maximum to the model. In a future climate, bio14 will be double in 2050 compared to present climate scenario in NE, whereas bio19 will increase by 35% in WG in the same timeframe. This suggests better climatic suitability for *Hevea* species in NE region of India by 2050. Altitude, village population, rooting (soil) conditions and labour availability were the major non-climate parameters that showed higher contributions to the model in NE than in WG. The experimental results are indicative of existence of unfilled niches of *Hevea* species in India. Therefore, the species is found to grow in regions with wide variation of environmental conditions.

5. Economics

5.1. Commercial yield evaluation of NR in Tripura

The data base is composed of identified growers from West Tripura and South Tripura, as more than three-fourth of the area under NR is in two districts. Out of the 23 RPS selected, 14 were from west Tripura and 9 from South Tripura for selection of 187 growers. The data was collected for the most prevalent clone of *Hevea* (RRIM 600) from 2003-04 onwards. Average annual yield over 22 years was 1343 kg/ha.

5.2. Trends in area under NR and food crops in Tripura: an assessment

A sample survey covering 544 NR growers was completed. The preliminary result shows that the NR has transformed the livelihood of the growers from a labour centric to a self-sufficient commercial cultivation of Natural Rubber. It was found that 73 per cent of the surveyed households are currently farmers (Table Agar. 2). Before initiating NR cultivation the 45 per cent of the surveyed household were engaged as labourers. The economically better position of NR and failure of other crops in the tilla land were found to be the main reasons for encouraging NR cultivation.

Table Agar. 2. **Trends in primary occupation of respondent households**

Category	Present	Prior to NR plantation during immature period
Farmer	398 (73.2)	90 (46.5)
Labourer	37 (6.8)	242 (44.5)
Shopkeeper/ Businessman	34 (6.3)	66 (12.1)
Job	73(13.4)	70 (12.9)
No clear arrangement	2 (0.4)	76 (14.0)
Total	544 (100)	544 (100)

Note: Figures in parentheses show percentage to total

6. Advisory work

Discriminatory fertilizer recommendation based on soil was offered to 336 rubber growers in the state of Tripura. A total of 312 latex samples were analyzed for DRC and other latex parameters. Total 1005 m of bud-wood of high yielding clones were supplied to the growers.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

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

1. Crop improvement

In the 2008 polycross progeny evaluation trial, the selected ortets were bud grafted and healthy poly bag plants were planted in the field of Rubber Board Campus, Dakopgre, Tura.

A Clonal nursery trial was initiated with the selected primary ortets collected from Tura, Agartala and Guwahati along with RRIM 600 as the check clone. Girth data of all the ortets were either at par or lower than the check clone RRIM 600. The juvenile yield of RRST 37 was the highest and RRST 37 and RRSG 9 showed significantly higher yield than RRIM 600. The juvenile yield ranged from 87.1 g $t^{-1}10$ $taps^{-1}$ to 5.8 g $t^{-1}10$ $taps^{-1}$ (Table Tura 1).

Table Tura 1. **Girth and juvenile yield of primary ortets**

Mother Ortets	Girth (cm)	Juvenile yield (g $t^{-1}10$ t^{-1})
RRSG 1	19.4	27.7
RRSG 2	23.5	24.1
RRSG 3	19.8	15.5
RRSG 9	23.3	39.8*
RRST 24	23.0	18.7
RRST 37	23.8	45.3**
RRSA 121	20.7	38.1
RRSA 315	18.0	6.7
RRSA 461	22.5	29.7
RRIM 600	23.0	22.8
CD (P > 0.05)	NS	12.7

Among the three on farm trials started during 2009 and 2010, RRII 429 recorded a maximum girth of 47.3 cm while RRII 422 (28.1 cm) minimum girth. In West Garo Hills, in terms of mean girth and number of branches RRII 417 and RRII 422 performed better.

In the evaluation of polycross progenies trial test tap yield was recorded in polyclonal seedlings collected from RES Nagrakatta, RRS Agartala, RRS Guwahati and RRS Tura.

In the Fundamental studies on the nature of wintering, flowering and seed germination in *Hevea* clones, clonal variation was observed in wintering, refoliation and flowering pattern in Garo Hills of Meghalaya. Seed set was poor due to heavy hail storm this year. Defoliation and refoliation was early in clones, *viz.*, RRII 105, RRII 118 and PB 5/5. While all the clones refoliated during the last week of February, RRII 105 refoliated during the third week of February. In the Nursery Evaluation of polycross seedlings trial-2013 and 2014, poly clonal seeds collected from Poly Clonal Seed Garden, Mizoram were planted at two locations RRS, Ganolgre farm and at R.B. campus, Dakopgre, Tura. In the experiment on evaluation of potential ortets under the Agro-climatic conditions of Garo Hills of Meghalaya, average plant height and girth 1.25 m and 2.9 cm respectively. For raising of Germplasm Arboretum at Teksragre Farm, 2600 budded stumps of 219 accessions and 25 control clones were planted in the polybag nursery.

2. Crop Physiology and Latex Harvesting Technology

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

Low temperature adversely affected the yield and DRC. Early defoliation and refoliation was observed and during winter DRC ranged from 28.2 to 28.9 %. The soil moisture content was lowest during the months of February and March.

2.2. Study on controlled upward tapping system

Treatment-wise monthly yield was recorded in controlled upward tapping (CUT) trial. Data showed that maximum average annual yield of 78.6 $gt^{-1}t^{-1}$ was recorded in T1 (S/4U d2 5% ET 21 days interval - S/2 d2 2.5% ET) followed by T2 (S/4U d2 5% ET monthly interval - S/2 d2 2.5% ET) and T3 (S/3U d2 5% ET monthly interval - S/2 d2 2.5% ET) and minimum in T4 (S/3U d2 5% ET 45 days interval - S/2 d2 2.5% ET). Under these, yield was 74.4, 72.8 and 65.5 $gt^{-1}t^{-1}$ respectively. TPD (%) was highest in T1 (7.6%) and lowest in T4 (5.98%). Under CUT 33 to 60% increased yield was observed over normal tapping.

2.3. Shallow tapping for stress alleviation in *Hevea* plantations during winter in NE

There was no significant difference between treatments. Maximum yield was recorded in normal tapping system followed by Normal continuous tapping and LFT + normal tapping and lowest was in shallow + normal tapping system. Normal continuous tapping system showed higher TPD followed by the shallow + normal tapping system and LFT + normal tapping and minimum was in normal tapping system.

2.4. Location specific stimulant application

Study on ethylene induced stress response in the tapping panel of the *Hevea* trees was initiated with the aim to reduce the stress effects in tissues in the tapping panel. In clone RRIM 600, six treatments were adopted with bark applications of ethephon. Result showed maximum yield in treatment of bark application of 5% ethephon at 150 cm above from bud union and near bud union and minimum yield in T6 (Unstimulated trees). There was no significant difference in DRC between treatments.

3. Crop Management

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

The result over 11 years showed the highest girth and yield in the treatment combination of $N_{60}P_{30}K_{45}$ kg ha^{-1} compared to other treatments. Positive linear response to N, P and K fertilizer application on dry rubber yield was observed. Continuous application of N, P and K fertilizer was found to improve the fertility status of the soil.

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 recording soil moisture. Soil moisture content increased with increasing depth. Maximum moisture content was in September and minimum was in January - February. Annual mean soil moisture content in the three depths 0-15, 15-30 and 30-60 cm were 23.0, 23.8 and 25.1% respectively. The data revealed that 31.4 % of available moisture was desorbed at - 0.033 MPa and 20.6 % at - 1.5 MPa.

3.3. Analytical / advisory work for fertilizer recommendation

Thirty two soil samples were collected from the rubber growing areas and analyzed. The results indicated that the organic carbon content was in the medium range (0.98 to 1.36 %) in the surface soil (0-30 cm), available phosphorus was in low range (3.2 to 6.3 mg kg^{-1}) and available potassium was in medium range (78.0 - 92.0 mg kg^{-1}). The soil is acidic in nature with pH ranging from 4.6- 5.3; fertilizer recommendation given accordingly.

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

Soil samples were air dried and processed. Collection of soil sample is in progress.

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

This experiment was started with 7 treatments in which coir pith, FYM, areca nut shell and top soil were used. An additional

treatment consisting of the dry fish was also tested on the same day planting of the germinated seeds. The seedlings were protected from cold during winter season using poly house. During winter, some of the leaves were affected with powdery mildew disease for which remedial measure was undertaken. The study is in progress.

3.6. Weed flora studies

During the year some weed samples were collected from the immature rubber fields from Garo hills of Meghalaya using GPS system and found that there was not very much variability among the weeds. The study is in progress.

REGIONAL EXPERIMENT STATION, NAGRAKATTA, BENGAL

1. Crop Improvement

1.1. Evaluation of clone

Experiments on multidisciplinary clone evaluation was initiated in 1990, 1991 and 1993 in the non-traditional area of Sub-Himalayan West Bengal with the aim of screening promising clones suitable for the area in terms of growth, yield and other attributes. In trial I and II out of eighteen clones, eleven clones showed significantly higher girth in comparison to the check clone RRII 105. In trial III, SCATC 93/114 showed highest girth followed by PB 235. However, in trials III and IV, clone RRIM 600 was found superior than the check clone.

The mean yield of RRIM 703 was significantly higher than RRII 105 in trial I and II. In trial III and IV PB 235 and Haiken 1 respectively were found significantly superior to RRIM 600 (Table Nag. 1).

Table Nag. 1. **Yield pattern 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 ($g\ t^{-1}t^{-1}$)
Gl 1	23.0	RRIM 612	30.3	SCATC 93/114	26.9
RRIM 605	29.8	RRIC 102	31.9	RRII 300	29.9
Haiken 1	34.4	Haiken 1	33.3	PR 261	30.1
PB 5/51	34.6	SCATC 93/114	34.3	RRII 208	30.6
RRII 203	35.2	PB 260	34.5	RRIC 104	32.4
PB 86	35.5	PB 86	35.9	PB 280	32.6
RRIM 612	36.7	PR 107	37.2	RRII 105	33.5
PB 311	40.5	RRII 208	41.8	RRII 308	33.7
GT 1	41.1	PB 310	45.77	PB 235	35.0
RRII 118	41.5	PB 235	57.5**	Haiken 1	40.8*
SCATC 88/13	41.7	RRIM 600	40.9	RRIM 600	27.7
PB 235	42.2	CD (Pd"0.05)	11.9	CD (Pd"0.05)	11.9
PR 107	42.5				
SCATC 93/114	43.3				
RRII 208	44.0				
RRII 300	45.3				
RRIM 703	46.1*				
RRII 105	31.2				
CD (Pd > 0.05)	14.3				

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

In the germplasm evaluation trial, girth of fourteen accessions were higher than the check clone RRII 105. In terms of yield, only RO 5363 was found significantly higher than the check clone. In general, the performance of Rondonia was better compared to the Acre and Mato Grosso accessions.

In the experiment on performance of polyclonal seedlings, the mean girth and yield of the population after 24 years of planting was 72.9 cm and 40.9 g t^{-1} t^{-1} respectively in which 34.8% plants showed above average yield. Selected Ortets were maintained in the source bush nursery for further evaluation.

In the experiment on multi trait screening of half sib progenies for cold tolerance and yield attributes (2012), RRII 208 plants were significantly taller while clones RRII 429 followed by RRII 208 and RRII 417 were superior in girth. However, SCATC 88/13 was found taller than RRIM 600. In the on farm evaluation of promising clones, no significant difference in girth could be observed among the clones. Another trial with nine promising clones has been initiated.

In the study on tree to tree variability in yield and yield related component (2011) among the three clones, volume of latex in RRII 105 was more variable than RRIM 600 and PB 311. DRC and yield of RRIM 600 and RRII 105 was more stable when compared to PB 311 (Table Nag. 2).

2. Crop Management

2.1. Inter-planting trial

Green tea leaf yield in inter-planted plots were 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 tea area of the inter-planted plots. Rubber yield in pure plot was better than the inter-planted plots due to more plant stand.

3. Crop Physiology

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

Fourteen seedling trees were screened on the basis of their juvenile yield potential (above 0.7 g cm^{-1} t^{-1} (t^{-1}). The climatic influence seemed to

Table Nag. 2. **Intra-clonal variability studies**

Parameter	Clone	Population mean	Range (R)			CV% (Between trees)
			Maximum	Minimum	Variation	
Volume	RRIM 600	72.8	170.6	10.0	160.6	45.8
	PB 311	105.4	256.7	24.0	323.7	35.3
	RRII 105	109.9	270.3	10.0	260.3	46.7
DRC (%)	RRIM 600	33.3	42.4	27.5	14.9	9.3
	PB 311	34.7	41.6	9.9	31.7	16.2
	RRII 105	34.5	43.2	25.6	17.6	9.1
Yield (g t^{-1} t^{-1})	RRIM 600	31.3	77.4	3.3	74.0	37.6
	PB 311	40.0	94.8	7.7	87.1	45.0
	RRII 105	52.0	108.4	13.4	94.5	38.0

be one of the important factors that ensure better growth of seedlings in non-traditional areas.

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

The clone RRII 208 was better adapted to the high alkaline pH calcium rich soil during immature phase followed by RRIM 605 and RRII 429. Growth of RRII 429 was better followed by RRIM 605 and RRII 417 in normal soil.

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

The data on girth increment did not show any significant difference among ortets collected from different regions.

3.4. Effect of stimulant application away from tapping panel

Experiment was initiated with six different treatments along with unstimulated trees as control under S/2 d3 system of tapping in blocks of 40 plants for each treatment in RRIM 600.

The yield data over four years did not show any definite advantage on applying ethephon at other locations in terms of yeild. TPD was high in two combinations (5.0% ET at 1 cm above bud union region and 5.0% ET above 125 cm from bud union) compared to control and application of 2.5% ethephon on the panel (Table Nag. 3).

3.5. Shallow tapping - an option to stress alleviation in *Hevea* plantations during winter season in NE regions

Adopting shallow tapping during winter rest period along with other treatments showed that there was no significant difference between the treatments. The percentage of plants showing above 75% TPD was more in continuous (S/2 d2 as well as S/2 d3) tapping compared to normal tapping with rest.

Table Nag. 3. **Effect of stimulation on yield of rubber**

Treatments	Yield (Kg ha^{-1} – 420 plants)	Percentage of plants showing 80-100 % TPD
5.0% ET at 125 cm height	2405*	5
5.0% ET at 1 cm above bud union region	2430*	13
5.0% ET at 125cm from bud union	2594*	12
2.5% Panel application	2343	8
Base material (Oil)	1993	5
Control (No ET)	2343	8
CD (P > 0.05)	397.00	NS

REGIONAL RESEARCH STATION, DAPCHARI, MAHARASHTRA

The major objectives of this station are to develop suitable clones and location specific agro technology for prevailing drought condition. The experiments on crop improvement (screening of wild *Hevea* accessions, evaluation of clones, polyclones, pipeline clones, selected ortets and wild *Hevea* accessions for growth and yield performance under North Konkan condition, study on unique characteristics of each clones to file a DUS norms) , environmental physiology (irrigation requirement and irrigation methods, drought studies, physiological evaluation of selected ortets from various agroclimates of India) and crop management (practices to mitigate the drought).

1. Environmental physiology

1.1. Drip and basin method of irrigation

The experiment started during 1987 with ET_c based irrigation scheduling with basin and drip methods. The objective is to standardize and evaluate the advantages of drip irrigation over basin method of irrigation in terms of water saving with out compromising yield. Basin irrigation at 1.0 ETc treatment registered the highest girth of 79.9 cm followed by 76.2 cm and 73.8 cm in basin 0.50 ETc basin and 0.3 ETc drip irrigation, respectively.The trees under different levels of basin irrigation registered higher girth as compared to drip system and control (rainfed). Reducing the irrigation had not affected either girth and yield of rubber in both the irrigation methods (Table Dap. 1).

Table Dap. 1. **Effect of different irrigation methods and irrigation scheduling on girth and yield of rubber in a dry sub-humid region**

	Treatment	Girth (cm)	Yield ($g\ t^{-1}t^{-1}$)
Control		67.6	32.8
Basin	1.00 ETc	80.0	32.2
	0.25 ETc*	76.0	37.7
	0.50 ETc	76.2	39.8
Drip	0.75 ETc	55.6	30.4
	0.25 ETc**	73.8	29.7
	0.25 ETc	71.5	27.5
±SE		8.1	3.8
CD (0.05)		25.0 (NS)	11.76 (NS)

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

1.2. Cost evaluation trial

Trial started during 1987 to find out the expenses incurred towards various inputs, farm practices and irrigation. The treatment contains irrigated (reduced irrigation of 1/5th ET_c -deep soil and 1.0 ET_c- shallow soil) and unirrigated rainfed trees of RRIM 600. The results indicated better summer (809 kg ha^{-1}) and annual (1885 kg ha^{-1}) yield in trees under 1/5th ETc irrigation under deep soil area than 1.0 ETc irrigation (Table Dap. 2).

Table Dap. 2. **Effect of different depth of soil to different irrigation schedule on yield of rubber** (kg ha^{-1})

	Rainfed	1/5 ETc + deep soil	1.0 ETc + shallow soil
Summer yield	367	809	581
Annual yield	888	1885	1115

1.3. 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 basis of adaptation and common mechanisms involved in cold and drought tolerant traits in ortets selected from different agro-climatic regions.

The objective is also to study the G x E interaction for growth and yield under varying agro-climates. A significant higher girth was noticed in RRSA 98 (14.9 cm) while the lowest recorded in Dap 36 (8.1 cm). Among the check clones, the highest girth was recorded in RRII 414 (15.4 cm) followed by RRII 430 (14.6 cm). Among the ortets RRSA stand first rank in terms of girth (12.4 cm) and height while Dap stands IV rank. Clone RRII 414 registered a higher girth increment (GI) of 5.1 cm whereas the lowest GI noticed in RRII 105 (3.4 cm). Ortet RRST 39 registered the highest GI of 6.9 cm while the lowest GI was seen in GH 3 (2.7 cm). The lowest height was registered in RRST 24 (223 cm) and highest in RRSA 98 (452 cm) followed by RRII 430 (442 cm). Clone RRII 105 recorded the lowest height among the check clones (316 cm). Total chlorophyll content did not show significant difference and ranges from 52.9 to 36.6 mg gm fr. wt.$^{-1}$ in RRII 429 and RRII 417. In general, ortet RRSA is superior in all growth characters studied followed by NGK, GH, DAP and RRST. The lowest yellowing was seen in Dap 34 while the highest was recorded in NGK 1 (9.8%) at pre drought stage. At post drought, Clone RRII 417 is more prone to yellowing (51.4%) followed by RRII 430 (17.2 %) while RRIM 600 recorded only 20.5 per cent yellowing. The lowest yellowing was observed in RRST 24 (7.0 %). In general RRSA ortets are more prone to yellowing in both stages while NGK ortets recorded less yellowing in both stages (Table Dap. 3).

2. Latex harvest technology

The demonstration trial on CUT (Controlled upward tapping) initiated during 2009 is being continued with same trend for yield as in previous years. Higher yield was recorded with S/4 and S/3 cuts with once in three weeks yield stimulation (1227 and 1284 kg ha^{-1}) and was lower under once in a month Ethephon application (948 and 917 kg ha^{-1}).

3. Crop improvement

A total of ten experiments are being conducted with major thrust on development of drought tolerant clones and screening of wild *Hevea* accessions for drought tolerance and future evaluations from modern clones, ortets selection, clones from half sib progeny of prepotent clones along with newly released 400 series and on unique characteristics of each clones to file DUS norms.

3.1. Ortet selection

Trial started during 2008 to evaluate the growth and yield performance of ortets selected from polycross seedling planted at this station with control clones. OS 37 recorded higher girth than clone RRII 105. Among the control clones, RRII 430 recorded highest girth. Except ortet OS 8 all ortets were superior in girth than clone RRII 105.

3.2. Germplasm screening

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

Table Dap. 3. **Growth parameters, chlorophyll index and visual scoring of leaf yellowing in various ortets selected from various locations of India**

Clones	Girth (cm)	GI (cm)	Height (cm)	Drying (%) (Pre drought)	Drying (%) (Post drought)	Chlorophyll index
RRII 105	8.0	3.4	313.6	8.9	23.9	51.1
RRIM 600	10.8	4.3	382.9	9.2	20.5	47.4
RRII 414	15.4	5.1	432.4	7.9	26.7	50.1
RRII 417	11.8	3.4	328.8	10.4	51.4	36.6
RRII 422	13.5	4.6	368.7	6.7	26.8	49.7
RRII 429	13.5	4.5	380.6	9.7	33.1	52.9
RRII 430	14.6	4.7	441.9	6.4	30.2	50.1
DAP 1	8.9	2.9	282.3	8.1	34.4	40.0
DAP 34	9.4	3.3	325.6	0.4	20.0	42.3
DAP 35	11.1	4.1	384.3	2.9	23.6	46.0
DAP 36	8.1	3.8	245.3	3.3	6.5	47.9
RRSA 98	14.9	4.8	451.4	3.7	26.0	48.0
RRSA 315	11.8	4.0	324.4	8.7	40.2	47.8
RRSA 585	10.4	3.3	379.5	8.5	45.2	40.9
NGK 1	9.9	3.5	331.4	9.8	23.9	46.9
NGK 47	9.7	3.9	350.4	9.0	17.4	46.5
NGK 69	10.2	3.7	371.4	1.0	14.3	51.0
GH 1	9.6	3.3	297.9	2.7	27.4	45.2
GH 3	8.8	2.7	295.1	3.7	30.3	46.7
GH 9	8.5	3.1	298.1	2.5	21.0	47.4
RRST 24	6.7	2.7	223.9	2.9	41.5	42.2
RRST 37	10.1	4.2	318.1	3.0	22.6	49.0
RRST 39	11.6	6.8	313.6	4.6	24.5	45.6
SE.d	1.4	0.99	31.9	15.8	13.3	4.2
CD (0.05)	4.0**	2.9	92.4**	45.8	38.5	12.2
Dap	9.4	3.5	309.4	3.7	21.1	44.1
RRSA	12.4	4.0	385.1	9.0	37.1	45.5
NGK	9.9	3.7	351.1	2.5	18.5	48.1
GH	9.0	3.0	297.0	3.5	26.2	46.5
RRST	9.5	4.6	285.2	3.7	29.6	45.6

3.3. Further evaluation of selected wild *Hevea* accessions

FET was laid out in July 2007 using 25 selected drought tolerant clones of wild *Hevea* accession for drought along with 5 HP clones *viz.* RRII 105, RRIM 600, Tjir 1, RRII 430, RRII 208 in Rectangular Lattice Design. The Accessions showed wide variability for all characters studied. A clonal nursery experiment with clones selected from half sib progeny of prepotent clone was initiated in 2010 with an objective to evaluate the clones in a clonal nursey and to advance the potential ones to LST and PCE to reduce the breeding cycle.

3.4. Clonal nursery evaluation

Evaluation of half sib progeny of 15 clones (started in 2011) based on dependable juvenile traits under rain fed condition is progressing. Trial with evaluation of pipeline clones for ought tolerance (started in 2011) using 50 pipeline clones and two check clones in rectangular lattice experiment is also progressing.

In connection with the studies on standardization of Distinctiveness, Uniformity and Stability (DUS) testing norms for evolving specific guidelines for varietal registration in rubber (2011) started with 50 divergent clones, morphological data on individual plants in the juvenile stage covering 40 traits were recorded in all three locations at the age of one year. Data was also recorded for the same set of traits in mature plants of the popular RRII clones (RRII 5, RRII 105, RRII 118, RRII 203, RRII 208 and RRII 400 series). Quantitative data on girth and height were also recorded to measure GxE effects in these clones.

4. Crop Management

Study initiated in 2008 to find out the effect of vertical mulching and Kaoline 6% spray on growth and yield of rubber was continued. There was no significant difference in growth between treatments with soil moisture conservation practices and control. The girth of the plants in the treatment with vertical mulching during summer and post monsoon season were 27.5 and 31.3 cm respectively while in control the girth were 27.8 and 32.2 cm. The average soil moisture contents at depth levels 0.30 and 30-60 cm under soil moisture conservation practice was 17.7 and 17.4 and the corresponding values under control were 18.2 and 19.8 cm respectively.

REGIONAL RESEARCH STATION, DHENKANAL, ODISHA

The Station location represents dry sub humid climate. The station continued its research activities with the particular objective of identifying clones suited to the drought prone conditions and crop management for the prevailing dry sub humid climate of the state.

1. Crop Improvement

There are five clone evaluation trials (*Hevea* clones and polyclonal population) to screen and evolve the most suitable and high yielding clones under dry sub humid climate.

1.1. Clone evaluation

In the trial 1(1987), the elite clone RRII 105 has recorded highest mean yield of 47.0 g $t^{-1}t^{-1}$. GT 1 recorded the lowest yield (32.6 g $t^{-1}t^{-1}$) while it had recorded significantly higher mean girth (81.7 cm) over RRII 105 and RRIM 600. In terms of growth all the three clones performed well.

In another clone trial (1990), RRII 208 (70 g $t^{-1}t^{-1}$) and SCATC 88-13 (79.4 g $t^{-1}t^{-1}$) showed superior performance both in terms of yield and growth. However, SCATC 93-14 (39.0 g $t^{-1}t^{-1}$) and RRIM 700 (47.4 g $t^{-1}t^{-1}$) were found yielding low in this region.

In the 1991 experiment, RRII 208 (86.5 g $t^{-1}t^{-1}$) and PR 255 (72.4 g $t^{-1}t^{-1}$) recorded higher yield among the clones studied. GT 1 (53.2 g $t^{-1}t^{-1}$) and polyclonal (50.4 g $t^{-1}t^{-1}$) seedlings had lesser yield in spite of achieving better growth

and adaptability under the prevailed stress conditions (Table Odi. 1).

Table Odi. 1. **Performance of different clones in the region**

Clone	Yield (g $t^{-1}t^{-1}$)	Girth (cm)
GT 1	53.2	95.3
RRII 105	56.4	82.8
RRII 208	86.3	91.3
RRII 5	61.21	85.0
RRII 300	53.2	89.3
PR 261	56.2	83.0
PR 255	72.4	88.7
RRIC 102	65.8	86.3
RRIM 600	56.4	81.2
Polyclonal	50.4	109.0
C.D. (P = 0.05)	17.5	8.47

In the other modern clones trial (2000), higher mean yield was observed in PB 28/59 (62.8 g $t^{-1}t^{-1}$), IRCA 109 and RRII 357 while lowest mean yield was recorded in RRII 51. Highest girth was observed in RRII 300 (Table Odi. 2).

Table Odi. 2. **Yield and growth performance of clones**

Clones	Yield (g $t^{-1}t^{-1}$)	Girth (cm)
RRII 300	45.8	65.5
RRII 208	39.2	56.0
RRII 357	55.3	55.2
RRII 352	50.5	53.7
RRII 28/59	62.8	57.0
RRIM 600	56.4	58.9
RRII 357	45.8	55.0
IRCA 109	59.6	47.9
RRII 105	43.0	52.1
RRII 51	30.7	55.5
IRCA 111	48.7	60.4
C.D.(P = 0.05)	15.8	-

1.2. Polyclonal ortet evaluation

In a trial with selected polyclones, ortet clones OR 3, OR 4, OR 1 and OR 6 recorded comparatively higher yield among ortets and was on par with high yielding clone RRII 208, under test tap condition. The ortets OR 4 (35.4 cm) followed by OR 8 (34.0 cm) exhibited superior girth (Fig. Odi. 2).

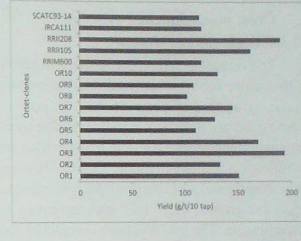


Fig. Odi. 2. **Yield performance of ortet-clones**

2. Latex Harvest Technology

2.1. Controlled upward tapping trial

CUT with ethephone application in clone RRIM 600 showed appreciable rubber yield under the dry sub humid region (Fig. Odi. 3). As observed and reported in previous year, T3 (S/3U d2 ET 5% La (M), S/2 d2 ET 2.5% Pa (2/y) contiuned to give the highest yield.

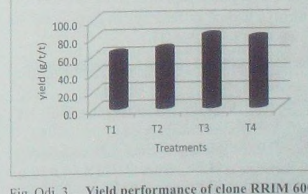


Fig. Odi. 3. **Yield performance of clone RRIM 600 under controlled upward tapping**

REGIONAL RESEARCH STATION, PADIYOOR, KERALA

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

1. Crop management

1.1. Water requirement studies

The impact of irrigation at different levels in immature rubber continued into the mature phase. Growth and yield at the 15th year of planting did not show any significant differences between treatments. Deficit irrigation as an optimization strategy in rubber, practiced during the immature stage of tree growth has no adverse effect on growth and yield once irrigation is discontinued. The trial has been completed and the data is being analyzed.

1.2. Response to applied fertilizers in high yielding clones

The experiment was initiated in June 2002 with budded stumps as planting material. The experimental site was demarcated into blocks for treatment imposition in the mature trees. The treatments comprised of 3 clones (RRII 105, RRII 414 and RRII 429) and four levels of applied fertilizers (recommended dose, twice and thrice the recommended dose and a zero fertilizer control). Clonal differences in girth were not significant (Table Pad. 1).

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

Treatment	Girth (cm)		
	RRII 105	RRII 429	RRII 414
D1	59.7	55.9	54.3
D2	53.3	51.6	54.7
D3	54.3	48.7	64.9
Control	52.1	49.2	59.7
CD (P=0.05)		NS	

1.3. Water consumption in rubber nurseries

Data collected on water use at different stages of growth for the different planting materials in the nursery stage was compiled. Trial to arrive at optimum use of water to be initiated.

2. Crop improvement

2.1. Large scale evaluation of clones

IRCA 18, IRCA 130 and PB 330 exhibited significant superiority with respect to girth over other clones. Mean annual yield of IRCA 130 was the highest and was on par with clones RRII 105 and PB 255. Summer yield of IRCA 130 was significantly superior to all the other clones tested (Table Pad. 2).

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

Clone	Mean annual yield (g $t^{-1}t^{-1}$)	Summer yield (g $t^{-1}t^{-1}$)
PB 255	63.9	50.4
PB 314	46.7	33.2
PB 330	47.7	37.2
PB 28/59	43.4	39.6
RRIM 703	34.8	21.5
IRCA 18	33.8	25.9
IRCA 109	45.9	43.3
IRCA 111	41.6	36.2
IRCA 130	77.3	67.3
IRCA 230	33.9	25.3
RRII 105	64.4	40.6
CD	16.6	14.5

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

RRII 203 recorded the highest mean annual yield and summer yield at 54.9 and 51.2 g $t^{-1}t^{-1}$ respectively and was on par with the mean annual and summer yield of RRIC 100, P 270, P 213 and Iritty. Yield of RRII 203 was significantly superior to that of RRII 105 (Table Pad. 3).

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

Clone	Mean yield ($g\ t^{-1}t^{-1}$)	
	Annual	Summer
RRII 105	31.8	28.4
RRII 203	54.9	51.2
RRIC 100	45.0	39.5
RRIC 102	13.0	10.5
PB 86	40.8	33.7
P 1	27.5	25.5
P 2	25.9	22.7
P 90	23.9	21.2
P 121	25.7	19.9
P 155	25.1	25.1
P 213	48.9	42.9
P 270	47.0	42.2
P 280	29.1	26.2
P 296	31.8	28.4
Iritty	47.0	37.5
CD (P =0.05)	16.5	15.2

HEVEA BREEDING SUB-STATION, KADABA, KARNATAKA

Hevea Breeding Sub-station (HBSS) with a research farm at Nettana was established in 1986. The major constraints in commercial rubber cultivation in this region include drought in summer months and occurrence of *Phytophthora* and *Corynespora* leaf fall diseases. The research programmes in the station are envisaged to identify clones tolerant to different biotic and abiotic stress factors and to identify locally adapted clones for South Konkan region. There are 11 clone evaluation trials, a source bush nursery of 106 clones for generating nucleus planting material and a Class B Agro-meteorological Observatory in the research farm.

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

In the small scale ortet evaluation trial, the compilation of the previous 12 years' yield data revealed that the ortets T 2, O 17, O 15, C 42, O 41, C 70, T 1, O 53, O 40, O 55, O 56 and C140 were superior in yield than the control clones. Hence, these ortets were shortlisted for further evaluation.

2. Large scale clone trials (1989)

Among the 14 clones evaluated in the large-scale trial initiated during 1989, yield of clones RRII 203 and PB 255 was superior than the check clone RRII 105 (Table Kad. 1). Clone RRII 203 recorded highest girth.

Table Kad. 1. **Yield and growth performance of clones in Large Scale Trial (1989)**

Clone	Mean yield 2014 (g $t^{-1}t^{-1}$)	Mean girth 2014 (cm)
Haiken 1	29.0	63.8
KRS 128	22.3	84.7
KRS 163	25.1	81.8
KRS 25	33.0	93.5
PB 255	46.0	82.1
PR 255	35.8	68.0
PR 261	21.5	76.4
RRII 105	38.4	77.1
RRII 203	50.0	101.0
RRII 300	34.1	83.0
RRII 308	30.7	88.4
RRIM 600	35.5	80.4
SCATC 88-13	38.3	69.5
SCATC 93-114	17.0	72.5
CD (P = 0.05)	15.74	7.65

Table Kad. 2. **Yield and growth performance of clones in Large Scale Trial (1990A)**

Clone	Mean yield 2014 (g $t^{-1}t^{-1}$)	Girth (cm) 2014
GI 1	32.1	70.4
GT 1	60.4	82.8
Hil 28	42.7	78.5
HP 185	50.2	82.6
HP 187	38.7	79.5
HP 204	41.3	72.6
HP 223	67.5	94.8
HP 372	60.8	101.0
Mil 3/2	41.6	86.2
PB 217	62.5	84.5
PB 235	59.8	95.6
PB 260	67.9	90.4
PB 311	59.4	81.8
RRII 105	57.1	76.1
Tjir 1	29.9	72.2
CD (P = 0.05)	17.12	6.39

3. Large scale clone trials (1990 A)

In the large scale clone trial planted in 1990, PB 260 recorded the highest mean yield followed by HP 223 while check clone RRII 105. (Table Kad. 2).

4. Trial on estimation of genetic parameters (1990 B)

Twelve parent clones and their half-sib progenies were evaluated in the trial planted in 1990. Among the parent clones, PB 235 (73.1 g $t^{-1}t^{-1}$) had highest yield followed by RRII 203 (67.5 g $t^{-1}t^{-1}$). Mean yield of progenies was highest for RRII 203 (47.3 g $t^{-1}t^{-1}$) followed by PB 235 (45.6 g $t^{-1}t^{-1}$).

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

Three Small Scale Trials were planted in 1991 with 62 clones including RRII 105, GT 1 and RRIM 600 as control clones. In the trial 1991A with 36 clones, RRII 203 (84.5 g $t^{-1}t^{-1}$) and PB 280 (76.5 g $t^{-1}t^{-1}$) recorded the highest mean yield while PB 235 (91.8 cm) followed by RRII 300 (91.3 cm) recorded highest girth. Among the 13 clones evaluated in the trial 1991 B, RRII 5 (68.5 g $t^{-1}t^{-1}$) recorded highest mean yield and NAB 17 (57.7 cm) recorded the highest mean girth. Control clone RRII 105 recorded 47.1 g $t^{-1}t^{-1}$ and 64.2 cm yield and girth respectively.

6. Large scale trial 2000

The large scale trial for evaluation of RRII 400 series clones was planted in the year 2000 with hybrids RRII 414, RRII 430, RRII 422, RRII 429, RRII 403 and RRII 407 as male and RRIC 100 and RRII 105 as female parents. The data revealed superior growth for clones RRII 414 and RRII 430 and mean yield for RRII 430 followed by RRII 429 (Table Kad. 3).

Table Kad. 3. **Performance of clones in Large Scale Trial (2000)**

Clone	Mean yield 2014 (g $t^{-1}t^{-1}$)	Mean girth 2014 (cm)
RRIC 100	49.7	72.4
RRII 105	56.6	64.8
RRII 403	46.3	60.0
RRII 407	48.5	66.3
RRII 414	62.8	79.2
RRII 422	66.0	56.1
RRII 429	67.8	67.2
RRII 430	71.9	76.3
CD (P=0.05)	27.19	7.02

7. Polycross garden 1995

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 in a polyclonal seed garden is being maintained for collection of open pollinated seeds for different studies.

HEVEA BREEDING SUB-STATION, THADIKARANKONAM TAMIL NADU

1. Genetic improvement of *Hevea brasiliensis* for developing ideal clones

1.1. Conventional breeding

Four projects were pursued, viz., clone evaluation, hybridization and clonal selection, new generation polyclonal seed garden and participatory clone evaluation. Training on the root trainer planting technique was imparted to planters and technical staff from Orissa and Vietnam during the year.

1.1.1. Clone evaluation

This project consists of seven clone evaluation experiments initiated under the agro-climate of Kanyakumari region. Field performance of the modern high yielding clones in these trials was studied. One large scale trial experiment on G x E interaction in *Hevea* was concluded. In this experiment, among twelve clones, RRII 203 and RRII 105 consistently performed better over 12 years of tapping. Among the 400 series clones, RRII 430, RRII 422 and RRII 417 performed better than RRII 414 and RRII 429 (Table Par.1).

Table Par.1.**Performance of clones (panel wise) in the LST of G x E interaction trial (1996)**

Clone	Mean yield (g t^{-1} t^{-1})		
	BO-1	BO-2	Over 12 yrs.
RRII 414	45.4	41.6	43.7
RRII 417	46.0	51.6	48.5
RRII 422	49.9	50.4	50.1
RRII 429	37.7	46.0	41.5
RRII 430	52.2	47.7	50.0
RRII 51	38.5	36.7	37.6
RRII 176	39.5	63.3	50.3
RRII 203	55.8	60.3	57.9
RRII 105	55.4	60.5	57.7
RRIC100	43.2	44.9	44.0
PB 217	38.8	51.0	44.4
RRIM 600	42.6	49.2	45.6

In a study on the performance of the 400 series clones, a total of five block experiments were conducted in five diverse agro-climatic conditions in Kanyakumari District. Among them, trees in Palazhi and Bethany estates came under tapping during the period under report. The initial yield trend over one year of tapping indicated RRII 430 to be superior over RRII 105 in both the estates, while RRII 422 was highest yielder in Bethany estate.

In an observational trial of 400 series clones laid out in 2000 consisting of six hybrid clones and RRII 105, ten years of yield data indicated RRII 429 (68.7 $t^{-1} t^{-1}$) as the best yielder, followed by RRII 422 (63.7 $t^{-1} t^{-1}$). The high yielding clone RRII 429 had higher incidence of TPD also (28.8%).

1.2. Hybridization and clonal selection

The breeding orchards consisting of 51 parental clones were properly maintained and 960 hand pollinations were attempted with two different parental combinations.

1.2.1. 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 2013 were test tapped and the promising ones were pollarded for multiplication and further evaluation.

2. Participatory clone evaluation experiments

Most of the 11 pipeline clones and the three check clones planted at Tharuvaiyar estate were found vigorous.

LIBRARY AND DOCUMENTATION CENTRE

Rubber Research Institute of India has a well maintained Library and Documentation Centre with a collection of 22882 books, 23901 bound volumes of periodicals, 6000 standards, 1563 reprints, 170 Theses/Dissertations and 1200 Microfiche/Microfilms. Computer based bibliographic databases of all books, research articles, standards, theses and subject bibliographies are also available.

Library continued the information and literature support to its in-house and institutional users by providing reference services, current awareness services and reprographic services. Eighty one books were added to the stock. Received and registered 715 issues of journals as subscription/exchange. Compiled and disseminated the information bulletins, *viz.*, New Additions List 2014, three issues of Documentation List and two issues of Rubber Alert. Staff Publications list 2011-2014 was also compiled during the period. Databases were updated with the details of 88 books and 270 articles. Classified and catalogued 199 books, circulated 1180 books, filed 2220 press clippings of relevant articles, issued 524 SDI bulletins and provided 11473 photocopies. Library membership given to 43 members and 539 outsiders used our library resources.

As a part of sales promotion of RRII publications, library organized the sale and distribution of 512 copies of the journal *Rubber Science*, 154 RRII Annual Report and 380 other publications. Library conducted exhibition cum sale of RRII publications in connection with *Parliamentary Standing Committee visit* and at the venue of *National Seminar on Indigenous Warships: Avenues & Opportunities for Indian Rubber Industry* organized at RRII.

SCIENTIFIC ADVISORY COMMITTEE RECOMMENDATIONS 2014-15

- Approved upgrading the clone RRII 208 to Category I for North Eastern Region.
- Recommended clones RRSA 114, RRSA 315, RRSA 98, RRSA 585 and RRSSA 144 to include in category III for North Eastern Region.
- Recommended elephant dung as potting medium for root trainer plants of rubber.
- Recommended two tuber crops namely, elephant foot yam and colocasia as intercrops for the North Eastern Region.
- Recommended to reduce the current does of fertilizer to 250: 125:50 kg of $N:P_2O_5:K_2OH$/ ha as urea, rajphos and potash.
- Ethephon products as latex yield stimulants in rubber (M/s. Vasudha Bioteck Pvt. Ltd. Vengal Rao Nagar, Hyderabad are recommended).
- Recommended the KAMCO TERA TRAC 4W Mini tractor using Aspee-RRII Tractor mounted high tree mist blower for low volume spraying.
- Recommended new MAK eco-friendly rubber spray oil supplied by M/s. Bharat Petroleum Corporation Ltd. Mumbai.

ANNUAL EXPENDITURE

Expenditure at a glance (2014-15)

Head of Account	Expenditure (Rs. in lakhs)
Non-Plan	
RRII HQ	652.26
CES, Chethackal	536.44
Total	**1,188.70**
Plan	
Research other than NERDS	2,257.64
North East Rubber Development Scheme	411.18
Total	**2,668.82**
Grand Total	**3,857.52**

LIST OF MAJOR EQUIPMENTS

Air permeability tester
Atomic Absorption Spectrophotometer
Autoclave (Cylindrical and Horizontal)
Ball mill
Bio – Cabinet
Bio safety cabinet
Biomedical Freezers (-30°C)
BOD Incubator
Brook field viscometer
Carbon Nitrogen Analyser
Centrifuges (High speed refrigerated)
Chemical fume hoods
Chlorophyll Content Meter
Chlorophyll index meter
Climatic chamber
Coir foam testing equipment
(indentation hardness, flexing, compression set A & B testers)
Compression set apparatus (25% strain)
De- mattia Flexometers
Deep freezer (-20° & -80°)
Deep freezers
Differential scanning calorimeter
Din Abrasion machines
Disper grader
DMA 50
DNA electrophoresis unit
DNA isolation machine
Eddy covariance system
ELISA reader
Environmentally controlled shakers
Flame photometer
Flash chromatograph & accessories
Fluorescence Monitoring System
Fluorescence spectrophotometer
Freeze Driers
FTIR spectrometers
Gas chromatograph–mass spectrometer
Gel blotting apparatus
Gel documentation & image analyzer
Gel documentation systems
Gel Dryer & Pump
Gel electrophoresis apparatus - 2D
Geldoc systems
Genetic Analyzer 3500 XL
Goodrich Flexometers
GPC
Hardeness tester (shore A,D,M,0)
High speed microcentrifuge
High speed Table Top Centrifuges
High Voltage Power Pack
High-Speed Centrifuge
Histo Embedder
HPLC system
Hybridization oven – Incubator shaker
Hydraulic press (14" × 14",)
Image processing and Analysis System
Incubator & Accessories
Incubator shaker with cooling
Information System (RS & GIS)
Infrared thermometer
Inverted Microscope
IRGA- Portable photosynthesis system
Isoelectric focusing unit
Laminar Air Flow Hoods
Latex foam testing equipment
(indentation hardness, flexing, compression set A & B testers)
Leaf Area Meter
Linear PAR Ceptometers
Liquid scintillation system
Luminometer measurement system
Measuring mixer (80 cc)
Micro centrifuge with cooling
Micro pH meter
Microtome – Base sledge
Microtome – Rotary with knife sharpener
Mini IEF electrophoresis unit

Mooney viscometer
Moving die rheometer
Muffle furnaces
Nanodrop Spectrophotometers
Nitrogen Analyzer
Oxygen electrode
Ozone chamber
P700 chlorophyll fluorescence
PAM-2000 portable flurometer
PAR/LAI Ceptometer
Particle size analysers
PCR machines
Phase contrast Microscope & accessories
Phosphor Image Analyser
Phosphor imager
photosynthesis system
Plant growth chamber
Plate reader
Polarizing Microscope & accessories
Portable photosynthesis system
Projection microscope with accessories
Protein separating systems - 2D
Real time PCR machines
Recirculating Cooler
Refrigerated and Heating Circulator
Refrigerated high speed micro centrifuge
Refrigerated shaker
Refrigerated Table Top Centrifuge
Remote Sensing and Geographical Information System (RS & GIS)
Research microscope and image analyser
Ross flexing Machine
Rotary Evaporator
Rubber Process Analyser
Sap flow meter
Sequencing gel electrophoresis unit
Sequi-Gen GT system (sequencing system)
Soil Respiration Analyser
Sonicator
Specific gravity balance
Spectro radiometer
Spectronic 20D Spectrophotometer
Spectrophotometer – nanodrop
Spectrophotometers
Speed vac concentrator system
Stereo microscope
Submerged Electrophoresis System
Temperature controlled incubator shaker
Temperature Recorder
Thermo gravimetric analyzer
Thermocouple psychrometer
Tissue processor
Two-roll mixing mill (6" × 13")
Ultracentrifuges
Universal testing machines (50N, 100N, 5 kN)
UV Spectrophotometer
Vertical electrophoresis unit
Walk in environmental Growth Chamber
Walk in Fume Hood
Water Potential meter WP4-T
Water potential system
Water purification system
Wet sieving apparatus
Zeta potential analyzer

NORTH EAST

Atomic Absorption Spectrophotometer
ChlorophyII content meter
Flame photometer
Fluorescence Monitoring System
Leaf Area Meter
Luminometer
Nitrogen Analyzer
PAR/LAI Ceptometer
Portable photosynthesis system
Spectronic 20D Spectrophotometer
Stereo microscope
UV-spectrophotometers
Water Potential meter WP4-T

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Nedumangadu- 695 541

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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Satellite Image of Rubber Research Institute of India

Back Cover

1. Launching of Mini Tractor Mounted Mist Blower jointly developed by RRII, ASPEE and VST Tiller Tractors.
2. IRRDB - RRII Workshops on International Rubber Health Clinic and Multilateral Clone Exchange 1 - 7 June, 2014, RRII, Kottayam.
3. National Seminar on Indigenous Warships: Avenues and Opportunities for Indian Rubber Industry, 6 June 2014, RRII, Kottayam.

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