

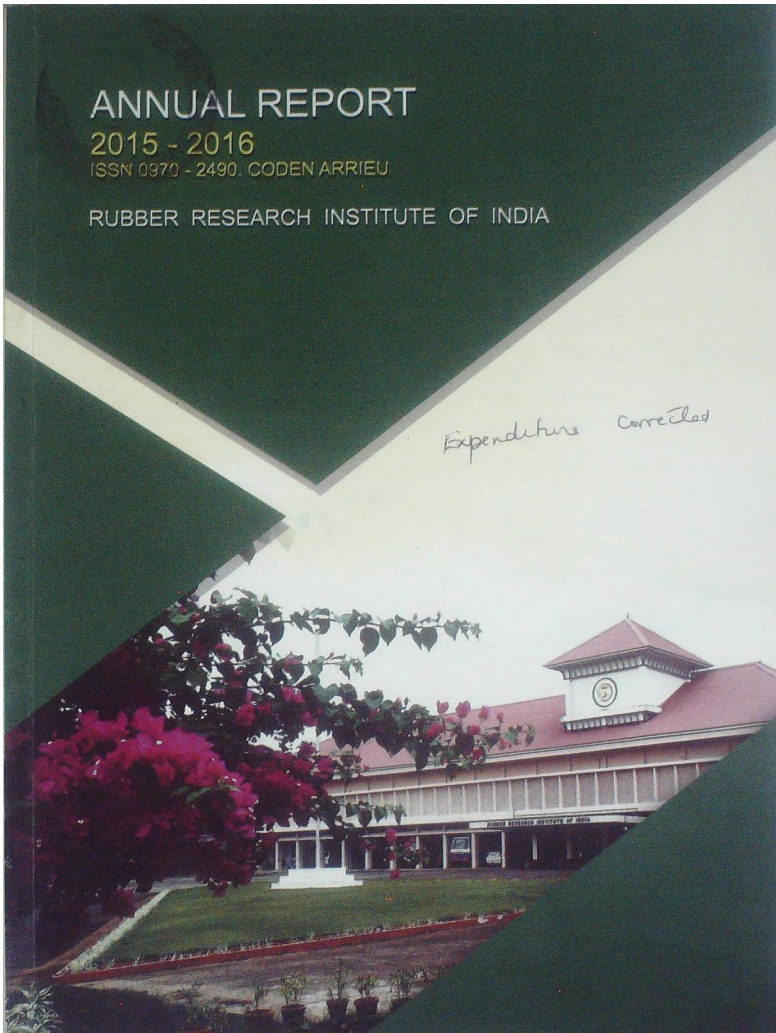
# ANNUAL REPORT

2015 - 2016

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RUBBER RESEARCH INSTITUTE OF INDIA

*Expenditure Corrected*



### **Rubber Research Institute of India**

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

### **Location**

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

### **Organization**

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

*Continued on p.112*

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RUBBER BOARD**  
(Ministry of Commerce & Industry, Government of India)  
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## CONTENTS

The Rubber Board .....	iv
Preface .....	vii
Director's Review .....	ix
Agronomy and Soils Division .....	1
Fertilizer Advisory Group .....	6
Climate Change and Ecosystem Studies .....	6
Botany Division .....	10
Germplasm Division .....	23
Biotechnology Division .....	28
Genome Analysis Laboratory .....	35
Plant Pathology Division .....	39
Plant Physiology Division .....	44
Latex Harvest Technology Division .....	52
Rubber Technology Division .....	56
Technical Consultancy Division .....	65
Economics Division .....	71
Central Experiment Station, Chethackal, Kerala .....	76
Regional Research Station, Guwahati, Assam .....	76
Regional Research Station, Agartala, Tripura .....	76
Regional Research Station, Tura, Meghalaya .....	79
Regional Experiment Station, Nagrakatta, West Bengal .....	83
Regional Research Station, Dapchari, Maharashtra .....	86
Regional Research Station, Dhenkanal, Odisha .....	88
Regional Research Station, Padiyoor, Kerala .....	89
Hevea Breeding Sub-Station, Kadaba, Karnataka .....	90
Hevea Breeding Sub-Station, Thadikarankonam, Tamil Nadu .....	93
Library and Documentation Centre .....	94
Scientific Advisory Committee Recommendations .....	94
Annual Expenditure .....	95
List of Major Equipments .....	96
Publications .....	98
Scientific and Senior Supporting Personnel .....	106
Research Establishments .....	111

## 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 22<sup>nd</sup> January 2010.

### Organization

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

### Chairman

Dr. A. Jayathilak IAS

### Rubber Research Institute of India

Dr. James Jacob  
Director

### Crop Improvement

Dr. Kavitha K. Mydin  
Joint Director

### Botany

Deputy Director (Vacant)

### Biotechnology

Dr. A. Thulaseedharan  
Joint Director (on Deputation w.e.f.  
14.08.2014)

### Genome Analysis

Dr. Thakurdas Saha  
Principal Scientist

### Germplasm

Dr. C.P. Reghu (upto June 2015)  
Joint Director

### Crop Management

Joint Director (Vacant)

### Agronomy

Dr. M.D. Jessy  
Joint Director

### Fertiliser Advisory Group

Dr. Mercykutty Joseph  
Principal Scientist

### Crop Physiology

#### Climate Change and

#### Ecosystem Studies

Dr. R. Krishnakumar  
Joint Director

### Plant Physiology

Dr. K. Annamalaiathan  
Joint Director

**Crop Protection**

Mr. Sabu P. Idicula  
Joint Director

**Mycology / Plant Pathology**

Dr. Jacob Mathew  
Joint Director

**Economics Research**

Dr. Tharian George K.  
Joint Director

Toms Joseph

Deputy Director

**Latex Harvest Technology**

Dr. K.U.Thomas  
Joint Director

**Rubber Technology**

Joint Director (Vacant)  
Deputy Director (Vacant)

**Technical Consultancy**

Dr. Siby Varghese  
Joint Director

**Central Experiment Station, Chethackal**

Dr. P. Mallinath Priyadarshan  
Deputy Director

**Regional Research Station, Guwahati**

Dr. Gitali Das  
Deputy Director

**Regional Research Station, Agartala**

Dr. Sushil Kumar Dey  
Joint Director

**Regional Research Station, Nagrakata**

Deputy Director (Vacant)

**Regional Research Station, Tura**

Deputy Director (Vacant)

**Regional Research Station, Dapchari**

Dr. Meena Singh  
Senior Scientist (Officer-in-charge)

**Regional Research Station, Dhenkanal**

Dr. Bal Krishan  
Senior Scientist (Officer-in-charge)

**Regional Research Station, Padiyoor**

Dr. Radha Lakshmanan  
Principal Scientist (Officer-in-charge)

**Hevea Breeding Sub-station, Kadaba**

Dr. P. Deepty Antony  
Scientist (Officer-in-charge)

**Hevea Breeding Sub-station,  
Thadikarankonam**

Dr. T.A. Soman (upto August 2015)  
Principal Scientist (Officer-in-charge)

**Administration**

Mr. Raveendran Nair K.  
Deputy Secretary

**Finance and Accounts**

CA. Zachariah Kurian  
Joint Director

**Instrumentation**

Dr. Thomas Baby  
Deputy Director

**Library and Documentation Centre**

N. Latha  
Documentation Officer

**Statistics and Computer**

B. Biju  
Assistant Director (Systems)





## PREFACE

It is a matter of satisfaction that soil fertility mapping of rubber growing soils is progressing as per schedule. Geo-referenced soil fertility maps are already made in GIS platform for all rubber growing districts of Kerala and Tamil Nadu which is a step close to developing online fertilizer recommendation for rubber growers. During the reporting year, field data on growth, yield and disease response of RR11 208 from different field trials from North Eastern states lasting almost 25 years were consolidated, paving the way for release of this clone for exclusive cultivation in cold prone North Eastern states. Satellite based remote sensing techniques have been successfully applied to locate, map and estimate the extent of area under natural rubber cultivation in the state of Assam. This technique is also being made use of to identify lands where natural rubber cultivation can be extended without causing deforestation or conversion of food cropped areas in non-traditional areas.

It is impressive that about 15000 hand pollinations were made during this year between parents derived from wild accessions of *Hevea* and high yielding RR11 hybrid clones resulting in the production of 353 new hybrid seeds. This indicates that seed set is very low in this species. Several thousands of such hybrid seeds need to be evaluated at different stages including in large scale, multi-locational field trials before a new clone can be successfully released for cultivation by growers and the whole exercise could take about quarter of a century or longer. These difficulties can be addressed to a large extent by developing genetic and physiological markers for early selection for which the Institute should complete the pending work of whole genome sequencing of natural rubber. It is understood that the genome sequencing using three platforms has been completed and the assembly and bioinformatics works are in progress which should be expedited quickly.

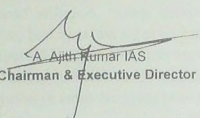
Several genes associated with the rubber biosynthesis pathway were expressed more in high yielding clones indicating that these genes are potential markers for high yield. But this needs to be validated for which whole genome sequencing assembly and bioinformatics data are critical. A small Heat Shock Protein (HSP) of chloroplast origin was validated as a physiological marker for drought tolerance. Water use efficiency of natural rubber trees (defined as the amount of dry rubber produced per

unit amount of water consumption by a tree) was comparable in the traditional rubber growing regions and cold prone non-traditional regions. However, this was reduced by almost 50 per cent in the hot and dry climatic conditions of the North Konkan. This finding has significant implications for extending natural rubber cultivation to regions where rainfall distribution is skewed and summer temperatures are very high.

During the reporting year, prototypes of three mechanized/modified tapping devices, designed and developed by private entrepreneurs were tested for their performance and this work should continue. Good progress has been made in developing a naturally degradable polythene that can be used for rain guarding rubber trees. Natural rubber latex-carbon black master batch developed in the past failed during scaling up trials. However, a new protocol for preparing natural rubber latex-carbon black master batch is being attended. New proteolytic enzymes were tried to make deproteinized natural rubber latex. During this year two interesting new studies were initiated namely, identification of genes associated with Phytophthora diseases in natural rubber and evaluating the quality of water in watersheds dominated by rubber, tea and cardamom.

The Institute should continue its scientific pursuit blending and balancing both basic and applied research with the ultimate objective of providing the best service to the stakeholders.

Kottayam  
30 October 2017

  
A. Ajith Kumar IAS  
Chairman & Executive Director

## DIRECTOR'S REVIEW

One of the major achievements during 2015-16 was consolidation of more than two decades of field data of trials conducted with the hybrid clone, RR11 208 in North Eastern Region (NER). Based on detailed analyses of growth, yield, response to low temperature stress and diseases made by the Breeding team of RR11, the Scientific Advisory Committee of the Institute upgraded RR11 208 to Category 1 for large-scale cultivation in NER, but its formal launching did not happen during the reporting year.

Another highly credible achievement during this year was completing the kriging analyses of data for 13 soil fertility parameters. One composite sample (based on three soil samples) was collected from every 50 ha of the entire rubber growing regions in South India and the fertility status was chemically analysed in the laboratory. These data available at the 50 ha scale were kriged to the scale of 30mts by 30mts and district-wise maps showing spatial variability in the status of the 13 soil fertility parameters were prepared. These maps will be uploaded in a web portal for making site-specific online fertiliser recommendation system for rubber growers for which work is in progress.

Breeding for high yield and secondary traits is a constant priority at RR11. The rather poor rate of seed set after hand pollination (often in the range of 2-3%) is a persistent concern for Breeders. This calls for innovative approaches in classical breeding through selective bi-parental crossing. I would strongly urge the Breeding

group to make better use of open pollinated seeds, either from designed breeding gardens or from other fields where there is a good chance of natural mixing of genes. A large number of naturally produced hybrid

seeds should be collected every year from different agro-climatic regions and subjected to intensive selection with the best molecular tools available for identifying advanced genetic materials right at the seedling stage. Increasing the sample size will increase the probability of identifying a better genotype. But handling large numbers of seedlings in the field poses many difficulties. Efforts to develop molecular markers and validate them should be strengthened as this can help in screening large number of seedlings quickly and reliably. During the reporting year the Molecular Biology/Genomics and Physiology groups made some significant progress in this direction.

The Institute has embarked on a "smart clone vision": to release in every five year or so at least one new clone with high rubber and timber yields, fast growth rate, reduced immaturity period, improved climate resilience and tolerance to pests and diseases. The present long breeding cycle of almost a quarter century, heterozygous genetics of the species, short window of time for hand pollination each year, poor seed set, non-synchrony of flowering in the selected parents, long gestation period and economic life of the

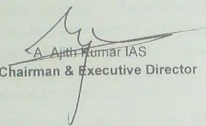


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crop, requirement of large areas of land for the long field trials etc. make the smart clone vision extremely difficult to realise, but not insurmountable. The Institute has put in place a strategy to make the smart clone vision a reality. This includes combining principles of classical and molecular breeding for early selection of promising seedlings and farmer participatory clone evaluation trials taken up concomitantly at different locations. Smart clones will always remain a priority because of the imperative to increase productivity on the face of constantly emerging new threats and challenges such as climate change, new pests/diseases, declining soil health, land fragmentation, labour scarcity etc. Completing the whole genome sequencing of rubber and assembly and annotation of genes is central to the smart clone vision, but shortage of funds and manpower is seriously hampering this project.

A total of 39 promising clones from Vietnam, China, Thailand, Brazil, Indonesia, Côte d'Ivoire and Sri Lanka were imported under the multilateral clone exchange program of the IRRDB. There are at present 71 pipeline clones under evaluation in 38 locations under the first four phases of the participatory clone evaluation project. Under the fifth phase of the project 22 pipeline clones were established in polybags in seven locations. Post release, RRII 400 series clones continued to perform well even as there are certain region specificity in the response of the clones. This is something that will be examined closely in the coming years.

A set of 200 wild accessions of *Hevea* germplasm and Wickham clones was planted in

the Arboretum established at Tura, Meghalaya. Crown budding with Fx 516 on PB 311 and PB 260 gave effective control to *Phytophthora*. Also the girth of the middle portion (PB 311 and PB 260) increased with regard to their respective controls in crown budded plants. Similarly crown budded plants gave more yield than the controls. These are significant findings which should be consolidated for popularizing among growers. Similarly studies on QTL mapping for *Phytophthora* tolerance also need to be expedited.

About 121 permanent soil health monitoring sites including forest sites adjacent to rubber plantation were identified and chemical analyses of the soil samples are in progress. Intercropping in immature rubber improved growth of rubber confirming several earlier findings from India as well as other countries. This finding is almost akin to some kind of a "symbiotic" association between the crops. Reasons for the beneficial impacts of intercrops on growth of rubber deserve further attention of Agronomists and Soil Scientists which can unravel the dynamic interactions among the different crops. Similarly, several studies have shown that mature rubber trees growing in healthy soils can continue to yield well even if fertilisers are skipped for a couple of years making mature rubber holdings a "closed" ecosystem, the dynamics of which are still unclear. Retaining pineapple after four years did not adversely affect rubber cultivation. Mulching with newspaper and gunny sheets coated with latex could control weed growth and conserve soil moisture.

As the mean size of rubber holdings is increasingly becoming smaller, rubber based homestead farming systems need to be evolved by assimilating different crops. In this context, the present planting design/system needs to be revisited. Rubber planted in the "twin planting system" maintained higher growth than control even after seven years. Three whorl poly bag plants appeared to be a better planting material. Growth of young rubber seedlings five months after treating with plant growth promoting bacteria was statistically higher than untreated control seedlings. But the difference was only 2-3 mm which makes the result insignificant from a practical point of view.

There was an interesting observation on the relationship between photosynthetic carbon assimilation at the ecosystem level (estimated by the Eddy covariance method) and water mining by rubber trees (measured by sap flow meter) with ambient light intensity. As light intensity increased in the morning, both  $\text{CO}_2$  assimilation and water mining increased steadily. During periods of clouds and also during sun set  $\text{CO}_2$  assimilation dropped much faster than transpiration. This observation points towards more complex and intricate physical and chemical control of photosynthesis than transpiration which is by and large controlled by the physical processes associated with movement of the stomatal guard cells in response to light.

The water use efficiency of rubber trees in terms of dry rubber yield per unit water consumption was the highest in the traditional region, closely followed by the cold prone region. This was drastically reduced in the hot and dry

North Konkan region. A naturally degradable polythene was developed in collaboration with a private entrepreneur which needs to be further tested in the field for its utility as a rainguarding material. Prototypes of two tapping devices, one mechanical and another one motorised were identified for detailed commercial evaluation.

During the reporting year rubber prices continued to decline forcing many growers to stop tapping the trees. There was close to 35 per cent drop in total domestic production compared the highest production of approximately 9 lakh tons during 2012-13. Several projects aimed at reducing cost of cultivation/production were continued. Reducing cost of cultivation is central to sustaining profitability of rubber cultivation.

Approximately 50 per cent of the tapped area seems to have been tapped for 14 years or more indicating that a substantial proportion of the area must be old and senile. This was an import consideration in the conceptual framework developed by the Economics team towards developing a national rubber policy in the country. The census data of rubber tappers showed that almost 90 per cent of the tappers are engaged in either daily or alternative daily tapping which clearly indicates that a given tapper must be working for more than one grower and many growers are still following high frequency tapping such as daily tapping or alternate daily tapping. Only less than 5 per cent of the tappers were doing d3 tapping and those who were doing weekly tapping must be an insignificant number.

A modified coagulant was developed to prepare air dried sheet rubber and one proteolytic



enzyme was identified for effective deproteinization of latex. A new method was developed to prepare high quality solid deproteinised dry rubber from field latex within a couple of hours. This method completely eliminates the creaming/centrifugation steps, facilitating high volume production at low cost. Latex-carbon black master-batch and latex-silica master-batch are two projects that are actively being pursued which can make significant impact on the industry. Similarly the project on devulcanization also holds considerable potential for recycling of used rubber. Know-how for a large number of products were developed and transferred to the clients as per their demand and requirement.

During the reporting year, a new study was initiated to assess water quality in water sheds dominated by rubber, tea and cardamom. Early results indicated that water samples collected from all the three watersheds contained coliforms above the limits prescribed for drinking water. Yet another new study initiated during the

reporting year was assessing the agro-climatic suitability of left wing extremism (LWE) affected states for rubber cultivation. Various meteorological indices are being tested. The reporting year experienced *El Nino* effect and there was more evidence for regional climatic warming in rubber growing regions of India. A new study on vegetation dynamics of rubber ecosystems was also initiated during the reporting period.

During the reporting year, scientists of this Institute published 51 peer reviewed articles, 21 research papers in seminars/symposia and four PhD theses were also produced from RRII. There were six book chapters written. The number of popular articles published was 44. As on 31 March 2016, the physical strength of scientists was 85 as against the sanctioned strength of 116. Amount of money spent on research schemes during 2015-16 was Rs.71.68 Lakhs which was highly insufficient to meet the routine expenses of the Institute.

Dr. James Jacob



## AGRONOMY AND SOILS DIVISION

Generating additional income from rubber plantations through crop diversification and reducing cost of cultivation through refinement of agromanagement techniques continued to be the thrust areas of research of the Division. Shortening the gestation period through good agricultural practices, management of biotic and abiotic stress and ground cover management also were priority areas. Soil fertility maps of all districts of Kerala, rubber growing regions of Tamil Nadu and Karnataka were prepared. For soil health monitoring, profile samples were collected from 22 forest sites adjacent to rubber plantations and analysis is 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, under took feasibility studies on cultivation of rubber and intercrops in various agroclimatic regions and investigated specific field problems.

### 1. Nutrient management

The experiment initiated at Central Nursery, Karikattoor for the evaluation of an organic manure, 'Geo-green' in seedling nursery was continued. Four months after planting, the diameter of seedlings which received basal application of Geo-green @ 2500 kg ha<sup>-1</sup> and 500 kg ha<sup>-1</sup> were comparable with that of current recommended practice and significantly superior to all other treatments. However, eight months after planting, these two treatments were significantly superior to all others.

The percentage buddability was also comparable in the treatments with Geo-green @ 500 kg ha<sup>-1</sup>, 2500 kg ha<sup>-1</sup> and current recommended practice. A field experiment was

initiated to evaluate Geo-green in young rubber plantation.

The field experiment to study the effect of long term use of inorganic and organic manures on the growth and yield of rubber and on the physico-chemical properties of soil was continued. The treatments viz. standard practice, 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 higher than the FYM alone and no fertilizer / no manure treatments (Table Ag.1). Higher available Zn and Mn status were found in FYM applied plots than in fertilizer alone applied plots.

Table Ag. 1. Effect of integrated nutrient management on yield of rubber

Treatments	Yield (g t <sup>-1</sup> t <sup>-1</sup> )
No fertilizer, no manure (control)	52.4
Farm yard manure (FYM) alone	64.2
Fertilizers alone (standard practice )	76.1
25% fertilizers + 75% FYM	81.7
50% fertilizers + 50% FYM	76.5
75% fertilizers + 25% FYM	69.7
SE	2.8
CD(P=0.05)	8.3

The multi-locational trials initiated in the first year plantations in three different locations during 2011 to study the effect of supplementing secondary and micronutrients on growth and yield of rubber, in areas low in their status were in progress. Experiment initiated at New Ambady Estate was discontinued due to logistics problems. Difference in growth was not significant between treatments in any location. Secondary and micronutrients applied during the 1<sup>st</sup> year of planting maintained soil nutrient status in the

sufficiency level during the 5<sup>th</sup> year also in all the locations.

The field experiment at Thanneermukkom, Cherthala, to study the performance of rubber in marginal soils was continued. Observations on growth and yield showed that by adopting appropriate agro-management practices, growth of rubber in coastal sandy soil is comparable to that in conventional rubber growing soils. Yield recorded during the initial year of tapping was 53 g t<sup>-1</sup>r<sup>-1</sup>.

Through Boundary Line Approach (BLA) the optimum level of soil and leaf nutrient for young rubber (clone RRII 105) was calculated. The optimum ranges for soil Ca and Mg were substantially higher than the current optimum ranges followed for rubber.

In the study on rhizosphere chemistry and growth of rubber in different pH and base status, the nutrient content in the rhizosphere and bulk soil varied and the rhizosphere pH was maintained around 5.0. The availability of P and K was significantly higher and organic carbon was low in the rhizosphere.

A glass house study to compare the growth of rubber seedlings in three different soil pH viz. pH 4.3, 5.5 and 7.3 indicated that the growth of seedlings was better in soils with pH 5.5 and was on par with soil having pH 7.3. Higher contents of N, P, K, Ca, Mg and micronutrients in the shoot and root were observed in seedlings grown in soil pH of 7.3. The nutrient availability at pH 4.3 and 5.5 were comparable.

The study on leaf nutrient status in low and high yielding trees in a plantation was continued and the results showed that there was no significant difference in the leaf nutrient status between low and high yielding trees. However, the nutrient ratios (N/Mg, N/Mn, P/Fe, Ca/Mn, Ca/Cu, Mg/Zn, Mg/Fe, Mn/Fe, Cu/Ca, Cu/Mg,

Cu/Fe) were significantly different between the low and high yielding trees.

Study on the effect of excess soil Cu on growth of young rubber plants showed that low levels of soil Cu (10 mg kg<sup>-1</sup> soil) has a positive effect on sprouting, growth, and dry matter production. Beyond 30 mg Cu kg<sup>-1</sup> soil, height, leaf area and total dry matter were significantly decreased. Calcium status of leaf, stem and root declined with increasing levels of soil Cu. Copper @ 100 mg kg<sup>-1</sup> soil adversely affected growth and dry matter production of plants. Excess Cu was accumulated in the roots, and was not translocated to the shoot, indicating some tolerance mechanism. Available Cu content of 20 mg kg<sup>-1</sup> soil or less did not have any adverse effect on the growth of young rubber plants.

## 2. Soil and water conservation

The experiment on evaluation of biological/vegetative hedges for soil and water conservation in rubber plantation was continued. Significant difference in soil erosion was not observed, six years after the establishment of vegetative hedges viz. vetiver, guinea grass, pineapple and *Strobilanthes* sp. The growth of rubber was not significantly influenced by the establishment of vegetative hedges.

## 3. Intercropping and cropping systems

The field experiment initiated to develop a multispecies rubber based cropping system for Tamil Nadu region was in progress. Four years after planting, the establishment of intercrops viz. pineapple, three varieties of banana, cinnamon and cocoa had significant positive effect on the growth of rubber. The growth of rubber was significantly higher in all intercropped areas except cocoa, compared to control. The experiment initiated in 2001 to evaluate the feasibility of growing perennial intercrops with rubber was also in progress. The growth and yield

of rubber continued to be not influenced by cultivating the perennial intercrops. Coffee yield was 60 per cent of that of monoculture. Cocoa and medicinal plants were planted in the space earlier occupied by *Garcinia* and vanilla. *Dracaena massangeana* continued to yield well in mature rubber plantation. The leaves were harvested once in 45-60 days, and sold in the local market @ Rs. 2.50/- per leaf.

Experiment on inter-planting of rubber with timber trees viz. teak, wild jack and mahogany was continued. Growth of rubber was not significantly influenced by row spacing and type of timber intercrops whereas the interaction showed significant variation. Under normal spacing, the growth of rubber intercropped with wild jack was significantly lower compared to all other treatments. However, significant difference in yield was not observed between spacing, type of intercrops and interactions.

With the objective to find out the effect of retaining pineapple as intercrop on performance of rubber and soil properties, a block trial was initiated at Kaliyar estate, Thodupuzha with one block having pineapple retained for four years after planting and another block where pineapple was removed four years after planting. Yield recording was carried out on a monthly basis. Preliminary results showed that retaining pineapple after four years did not adversely affect rubber yield.

#### 4. Ground cover management

The field experiment to compare the effect of legume covers and natural flora on the growth of rubber, soil physico-chemical and biological properties, biomass and nutrient turnover laid out during 2010 at CES, Chethackal was continued. The growth of rubber plants was affected significantly by allowing natural flora with life-saving weeding and the reduction in girth over a period of four years was to the tune of 27 per

cent compared to rubber with cover crops, whereas, there was only a slight reduction (7%) in the growth of rubber plants with one m<sup>2</sup> weeding (Table Ag. 2).

Table Ag. 2. Effect of different weed management methods on growth of rubber

Treatment	Girth at 150 cm from the bud union (cm)
Rubber + <i>Pueraria</i>	25.8 <sup>a</sup>
Rubber + <i>Mucuna</i>	25.2 <sup>a</sup>
Rubber + Natural cover (1 m <sup>2</sup> area weed free)	23.7 <sup>b</sup>
Rubber + Natural cover (life-saving weeding)	18.7 <sup>c</sup>

An observational trial was initiated to study the efficacy of latex coated mulch materials in preventing weed growth in immature rubber. Latex coated mulches were prepared using low cost materials such as newspaper and gunny, and laid in the plant basin of young rubber plants. Observations on weed growth and soil moisture were made. Results showed that latex coated newspaper and gunny were effective in preventing weed growth and conserving soil moisture. The durability of these materials under field condition is being evaluated.

The field experiment initiated at CES, Chethackal for the evaluation of *Calopogonium caeruleum* was continued. The N content was less and P, K, Ca and Mg contents were more in *C. caeruleum* than that in *Mucuna bracteata*. Soil pH under different ground covers decreased in the order: natural cover > *Pueraria phaseoloides* > *Calopogonium caeruleum* > *Mucuna bracteata*.

#### 5. Planting techniques

The field experiment to assess the impact of mechanized land preparation on soil erosion



and physical and chemical properties of rubber growing soils was continued. Pitting, terracing and tilling inter rows by earth mover, pitting and terracing by earth mover, pitting by tractor-mounded hole digger and manual terracing and standard practice - manual pitting and terracing (control) were the different land preparation methods. The rate of soil erosion was significantly higher in plots where pitting, terracing and tilling inter-rows were done by earth mover than all other methods. The experiment to evaluate different planting designs was in progress at Cheruvally estate and the canopy of rubber has an asymmetrical growth pattern in the modified planting designs. Girth of plants in the twin planting system was superior to control.

#### **6. Development of agro-management techniques for shortening the gestation period**

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

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

An experiment investigating the comparative field performance of one-whorl, two-whorl and

three-whorl polybag and root trainer rubber plants initiated at CES, Chethackal during 2008 was continued. The superiority of three-whorl polybag plants over all other planting materials continued. The experimental area was marked for tapping.

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

#### **7. Rubber growing soils**

The project on soil CO<sub>2</sub> flux measurements in mature rubber plantations in different agro-climatic zones was continued. The data indicated that soil respiration rate was more in Agartala compared to Dapchhari during the winter period. In Dapchhari, though soil organic carbon was more and soil acidity was less than Agartala, soil respiration rate was less. The reason could be the lower soil moisture status in Dapchhari during the winter period.

The experiment to assess the contribution of root respiration towards soil respiration was in progress. It was observed that in mature rubber system, soil respiration rate was comparatively more during the summer period and less during the winter period. The contribution of root respiration towards soil respiration was more in rubber-pineapple system than to rubber alone while the highest rate was recorded in rubber-banana system. In all the systems studied, soil moisture was comparatively low during the winter period compared to all other seasons.

#### **8. Stress management**

##### **8.1. Drought management**

Two field experiments, one with RRII 105, a drought susceptible clone and the other with RRII 430, 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 concluded. Polypropylene woven fabric improved soil moisture retention, modified the surface temperature regime as well as suppressed weed growth. Significantly higher growth was recorded by the plants under polypropylene woven fabric irrespective of clones. Based on the study, polypropylene woven fabric was recommended for use in rubber plantations.

Pot culture studies conducted in the glass house to compare the K concentration of leaf between drought tolerant and susceptible clones indicated that leaf K concentration was higher in RRII 400 series clones and RRII 600 compared to RRII 105 (Table Ag. 3). It was also observed that the soil K content in the root zone of RRII 430 was the highest among the clones.

Table Ag. 3. Leaf K concentration in different clones

Clone	Leaf K (%)
RRII 414	1.17
RRII 429	0.90
RRII 417	1.04
RRII 430	1.26
RRII 422	0.99
RRII 600	0.97
RRII 105	0.86
CD (P=0.05)	0.08

## 8.2. Disease management

Polybag experiments initiated to study the effect of supplementing Ca (through different sources), K and B on *Phytophthora* infection in young rubber plants of clone RRII 105 was in progress. Preliminary results indicated that among the different treatments, plants supplied with Ca

through quick lime integrated with K and B showed comparatively less lesion size and leaf fall when inoculated with *Phytophthora* strain.

## 9. Environmental aspects of rubber cultivation

A study on assessment of water quality in watersheds of different plantation crops, viz. rubber, tea and cardamom was initiated. Watersheds were delineated using Survey of India topographic map and satellite image, at Poonjar (rubber area), Vandipperiya (tea area) and Udumbanchola (cardamom area). Sampling sites (Rubber-37, Tea-30, Cardamom-34) were identified for groundwater and surface water, mapped the sites and verified the distribution. Water samples were collected during the post-monsoon season of 2015 and analyzed for important physico-chemical and bacteriological parameters. Preliminary results showed differences in electrical conductivity, total dissolved solids, salinity and hardness between different systems. Bacteriological analysis showed that water samples from all the three watersheds contained total Coliforms and *E. coli* above the limits specified for drinking water.

The experiment to study the changes in soil properties during a cycle of rubber cultivation was continued and soil samples collected from RRII field which was in the end phase of second cycle are being analyzed.

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

Soil fertility maps of all the districts of Kerala, rubber growing regions of Tamil Nadu and Karnataka were prepared. For soil health monitoring, profile soil samples were collected from 22 forest sites adjacent to rubber plantations and analysis is in progress.

### FERTILIZER ADVISORY GROUP

Site specific nutrient management recommendations to small growers and large estates were provided on the basis of analysis of soil and leaf samples received at the laboratory. Soil and leaf analysis services were provided through laboratory at RRII and the seven regional laboratories. Advices on fertilizer use were provided during the visit of the growers to the laboratory or as clarifications on telephonic enquiries. Estimation of dry rubber content of the latex samples were continued in the regional laboratories.

- Offered site specific fertilizer recommendation to 700 individual fields from 25 large estates on the basis of analysis of 503 leaf samples and 996 soil samples.
- 149 leaf samples and 1115 soil samples from smallholdings were analyzed and offered site specific fertilizer recommendations to smallholders.
- 70720 latex samples were tested for dry rubber content.
- Advices were provided to 114 smallholders through telephone and during visit of the farmers to RRII.

Table FAG 1. Details of soil, leaf and latex analysis

Parameter	Number	Revenue (Rs.)
Soil	2011	1,59,474.00
Leaf	652	1,48,612.00
DRC of latex samples	70720	36,19,946.00
Total revenue (Rs.)		39,28,032.00

### CLIMATE CHANGE AND ECOSYSTEM STUDIES

Studies are being carried out for development of information system on rubber cultivation using Remote Sensing (RS) and Geographic Information System (GIS) to identify rubber growing areas and also to identify suitable areas where rubber cultivation can be extended in the country. Studies on impact of extreme weather events on rubber cultivation, carbon sequestration and bio-diversity conservation are continuing. Agroclimate data in traditional and non-traditional rubber growing regions were updated and being studied to address climate change issues in rubber growing regions.

#### 1. Development of rubber based information system using RS and GIS

Estimation of NR growing areas of Andhra Pradesh was completed using Landsat satellite

data for March 2014 with spatial resolution of 30m. Satellite based NR acreage of East Godavari district in Andhra Pradesh for the year 2014 was 240 ha. Majority of the NR holdings were distributed in Rampachodavaram taluk of East Godavari district.

#### 1.1. Leaf area index (LAI) of rubber trees

For understanding the general trend of LAI of rubber trees, month-wise measurements were taken from mature rubber trees from the field from January to December 2015 using Sunscan Canopy Analyzer. During defoliation (wintering period) LAI was below one. LAI of the rubber tree was found to attain peak during April (5.4) and August (5.1) seasons after full refoliation.

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

Rubber plantation area and waste lands suitable for NR cultivation were estimated from Karbi Anglong, Hailakandi and Golaghat districts of Assam using high resolution satellite images. Satellite data pre-processing such as ortho-rectification, mosaicking, clipping and PAN sharpening were carried out. Detailed interpretation of NR and waste lands were carried out using PAN sharpened Cartosat satellite images (Table CCES. 1). Geo-spatial distribution of NR holdings and waste lands suitable for rubber cultivation in these three districts of Assam were also carried out.

Table CCES. 1. Satellite based NR area and waste lands suitable for NR cultivation in three districts of Assam

State/District	NR area (ha)	Suitable lands (ha)
Karbi Anglong	3872	6052
Hailakandi	293	619
Golaghat	500	98

### 1.3. GIS based soil fertility mapping

Collaborative project on GIS based soil fertility mapping of the traditional and Konkani region was completed during the year. Kriging analysis was carried out to interpolate soil fertility parameters of rubber growing districts of Kerala, Tamil Nadu, Karnataka, Maharashtra and Goa. District-wise interpolation maps were prepared for major, micro and secondary nutrients for the region. About 352 soil fertility maps have been prepared in GIS platform.

### 1.4. GIS based watershed delineation of tea, cardamom and rubber ecosystems

Tea, cardamom and rubber watersheds were delineated using Survey of India (SOI) toposheets,

satellite data, Digital Elevation Model (DEM) and ground truth data for the collaborative project of Agronomy and Soils Division on water quality. Watershed boundary, drainage network, road and locations were vectorized in GIS platform. Global positioning system (GPS) based field survey was conducted in Poonjar watershed and a total of 34 water sampling sites were located for water quality monitoring.

## 2. Climate change studies

### 2.1. Climate change studies in the rubber growing tracts of India

The study aims at characterizing and comparing the temperature and rainfall extremes with the help of change points statistically detected in observed time series observations for the traditional rubber growing regions.

In the absence of a long term dataset (>30 years) for most rubber growing locations, the study utilizing the technique of climate change point identification could identify significant changes in the available datasets for these parameters. By fitting appropriate frequency distributions to the 'before' and 'after' change points, the probability of exceedence for threshold values with corresponding return periods (time period of occurrence) was determined. This could predict how the respective variability and extremes would behave in future, so as to know its impact on rubber. The study has been conducted by way of cumulative sum charts (CUSUM) and bootstrapping methods for the detection of abrupt changes in the time series data known as change points. Seasonal mean maximum temperature, minimum temperature and rainfall data of RRII, Kottayam, Kerala (from 1956 to 2016) and Regional Research Stations viz. Chethackal, Kerala (from 1994-2014), Padiyoor, Kerala (from 1998-2016), Paraliar, Tamil Nadu (from 1995-2016), Nettana,



Karnataka (from 1995-2016) and Dapchhari, Maharashtra (from 1998-2016) were used.

Warming tendencies were consistently observed in majority of the stations during the winter season from 1998 onwards. Kottayam showed increase in maximum and minimum temperatures during the winter season. With increasing frequency of hot seasons, the intensity of warming was observed to be high for Kottayam during winter. The frequency of winter seasonal maximum temperature occurrence of  $>33.5^{\circ}\text{C}$  increased from a probability of one in 10 years to that of once every year after 2002. During the post-monsoon season, Dapchhari station registered two change points during the pre and post-monsoon seasons in the years 2002 and 2000, respectively. Paraliar and Chethackal showed a decrease in the minimum temperature after the change point in 2009 and 2004, respectively during winter. Extreme values were observed in maximum and minimum temperatures for Padiyoor during the winter and post-monsoon season, respectively. While in the traditional belt, the pre-monsoon rainfall showed an increase in the occurrence at Chethackal, it showed a decrease during the post-monsoon season at Nettana. Earlier, it was detected that rainfall during the winter months in Agartala and Guwahati showed a significant decrease in the occurrence.

## 2.2. Identification and mapping of suitable areas for NR cultivation in the Left Wing Extremism affected (LWE) and NE states in India

Identification of agroclimatic suitability for NR cultivation for the 10 Left Wing Extremism affected states (LWE) in India is being undertaken. Initially seven climatic parameters were tested for 30 districts of Odisha state. These were maximum temperature, rainfall,

rainy days, vapour pressure deficit, potential evapotranspiration, aridity index and annual degree days. These data, obtained for the period of 1901-2002 were plotted and compared based on the Area-Under-Curve (AUC) comparison with the respective parameter of Kottayam district. The overall climatic suitability in Odisha was also evaluated based on the percentage differences in AUCs of each district at  $<10$  and  $<20$  per cent favourability from the Kottayam AUC. The AUC differences were observed to be very high for the rainfall, rainy days and therefore the Aridity Index. None of the districts in Odisha can be classified as suitable when compared to traditional areas or non-traditional North East districts where rubber is cultivated as a rainfed crop. This analysis also showed that the single parameter of Aridity Index which is defined as the ratio of monthly precipitation and potential evapotranspiration could be utilized to define the suitability of any place for NR cultivation. This parameter is utilized to identify favourable districts in all the 10 LWE states. In order to detect any long-term differences in Aridity Index, two periods viz. 1901-1955 and 1956-2002 are separately analyzed for climate suitability. A total of 287 districts from 10 LWE states have been considered for the analysis. Work has been carried out for 172 districts spanning eight states and analysis for the rest of the districts is progressing.

## 2.3. Weather conditions in the NR growing regions during 2015

The principal meteorological station in Kottayam recorded an annual rainfall of 2629 mm (138 rainy days) with 32 per cent less rainfall obtained during the SW monsoon season from the long-term average. The Kerala region received 26 per cent less rainfall during the period likely



due to the El Nino effect, while Karnataka and Tamil Nadu received normal rainfall. Rainfall during the SW monsoon period was lower than the long-term average for most traditional rubber growing stations. All the stations in the traditional research regions received less than average annual rainfall except for Chethackal, which received 10 per cent more annual rainfall than its long-term mean. Dapchari in Maharashtra received a low annual rainfall which was 34 per cent less than the long-term average of 2506 mm. Maximum temperature of 37.0 °C for two days was recorded during the last week of March and first week of April. A heat wave condition existed in the state of Kerala for the first time during the last week of March and first week of April, 2015. During the ensuing pre-monsoon season the maximum and minimum temperatures recorded for the season were higher than the average in Kottayam and Chethackal, while Paraliar and Padiyoor almost recorded the respective long-term mean annual rainfall for 2015. Maximum temperature fluctuated from 30.0 to 30.4 °C and minimum temperature fluctuated from 20.4 to 24 °C for the stations situated in Kerala.

#### 2.4. Climate resource characterisation of rubber growing areas

Keeping the temperature profile of traditional region as standard, suitability of temperatures were classified separately for maximum temperature ( $T_{max}$ ) and minimum temperature ( $T_{min}$ ), grouped the identical locations and ranked the locations in order of suitability for each season for each agroclimatic region.  $T_{max}$  profile during SW monsoon was most favourable in Nettana, followed by Kottayam and Padiyoor, which were on par.  $T_{max}$  profiles were not found optimum in Guwahati, Agartala and Nagrakata.  $T_{min}$  profiles

were also found not optimum in Agartala followed by Nagrakata, Depchari and Guwahati.  $T_{min}$  during SW monsoon was most favourable in Nettana followed by Padiyoor and Kottayam.

It was observed that temperature regime prevailing in NE during June-September remains fairly high compared to that of traditional area against the accepted notion that low minimum during winter is the only abiotic stress acting in NE. Maximum and minimum temperature (mean) profiles in the eight locations could be served as an indicator of multi-seasonal abiotic stress in different NR growing areas in India.

Another interesting observation is that  $T_{max}$  profiles during winter season were optimum in Agartala, Guwahati and Nagrakata and not in optimum range in Padiyoor followed by Nettana. Stations like Parliar, Kottayam and Depchari received moderate winter temperatures. During winter  $T_{min}$  is optimum in Parliar, Kottayam and Padiyoor. Unfavorable  $T_{min}$  profiles were observed in Nagrakata followed by Guwahati and Agartala.

Analysis of the precipitation regime revealed that rainfall received during SW monsoon may not be able to combat the high temperatures in addition to the fact that entire NE is land locked. Hence proper agro management solutions need to be evolved, so as to keep the microclimate near optimum for better yield.

### 3. Ecosystem studies

#### 3.1. Vegetation dynamics in rubber ecosystems

A study has been initiated on the vegetation dynamics of rubber ecosystems which analyses spatial and temporal variation of vegetation structure and composition.

## BOTANY DIVISION

One of the major thrusts of crop improvement efforts during the year was introduction of elite domesticated *Hevea* germplasm via multilateral clone exchange programmes with other rubber growing countries. A total of 39 promising clones evolved in IRRDB member countries viz. Vietnam, China, Thailand, Brazil, Indonesia, Philippines, Cote de Ivoire and Sri Lanka were introduced. 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 fifth phase of the Farmer Participatory Clone Evaluation programme with 22 pipeline clones was launched with the establishment of field experiments in seven locations. Studies on response to stimulation in pipeline clones were continued.

## 1. Evolving high yielding clones for the traditional area

### 1.1. Hybridization and clonal selection

Twenty six hybrids from the 1990 hybridization programme (Wickham x Wild germplasm) were assessed in the 12<sup>th</sup> year of tapping. Selections continued to show superior performance in terms of yield compared to parental check clone (Fig. Bot. 1). Top selection 90/10 (RRII 105 x RO 142) showed maximum yield of more than 67 g t<sup>-1</sup>. Mean yield over 12 years also established superior performance of the selections. An assessment was made to find clonal variation with reference to abnormal leaf fall disease. Hybrids with better leaf retention were identified. Maximum (>59%) leaf retention was recorded in progenies of family RRII 105 x RO 142.

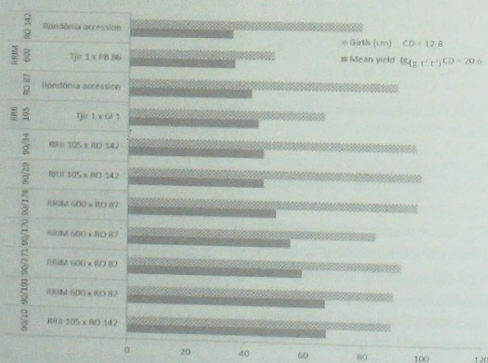


Fig. Bot. 1. Girth and annual mean yield of WxA selections in SST (1995)

Out of 34 hybrid clones under evaluation in an SST (1995), 23 clones had better girth than the check clone. Mean yield ranged from 23.8 to 101.1 g t<sup>-1</sup> t<sup>-1</sup> with check clone RR11 105 having 45.7 g t<sup>-1</sup> t<sup>-1</sup>. Four years' mean yield data revealed two clones (89/27 & 89/145) with significantly higher yield than the check clone RR11 105. These clones also had better tolerance to ALF. Clone 89/27 which continued to yield more than 100 g t<sup>-1</sup> t<sup>-1</sup> with superior girth was included in the Participatory Clone Evaluation (Phase IV) for further evaluation and selection.

Among the 17 hybrids in SST 1995 C (W x A hybridization programme), highest girth was recorded in 90/55 (96.1 cm) followed by 90/109 (88.3 cm) and 90/129 (88.0 cm). Highest yield was recorded in 90/109 (58.4 g t<sup>-1</sup> t<sup>-1</sup>) followed by 90/55 (55.7 g t<sup>-1</sup> t<sup>-1</sup>) and RR11 105 (54.1 g t<sup>-1</sup> t<sup>-1</sup>). In the small scale evaluations of hybrid clones laid out at CES in 1998, clones 93/98 and 93/48 were found superior to the check clone RR11 105 in terms of mean yield of four years. In SST 1999A, highest girth was recorded in hybrid clone 95/62 (86.8 cm) followed by 95/124 (85.5 cm) and 95/575 (81.8 cm). Highest yield was recorded in RR11 203 (53.8 g t<sup>-1</sup> t<sup>-1</sup>), 95/131 (48.6 g t<sup>-1</sup> t<sup>-1</sup>) and RR11 105 (46.5 g t<sup>-1</sup> t<sup>-1</sup>). Analysis of data on annual mean yield in 1999 SST B showed that there was no significant variation for the trait among the hybrids in the 9<sup>th</sup> year of tapping. Top hybrid selections 95/306 and 95/346 exhibited more yield than RR11 105. These selections maintained superior yield trend based on mean yield over nine years of tapping.

In another SST laid out in 1999, out of 24 hybrid clones, five registered superior performance based on mean yield over seven years and based on the growth over 16 years. The selected clones were 94/87, 92, 296, 567, 560 and 92. Highest mean yield was 63.0 g t<sup>-1</sup> t<sup>-1</sup>

registered by clone 92 when compared to RR11 105 (38.0 g t<sup>-1</sup> t<sup>-1</sup>) as control. Selected clones were pollarded for multiplication and their budwood nursery was raised for further trials.

Out of 24 hybrid clones under evaluation in SST 2001A, two clones viz. 95/413 (RR11 600 x RR11 203) and 95/425 (RR11 600 x RR11 203) with 59.0 g t<sup>-1</sup> t<sup>-1</sup> registered superior yield in the 6<sup>th</sup> year of tapping and growth after 14<sup>th</sup> year while checks registered 29.0 g t<sup>-1</sup> t<sup>-1</sup> (RR11 105) and 43.0 g t<sup>-1</sup> t<sup>-1</sup> (RR11 203). In SST 2001B, out of 14 hybrid clones, 95/121 (RR11 105 x PB 28/59) registered superior yield (50.0 g t<sup>-1</sup> t<sup>-1</sup>) over six years and 14<sup>th</sup> year of growth when compared to check RR11 105 (35.0 g t<sup>-1</sup> t<sup>-1</sup>). In SST 2001C, clones 95/304 (82.9 cm), 95/514 (74.0 cm) and 95/410 (66.0 cm) registered exceptional growth when compared to control (RR11 105, 57 cm). Clone 95/410 (RR11 600 x RR11 203) and 95/304 (RR11 x RR11 118) maintained higher yield continuously for six years. In the small scale evaluation (2003), out of 36 hybrid clones, two clones viz. 96/417 (58.0 g t<sup>-1</sup> t<sup>-1</sup>) and 96/459 (51.0 g t<sup>-1</sup> t<sup>-1</sup>) registered superior yield than check clone RR11 105 (38.0 g t<sup>-1</sup> t<sup>-1</sup>) over three years.

In order to widen the genetic base of breeding populations and to develop improved clones by crossing promising Wickham x Amazonian hybrids (W x A) with the RR11 400 series clones and RR11 105, progenies were developed every year between 2011 and 2014 followed by planting in seedling nurseries along with half sibs collected from female parents. Female parents used were RR11 105, RR11 414, RR11 429, RR11 430 and male parents included three W x A hybrids viz. 95/10, 95/34 and 95/274. A total of 353 hybrid seedlings were obtained from 14703 hand pollinations (Table Bot. 1) which are being maintained along with 1000 half-sib seedlings in the seedling nursery.



Table Bot. I. Details of hand pollinations performed and hybrids obtained using W x A hybrids as male parents

Female parents	Male parents (W x A)					
	Clone 95/10		Clone 95/34		Clone 95/274	
	No. of hand pollinations	No. of hybrids obtained	No. of hand pollinations	No. of hybrids obtained	No. of hand pollinations	No. of hybrids obtained
RRII 414	4016	62	1566	81	240	-
RRII 417	61	-	38	-	124	-
RRII 422	92	-	367	-	162	-
RRII 429	362	-	699	6	-	-
RRII 430	883	63	260	6	420	23
RRII 105	1736	13	3150	99	527	-

Seedlings planted in 2012 were test tapped consecutively for two years during 2014 and 2015. Out of 275 seedlings (3<sup>rd</sup> year of planting), five seedlings yielded more than 100 g 10 tap<sup>-1</sup> and 25 seedlings yielded more than 50 g 10 tap<sup>-1</sup>. Seedlings planted in 2013 were also test tapped during 2015 in which three half-sib seedlings yielded more than 50 g 10 tap<sup>-1</sup>, two from RRII 414 and one from RRII 105. Out of the 175 HP and 302 half-sib seedlings, 18 and 45 seedlings respectively showed more than 20 g 10 tap<sup>-1</sup>.

In order to study and document characteristic features of different clones with regard to flowering attributes and to develop improved clones by HP, a breeding orchard was maintained at RRII consisting of 48 elite clones. Flowering was observed in 24 clones during the normal flowering season within a window period from February to March. Female phase lasted for approximately two weeks in each clone. Recording of the flowering pattern was carried out in clones which flowered. Clones with synchrony in flowering were identified in order to select parents for crossing. Preliminary observations regarding breeding in different clones especially wintering pattern, off-seasonal

flowering, flower initiation, maturation of male and female flowers and duration of fruit maturity were recorded in order to generate a database and to strategize future breeding programmes. A total of 6056 hand pollinations were carried out involving 22 cross combinations.

## 1.2. Ortet selection

In the clonal nursery evaluation trial (2012) of 11 ortets (selected from South Andamans), ortet 99/25 showed the highest girth (12.0 cm) compared to RRII 430 (11.0 cm) when eight ortets were found superior to RRII 105 (8 cm). In the clonal nursery of ortets/hybrids at RRII (2007B), the secondary traits including growth and bark anatomy were studied. Among the promising selections for yield, Par 18 and Par 10 from Paraliar and Guw 4 and Guw 9 from Guwahati had growth on par with RRII 414 (the check clone with superior girth) and except Guw 9 all the clones recorded more latex vessel rows that were comparable to the check clone RRII 430. The clones Par 9 and Guw 9 exhibited better response to stimulation.

In the SST of ortets (1995), two ortets continued to show consistently superior performance



than RRII 105 (50-64 % yield improvement) of which one clone was found suitable as latex timber clone. The ortets 072 (83.4 g t<sup>-1</sup> t<sup>-1</sup>) and 073 (76.5 g t<sup>-1</sup> t<sup>-1</sup>) exhibited significantly superior yield (11<sup>th</sup> year of tapping) over RRII 105 (45 g t<sup>-1</sup> t<sup>-1</sup>). In terms of mean yield over 11 years of tapping also they were significantly superior (73-77 g t<sup>-1</sup> t<sup>-1</sup>) when compared to RRII 105 (47.5 g t<sup>-1</sup> t<sup>-1</sup>). Highest girth (90 cm) was exhibited by the ortet P 073.

## 2. Evaluation of clones

### 2.1. Large scale evaluation

There was significant clonal variation in annual mean yield and summer yield in both the large scale trials of RRII 400 series clones at CES. Clones RRII 430 and PB 330 were superior to RRII 105 with respect to yield over three years in the renewed bark panels. RRII 105 had 70.1 and 57.3 g t<sup>-1</sup> t<sup>-1</sup> yield in Trial A and B, respectively. Clones RRII 417, RRII 52, RRII 410, RRII 55, RRII 403, RRII 429, RRII 414 and RRII 422 maintained yield over 60.0 g t<sup>-1</sup> t<sup>-1</sup> in the renewed bark and were on par with the check. Clone PB 330 responded well to stimulation and was superior to the rest of the clones with an yield of 90.5 g t<sup>-1</sup> t<sup>-1</sup> in the 15<sup>th</sup> year of tapping under stimulation thrice a year.

In order to assess the performance of RRII 400 series clones (BI 1 panel) yield parameters were studied. In Trial 1, RRII 414 showed highest total latex volume during peak (283 ml t<sup>-1</sup> t<sup>-1</sup>) as well as summer (122 ml t<sup>-1</sup> t<sup>-1</sup>). RRII 417, RRII 403, RRII 429 and RRII 105 showed statistically comparable total latex volume with RRII 414 during peak season, whereas, during summer only RRII 417 was statistically comparable. RRII 430 showed highest DRC both during summer as well as peak. DRC in RRII 414, RRII 429 and RRII 417 were comparable with that of RRII 105 in both seasons except in RRII 417 in which summer DRC was inferior. RRII 414 showed

highest dry rubber yield during peak (112.0 g t<sup>-1</sup> t<sup>-1</sup>) as well as summer (52.0 g t<sup>-1</sup> t<sup>-1</sup>). RRII 417, RRII 429 and RRII 105 showed statistically comparable dry rubber yield with RRII 414 during peak season, whereas during summer RRII 429 was statistically inferior.

In Trial 2, among RRII clones, RRII 430 showed highest total latex volume during peak (260 ml t<sup>-1</sup>) as well as summer (101 ml t<sup>-1</sup>) which were statistically comparable with PB 330 having 275 and 115 ml t<sup>-1</sup> respectively. RRII 105 and RRII 410 also showed statistically comparable total latex volume with RRII 430 during peak as well as summer season, whereas RRII 422 was statistically comparable during summer only. Among RRII clones, RRII 430 showed highest DRC both during summer as well as peak seasons. Dry rubber content in RRII 430, RRII 422, RRII 410 and PB 330 were statistically comparable during both the seasons whereas DRC was comparatively inferior during peak season in RRII 105. Among RRII clones, RRII 430 showed highest DRC during peak (122.0 g t<sup>-1</sup> t<sup>-1</sup>) as well as summer (50.0 g t<sup>-1</sup> t<sup>-1</sup>). Dry rubber yield of RRII 105 and RRII 422 were statistically inferior to RRII 430 during peak season. In the Large Scale Trial (1989) at CES on stimulation response, PB 280 continued to maintain superior yield with more than 80 g t<sup>-1</sup> t<sup>-1</sup> followed by PB 217 as compared to control RRII 105 (54.0 g t<sup>-1</sup> t<sup>-1</sup>) during 18<sup>th</sup> year of tapping. When clones were assessed after stimulation, significant difference in yield could not be observed. Though response to stimulation varied among clones, PB 312 responded better with 83 per cent increase over mean yield.

In the 1994 LST at CES (11<sup>th</sup> year of tapping and 21<sup>st</sup> year after planting), highest girth was recorded in 86/120 (96.3 cm) followed by O 65 (93.4 cm) and 55/180 (79.8 cm). Superior yield

was observed in 86/120 (63 g t<sup>-1</sup> t<sup>-1</sup>) followed by RRII 722 (61 g t<sup>-1</sup> t<sup>-1</sup>), RRII 712 (55.6 g t<sup>-1</sup> t<sup>-1</sup>) and O 65 (54.6 g t<sup>-1</sup> t<sup>-1</sup>). In the LST (1996 B at RRS, Padiyoor), containing a set of 13 hybrid clones, three clones *viz.* 86/468, 613 and 674 showed better yield than RRII 105 in the BO-1 (50.0 – 60.0 g t<sup>-1</sup> t<sup>-1</sup>) and BO-2 (60.0 g t<sup>-1</sup> t<sup>-1</sup> over 3 years) panels. Clone 86/468 was the highest yielder over eight years of tapping. In the Large Scale Trial of ortets from Koney and Mundakkayam estates, in terms of girth, RRII 430 was superior. Ortets MO 28, MO 45, MO 12 and KO 7 were found on par with RRII 430. Based on second year yield data, control clones *viz.* RRII 105 and RRII 430 were found superior (about 50.0 g t<sup>-1</sup> t<sup>-1</sup>) while ortets KO 27 and MO 7 were found on par.

In another LST of nine promising ortets from Cheruvally estate, three ortet clones (Cy 48, Cy 41 and Cy 35) showed significantly superior girth compared to the check clone RRII 105. Mean yield in the first two years of tapping ranged from 12.3 to 38.6 g t<sup>-1</sup> t<sup>-1</sup> with RRII 105 having

28.3 g t<sup>-1</sup> t<sup>-1</sup>. In terms of mean yield (first two years of tapping), six ortet clones were comparable with check clone RRII 105.

## 2.2. On-farm evaluation

Post release observations on RRII 400 series clones in smallholdings across North, Central and South Kerala were continued. The performance of RRII 400 series clones was assessed in four smallholdings in the Malabar Region at Ottapalam, Thrissur, Wandoor and Mannarakadu (Tables Bot. 2 & 3). At Thrissur, yield of RRII 414 (53.6 g t<sup>-1</sup> t<sup>-1</sup>) was comparable with RRII 105 (59.2 g t<sup>-1</sup> t<sup>-1</sup>). Bark thickness of RRII 414 was 8.7 cm while that of RRII 105 was 9.1 cm. Among other locations, RRII 414 at Wandoor, RRII 417 at Ottapalam and RRII 429 at Mannarakadu showed better yield. Over the locations, bark thickness of RRII 400 series clones was comparable with RRII 105.

As part of the post release study, yield recording was carried out from eight locations in Central Kerala. Location - wise variation for yield was evident for the RRII 400 series clones (Table

Table Bot. 2. Mean bark thickness (mm) of RRII 400 series clones in Malabar region

Location	RRII 403	RRII 414	RRII 417	RRII 422	RRII 429	RRII 105
Wandoor	7	9.1	-	9.8	-	9.5
Thrissur	8.0	8.7	-	8.7	-	9.1
Mannarkadu	-	8.0	-	-	6.8	7.7
Pallaramangalam	-	-	8.4	-	-	9.5
Mean	7.5	8.6	8.4	9.3	6.8	9.0

Table Bot. 3. Mean yield (g t<sup>-1</sup> t<sup>-1</sup>) of RRII 400 series clones over three years of tapping

Location	RRII 403	RRII 414	RRII 417	RRII 422	RRII 429	RRII 105
Wandoor*	47.6	54.9	-	44.0	-	64.2
Mannarkadu*	-	43.3	-	-	43.6	48.0
Thrissur**	46.3	53.6	-	54.6	-	59.2
Pallaramangalam**	-	-	44.7	-	-	39.5
Overall mean yield	46.9	50.6	44.7	49.3	43.6	52.7

\*Mean yield over 3 years; \*\*Mean yield over 4 years

Bot. 4). Of the eight locations, RRII 400 series clones recorded superior yield over RRII 105 in six locations. Mean yield of RRII 400 series clones across the locations showed that all clones except RRII 429 recorded higher yield than the control clone RRII 105. Highest girth was recorded for RRII 414.

Table Bot. 4. Mean yield of RRII 400 series clones across locations in Central Kerala

Clone	Yield ( $\text{g t}^{-1} \text{t}^{-1}$ )
RRII 414	54.3
RRII 417	58.7
RRII 422	52.8
RRII 429	42.2
RRII 430	57.4
RRII 105	48.9

In an on farm trial of six clones (1992) at Shaliackary Estate, Punalur, considerable increase in yield was found under the combined system of CUT with stimulation followed by normal tapping with stimulation in lower panel. Overall mean yield of the clones in the higher panel was  $2166 \text{ kg ha}^{-1} \text{yr}^{-1}$  with 59 per cent increase over the yield in lower panel under normal tapping system. The experimental clones maintained almost similar yield trend comparable to yield of the virgin panel. Clone RRII 5 followed by PB 280 and PR 255 had better yield compared to RRII 105. Clones RRII 5, PB 280, PB 260 and PR 261 showed better response to CUT with increase in yield ranging from 54 to 76 per cent compared to normal tapping in BO-I panel. In another OFT at Ayiranallur, PB and PR clones (7<sup>th</sup> year of tapping), PB 217 and PB 280 showed comparable yield of more than  $1500 \text{ kg ha}^{-1} \text{yr}^{-1}$ . However, the check clone RRII 105 showed higher yield with more than  $2000 \text{ kg ha}^{-1} \text{yr}^{-1}$ .

### 2.3. Genetic studies and investigations on genotype x environment interactions

In the G x E interaction studies (1996) across five locations, over 12 years of yield data has been generated from three locations viz. Kanyakumari (KYN), Agartala (AGR), Nagrakata (NGK), and yield over nine years from Padiyoor (PDR) and over four years from Bhuvanewar (BBN). Mean yield of high yielding clones of RRII 400 series across locations along with check clones RRII 105 (traditional region) and RRIM 600 (non-traditional region) is given in Fig. Bot. 2. Single site analysis followed by mixed model analysis for G x E interaction for long term yield data was processed.

For the study of G x E interaction in pipeline clones, growth and yield of pipeline clones was assessed under cold and drought conditions at Agartala and Dapchhari, respectively. At Dapchhari, girth of clones ranged from 7 to 14 cm after three years of growth. Among the pipeline clones, P 110 excelled with more than 14 cm followed by P 102, P 63 and P 70. RRII 208 (13 cm) and RRII 430 (12 cm) performed better than RRII 105 (11 cm). In the first test-tap, P 26 topped with  $25 \text{ g t}^{-1} \text{tap}^{-1}$  followed by P 70, P 92 and RRII 430. RRII 208 gave  $18 \text{ g t}^{-1} \text{tap}^{-1}$ . At Agartala, 13 pipeline clones showed better yield than RRIM 600. P 107 excelled with  $80 \text{ g t}^{-1} \text{tap}^{-1}$  followed by P 17 and RRII 430, in the first year. P 107 had maximum girth. The growth of P 26 and P 20 were comparable to RRII 430 at both locations, indicating their adaptability to cold and drought conditions.

Under the project on physiological evaluation for drought/cold tolerance in pipeline clones, 45 pipeline clones grown in polybags at Regional Research Stations at North Konkan Region (Maharashtra) and Agartala (Tripura) were allowed to experience drought stress during summer and low temperature stress during winter



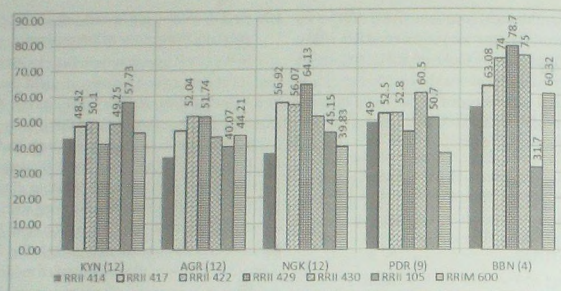


Fig. Bot. 2. Mean yield of RRII 400 series clones across different locations

respectively. Gas exchange parameters were measured in 12 clones selected based on membrane stability, chlorophyll index and growth parameters. In terms of  $CO_2$  assimilation, the pipeline clones P 20, P 73 and P 57 performed better than the tolerant check clones RRIM 600, RRII 430 and RRII 208 under drought conditions. Percentage of drought influenced inhibition in  $CO_2$  assimilation was at minimal in these clones. In terms of transpiration and photosynthetic efficiency, the clones P 20 and P 73 performed better while P 20, P 21 and P 73 displayed least membrane injury and stayed green under drought stress. In terms of overall performance, P 20 and P 73 were found superior in drought prone region. Similarly under cold stress, performance of P 078, P 083, P 01 and P 101 was found on par with the check clones RRII 429 and RRII 208 in terms of  $CO_2$  assimilation. In terms of photosynthetic efficiency, P 01, P 71, P 83 and P 76 were found superior under cold stress. In total, the clone P 01 was found superior under cold stress in terms of parameters investigated. Among the check clones, RRII 429 was found superior both in terms of drought and cold tolerance. This multi-

locational study could identify potential drought and cold tolerant pipeline clones.

### 3. Participatory evaluation of rubber clones in the pipeline

#### 3.1. Source bush nurseries of pipeline clones

Thirty promising selections from half-sib progenies were added to the list of pipeline clones during the reporting period and planted in source bush nurseries at CES, raising the number of pipeline clones to 267. These pipeline clones are maintained in 12 source bush nurseries at CES, Chethackal.

#### 3.2. Phase 1 trials

Under Phase 1, 20 pipeline clones and three checks viz. RRII 105, RRII 414 and RRII 430 are under evaluation in 13 field trials at 12 sites. Girth of clones was recorded and tapping was initiated (7 years after planting) in the Central Large Scale Trials at CES. Yield recording has been initiated. In general, the check clones RRII 414 and RRII 430 exhibited very high girth and tappability.

In the Central Large Scale Trials, there was significant clonal variation for growth in both the



batches of clones in the year of opening. In LST 1, among clones in batch 1, RRII 414, P 021, P 061 and RRII 430 recorded girth at opening superior to RRII 105. Clones RRII 414, RRII 430, P 061 and P 088 recorded high tappability of > 70 per cent. Among the second batch of clones in LST 2, four clones viz. RRII 414, P 070, P 044 and P 026 were found comparable in girth and superior to RRII 105 in the year of opening. Tappability was highest in RRII 414 followed by P 044, P 070, RRII 430, P 026 and P 064 with < 70 per cent tappable trees.

In the multilocal on farm trials also, there was significant variation in growth of clones in both the batches. Among clones in Batch 1, RRII 414, RRII 430, P 021 and P 061 were found superior in girth to RRII 105. Clone RRII 414 exhibited highest girth and tappability across locations. Clones RRII 430, P 061, P 021, P 010, P 074 and P 068 attained more than 50 per cent tappability across locations in the 7<sup>th</sup> year. In Vithura, these clones attained tappability in the 6<sup>th</sup> year after planting. Tapping was initiated in all the OFTs at Calicut, Mooply, Manikkal and Adirappally estates. Among clones in Batch 2, clones RRII 414, RRII 430, P 065, P 078, P 026, P 072 and P 069 registered girth of above 50 cm in the 7<sup>th</sup> year. RRII 414, RRII 430 and P 063 were vigorous across locations. In terms of tappability, clone RRII 414 attained < 78 per cent tappability across locations and clones P 026, RRII 430 and P 063 had more than 60 per cent tappable trees across locations. Tapping was also initiated in Batch 2 clones after seven years of planting in the on-farm trials in Kootickal and Be Be estates.

### 3.3. Immature trials under Phases 2 to 5

When growth of Central Large Scale trial at CES and On-Farm Trials in eight locations under Phase 2 established in 2010 was monitored (6<sup>th</sup> year after planting), check clones RRII 430 and RRII 414 were found most vigorous (CES,

Chethackal). Pipeline clones P 044, P 080 and P 070 were comparable to the vigorous checks. In terms of girth, clones RRII 414 and RRII 430 were the most vigorous in six out of eight locations. Pipeline clones P 044, P 054 and P 070 exhibited better growth. Girth of clones in the fourth year in the Central LST at CES and the six OFTs under Phase 3, laid out in 2012 was monitored. The Central LST under Phase 4 laid out at RRII farm, Kottayam and the seven OFTs in various locations were also monitored for proper growth and maintenance.

Polybag nurseries of 22 pipeline clones under Phase 5 raised for field planting were maintained in seven locations for the central LST at CES, Chethackal, and for on farm trials in six locations (RPL, Kulathupuzha, SFCK, Chithalveti, SFCK, Cherupittakkavu, KFDC, Subramanya Estate, Karnataka; Paalali Estate, Kulasekharan, ARC Estate, Kanyakumari). Growth of pipeline clones planted in various locations under the first four phases of the project was monitored.

## 4. Breeding for other specific objectives

### 4.1. Breeding for drought tolerance

Evaluation of 13 ortets selected from Dapchari, North Konkan for yield, growth and the structural and physiological components of yield over six years of tapping in panel BO-1 under 1/2S d3 6d/7 system of tapping without stimulation led to the identification of two clones viz. D 111 and D 37 which were superior for most of the traits. Ortet clone D 111 gave a very high yield of 90.8 g t<sup>-1</sup> t<sup>-1</sup> in panel BO-1 and had the highest summer yield of 64.4 g t<sup>-1</sup> t<sup>-1</sup> which was significantly superior to the check and the rest of the ortet clones. This was followed by clone D 37 (62.7 g t<sup>-1</sup> t<sup>-1</sup>) which was comparable to the check RRII 105 (63.9 g t<sup>-1</sup> t<sup>-1</sup>) in terms of annual mean yield in panel BO-1 and was superior to the check in summer yield which was

as high as 44.11 g t<sup>-1</sup> t<sup>-1</sup>. Four more ortet clones viz. D 236, D 95, D 35 and D 173 with yield of more than 50 g t<sup>-1</sup> t<sup>-1</sup> were on par with the high yielding check clone RRII 105. While D 111 also possesses high summer yield, D 37 was found to be a high girthing clone. Both these clones are promising in terms of yield components like a high number of latex vessel rows and high volume of latex. No incidence of tapping panel dryness was observed in these clones after six years of tapping. No association was found between the yield of the original ortets in the drought prone tract of North Konkan and that of their respective clones in Central Kerala as evidenced by positive but non-significant correlations.

In the SST (1998) consisting of 15 hybrids derived from hybridization in 1993 between high yielding and drought tolerant clones, analysis of annual mean yield in the 10<sup>th</sup> year of tapping showed highly significant variation. Selections 93/216 and 93/214, exhibited better yield than RRII 105. In general, hybrids of family RRII 105 x RRIC 52 showed good yield performance in terms of annual mean and summer yield. In a 1999 SST of hybrids from the 1994 HP, in the 9<sup>th</sup> year of tapping, among the hybrid clones, highest girth was recorded in 94/23 (82.2 cm) followed by 94/90 (81.5 cm) and 94/44 (81.2 cm). Highest yield was recorded in 94/90 (88.2 g t<sup>-1</sup> t<sup>-1</sup>) followed by 94/38 (68.0 g t<sup>-1</sup> t<sup>-1</sup>), 94/23 (50.0 g t<sup>-1</sup> t<sup>-1</sup>) and 94/50 (49.3 g t<sup>-1</sup> t<sup>-1</sup>).

In order to develop drought tolerant clones for the non-traditional area, the drought tolerance capacity of the selected progenies from a cross between high yielding parent (RRII 105) and a drought tolerant parent (PB 280) and also its reciprocal (PB 280 x RRII 105) was evaluated in a clonal nursery trial in the drought-prone area (RRS Dapchari). Forty trial clones along with nine control clones were established at RRS Dapchari. Girth of 22 experimental clones were

found statistically comparable with the top most check clone RRIM 600 out of which 12 clones (42, 58, 31, 38, 64, 83, 73, 37, 78, 100, 29 and 41) were found top yielders in the seedling nursery evaluation.

A study aimed at developing drought tolerant rootstocks for the non-traditional area by evaluating the drought tolerance capacity of the seedlings from non-traditional areas as against the seedlings from traditional areas was carried out in a nursery trial at RRS, Dapchari. Seeds were collected from three drought prone non-traditional rubber growing areas namely Maharashtra (RRS, Dapchari), Orissa (RRS, Dhenkanal) and Karnataka (HBSS, Nettana) and from traditional areas namely Kerala (CES, Chethackal) and Tamil Nadu (HBSS, Paralhar). 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. The plants were maintained under rainfed condition. Chlorophyll index was recorded from the leaves during summer as an indirect measurement of impact of drought. Shoot and root growth parameters were recorded in the plants that survived the drought. Shoot growth parameters like number of leaves, plant height, fresh and dry weight of the shoot were recorded. Root growth parameters like root length, fresh and dry weight of the root were also recorded. Budgrafting of the drought - survived seedlings at Dapchari was done using drought susceptible clone RRII 105 as scion. Budgrafting of the unselected seedlings at Central Nursery, Karikkattoor was also done using RRII 105. Both groups of plants were raised in polybags for field evaluation of the drought - survived stock as against the unselected stock at RRS, Dapchari.

In the screening of progenies of crosses involving drought tolerant parents for abiotic stresses, significant clonal variability was observed for seven major xylem vessel features among 47 clones. Vessel diameter was the most variable

trait (109-180  $\mu\text{m}$ ). Solitary vessels per unit field ranged from 2-10 whereas tylosis incidence varied from 0.4 to 13. Number of solitary vessels, grouping pattern and incidence of tylosis recorded moderately higher heritability ( $H^2$  0.42).

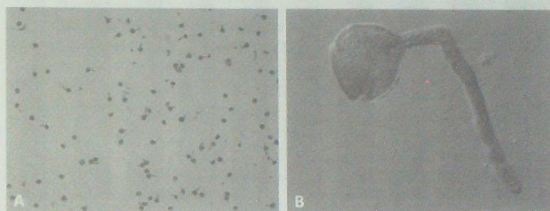


Fig. Bot. 3. Germination of pollen stored under  $-80^{\circ}\text{C}$  (a) Several pollen showing germination.  
(b) An individual pollen showing well developed germ tube photographed using Normarski's Interference Contrast Prism of Nikon Eclipse.

#### 4.2. Breeding for disease tolerance

Under the project on development of disease tolerant genotypes in *Hevea* through preferential hybridization, HP was carried out between selected parental clones. Stored and fresh pollen were used for the HP. Thirty two hybrids of specific combinations were recovered and were planted in seedling nursery. Under the experiment on evaluation and selection of open-pollinated progenies for disease tolerance, OPs were recovered from clone Fx 516. The OP progenies were planted in seedling nursery at RRII, Kottayam and CES, Chethackal. Phenotypic observation was carried out on disease incidence. In general, OPs of Fx 516 were apparently free of diseases. In order to generate *Hevea* mapping population for identification of QTL for rubber yield, timber and disease resistance traits, using RRII 414, RRII 430 and RRII 105 as female parents and Fx 516 as male parent, hand

pollination was carried out at CES, Chethackal. So far, a total of 58 putative hybrids have been recovered and planted in seedling nursery for evaluation. Hybridizations were carried out using stored and fresh pollen of Fx 516. In an experiment to find out the viability of stored pollen in different storage conditions, pollen stored at  $-80^{\circ}\text{C}$  were found to retain viability of more than 5 per cent germination (Fig. Bot. 3) even after 16 months.

#### 4.3. Polycross progeny evaluation

In the 1993 polycross progeny trials at CES, yield of selections was monitored in the renewed panel. Twenty four clones recorded higher yield in the renewed panel than the high yielding check, RRII 105. Of these, 19 clones (172, 147, 106, 27, 116, 151, 37, 117, 112, 161, 180, 132, 69, 82, 93, 19, 199, 52 and 188) with over  $60 \text{ g t}^{-1} \text{ t}^{-1}$  registered more than 20 per cent improvement over the check. Ten clones viz. 27, 116, 172, 147, 37,





Fig. Bot. 4. Growth and yield performance (3<sup>rd</sup> year of tapping) of polycross progenies

4, 117, 180, 112 and 161 showed a rise in yield in the renewed panel indicating response to stimulation. Results from the clonal nursery evaluation, 2007 at RRII farm showed clones viz. HS BD/27, HS PB/86/57, LCB 1320/30 and HS GT1/56 to perform better than RR11 105. Trees of the selections were pollarded for the subsequent production of poly bag plants.

In the observational trial of polyclonal seedlings (2005) at Chethackal, polyclonal seedlings had better girth and yield in the 3<sup>rd</sup> year of tapping (Fig. Bot. 4). Ninety four polyclonal progenies had better yield than clone RR11 105. Three progenies had more than 50 g t<sup>-1</sup> t<sup>-1</sup>. Sixty two progenies performed well above population mean of 39 g t<sup>-1</sup> t<sup>-1</sup>. Family-wise, in terms of yield RR11 203 excelled followed by Ch 26.

#### 4.4. Genetic linkage mapping using stress responsive SSR markers

In connection with this work, out of 150 primer pairs tested for PCR amplification using

the template DNA of interspecific cross (*Hevea brasiliensis* and *Hevea benthamiana*), about 60 primer pairs were found to be informative. These primers would be further employed for the construction of genetic linkage mapping.

#### 5. Anatomical investigations

The potential of the multi-layered pericycle of the callus tissue developed during the hardening process of root trainer plants is being utilised for modification of the root architecture with the expected advantages of better anchorage and absorption of soil resources. Callus tissues developed during hardening have a unique anatomy to the parental root. The pericycle of the callus may have immense potential to be used as explant in tissue culture experiments as large number of roots emerged from the pericycle maintain the polarity and microfibrillar orientation similar to the parental tissue.



Table. Bot. 5. Fibre traits ( $\mu\text{m}$ ) and specific gravity (SG) of Prang Besar (PB) clones

Clone	Parentage	Fibre traits ( $\mu\text{m}$ )			SG
		Length	Width	Wall thickness	
PB 235	PB 5/51 x PB S/78	1058.9	24.3	9.5	0.65
PB 311	RRIM 600 x PB 235	1014.1	23.7	9.1	0.66
PB 280	Primary clone	1036.3	25.0	9.8	0.66
PB 314	RRIM 600 x PB 235	1043.8	23.2	8.9	0.65
PB 312	RRIM 600 x PB 235	965.7	23.3	9.2	0.66
PB 217	PB 5/51 x PB 6/9	1017.4	24.3	9.5	0.63
PB 260	PB 5/51 x PB 49	1133.6	24.3	9.3	0.63
PB 255	PB 5/51 x PB 32/36	1033.9	25.2	10.0	0.62
RRII 105	Tjir 1 x GI 1	999.8	23.9	9.4	0.67
S.E.		25.6	0.5	0.3	
C.D. (P=0.05)		76.9	NS	0.5	NS

The root system of the plants was modified chemically to thrive over the adverse climatic conditions. Both morphology and root anatomy of the chemically modified plants were found altered. Cortical cells proliferated with irregular out line and the cells were found devoid of starch grains. Thick deposition of phenolic contents was found both in the cortical and pith cells, and 4-6 layered pericycle could be observed. The modified roots showed early symptoms of senescence.

Nine Prang Besar (PB) clones were assessed for their wood traits. Wood core samples were collected from standing trees using increment borer following non-destructive method of wood specific gravity assessment technique. Specific gravity of the clones did not show any significant difference among the clones. Specifically, the wood specific gravity ranged from 0.634 in PB 217 to 0.652 in PB 314. There was variability for fibre traits (Table Bot. 5).

#### 6. Studies on propagation

By adopting the recently standardized protocol for developing Modified Root Trainer

Plants (MRTP), planting material was raised and planted in a drought prone region, Dapchari. Modified root trainer plants with  $\frac{1}{2}$  the recommended dose of life saving irrigation recorded the highest survival rate (90%). Two year old plants were found vigorous with a height of 363 cm and 10.5 cm girth.

Following the recommendation of elephant dung as potting medium for rubber, commercial level production of root trainer plants using elephant dung was initiated in two private rubber nurseries and in the Central Nursery of the Rubber Board. Steps were taken to popularise the technology among various stakeholders through outstation training programmes. Data collected from the Forest Department revealed that the highest population density of tamed elephants in Kerala is in Kottayam district. The result can help the nursery owners for the timely collection of dung from the nearest available site. Chemical analysis of the potting media revealed that elephant dung has higher NPK content than coir pith. Ca and Mg contents were more in coir pith compared to elephant dung.

Table Bot. 6. Effect of different age of bud source on growth and yield

	Age of budwood source	Girth 7 <sup>th</sup> yr. (cm)	Tappability 7 <sup>th</sup> yr. (%)	Mean Yield. over 2 yrs. (g t <sup>-1</sup> t <sup>-1</sup> )
T1	Vdkl. 20 yrs	42.0	59.5	31.8
T2	Vdkl. 4 yrs	46.3	59.3	33.1
T3	Ktkl. 4 yrs	46.2	70.7	34.7
T4	CN K. 4 yrs	44.5	51.3	34.7
T5	CN K. 20 yrs	42.5	38.5	30.6
T6	Trees 10 yrs*	34.0	3.7	28.2
T7	Fresh stocks Ktkl. 1yr	45.1	59.3	37.0
	CV	6.9	27.4	8.7
	CD (P = 0.05)	4.3	19.5	4.2

\* Buds collected directly from the terminal branches

Results from the studies on the effect of different age of bud source on growth and yield shows that the age of budwood stock has no role in the production of quality planting materials (Table Bot. 6). Direct use of buds from field trees must be avoided as the treatment (T6) imparted significantly inferior growth (girth 34 cm),

tappability (3.7%) and yield (28.2 g t<sup>-1</sup> t<sup>-1</sup>) when compared to trees raised from the fresh stock of age one year over two years of observation. In the studies on effect of age of budwood stock and production of quality planting material, bud was found to have no role in determining quality of planting materials and there was no significant difference between treatments with respect to trunk growth as well as initial yield (Table Bot. 7).

Table Bot. 7: Effect of different age of bud source on growth and yield

	Type of buds source	Girth 7 <sup>th</sup> yr. (cm)	Mean yield over 2 yrs. (g t <sup>-1</sup> t <sup>-1</sup> )
T1	Brown	43.6	42.7
T2	Conventional	45.6	41.8
T3	Semi-green	45.3	43.3
T4	Scale bud	42.0	40.2
T5	Whorl bud	43.0	37.9
T6	Light green	43.0	43.2
T7	Unhealthy buds	42.0	37.1
	CD (P = 0.05)	NS	NS

## GERMPLASM DIVISION

The *Hevea* germplasm maintained at RRII includes the domesticated gene pool with clones derived from the original Wickham collection of 1876, the wild germplasm belonging to the 1981 IRRDB collection, and the collection of other *Hevea* species. Maintenance of the domesticated gene pool collection, introduction and conservation of remaining *Hevea* species, conservation of the wild germplasm, its agronomic evaluation, screening for diseases, drought and cold stress resistance, timber latex traits and molecular characterization are the major activities of the division.

### 1. Introduction, conservation and documentation

#### 1.1. Domesticated gene pool (Wickham collection) from secondary centers

The 183 Wickham clones belonging to this gene pool are being conserved in a budwood nursery (the clone museum) at RRII, Kottayam, and three arboreta (Germplasm Gardens) at CES, Chethackal. The arboreta serve the primary purpose of conservation and scientific data collection as and when necessary, and are a source of clonal flowers for breeding when required.

Fifty one introduced clones are being conserved in Germplasm Garden 77. This arboretum was planted in 1977 and regenerated in 2000 by ratooning at a height of 20 cm and gap filling the vacancies with the corresponding polybag clones. Among the five IRCA clones in the Germplasm Garden 92, IRCA 111 and IRCA 130 continued to be superior to RRII 105 for vigour, while the remaining three clones were on par with it. In the 14<sup>th</sup> year of tapping, dry rubber yield of IRCA 130 was far superior to that all of other clones, which were on par with RRII 105.

In the Germplasm Garden 94, statistically significant clonal differences were recorded for girth and yield. In the 13<sup>th</sup> year of tapping, among the 20 clones, RRII 609, RRII 23 and RRII 100 were superior to the remaining clones (85.4 - 79.1 g t<sup>-1</sup>t<sup>-1</sup>) for yield. Control (RRII 105) had an average yield of 42.2 g t<sup>-1</sup>t<sup>-1</sup>.

#### 1.2. IRRDB 1981 wild gene pool

3576 wild accessions are being conserved in field gene banks in the form of budwood nurseries and arboreta in the traditional area.

##### 1.2.1. Conservation nurseries

The wild accessions originally introduced from 1984-1990 have been reestablished in compact new conservation-cum-source bush nurseries (SBNs) from 2003 to 2008. Characterization and preliminary evaluation in the juvenile stage identified a large number of potentially useful accessions for further evaluation. Accessions showing potential for yield, yield contributing traits like latex vessels, disease and drought tolerance traits, are being established in a separate working collection. The first set of 32 accessions has been planted in the Germplasm Working Collection nursery.

##### 1.2.2. Arboretum

The second set of 200 accessions comprising wild and Wickham clones was planted at Tura, Meghalaya. So far, 242 wild and 29 Wickham clones have been planted. The third set of 220 accessions was multiplied at the Central Nursery in Kerala and established in the polybag nursery at Tura. Another arboretum established earlier and comprising 120 accessions, is being maintained at Central Experiment Station, Chethackal.



### 1.3. Other *Hevea* species

An arboretum established in 2006 at CES conserves six accessions of five other species available at RRII (*H. benthamiana*, *H. spruceana*, *H. nitida*, *H. camargoana* and two accessions of *H. pauciflora*), along with five natural putative interspecific hybrids, two *H. brasiliensis* clones, and FX 516 (an interspecific cross between *H. brasiliensis* and *H. benthamiana*). Tapping was initiated this year. *H. nitida* and *H. camargoana* are shrub-like and could not be tapped. In the first eight months of tapping, among the other *Hevea* species and hybrids, FX 516 had the highest yield of 34.5 g t<sup>-1</sup> t<sup>-1</sup>, followed by *H. pauciflora* with 16.0 g t<sup>-1</sup> t<sup>-1</sup>. FX 516 also had the highest girth of 71.1 cm and the lowest ALF incidence. *H. camargoana* has been showing precocious flowering from the first year of growth, with pigmented flowers and small fruits.

## 2. Characterization and preliminary evaluation

At RRS, Padiyoor, relatively high yielding accessions (AC 3131, AC 552, RO 2136, RO 1313, AC 567, AC 1964) and vigorous accessions for timber traits (MT 4219, AC 4140 and MT 387) were selected in PET 2000A and 2002. 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 2883 were identified as potential timber clones. These accessions are conserved as male parents for future W x A hybridization programmes at RRS, Padiyoor.

### 3. Further evaluation and selection

Detailed evaluation of selections from preliminary evaluations are being carried out in clonal nurseries (CNs) for accessions with 50-80 per cent of the test tap yield of the controls, while those with more than 80 per cent yield are

evaluated in field trials (FETs) at normal spacing.

#### 3.1. Clonal nursery evaluation

The clonal nursery planted in 2010 at Central Experiment Station comprising 15 wild accessions in RBD at 2.5 x 2.5 m spacing, was subjected to the second round of test tapping. The highest yielder was AC 2027 (8.3 g t<sup>-1</sup> t<sup>-1</sup>) followed by MT 1056 (7.4 g t<sup>-1</sup> t<sup>-1</sup>). Among the check clones, RRII 414 recorded the highest yield (18 g t<sup>-1</sup> t<sup>-1</sup>) followed by RRII 430 (16 g t<sup>-1</sup> t<sup>-1</sup>). RRII 105 yielded 5.6 g t<sup>-1</sup> t<sup>-1</sup>. The highest girth was recorded by MT 5078 (35.5 cm). Among the check clones RRII 414 had the highest girth (43.5 cm).

#### 3.2. Further evaluation trials

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

Out of 22 wild accessions in the FET 2003, RO 2629, AC 626 and MT 2233 recorded the highest annual girth while RO 2629, AC 4149, RO 3804 and AC 716 recorded the highest yield.

The FET 2005 comprising 22 wild accessions and three controls was opened for regular tapping in 2015. In the first nine months of tapping, AC 2004 showed an average yield of 31.7 g t<sup>-1</sup> t<sup>-1</sup> compared to the yield of controls RRII 105, PB 260 and RRIM 600, which ranged from 40.9 to 32.7 g t<sup>-1</sup> t<sup>-1</sup>. MT 4788 yielded 25.2 g t<sup>-1</sup> t<sup>-1</sup>. AC 2004 followed by MT 43 and MT 4788 had the highest girth, on par with the best check PB 260.

In FET 2008, AC 176 (54.0 cm), RO 2846 (52.60 cm) and RO 4149 (51.1 cm) recorded the highest girth in the eighth 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 three control clones are in the second 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, are in the second year of growth in a further evaluation trial (FET 2014), at RRS, Dapchhari.

### 3.3. On-farm trials

Selections from FETs are subjected to multi location evaluation in On-Farm Trials for confirmation of yield potential. The first OFT, established at five locations viz. B.C. Cheruvally estate in Erumely, Malankara estate in Thodupuzha, Mooply estate in Trissur, Calicut estate in Kozhikode and Bethany estate in Kanyakumari for evaluating the performance of the three selected IRCA clones (IRCA 130, IRCA 111, IRCA 109) and one wild accession (AC 166), is in the sixth year of growth. Girth of the clones was recorded in all the five locations. At Mooply estate, among the test clones wild accession AC 166 recorded the highest girth 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 BC Cheruvally estate, highest girth was recorded by IRCA 130, followed by IRCA 111 and AC 166. At Calicut estate, the clone IRCA 130 showed better growth performance followed by IRCA 111, IRCA 109 and the least in AC 166. At Bethany estate, Kanyakumari, the best growth was recorded for clones IRCA 109 (42.2 cm) and IRCA 111 (40.1 cm), compared to the girth of the check clones RRII 105, RRII 414 and RRII 430 (42.6 cm, 44. cm and 43.0 cm respectively).

## 4. Screening for stress tolerance

### 4.1. Screening for biotic stress tolerance

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

### 4.2. Abiotic stress resistance

#### 4.2.1. Drought tolerance

In the clonal nursery of 40 potential half-sibs of nine clones and seven hybrid seedlings at RRS, Dapchhari, the highest average girth in the fifth year of growth was recorded by the family of PB 5/51. Among the three check clones, girth was on par for RRII 430 and RRII 414. There was significant difference in clonal response towards drought stress. The clones were subjected to 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 eight clones and two hybrid progenies, 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 242.

In the further field evaluation of selected *Hevea* clones at RRS, Dapchhari 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 eight summer periods from 2008-2015, four wild accessions recorded girth higher than the proven drought tolerant clone RRIM 600.

Accession MT 40 recorded highest girth at 8<sup>th</sup> 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.

#### 4.2.2. Cold tolerance

Two cold evaluation trials comprising of sixty four wild *Hevea* accessions were under evaluation for growth and yield at Regional Experiment Station, Nagrakata, 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

Twenty five genotypes were evaluated for annual girth and monthly yield 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

At CES, Chethackal, in the 2009 hand pollination programme involving three wild accessions and six cultivated Wickham clones, 75 progenies along with the OP seedlings of the Wickham parents were continuously monitored for their performance. Five cross combinations with test tap yield higher than the population average, were identified and required budwood is being raised for further evaluation.

At RRS, Padiyoor, 29 seedling progenies derived from two cross combinations in 2009,

along with 25 OP seedlings of RRII 105, are under evaluation in a seedling nursery. Seven hybrid progenies and five OP seedlings were found to be promising for test tap yield. They were multiplied at Ulickal nursery in order to raise a budwood nursery of these high yielding hybrids.

At RRII, growth and test tap yield were monitored in the hybrid progeny of the 2013 W x A HP involving two cross combinations: of the 33 seedlings, six showed more than 2 g t<sup>-1</sup> t<sup>-1</sup> in the second year of growth, with a maximum of 3.5 g t<sup>-1</sup> t<sup>-1</sup>.

Twenty nine surviving seedling progeny of the 2014 HP involving four potential wild accessions and RRII 105 were monitored for growth. The cross AC 4833 x RRII 105 showed the highest mean girth. The performance of the 255 surviving interspecific progenies of the 2009, 2013 and 2014 HPs are reported in the next section.

In the 2016 HP, 1960 W x A pollinations were carried out with an initial fruit set of 56 (2.9 %). These involved four wild accessions including one with relatively high *Oidium* tolerance and RRII 105.

#### 6.2. Phenotyping of mapping population for QTL identification

The total number of seedlings planted in the nursery from all the HP programs (HP 2009, 2010, 2013 and 2014) was 306, of which 255 are now surviving. The growth in the seedling nurseries was monitored. Being two different species, the two parental clones differ markedly in many traits, including yield, girth and disease resistance. After accurate pheno-typing, all segregating traits can be mapped onto the same genetic map.



The HP 2009 seedling nursery has been subjected to a detailed study: data on growth, bark anatomy, three rounds of test tapping, and ATP profiles have been recorded. Three potential high yielders have been identified so far in the population based on three rounds of test tapping. Simple correlations were worked out in this population between these traits at the nursery stage. All the correlations were significant at  $P > 0.01$  ( $n=81$ ).

The population has been phenotyped for *Phytophthora* resistance in the laboratory and transgressive segregants have been obtained. Mapping for this trait is in progress. As the phenotypic evaluation in this nursery is complete, the plants were cut back to raise budwood for multiplication for the field trial for phenotyping of yield. DNA extraction for genetic mapping commenced (Genome Analysis lab) using 86 progeny that were confirmed as hybrids. The results will be confirmed in the remaining 2013-14 populations as and when they are ready for multiplication, after completing nursery phenotyping.

Growth of the 2013 and 2014 HP interspecific progenies was monitored. Girth of the 70 progeny in the 2013 HP ranged from 5 to 24 cm with an average of 11.7 cm. Of the 61 seedlings test tapped in the second year of growth, three gave more than  $2 \text{ g t}^{-1} \text{ t}^{-1}$ . Girth of the 79 progeny in the 2014 HP ranged from 4-14 cm with an average of 9.8 cm.

## 7. Other studies

### 7.1. Assessment of the performance of new rubber plantations of ITDA, AP

12 selected rubber farmers' fields (2008 plantings) were assessed for growth at RC Varam, A.P., a collaborative project with the Integrated Tribal Development Agency (ITDA), Govt. of Andhra Pradesh. In the 8<sup>th</sup> year of growth, Farm 7 and Farm 1 recorded the highest growth.

### 7.2. Studies on alternative sources of natural rubber yielding plants

Eleven accessions of Ceara rubber (*Manihot glaziovii*) are being conserved at RRII.

## BIOTECHNOLOGY DIVISION

The research focus of the Division is mainly on developing genetically modified *Hevea* plants with desirable traits. Experiments on genetic transformation were fine tuned for producing transgenic plants with enhanced biotic and abiotic stress tolerance and improved latex yield. Attempts were also made for developing antibiotic marker-free transgenic plants. Development of efficient protocols for *in vitro* regeneration of haploid plants, propagation of elite *Hevea* clones, *in vitro* approaches to complement conventional breeding programmes, molecular cloning and characterization of somatic embryogenesis related *SERK* gene from different tissues of *Hevea brasiliensis* were the other research programmes carried out in the division during the reporting period.

### 1. Development of transgenic plants

#### 1.1. Genetic transformation of *Hevea brasiliensis* with *MnSOD* gene construct for producing transgenic plants with enhanced stress tolerance

Genetic transformation experiments were carried out with two *MnSOD* gene constructs harbouring CaMV35S and FMV34S promoters, for improving tolerance to environmental stress. *MnSOD* gene was integrated in *Hevea* callus of clones RR11 430 and 105 using different target

tissues viz. proliferated friable anther callus and embryogenic callus, proliferated friable leaf callus and embryogenic callus and zygotic embryo derived embryogenic callus. The *Agrobacterium* infected target tissues were periodically cultured over kanamycin containing medium. Two transgenic cell lines emerged from the infected anther callus after 40 days of culture. These callus lines were proliferated and cultured for somatic embryo induction. Eight transgenic cell lines were obtained from leaf embryogenic callus. All the cell lines were cultured individually over proliferation medium and proliferation was observed from two callus lines.

#### 1.2. Genetic transformation of *Hevea brasiliensis* for yield enhancement

##### 1.2.1. Transformation and plant regeneration

*Agrobacterium* mediated genetic transformation experiments were carried out using the friable callus of RR11 105 and 430. Transgenic cell lines were obtained from the callus of both clones. The cell lines of 430 proliferated very slowly, whereas high proliferation was noticed in callus from RR11 105. The proliferated cell lines were cultured for somatic embryogenesis and embryogenesis was observed in three transgenic cell lines. Plant regeneration was achieved from one cell line (Fig. Biotech. 1).



Fig. Biotech. 1 (a-d). Various stages of plant regeneration

1a. Emergence of transgenic cell lines

1b. Somatic embryo induction

1c. Somatic embryo germination

1d. Transgenic plant

### 1. 2. 2. HMGR enzyme assay in *Hevea transgenic* plants

Transgenic plants integrated with *hmg1* gene were developed earlier from the zygotic embryo derived embryogenic callus of *Hevea*. HMGR enzyme activity was quantified in the selected transgenic plants as well as control. Bark and leaf samples were collected in ice and the enzyme activity was determined by spectrophotometric assay (Liang *et al.*, 2015). The HMGR activity was quantified as  $\mu$  mol NADPH oxidized by one mg of the enzyme per minute. The HMGR activity in the bark samples of nine transgenic plants and one control is given in Table Biotech. 1.

Table Biotech. 1. HMGR activity in the bark samples of transgenic and control plants

Sample	Protein ( $\mu$ g ml <sup>-1</sup> )	HMGR activity ( $\mu$ mol mg <sup>-1</sup> min <sup>-1</sup> )	
T <sub>1</sub>	208.27	0.1988±	0.02489
T <sub>2</sub>	366.21	0.2972±	0.0228
T <sub>3</sub>	198.42	0.1881±	0.02345
T <sub>4</sub>	357.90	0.2535±	0.01303
T <sub>5</sub>	214.20	0.1876±	0.01703
T <sub>6</sub>	204.10	0.1843±	0.02366
T <sub>7</sub>	327.06	0.30935±	0.0189
T <sub>8</sub>	239.90	0.22872±	0.0376
T <sub>9</sub>	191.32	0.1761±	0.02121
Control	215.15	0.1621±	0.027

From the table it is clear that five transgenic plants expressed higher HMGR activity than the control plant and out of the five, three plants recorded moderately higher values.

The HMGR enzyme activity in the leaf samples was also quantified and the results are given in Table Biotech. 2.

Table Biotech. 2. HMGR activity in leaf samples of transgenic and control plants

Sample	Protein ( $\mu$ g ml <sup>-1</sup> )	HMGR activity ( $\mu$ mol mg <sup>-1</sup> min <sup>-1</sup> )
T <sub>1</sub>	52.3	0.2558± 0.0359
T <sub>2</sub>	43.3	0.2939± 0.01612
T <sub>3</sub>	33.5	0.3420± 0.0242
T <sub>4</sub>	31.2	0.2533± 0.02024
T <sub>5</sub>	24.6	0.2125± 0.0214
T <sub>6</sub>	37.2	0.2756± 0.0176
T <sub>7</sub>	42.2	0.2023± 0.0134
T <sub>8</sub>	61.3	0.1796± 0.01
T <sub>9</sub>	34.7	0.2332± 0.0089
Control	54.2	0.224± 0.0114

Five out of nine transgenic plants showed higher enzyme activity than the control in the leaf samples.

### 1. 3. Genetic transformation of *Hevea* with osmotin gene

New *Agrobacterium* infections were carried out with clonal as well as zygotic explants for developing new transgenic events. From the two month old anther callus 40 transgenic callus lines were developed and transferred to proliferation medium out of which three cell lines were proliferated. The proliferated callus lines were further cultured for the induction of embryogenic callus. Embryo maturation and germination was attempted from the transgenic cell lines developed earlier. A short liquid phase culture (3-5 days) was introduced before transferring the embryos to germination medium. Liquid culture could improve the size and appearance of embryos. The normal looking embryos were selected and transferred to germination medium. A few embryos were germinated and the plantlets developed were transferred for hardening.



### 1.3.1. Stress tolerance evaluation studies in osmotin gene integrated transgenic plants

The stress tolerance evaluation of the transgenic plants developed earlier was continued. Leaf discs from transgenic and control plants were subjected to salt and PEG stress for fixed time intervals and proline estimation was carried out. Results showed that transgenic plants recorded higher proline values under both salt and PEG stresses indicating their improved stress tolerance capacity. The plants were multiplied by bud grafting for further studies.

### 1.4. Genetic transformation of *Hevea* with *ipt* gene

Experiments were carried out for incorporating *ipt* gene in fresh and embryogenic callus obtained from RRII 105 leaf explants. One transgenic callus line could be proliferated. Few plants integrated with *ipt* gene were developed from transgenic callus maintained from earlier experiments but could not be hardened.

### 1.5. Multiple gene integration

Attempts were made for integrating *MnSOD* and osmotin genes in *Hevea* callus by repeated genetic transformation and co-transformation. Repeated genetic transformation was carried out by *Agrobacterium* mediated transformation with *MnSOD* gene construct using osmotin gene integrated embryogenic callus as target tissue. Since *npt II* gene is the selectable marker gene for both the gene constructs, kanamycin 300 mg L<sup>-1</sup> was used for the selection of transgenics. The *Agrobacterium* infected calli were periodically cultured over selection medium and emergence of transgenic callus is awaited. Attempts were also made for the integration of *MnSOD* and osmotin genes by co-transformation using embryogenic callus of clone RRII 105. The *Agrobacterium* infected calli were periodically

sub cultured over selection medium and emergence of transgenic cell lines is awaited.

New *Agrobacterium* infections were carried out for incorporating *MnSOD* and *ipt* genes using leaf proliferated friable fresh callus and embryogenic callus. Eight transgenic callus lines have been obtained from embryogenic callus and proliferation of the transgenic cell lines was also observed.

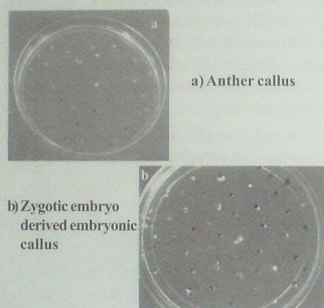


Fig. Biotech. 2 (a-b). Emergence of transgenic cell lines

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

*Agrobacterium* mediated genetic transformation experiments were carried out with proliferated anther callus derived from RRII 105 clone and zygotic embryo derived embryogenic callus. For the selection of putative transgenics, kanamycin 350 mg L<sup>-1</sup> was provided in the selection medium for proliferated anther callus and 300 mg L<sup>-1</sup> for embryogenic callus. From the proliferated anther callus, 12 kanamycin resistant callus lines were obtained and twenty two callus lines were selected from polyembryony derived embryogenic callus (Fig. Biotech. 2)

The kanamycin resistant callus lines obtained were cultured individually over proliferation medium. For the proliferation of callus lines from anther callus, the media optimized earlier for the proliferation of transgenic callus lines from other gene constructs were used and for embryogenic callus, media modifications were made for getting proliferated transgenic callus. Even though 10 proliferated callus lines could be obtained from the polyembryony derived embryogenic callus, the percentage of proliferation varied from callus line to line. For molecular confirmation of gene integration, genomic DNA was isolated from a portion of two randomly selected embryogenic callus lines. PCR was performed with gene specific primer pairs and amplification was obtained in both the lines. The PCR positive callus lines were individually cultured over embryo induction medium and embryo induction could be obtained.

## 2. Somatic embryogenesis of *Hevea brasiliensis*

### 2.1. Somatic embryogenesis from immature anther

Experiments were conducted for optimizing the conditions for plant regeneration via somatic embryogenesis from the clones RR11 105, 414 and 430. Immature anthers were inoculated from the flower buds of the above clones. After media refinements somatic plants could be regenerated from the RR11 105 anther callus. Extensive media modifications were made for getting embryogenic callus from RR11 414 and 430. Embryo induction was observed in callus obtained from RR11 430.

#### 2.1.2. Cloning and characterization of *SERK* gene

A partial *SERK* gene from genomic DNA was characterized and proved its relationship with somatic embryogenesis induction and acquisition of embryogenic competence. In order to study the tissue specific expression of *SERK* gene,

different tissues viz. explant anther, non-embryogenic callus, embryogenic callus and emerging shoot were subjected for expression analysis. RNA was isolated from all the samples and first strand cDNA was synthesized using isolation kit. RT-PCR was carried out using the optimized primer and PCR conditions. Results of the experiment showed that good quality RNA was isolated from all samples. With explant anther, no amplification was obtained with both gene specific primers as well as with endogenous control gene suggesting that the cDNA was not synthesized or an error was happened throughout the preparation. No amplification was obtained with non-embryogenic callus. However, a *HbSERK* gene ortholog was amplified with emerging shoot suggesting that in *Hevea* in addition to somatic embryogenesis, *SERK* gene has also played a role in shoot emergence.

Attempts were also made for characterization of full length *HbSERK* gene. Embryogenic callus was produced from immature anther of RR11 105. RNA was isolated from embryogenic callus and the first strand cDNA was synthesized. An attempt on full length characterization of *HbSERK* was also made and PCR was carried out with same conditions as in partial *HbSERK* gene isolation, but no amplification was obtained. In another attempt, PCR was carried out with different temperature regimes ranging from 50-60 °C and no amplification was produced. In the third experiment, the primer combination was changed. The forward primer designed for full length characterization along with a reverse primer designed for quantitative analysis was used and PCR was carried out at 60 °C. Under this PCR conditions, good amplification was obtained. Hence, further cloning and sequencing of this fragment will be required to amplify the *HbSERK* gene in full length.

## 2.2. Somatic embryogenesis from leaf explants

Somatic embryogenesis was attempted from leaf explants using glass house grown bud grafted plants of different clones viz. RRII 105, RRII 414 and RRII 430. Callus induction was obtained within one month from all the clones and callus proliferation was carried out in medium with inclusion of stress inducing compounds and optimized phytohormones. Sequential subculture of the callus was carried out during proliferation in media where concentration of major nutrients was reduced and cytokinin/auxin ratio was gradually increased. Embryo induction medium without charcoal, addition of silver nitrate and increased phytigel was used for the third subculture during proliferation. Callus texture could be improved considerably and helped in embryogenic callus induction in clones RRII 105 and RRII 414. Somatic embryo induction was obtained from RRII 105. Embryos were cultured individually and maintained in the dark. Slow desiccation of embryos in sealed petri plates for three days enhanced embryo germination and plant regeneration. Callus formed in clone RRII 414 was also proliferated and embryogenic callus was obtained. Minor modifications were made in the culture medium composition for embryogenic callus induction in clone RRII 414 and embryogenesis is awaited.

*In vitro* leaf cultures were also initiated from bud grafted plants (clone RRII 105 & 430) and from *in vitro* sprouted axillary buds for direct embryo induction. Explant pre-treatment such as auxin shock and liquid pre-culture were repeated. The pre-treated explants were subcultured in different basal medium with different hormone combinations. Experiments were repeated with different concentrations of picloram both in presence/absence of phytohormones. ½ MS and

ChuN6 medium responded well. Among the pre-treatment experiments tried, auxin shock was found to be good. Experiments are being continued for further development of proembryos induced from leaf explants cultured on ½ MS and ChuN6 medium containing silver nitrate and phytohormones.

Experiments with paclobutrazol for enhancing the root formation were continued. *In vitro* cultures were established with clonal shoot tips, auxiliary buds and zygotic embryos. Roots were induced both *in vitro* and *ex vitro* in the mature shoots obtained after hormone pulse for fixed time intervals. Effect of paclobutrazol was studied after root induction. Rooted plants exhibited symptoms of dwarfing and root thickening with side root induction.

## 3. Development of Hevea homozygous haploid plants

### 3.1. Microspore culture in Hevea brasiliensis

The callus obtained from the mature anthers of clone RRII 430 were proliferated and subcultured for embryogenic callus formation. Friable, embryogenic callus emerged from the proliferated callus. New inoculations were performed using the mature anthers of clone RRII 422 in the callus initiation medium.

### 3.2. Gynogenic haploids in Hevea brasiliensis

The callus obtained from the cultured embryo sac turned embryogenic within four months of culture. Embryo induction occurred at a low frequency in the medium supplemented with Kinetin and NAA. The embryos were separated and cultured for maturation and plant regeneration. Two plantlets of clone RRII 105 were transferred to small polybags for hardening (Fig. Biotech. 3a-k). Cytological studies to confirm the haploid nature of the plants is in progress.



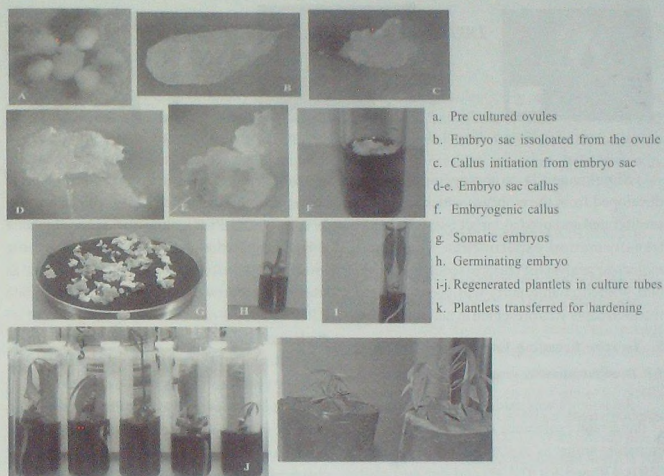


Fig. Biotech. 3 a-k. Different stages of plant regeneration and acclimatization

Unfertilized ovules from RR11422 and RR11105 were inoculated in semi-solid medium and embryo sac was isolated and exposed to temperature shock. The callus obtained from the haploid cells was separated and cultured for friable callus induction. The friable callus was further cultured for somatic embryogenesis.

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

##### 4.1. *In vitro* fertilization and plant recovery

Experiments for the standardization of an *in vitro* fertilization technique were continued. *In vitro* ovular pollination was carried out following the already standardized protocol. New media combinations for the recovery and continued

growth of the embryos were tried. Swelling of the fertilised ovules was observed and for further development, the swelled ovules were sub-cultured over different media combinations.

##### 4.2. Induction of polyembryony

Experiments for scaling up of the existing pathway and development of more embryogenic callus lines were continued. Three new polyembryonic lines were developed from the half ovule embryo culture carried out in the previous year. Uniform seedlings were produced from the multiple embryos (Fig. Biotech. 4 a-c). The uniform seedlings developed through this technique and their budgrafted counter parts were field planted for further evaluation.

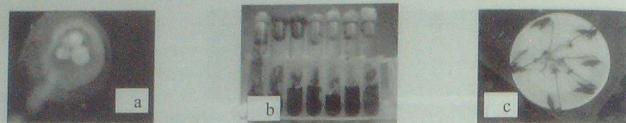


Fig. Biotech. 4 (a -c). Induction of multiple embryos and plant regeneration

Simultaneously, embryogenic callus was developed from the embryos which was further proliferated and used as target tissue for genetic transformation experiments. The data on molecular analysis of uniform seedlings and their bud grafted counterparts was compiled and a research paper was presented in national seminar.

#### 5. *In vitro* breeding for drought tolerance

##### 5.1. Drought tolerance screening by *in vitro* method

An attempt was made for *in vitro* screening of the drought tolerance of *Hevea* clones using immature anther as explant. Two tolerant clones (RRII 430 and RRIC 100) and one susceptible clone (RRII 105) were selected based on results of field performance and other physiological studies. The non-ionic water soluble polymer PEG (Molecular weight 6000) was used in the callus induction medium to stimulate water stress. The experiment consisted of six treatments with PEG stress level; 0, 5, 10, 15, 20 and 25 per cent. After sterilization, anther was dissected and inoculated in medium with the above treatments and experiment was replicated three times. Cultures were kept for stress period of one month under darkness. At the end of stress period, observations were made on callus induction efficiency and growth of callus for each genotype. Results

showed that under *in vitro* stress condition, the callus induction efficiency varied with genotypes. In control (0% PEG), almost all clones induced callus and growth was also good. With increasing level of PEG, efficiency of callus induction as well as callus growth was reduced. For the clone RRII 105, at 0 per cent PEG, 95 per cent of explants induced callus with good callus growth (rating scale 7). When PEG level was increased to 5 per cent, even though all explants induced callus, callus growth was very minimal (rating scale 2). Further increase of PEG to 10 per cent, explants just swelled but no callus induction was observed and beyond 10 per cent, all the explants died which indicated the relative susceptibility of the genotype to PEG induced stress. The clones RRII 430 and RRIC 100 showed almost similar response of callus induction and callus growth. Increasing the level of PEG from 0 to 15 per cent, all explants induced callus and growth was also good with a rating scale of 8. At 20 per cent PEG also, little callus formation was observed indicating their relative tolerance to drought stress. All these *in vitro* results showed that *in vitro* screening method is a potential tool for drought tolerance studies. Replicated studies with more number of clones are required to conclude the results.

## GENOME ANALYSIS LABORATORY

The genomic research is mainly focused on (i) development, optimization and validation of molecular tools for the assessment of genetic diversity and evolutionary relationships in rubber and genetic linkage mapping (ii) development of genetic markers for biotic and abiotic stress tolerance and understanding the stress adaptation processes through transcriptome analysis (iii) cloning and characterization of agronomically important genes and (iv) genome sequencing and *de-novo* assembly of rubber genome.

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

#### 1.1. Allelic distribution of SSR marker loci in cultivated Wickham clones and Amazonian (wild accessions) *Hevea* population

Genotyping of cultivated clones and wild accessions using 15 informative SSR markers was performed for understanding the allelic distribution in these two populations. Wild population of rubber, comprising representation of individuals from three different provinces: Acre, Mato Grosso and Rondonia of Brazil, showed more allelic diversities at any of the SSR locus than the cultivated population consisting of 40 rubber clones originating from South East Asian rubber growing countries including India. With these selected 15 SSR markers, 135 alleles were identified in the wild accessions, whereas only 59 alleles could be observed in cultivated clones. Therefore, 56 per cent allele loss or loss of genetic diversity was noted in cultivated Wickham clones, as a result of domestication

mainly through selective breeding over the past 100 years. When allelic diversity was estimated in cultivated population against the Mato Grosso population, only 28 per cent allele loss was detected indicating their closeness with the Mato Grosso sub-population.

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

##### 1.2.1. SNPs and haplotype structuring in the latex biosynthesis genes of *Hevea brasiliensis*

Two latex biosynthesis genes: *cis*-prenyl transferase (*CPT*) and phosphomevalonate kinase (*PMVK*) from five clones were sequenced for identification of SNPs. In *CPT* gene, 21 SNPs were identified. Consequently eight haplotypes were determined based on 17 SNPs having phase information. Two haplotypes present in RRII 105 (Hap 1 & Hap 2) were found to be unique as the same was absent in the other four clones: RRII 118, RRIM 600, GT 1 and RRIC 52.

Sequence analysis of *PMVK* gene revealed the presence of 22 SNPs. Out of the six coding SNPs identified, four were synonymous and two were non-synonymous. Six haplotypes were identified for *PMVK* gene in five experimental clones. Haplotype frequency was estimated and Hap 3 frequency was found more (40%). Presence of two allelic variants of *PMVK* gene was identified based on a large indel (1.2 kb) existing in the last intron at the 3' region. Allelic nature of these gene fragments was evidenced through the segregation pattern of *PMVK* gene in a mapping population derived from a cross between RRII 105 and RRII 118. Marker generation using SNPs from latex biosynthesis genes is in progress.



### 1.2.2. Expression analysis of rubber biosynthesis genes

Among the five clones, RRII 105 showed comparatively higher expression of *CPT* gene in latex. Therefore, an association of haplotypes existing in RRII 105 (Hap 1 & Hap 2) with gene expression may be presumed. Higher expression of *PMVK* gene was noticed in RRII 118 (homozygous for Hap 2) followed by RRII 105 (heterozygous bearing both Hap 1 & Hap 2). Lower expression was noticed in GT 1 compared to other clones, which was heterozygous for different haplotypes (Hap 2 & Hap 5). Therefore, Hap 2 in homozygous state probably contributed to higher expression of *PMVK* gene. Hap 2 in combination with Hap 1 also gave better expression as seen in RRII 105. To establish association between haplotypes of respective genes and their expression, more clones are to be analyzed at haplotype level as well as mRNA abundance of respective genes in latex.

### 1.2.3. Effect of non-synonymous coding SNPs in protein structure conformation

Positional assessment of each non-synonymous coding SNP in *HMGs* as well as *GGDPS* revealed that these SNPs had no direct interaction with the binding sites as none of them was found inside or near the protein-small molecule interaction interfaces of these two molecules.

### 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*)

High-density linkage map construction for F4542 (*H. benthamiana*) and RRII 105 with SNP and silico DArT markers is in progress. Initially two parental maps were generated (M1 & M2). The first map (M1: F4542) was constructed with

20,484 markers grouped into 18 linkage groups. The second map (M2: RRII 105) was constructed with 9813 markers, which also formed 18 linkage groups reflecting the haploid chromosome number of *Hevea*. Marker orders and distance in each linkage group were established. The map covered a total distance of 3665 cM in *H. benthamiana*. A consensus map was constructed with 24004 DArT markers.

An attempt was made to integrate seven rubber biosynthesis genes into the existing linkage map of RRII 105 and RRII 118 developed in the Genome Analysis Laboratory using SNP markers. *FDPS* (1380 C/T) and *GGDPS* (741 C/T) were integrated in RRII 118 map, whereas *MVK* (2628A/G) and *PMVK* (102C/T) were integrated in RRII 105 map. *HMGs* (3059A/G), *CPT* (1438C/T) and *PMVK* (1786C/T) couldn't be integrated in any of the parental map.

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

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

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

Transcriptome sequencing of *Phytophthora* challenged and control samples of RRIM 600 (susceptible) and Fx 516 (tolerant) were repeated, as sequence read participation in two assemblies was less in our earlier transcriptome sequencing. As a result, number of contigs in an assembly also reduced which became a problem while doing differential gene expression studies using bioinformatics tool. Library preparation for transcriptome sequencing was completed with fresh samples. Paired-end sequencing (2x150 bp read length) was performed using Illumina

NextSeq500 sequencing platform and 27-30 million filtered reads were generated. *De-novo* assembly is in progress.

#### 2.1.2. QTL markers for *Phytophthora* tolerance

An effort was made to identify QTL markers for *Phytophthora* tolerance with the disease response data generated for 86 interspecific hybrid progenies along with their parents through *in-vitro* screening at the Plant Pathology Division. Transgressive segregation of resistance trait against *Phytophthora* pathogen was noticed among interspecific hybrid population indicating the involvement of multiple genes/loci for resistance trait.

#### 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 transcriptome

The NAC proteins are plant-specific transcriptional regulators. They are involved in various biological processes, including both biotic and abiotic stress responses. Characterization of NAC gene and its prokaryotic expression were reported last year. NAC cDNA cloned from RR11 105 and RRIM 600 were analyzed for the presence of SNPs. Five non-synonymous SNPs in the coding regions were identified resulting in amino acid changes. Understanding the role of these SNPs in catalytic activity of NAC proteins is in progress.

NAC regulatory sequence from RRIM 600 was characterized to know the reason behind over-expression of NAC gene in RRIM 600 under drought stress. A fragment of 844 bp NAC regulatory sequence was identified from whole genome shotgun sequence and motif analysis was performed. Twenty four motifs were identified

including the characteristic motifs of a promoter. Cloning of the promoter sequence from RR11 105 for comparison is in progress.

##### 2.2.2. Methylation dynamics of *Hevea brasiliensis* genome

A growth chamber experiment was initiated to validate the impact of cold stress on the promoter region of selected rubber biosynthesis genes (*HMGR*, *HMGS*, *FDPs* and *REF*) in three popular clones (RR11 105, RRIC 100 and RRIM 600). The objective was to identify DNA methylation changes induced by cold stress in the promoter region of these five genes in cold susceptible (RR11 105, RRIC 100) and tolerant (RRIM 600) clones. Three poly bag plants of each clone were subjected to cold stress mimicking the winter climate of North East India for two weeks. Genomic DNA was extracted from these plants before imposing any stress (control), under the stress and post stress (one month after imparting stress) to study the impact of cold stress on the epigenome of rubber.

#### 3. Cloning and characterization of agronomically important genes

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

Altering the lignin monomeric composition *i.e.*, increasing syringyl to guaiacyl monomer (S/G ratio) without reducing total lignin content is an effective way to improve timber quality. Coniferaldehyde-5-hydroxylase (*CaId5H*) gene plays a key role in regulating of S/G ratio by channeling the intermediate compounds of guaiacyl precursor to syringyl lignin pathway without any influence on total lignin content. Therefore, cloning and characterization of *CaId5H* gene from rubber was initiated.

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

##### 4.1. *Hevea* genome sequencing

Whole genome sequencing of rubber was performed to generate a draft sequence of clone RRII 105 using Next Generation Sequencing (NGS) platforms. Sequence data generation of RRII 105 genome was completed with four sequencing platforms (Illumina HiSeq, MiSeq, Roche GS-FLX and PacBio). *De-novo* assembly was performed successfully using MaSuRCA software with MiSeq PE, Roche GS-FLX, PacBio and NextSeq 2Kb MP sequence data. Assembled total genome length was ~1.48 Gb (without gap), scaffold N50 was 19,799 bp. However, majority of the data (~250 Gb of HiSeq and other mate-pair libraries 4, 6, 8 & 20 Kb) were not included in this assembly. Rubber genome assembly using all cross platform sequence data is in progress.

##### 4.2. 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. Out of 15, 13 RNA-seq samples were subjected to in-house quality filtering analysis. In total 52 assemblies (multiple k-mer based

assembly for each sample) were subjected to length filtering, ambiguity filtering and clustering analysis.

##### 4.3. Bioinformatics resource generation

###### 4.3.1. Tools/software/pipelines setup

Bioinformatics tools/software/pipeline had been installed and setup in a server at genomics lab. Around 100 tools/dependencies had been installed since April 2015. These tools will be upgraded periodically. Most of these tools were subjected to benchmarking studies to understand their algorithm using publicly available or simulated data. Multiple in-house PERL scripts generated as and when required for data parsing.

Latest version of mySQL server (mysql-5.6.25-linux-glibc2.5-x86\_64) had been installed and configured for performing various database based analysis. OrthoMCL tool was installed and configured in mySQL database to identify gene orthologs in *Hevea* genome. Besides, a pipeline for gene expression analysis was established using mapping tool bowtie, read quantification tool RSEM & eXpress and gene expression detection tool DESeq. Benchmarking with MaSuRCA assembler was also carried out for its use in *de-novo* assembly of all cross-platform sequence data generated in rubber.



## PLANT PATHOLOGY DIVISION

The research programmes of the Division includes monitoring the occurrence and development of pest and diseases, their management through chemical and biological means, impact of protecting trees against various diseases *etc.* Evaluation of new clones for disease resistance, identification of genes involved in disease resistance, biotic etiology of tapping panel dryness, use of beneficial microorganisms for plant growth and drought tolerance *etc.* were other areas of research.

### 1. Leaf diseases

#### 1.1. Abnormal leaf fall diseases

Studies to assess the impact of Abnormal Leaf Fall in four modern clones *viz.* RR11 414, RR11 422, RR11 429 and PB 260 were continued. The severity of ALF disease in general during 2015 disease season was less. The difference between sprayed and unsprayed blocks was not significant. The girth of trees in sprayed blocks was significantly higher in clones RR11 414 and RR11 429, whereas girth increment did not give a clear trend. Bark thickness was better in sprayed blocks of RR11 414 and RR11 429 than unsprayed.

The impact of ALF was consistently more on clone RR11 414 recording yield drop in the current year and previous years also. The clones RR11 414 and PB 260 registered yield drop of 13.6 and 9.9 per cent, respectively, in unprotected blocks, whereas the impact of leaving unsprayed was not noticed in clones RR11 429 and PB 260 in the current season due to less ALF intensity. Dry rubber content (drc) of latex was not influenced by ALF. Tapping panel dryness (TPD) was high in PB 260 followed by RR11 414, RR11

422 and the lowest in RR11 429. Between sprayed and unsprayed, no definite trend could be seen.

In the crown budding experiment on clone PB 311 located in Malankara Estate, the crown-budded trees retained good canopy. The ALF severity was not high in general compared to previous years. The trees crown-budded with Fx 516 recorded higher girth and timber volume. The effect of crown modification in clone PB 311 on latex/raw rubber properties such as Plasticity ( $P_o$ ), Plasticity Retention Index (PRI), Mooney Viscosity (MV) *etc.* was studied for three seasons and no adverse effect due to crown modification was seen. In another experiment to evaluate the effectiveness of nursery crown budding in clone PB 260 at CES, the girth was found higher in crown-budded than control. However, girth increment was not influenced by crown budding. The ALF and *Phytophthora* shoot rot were mild in crown-budded plants. The crown-budded trees recorded higher yield, whereas drc did not show significant variation (Table Path. 1).

Table Path. 1. Yield and dry rubber content in crown-budded PB 260 trees

Treatment/ Crown	Yield (g t <sup>-1</sup> t <sup>-1</sup> )	DRC (%)
Crown-budded/ Fx 516	41.9*	35.0
Control/ PB 260	32.6	37.6
Yield increase over control (%)	21.4	-

\* Significant at 5% level

The effect of crown modification in clone PB 260 on latex/raw rubber properties was studied. The  $P_o$  and MV were found less in crown-budded trees whereas PRI was higher in latex from crown-budded trees. In another

experiment, attempts are being made to develop crown-budded root trainer plants. In this regard, plants were raised in root trainer cups of 1000 cc volume, but the required height for crown budding could not be achieved.

Evaluation of the crop loss due to the combined effect of ALF and powdery mildew diseases at RRS, Padiyoor was continued. Powdery mildew disease was mild and abnormal leaf fall disease was severe. The leaf retention was only 75 per cent in the RRII 105 unsprayed area and it was 20, 65 and 50 per cent for the clones RRIM 600, PB 235 and PB 5/51, respectively after the ALF disease season. Overall crop loss in RRII 105, RRIM 600, PB 235 and PB 5/51 for the year was found to be 28.8, 56.0, 29.9 and 45.4 per cent, respectively. Reduction in girth increment was also noticed in the unsprayed plots of all the clones.

Studies were initiated for identification of genes involved in *Phytophthora* disease resistance. *Phytophthora* susceptible (RRIM 600) and tolerant (Fx 516) clones were challenge-inoculated with zoospores of *Phytophthora meadii*, the predominant species of *Phytophthora* affecting rubber and transcriptome sequencing of healthy and infected leaf samples was performed using NGS platform (NextSeq 500). A large number of genes identified were annotated where the gene designations are known but even more number of transcripts/genes were identified for which the gene designations are unannotated, which needs to be studied in detail. Validation of putative genes associated with resistance is in progress through qPCR studies.

A fully saturated genetic linkage map was constructed using a mapping population derived from an interspecific cross between *H. brasiliensis* (clone RRII 105- commercially cultivated high

yielder clone with moderate disease resistance potential) and *H. benthamiana* (F4542-low yielder with high level of disease resistance). Genotyping the mapping population was carried out using 24004 markers derived from DArT sequencing (co-dominant SNP markers and dominant *silico* DArT markers), which assembled into 18 linkage groups, thus reflecting the basic chromosome number of *Hevea*. This genetic linkage map, showing relative positions of genetic markers on a chromosome helps in marker assisted selection for agronomic traits of interest.

Phenotypic assessment for disease response to *Phytophthora* spp. clearly discriminated progenies with different levels of resistance reactions. Progenies with extreme level of tolerance and susceptibility were identified along with the majority of the population showing moderate level of resistance / susceptibility. Frequency distribution of disease resistance among the progeny population was continuous, implying that disease resistance is a quantitative trait. Transgressive segregation for resistance trait in the population was evident, suggesting favourable alleles of multiple genes conferring resistance to *Phytophthora* are controlled by both parents, which has resulted in extreme phenotypes among hybrid progenies.

Efforts were in progress towards uncovering the genetic architecture of *Phytophthora* resistance through QTL mapping, which identified six QTL markers for resistance to *Phytophthora* spp., which need to be validated. These results are important for understanding genetic determinism of disease resistance and for marker assisted selection.

## 1.2. Powdery mildew disease

Experiment to evaluate the efficacy of two bacterial bio control agents along with

recommended fungicide (sulfex), a new fungicide (trifloxystrobin + tebuconazole) and untreated control was continued. Treatments were imposed in eight days interval and disease intensity was assessed on a 0-5 scale before each treatment. Among the various treatments, the fungicide trifloxystrobin + tebuconazole was the most effective one. RH 34 gave about 50 per cent reduction in the disease intensity over control and was comparable to the recommended fungicide sulfex.

### 1.3. *Colletotrichum* leaf disease

The level of disease tolerance in 185 Wickham clones to *Colletotrichum* spp. was assessed using crude toxin derived from *C. acutatum* under *in vitro* conditions. Consolidation of the results on inherent tolerance of these clones revealed that eight clones possessed over 70 per cent tolerance and 57 clones were in the highly susceptible group (> 80%). The remaining clones fell into the moderate level of resistance category. Second round of screening is in progress.

Three years data on *Colletotrichum* leaf disease assessment in the Phase I of PCE trial from various locations indicated that two clones P 078 and P 063 were having low disease intensity in many of the locations compared to RR11 105. The mean disease intensity was 49.0 and 68.7 per cent, respectively. The mean per cent disease intensity (PDI) of RR11 414 and RR11 430 was 51.6 and 53.2. However, it was 91.6 for the clone RR11 105.

### 1.4. *Corynespora* leaf disease

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 per cent was found significantly superior to all other treatments in controlling the disease (Table Path. 2).

Table Path. 2. Efficacy of fungicides on *Corynespora* disease intensity

Treatments	% Disease intensity	
	Sheradi	Nelliyadi
Mancozeb (0.2%)	1.8	1.1
Carbendazim (0.05%)	1.9	1.5
Untreated control	4.4	3.2
Pyroclotrabin+Metriam (0.06%)	1.4	1.4
Thiophanate methyl (0.07%)	1.2	0.4
CD (P = 0.05)	0.35	0.25

One new fungicide and organic compounds including antagonistic endophytic bacteria were evaluated against *Corynespora* leaf fall disease on budwood plants of RR11 105 at Ulickal nursery, Iritty. The results showed that the antagonistic endophytic bacteria (8LK) and new chemical, difenconazole were effective in nursery and on par with recommended fungicide carbendazim.

NGS-based RNA-Seq study was conducted to understand the differentially regulated genes involved in host tolerance to *C. cassicola* through transcriptome analysis of healthy and infected susceptible (RR11 105) and tolerant (GT 1) clones. The study identified differentially regulated genes being significantly over-expressed in tolerant clones, whereas these transcripts were either completely suppressed or down regulated in susceptible clones upon *C. cassicola* infection. Microsatellites with different repeat motifs were identified, which facilitated in the process of marker development for disease resistance. The NGS study provides a transcript resource for gene discovery and development of functional markers essential for



QTL mapping of genes involved in disease tolerance. Assessment of level of disease resistance among 185 Wickham clones of rubber as well as the six species of *Hevea* to *C. cassicola* was carried out using crude toxin derived from *C. cassicola* under *in vitro* conditions. Twelve clones showed higher levels of tolerance. Second round of screening is in progress.

## 2. Stem diseases

### 2.1. Pink disease

New generation fungicides were evaluated for their prophylactic and curative effect at TR and T estate, Mundakayam on three year old plants. Prophylactic and curative applications of fungicides were carried out by spraying with a high volume single man operated power sprayer. Observation on the recovery of plants showed that both the fungicides trifloxystrobin + tebuconazole (Nativo) and tebuconazole (Folicur) recorded better protection in preventing the pink incidence and also curative effect when applied during the initial stage of infection.

Consolidation of three years data on pink disease incidence in different clones in the PCE trials (phase 1) at different locations showed that the clones P 068, P 021, P 088, P 072, P 026, and P 076 recorded high incidence of pink disease and P 084, P 063 and P 065 recorded low incidence.

### 2.2. Tapping panel dryness

In order to characterize the organism (Phytoplasma Like Organism) associated with the TPD affected phloem sieve tube, bark samples of 15 healthy trees and one TPD affected tree were collected and DNA was isolated by modified MLO enrichment procedure. Amplification of the isolated DNA through nested PCR yielded ~1200 bp band from seven healthy plants (Fig. Path. 1). The PCR product was cloned and sequenced and all the sequence data from the amplified product showed homology to "Phytoplasma Like Organism" on optional phytoplasma blast analysis. However, out of the seven healthy (positive trees), six were later changed into TPD and one remained as healthy.



Fig. Path. 1. Phytoplasma amplification from healthy trees and TPD affected trees  
(M- Marker; 16- Positive control; 1-15 - Healthy trees)

### 3. Microorganisms for improving growth of young rubber plants

Four PGPR isolates selected from earlier studies and two consortium were evaluated in root trainer grown rubber seedlings at 25 and 50 per cent recommended levels of fertilizer. Uninoculated plants supplied with 25 per cent, 50 per cent and full fertilizer were kept as controls. Fertiliser application was carried out at weekly intervals. Growth of plants after five months showed highest girth in plants applied with PGPR (RH 104) at 25 per cent fertilizer application. In general, all the inoculated plants at 25 and 50 per cent level of fertilizer application did not show much difference in growth. Population of the inoculated cultures and total microbial population were more in potting mix than the controls in all the treatments (Table Path. 3).

Table Path. 3. Growth of plants inoculated with PGPR isolates at different fertilizer levels

Treatment	Girth (mm)	Height (cm)
Consort.1 + 25% fert.	12.7	133.6
Consort.2 + 25% fert.	11.9	129.6
RH 104 + 25% fert.	13.0	140.0
Ri 25 + 25% fert.	12.4	135.3
RH 34 + 25% fert.	11.7	137.8
K 24 + 25% fert.	11.8	142.7
50% fert. alone	10.5	118.8
Consort.1 + 50% fert	10.3	127.4
Consort.2 + 50% fert	11.7	131.2
RH 104 + 50% fert.	11.0	129.3
Ri 25 + 50% fert.	12.1	136.4
RH 34 + 50% fert.	11.1	144.3
K 24 + 50% fert.	12.0	137.3
50% fert. alone	10.8	135.1
Full fert. alone	9.2	96.3
CD (P=0.05)	1.44	13.4

### 4. Growth of plants inoculated with PGPR isolates at different fertilizer levels

The phosphofungi Pf 11, selected from previous study was again evaluated using rubber

seedlings in root trainers at 25 per cent, 50 per cent and full fertiliser application keeping uninoculated plants with the corresponding levels of fertilisers as controls. The experiment confirmed the efficacy of Pf 11 and the plants inoculated with Pf 11 at 50 per cent fertilizer showed more girth, height and root development followed by plants at 25 per cent fertiliser level (Table Path. 4). Morphological studies of the phosphofungi from potting mix showed highest population of inoculated phosphofungi at 25 per cent fertilizer application and was reduced as the fertilizer levels increased. The reisolated colonies showed clear zones of  $PO_4$  solubilisation around the colonies in Apatite agar medium.

Table Path. 4. Effect of treatments on the growth of plants

Treatment	Height (cm)	Girth (mm)	Root vol. (ml)	Root wt.(g)
Pf 11+25% Fert.	125.3	10.8	24.0	20.5
Pf 11+50% Fert.	160.0	12.4	25.7	28.6
Pf 11+100% Fert.	96.3	9.5	23.7	20.6
25% Fert. alone	118.8	10.5	24.7	19.3
50% Fert. alone	135.2	10.8	11.8	19.0
100% Fert. alone	96.3	9.2	13.3	10.8
CD (P=0.05)	13.8	1.4	7.2	7.1

In the biofarming trial, plants in the integrated treatment showed more girth (49.2 cm) followed by biological (45.2 cm) and chemical (44.7 cm) treatments which were on par, after six years of planting. At the height of 150 cm, 43 per cent of the plants attained tappable girth in the integrated treatment while it was 29 per cent in the biological and 23 per cent in the chemical treatments. Inoculated plots showed higher soil microbial population and enzyme activities. Soil micronutrients and leaf nitrogen were more in biological and integrated treatments than chemical treatment.

Ten ACC +ve selected isolates with more than 50 per cent survival of plants after the drought season in preliminary screening and a mixed inoculum from outside source were multiplied and further screened using drought susceptible rubber clone RRII 105. Forty replications were maintained for each isolate along with uninoculated control plants. The inoculation was carried out at fortnightly intervals. Girth, height, leaf numbers, root volume and weight of plants inoculated with the 11 selected isolates before imposing stress varied with

treatments and most of the treatments showed better growth of the inoculated plants than controls. The plants were maintained without watering for 10 days. Chlorophyll content and photosynthetic rate of the plants during the stress period and recoument capacity of the plants after drought, varied with isolates. Chlorophyll content was more for the plants treated with isolate 648. After five days of water stress, plants treated with isolate nos. 4, 526, 648 and 922 showed higher photosynthetic rate than others.

## PLANT PHYSIOLOGY DIVISION

The major areas of research in the 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*

A small heat shock protein (hsp) was validated as a physiological marker for drought tolerance along with certain crucial photosynthetic parameters like  $\phi$  PS II, photosynthetic oxygen evolution activities *etc.* after checking in many elite clones and germplasm accessions using Western blot technique. The stress protein was relatively more in stress tolerant clones and accessions than susceptible clones. On the basis of the stress protein expression and drought responsive physiological parameters in terms of relative stable level of activities, clones *viz.* RRIM 600, RRII 208 and RRII 430 and three wild

germplasm accessions were classified as relatively drought tolerant.

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

From transcriptome sequencing data, about 20 drought responsive transcripts were selected; primers were designed and synthesized for expression studies. A total of 17 transcripts were validated by qPCR analysis in clones RRII 105, RRII 208, RRII 414, RRII 430 and RRIM 600. Transcripts such as ferritin, DNA binding protein and NAC *tf*, were found significantly up regulated in drought tolerant clones *viz.* RRIM 600, RRII 208 and RRII 430 compared to RRII 105 and RRII 414 (susceptible clones). The expression level of aquaporin (Pip2.1) was significantly lower in water deficit tolerant clones compared to susceptible ones.

#### 1.2.1. Investigations on micro RNAs : Role in gene regulation during abiotic stresses

Analyses were carried out to find out miRNAs that show stronger association with cold tolerance. Next generation sequencing using



Illumina HiSeq method revealed that significant level of expression of 29 and 21 conserved miRNA families in cold stress exposed and control samples, respectively (clone RRIM 600). A total of 42 novel miRNAs were identified. From the differential expression analysis, eight conserved miRNAs were found common in both the samples. Expression analyses were performed with six selected miRNAs by qPCR method in two clones (RRII 105 and RRIM 600). miR169, miR 482 and miR159 showed strong association with cold tolerance. These miRNAs can be employed as markers for cold tolerance after extending the validation to larger number of clones.

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

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

In the field experiment being conducted at CES, Chethackal, RRII 400 series clones recorded better trunk girth (6<sup>th</sup> year) compared to RRII 105 and RRIM 600. Among the 400 series clones, the highest trunk girth was observed in RRII 414 followed by RRII 422.

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

Significant difference was observed for xanthophyll pigment pool size in drought imposed plants than the control plants. The xanthophyll cycle pigment composition showed low levels of anthroxanthin (A) and zeaxanthin (Z) in leaves of control plants compared to that of drought stressed plants. Xanthophyll cycle activity showed significant variation among clones when exposed to water deficit stress. RRII 430 and RRIM 600 showed high xanthophyll cycle activity compared to other clones studied. There was an increase in the level of neoxanthin, lutein and zeaxanthin in stressed plants compared to the control plants.

It was found that the level of lutein was high in the leaves of drought exposed plants compared to the control plants.

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

Six relatively drought tolerant and four susceptible wild *Hevea* germplasm accessions along with check clones were raised in polybags. Six month old plants were subjected to drought stress and analysed for osmolyte accumulation (proline and glycine betaine). Proline and glycine betaine showed significantly higher level of accumulation in tolerant accessions and tolerant check clones than susceptible clones and accessions.

#### 1.5. Ecosystem flux measurements

##### 1.5.1. Sap-flow measurements in mature rubber plants

Water mining of mature rubber plants was estimated using thermal dissipation probes (TDP-xylem sap flow system). The water use efficiency of mature trees as a function of dry rubber productivity was worked out in three agro-climatic conditions. In traditional region (RRII, Kottayam) the WUE (yield/unit water consumption) was  $8.0 \times 10^{-4}$  kg rubber/kg water whereas in a drought prone region (RRS, Dapchari) it was  $(3.7 \times 10^{-4})$  in rainfed trees and  $4.9 \times 10^{-4}$  in summer irrigated plants. In the case of cold stress prone region (RES, Nagrakata) the WUE was  $7.4 \times 10^{-4}$  kg rubber<sup>-1</sup> kg<sup>-1</sup> water.

##### 1.5.2. Relationship between canopy level CO<sub>2</sub> and water fluxes

The canopy level CO<sub>2</sub> and water fluxes were analyzed in mature rubber plantations. The CO<sub>2</sub> influx (photosynthetic carbon assimilation) in the canopy was directly controlled by light whereas, transpiration was not under strict regulation of solar light. As light intensity increased in the

morning, CO<sub>2</sub> assimilation increased and reached steady state. During periods of low light due to shade/cloud cover CO<sub>2</sub> assimilation decreased sharply while transpiration was little affected immediately. As sun started to set in the evening, CO<sub>2</sub> assimilation dropped quickly to zero (5.30pm), but transpiration reached zero much later (8.00 pm). This is because the transpiration is purely a physical process of diffusion and perhaps the underlying mechanism may be different during opening and closing of guard cells.

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

Under controlled condition, *Hevea* clones (RRII 105, RRII 600, RRII 208, RRII 422 and SCATC 88/13) were exposed to low temperature by gradual lowering of temperature to 15 °C / 7 °C (day/night) and 11h photoperiod. Clonal variation was noticed in low temperature responses. Severe foliar injury symptoms were noticed in clone RRII 422. Chlorophyll fluorescence measurement showed a substantial decline in maximum quantum yield ( $F_v/F_m$ ) and effective quantum yield of PSII ( $\phi$  PSII). Upon exposing to cold stress by gradual lowering of temperature non photochemical quenching (NPQ) tend to be reduced and reached a minimum. Drought imposition leads to the reduction of effective quantum yield in all the clones studied, however, this reduction was less in RRII 400 series clones compared to check clones. RRII 430 showed better chlorophyll stability index followed by RRII 600, RRII 422 and RRII 208. Anthocyanin content was found to be increased in response to the progress of drought stress and it was higher in relatively drought tolerant clones. Biochemical

analyses towards identifying clones with common adaptive mechanisms to drought and cold stresses are in progress.

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

A significant level of morphological difference in kaolin treated plants was observed. Leaf water potential was better in kaolin treatment. Plant biomass, leaf water content, level of chlorophyll and chlorophyll stability index were high under kaolin and KCl application. The study showed better chlorophyll retention with kaolin application followed by KCl and salicylic acid. Among the treatments, anthocyanin content and antioxidant enzyme (SOD) activity was high in kaolin applied plants. The preliminary results showed a positive effect of foliar applications of KCl and kaolin in the mitigation of drought stress in young polybag plants.

##### 1.7. Physiological evaluation of root trainer plants

A nursery level study with root trainer and polybag plants at RRS, Dapchari indicated that CO<sub>2</sub> assimilation rates declined by 96 per cent within three days of withholding irrigation in root trainer plants while the corresponding decrease in polybags were only 40-60 per cent. In the same location, field evaluation of root trainer and polybag plants of two clones (RRII 105 and RRII 430) was carried out during summer season. After one year of planting, casualty due to moisture stress was assessed and the percentage of casualty was more in polybag plants of RRII 105 whereas RRII 430 recorded relatively lesser casualty in root trainer as well as polybag plants (Table Phy. 1)

Table Phy. 1. Casualty percentage in the field under drought condition after one year of planting

Clones		Casualty (%)
RRII 430	Polybag	14
	Root trainer	20
RRII 105	Polybag	50
	Root trainer	36

**1.8. Drought survey in young rubber plantations**

A field survey was carried out for four years in one year old plantations of Kerala to study the management practices followed by farmers especially irrigation during summer. The survey indicated delayed planting in Central and South Kerala. Management practices like mulching, shading and cover cropping were least followed in South Kerala. Wherever water was available many farmers irrigated the plants at least once during peak summer period. It was found that nearly 18 per cent of the rubber growers irrigated their one year old rubber plants during summer in Kerala (Table Phy. 2). Management practices during early establishment of the crop have to be ensured in the plantations to counter the adverse effects of high temperature and moisture stress.

Table Phy. 2. Percentage of planters following good management practices in young rubber plantations

	South	Central	North-Central	North	All Kerala
Mulching	23	75	67	67	58
Shading	0	65	71	77	53
Weeding	93	98	95	88	94
Cover crop	3	10	15	3	8
Irrigation	3	30	19	20	18

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

Mid-day canopy temperature an indirect indication of drought tolerance was monitored in 31 accessions of SBN 2004 and 14 accessions of SBN 2005 groups during peak summer (March) in field grown nursery plants. Leaves fully exposed to direct sun light were measured using an infra-red portable remote thermometer (Raytek Corporation, USA) on clear sunny days. The canopy temperature varied from 35.7 °C for accession AC 2009 to 41.3 °C for accession RO 291 across genotypes. All accessions were sorted, ranked and the most tolerant ones maintaining low canopy temperature were identified. The accessions, AC 2009, RO 2554, MT 196, RO 211, RO 2151, AC 453, RO 2360, RO 2619 and AC 3146 were ranked top (low canopy temperature at peak mid-day hours) and accessions RO 291, AC 3207, RO 322, RO 2864 and AC 3913 ranked low (higher canopy temperature during summer). In SBN 2005 accessions MT 2210, RO 1421, RO 1406, AC 173 and MT 2231 were top ranking with low canopy temperature.

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

Sixteen ortets selected from different agro-climatic regions and seven check clones were planted at CES, Chethackal in 2012 as part of a multi-location trial. Girth was recorded at 50 cm height from the bud union during February 2016. Among the ortets RRSA 98 (25.3 cm) recorded the highest girth, whereas, the ortet GH 1 had the lowest girth. Among the check clones, RRII 430 showed higher girth than RRII 422 and RRII 417.



Cumulative dry rubber yield of 10 test tappings was recorded in ortets. The highest yield was recorded in clone RRII 417 followed by clone RRII 430 in the peak yielding season (Sep-Oct). Ortet RRSA 98 (33.8 g) followed by DAP 1 (29.1 g) and DAP 35 (26.9 g) recorded high yield, whereas, ortets NGK 69 (4.4 g), GH 3 (6.4 g) and RRSA 315 (6.0 g) recorded the lowest yield. In summer periods clone RRII 430 was the top most yielder followed by ortet RRSA 98. Ortet GH 3 recorded very low yield in summer tapping.

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

Data from photosynthetic measurements and biochemical analysis of the ortets from cold stress (Nagrakata) and drought stress (Dapchhari) prone regions during stress and stress-free periods and from Chethakkal (control location) were compared. Cold stress inflicted more detrimental effect on photosynthetic parameters than drought stress in most of the ortets/clones. Studies on biochemical parameters like contents of sugars, protein, malondialdehyde and assay of antioxidant enzymes (peroxidase and glutathione reductase) were also carried out in these ortets. A few ortets namely, GH 1, DAP 35 and GH 3 were found better adapted to cold as well as drought conditions which can be developed as clones for wider climate adaptability. Ortets RRSA 98, RRST 39 and RRSA 585 recorded better photosynthetic rate under drought, while DAP 1, RRST 24 and GH 9 performed better under cold stress.

#### *1.11. Experimental cultivation of high yielding varieties of rubber plants for establishment in higher elevation*

In a clone trial at Haileyburia Tea Estate, Elappara, the growth of rubber trees was not satisfactory and adversely affected by prevailing

environmental factors. The growth of polyclonal seedlings (38.6 cm) planted in between the tea rows was better than clonal plants at higher elevation.

#### *1.12. Proteomic studies in Hevea brasiliensis under abiotic stresses*

##### *1.12.1. Proteomic studies under cold stress*

Five *Hevea* clones were subjected to cold stress inside a growth chamber. Cold injury (necrotic spots on leaf) was noticed in susceptible clones RRIC 100 and RRII 414 as well as in cold tolerant clone RRII 429. However, net photosynthesis rate and  $\phi$  PSII were better in tolerant clones (RRIM 600 > RRII 208 > RRII 429). A total of around 50 differentially expressed proteins were found when total leaf proteins were analysed using 2D gel electrophoresis.

##### *1.12.2. Proteomic studies under drought stress*

Net photosynthesis rate and stomatal conductance were higher in clones RRII 430 and RRIM 600 followed by RRII 208, RRII 414 and Tjir 1 whereas RWC was higher in clones RRIM 600 followed by Tjir 1, RRII 430, RRII 208, and RRII 414 after nine days of drought exposure. Among the differentially expressed proteins eighteen were more abundant in the drought tolerant clones (RRII 430 & RRIM 600).

#### *1.13. Growth regulation and high density planting (HDP) for productivity enhancement*

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

Field planting of a new experiment on high density planting (HDP) and growth regulation was carried out in June-July 2015 as targeted with three densities and two types of planting material viz. polybag and root-trainer plants.

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

SEM micrographs showed less anisotropically oriented cuticle folds on leaf upper surface of *Hevea* plants pre-treated with PBZ (Fig. Phy. 1) In these plants, number of stomata were 23 per cent more (488 against 396 mm<sup>-2</sup> in control) with 33 per cent lesser stomatal aperture (8.0 against 12 mm in control) and more stomatal striations;

helpful for imparting drought tolerance that were subsequently confirmed by physiological and biochemical parameters. Net photosynthesis rate (A) was better in RRII 430, RRIM 600 and RRII 208 than drought susceptible clones Tjir 1 and RRII 414 after drought exposure and P<sub>n</sub> improved further in all the clones pre-treated with PBZ. Similar trends were noticed in biochemical parameters like SOD enzyme activity, proline, and leaf sugar contents.

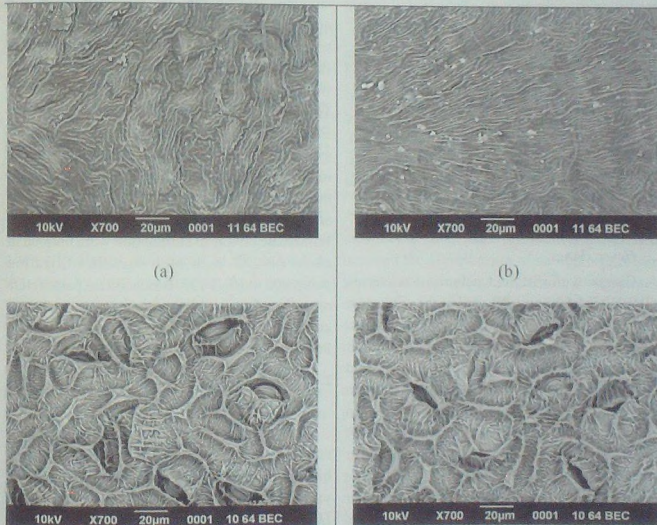


Fig. Phy. 1. SEM micrographs showing less anisotropically oriented cuticle folds on leaf upper surface (b) and more stomatal density with lesser stomatal aperture length and striations covering the stomates (d) of *Hevea* plants pre-treated with PBZ compared to un-treated plants (a & c)

Paclobutrazol (PBZ-50mg) pre-treated polybag plants had better intrinsic drought tolerance potential owing to modification of leaf anatomy. Field planting has been carried out with PBZ pre-treated plants along with control at RRS, Padiyoor to confirm the results.

## 2. Production physiology

### 2.1. Intercropping with tree crops in rubber

Mahogany (49.3 cm) was found growing better as intercrop than pathimugom (24.8 cm) in a rubber inter-crop trial at CES, Chethackal. The mean girth of rubber trees did not exhibit any significant difference between the treatments. The trees were continuously tapped for five years under S/2 d3 system of tapping and yield was recorded from field coagula. Mean rubber yield in rubber alone and rubber intercropped with tree species did not exhibit any significant variation. Among the intercrops the growth of pathimugom trees was largely affected due to shading by the mature rubber trees.

### 2.2. Wintering nature in double budded *Hevea* clones

Clones with different pattern of wintering traits (RRIM 600 and PR 107 early wintering; RRII 105 and GT 1 late wintering) were budded on to a single stock and planted along with their single budded plants (control). It was assumed that wintering pattern of the double budded scion would give an indication of the scion-to-scion communication. Girth data indicated significantly higher girth in the single budded plants of four clones compared to double bud plants. The scions of double budded plants did not differ from their single budded counterparts with respect to wintering pattern, indicating lesser communication of wintering trait between the two scions in a double budded plant. Latex yield from single and double budded plants was compared. Initial yield

(8 months) of two early wintering pairs (RRIM 600 and PR 107) was higher in double budded plants compared to respective single budded normal plants.

### 2.3. Clonal variation and effect of stimulation on latex regeneration mechanism

Based on earlier findings on latex regeneration mechanism in *Hevea*, a new experiment was started at CES, Chethackal in newly opened trees of clones with different latex metabolism (RRII 105, PB 217 and PB 260) to study the effect of tapping on latex regeneration and effect of stimulation using ethephon. Eighteen trees were selected (nine control and nine stimulated with 2.5% ethephon) and latex samples were collected monthly after each stimulation. The trees were under d3 system of tapping. Biochemical parameters related to rubber biosynthesis (ATP and sucrose), oxidative stress indicators (peroxidase, glutathione reductase and thiols) and protein content were measured. Yield and dry rubber content were also recorded. Immediate effects of stimulation were noticed in clone PB 217 with high sucrose utilization compared to RRII 105. Biochemical parameters related to latex regeneration process were not altered immediately after stimulation in clone PB 260.

### 2.4. Studies on rubber biosynthesis: Gene expression studies

Expression of 14 genes corresponding to enzymes/regulatory proteins involved in rubber biosynthetic pathway was analysed in high/low yielding clones. The level of expression of *HbSUT3* a sucrose transporter and enzymes like hydroxymethyl glutaryl-CoA synthase (HMGS), HMG-CoA reductase (HMGR) and mevalonate diphosphate decarboxylase (*MVD*) was found to be significantly higher in high yielding than low



yielding clones. The high magnitude of expression of these genes might result in an increased supply of IPP, the isoprenoid monomer, for rubber biosynthesis. Expression of genes in the downstream biosynthetic pathway like *FPPS*, *RuT* and *REF2* was also found to be significantly higher in high yielding than low yielding clones. The results suggest that high rubber yield is associated with higher level of expression of these genes and may be useful as markers for high yield potential of *Hevea*.

### 3. Tapping panel dryness

#### 3.1. Biochemical aspects of low frequency tapping

The analysis of biochemical data on low frequency tapping trial at Koney estate with clone RRH 105 under different tapping frequencies with recommended level of stimulation indicated balanced and activated metabolism in S/2 d4 7d/7 and S/2 d4 6d/7 with sufficient sucrose loading, high energy availability (ATP) and protection of laticiferous system. Tapping system S/2 d6 7d/7 and S/2 d7 6d/7 showed high utilization of sucrose after stimulation compared to d2 and d3 as indicated by very low sucrose, high invertase activity, normal level of thiols and sufficient energy availability. No sign of degradation of the laticiferous system was observed after frequent stimulation in S/2 d6 7d/7 and S/2 d7 6d/7 (indicated by high thiol, no accumulation of proline and phenol). Though the level of sucrose was

significantly low in d6 and d7 after stimulation, it was maintained throughout the experimental period.

### 4. Secondary metabolites

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

The relationship of inositols and other solutes with water relations of latex (osmotic potential) and their role in drought responses of different clones during peak yielding and stress seasons was assessed. Contribution of all these solutes to osmotic potential ( $\psi_s$ ) and osmotic regulation was also studied. Clonal variation was observed in latex osmolytes concentration. Inositol content showed significant clonal and seasonal variation. The variation in total inositol content in the latex was closely related to latex osmolality. High yielding clones generally showed increased production of inositols. Osmotic contribution of the solutes of latex showed the positive role of inositols to total latex osmolality. Differences between the contribution of soluble sugars and inorganic ions indicated that osmotic adjustment is mainly contributed by soluble sugars and inositols during stress periods. The study revealed that osmotic adjustment of *Hevea* plants to drought is mainly contributed by higher per cent contribution of inositol and total carbohydrate pool.

## LATEX HARVEST TECHNOLOGY DIVISION

The Division was very active in the research and advisory services on all aspects of crop harvesting. The programme on low frequency weekly tapping in smallholdings is progressing and is getting acceptance among growers. In the low price scenario, adoption of weekly tapping and controlled upward tapping for old and senile trees will empower the growers to continue successfully. Long term findings from the comprehensive trial on LFT were issued as recommendation to growers. Results from experiment and commercial evaluation of quarter spiral d3 tapping are promising. The naturally degradable polythene produced in a collaborative programme with a private entrepreneur has successfully completed repeated commercial evaluation. After field evaluation at CES, Chethackal, one mechanical and one motorized knife have been identified for further commercial evaluation.

#### 1. Low Frequency Tapping (LFT)

The division continued experiments, onfarm and advisory trials on low frequency tapping. The

comprehensive study on frequencies ranging from alternate daily to weekly tapping at Koney estate of Harrison's Malayalam Ltd. initiated during 2010 was concluded. Yield under all frequencies except in S/2 d3 without yield stimulation were comparable. Based on the results, modified comprehensive yield stimulation schedule has been formulated and recommended (Table. LHT. 1). Bark consumption was directly related to the frequency. Monitoring of the various latex biochemical parameters for three consecutive years, it was conclusively proved that there was no indication for accumulation of stress indicators like proline and phenol even under weekly tapping with higher rounds of yield stimulant application. For the successful performance of weekly tapping, it is essential to ensure regular and uninterrupted tapping, removal of bark shaving at 2.5 mm  $\text{tap}^{-1}$ , correct depth of tapping *i.e.* one mm near to the cambium in all tapping and application of yield stimulant (ethephon) at recommended schedules and levels.

Table. LHT. 1. Comprehensive yield stimulation schedule for different frequencies of tapping

Clone	Tapping system	Rounds/year	Schedule
RRII 105	S/2 d3 7d/7	2	April/May/June, September/ October/ November
RRII 105	S/2 d3 6d/7	3	April/May/June, September, November
RRII 105	S/2 d4 7d/7	4	April/May/June, August, October, December
RRII 105	S/2 d4 6d/7	6	April/May, June, August, September, November, December
RRII 105	S/2 d6 7d/7	10*	All months - after every 6 <sup>th</sup> tapping, 72 hours before the 7 <sup>th</sup> tapping
RRII 105	S/2 d6 6d/7 (weekly)	12**	All months - after every 4 <sup>th</sup> tapping, 72 hours before the 5 <sup>th</sup> tapping

\* During the initial two years of opening 20 rounds per year

\*\* During the initial two years of opening 24 rounds per year

### 1.1. Collaborative programme with Regional offices of Rubber Board in popularizing weekly tapping with stimulation in smallholdings

The programme initiated on implementing weekly tapping progressed well. All the participants under various regional offices are reporting good and steady performance. We have more than 40 participants, but later many others also have switched to weekly tapping after gathering information from these successful growers. In collaboration with the publicity and public relations Department, an exclusive video on weekly tapping prepared, telecasted through a popular channel and the CD was handed over to extension Department of Rubber Board for their future use.

In the demonstration plot at Central Experiment Station (CES) on weekly tapping with monthly application of 2.5 per cent ethephon, yield of 2137 kg 400 trees<sup>-1</sup>, was 5.3 kg tree<sup>-1</sup> year<sup>-1</sup> out of 45 tapping days. Incidence of tapping panel dryness was 4.5 per cent.

### 1.2. Low Frequency d10 tapping in clone RR11 105

In the trial at CES, trees were tapped under d6 frequency of tapping with monthly stimulation in BO-2 panel during 2002-2004. From April 2004 onwards the tapping frequency was changed to d10 with once in 20 day's stimulation. It continued to give promising yield during 2015-16 (Table LHT. 2). Yield of 2186 kg 400 trees<sup>-1</sup> was obtained in the fifth year of BI-1 panel. TPD per cent was low after 14 years of tapping (6.6 %).

In the large scale trial on d10 frequency with 10 tapping blocks at Kanthimathy Estate in Kulasekharan Region of Tamil Nadu mean dry rubber yield of 2095 kg block<sup>-1</sup> could be obtained as against the estate average of 1965 kg block<sup>-1</sup> during the year 2015-16.

## 2. Controlled Upward Tapping (CUT)

All India coordinated project on CUT continued successfully in all locations and the yield performance under periodic panel change system was similar to that in previous year. The long term information from different parts of the country has conclusively proved that CUT is the apt technology to enhance yield from old and senile rubber trees with below average productivity at least by 50 per cent in the long run.

### 2.1. Low Frequency Controlled Upward Tapping

The field experiment on Low Frequency Controlled Upward Tapping (LFCUT) under d10 frequency of tapping with four treatments and five replications laid out at EFU, RIT, Pampady was continued. Significant yield variation was observed among the treatments. Higher per tap yield could be observed under d10 frequency of tapping (Table LHT. 2). Highest mean dry rubber yield of 153 g t<sup>-1</sup> t<sup>-1</sup> could be obtained under d10 frequency of tapping with periodic panel change as against 65 g t<sup>-1</sup> t<sup>-1</sup> under d3 frequency of tapping. However, dry rubber yield (kg tree<sup>-1</sup>) was observed to be significantly higher under d3 system of tapping.

## 3. Other experiments

### 3.1. Onfarm evaluation of reduced spiral tapping

All the information from the experiment at EFU, RIT, Pampady and commercial plots in various locations were compiled. It is conclusively proved that in clone RR11 105, opening a quarter spiral cut from 45 cm girth onwards, and tapped under once in three days frequency with six rounds of yield stimulation/year (at 45 days interval during nine months except February, March & April) using 2.5 per cent ethephon will ensure equivalent yield of S/2



Table LHT. 2. Yield performance of clone RRII 105 under Low Frequency Controlled Upward Tapping (LFCUT) with d10 frequency of tapping ( 2015- 16)

Treatment	Yield		
	kg tree <sup>-1</sup>	kg 400 trees <sup>-1</sup>	kg tap <sup>-1</sup> 400 trees <sup>-1</sup>
1. S/2 d3 6d7 ET 2.5 % Pa (3/y), S/4 d3 6d7 U ET 5 % La (m) (Periodic CUT)	6.4 a	2560 a	25.8 c
2. S/3 d10 U ET 5 % Ga (20 d) (Continuous CUT)	4.1 c	1640 c	52.1ab
3. S/2 d10 ET 2.5 % Pa (20 d), S/3 d10 ET 5 % Ga (20 d) (Periodic CUT)	3.9 c	1560 c	47.9 b
4. S/2 d10 ET 2.5 % Pa (20 d), S/3 d10 ET 5 % Ga (10 d) (Periodic CUT)	5.3 b	2120 b	61.1 a
LSD(0.05)	0.9712	388.5	9.2

d3 tapping with three rounds of stimulant application. The adoption of S/4 d3 6d/7 ET 2.5 per cent Pa (6/y) will also enable to open trees almost one year prior to normal opening (50 cm), and continuing on the system will assure tapping on four virgin panels (7x4=28 years) instead of two virgin panels (7x2=14) meaning doubling the tapping period on virgin panel and ultimately longer economic life.

### 3.2. Comparative evaluation of various yield stimulants in clone RRII 105

The experiment was initiated at CES Chethackal in clone RRII 105. The experimental design was RBD with three replications comprising 60 trees per replication. Tapping system adopted in this trial was S/2 d3 6d/7 with panel application of 2.5 per cent ethephon (3/y and 6/y) and groove application of Vitex (3/y, 6/y and 9/y) and Agrowin gel (1/y) stimulant. Yield was comparable in all treatments.

### 3.3. Response of RRII 400 series clones to yield stimulation

In the yield stimulation experiment on RRII 400 series clones (RRII 414, RRII 422 and RRII 429) tapped under S/2 d3 6d/7, two rounds of stimulation with ethephon 2.5 per cent was given during 2015-16 and compared with the unstimulated control trees.

In clone RRII 422, significant yield increase was recorded in stimulated trees compared to unstimulated trees. No significant yield increase was noticed in clones RRII 414 and 429. In general, RRII 429 had higher yield than RRII 422 and RRII 414 (Fig. LHT. 1). Stimulation response was good in RRII 422, but poor in clone RRII 414 and 429.

### 3.4. Evaluation of non-conventional tapping methods

#### 3.4.1. Evaluation of vertical tapping

Observations on vertical tapping indicated that it was feasible to get comparable yield with

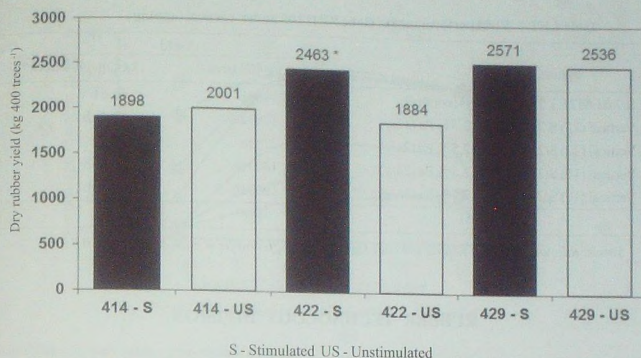


Fig. LHT. 1. Yield performance of RR11 400 series clones under yield stimulation

that of normal half spiral downward tapping with appropriate stimulation (Table LHT. 3). It is worthwhile to study vertical tapping in detail as it could be easy to develop mechanised tapping tools for it. There is less bark utilization compared to half spiral cut under vertical tapping.

#### 3.4.2. Comparison of needle tapping with conventional tapping system

Response of needle tapping in comparison with the conventional half spiral d3 frequency of tapping in clone RR11 105 was continued at EFU, RIT, Pampady. Three rounds of yield stimulant (ET.2.5%) were imposed in the conventional tapping, whereas 24 rounds were imposed in the needle tapping. Even with fortnightly stimulation, yield under needle tapping system was only

42 g t<sup>-1</sup> t<sup>-1</sup> compared to 59.5 g t<sup>-1</sup> t<sup>-1</sup> under conventional tapping.

#### 3.5. Development and evaluation of naturally degradable polythene for rainguarding

In collaboration with a private entrepreneur a naturally degrading polythene for rainguarding rubber trees was developed. This material will have an effective service period of 6-7 months *i.e.* it can protect the tapping panel from rainwater during both the monsoons. After 6-7 months it starts degrading, fragmenting and when littered on soil surface will totally disintegrate within next six months, *i.e.* before the onset of next monsoon. Thus the rainguard polythene littered in the field will not be a cause for water accumulation and mosquito breeding.

Table LHT. 3. Yield performance of clone RR1105 under vertical tapping

Treatment	Yield	
	kg 400 trees <sup>-1</sup>	kg tap <sup>-1</sup> 400 trees <sup>-1</sup>
1. S/2 d3 6d7 ET 2.5 % Pa (3/y) (control)	1480 b	16.6 b
2. Vertical (22) S/2 d3 6d7 ET 2.5 % Pa (12/y)	1760 ab	19.4 ab
3. Vertical (22) S/2 d3 6d7 ET 2.5 % Pa (24/y)	2280 a	25.1 a
4. Vertical (10) S/2 d3 6d7 ET 2.5 % Pa (24/y)	1800 ab	20.0 ab
5. Vertical (10) S/2 d3 6d7 ET 2.5 % Pa (36/y)	1960 ab	21.7 ab
LSD (0.05)	583.8	6.5

Values followed by same letter/s are not significantly different from each other at  $P < 0.05$

## RUBBER TECHNOLOGY DIVISION

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

### 1. Primary processing

#### 1.1. Development of a modified coagulant for NR latex processing

A modified coagulant developed at RRII was used to prepare 'Air dried sheet rubber' (ADS) and the storage behaviour of such sheets was studied. The variation of initial plasticity ( $P_0$ ) with storage time of ADS is given in Fig. Chem.1. The variation of  $P_0$  of sheets prepared with

conventional coagulant (control) was compared with the  $P_0$  of sheets prepared with the modified coagulant A, B, C, D and E on storage for a period up to six months.

The variation in Mooney viscosity with storage also has been evaluated and its variation trend was similar to that of  $P_0$ .

#### 1.2. Deproteinised natural rubber (DPNR)

##### 1.2.1. New proteolytic enzyme for deproteinization of NR

In earlier studies, deproteinization of NR latex was attempted using enzyme 'Papain'. Two new proteolytic enzymes were used for deproteinization studies of NR latex, of which one was found to be very effective. Through enzyme treatment - cum - creaming method of field latex, DPNR of good quality could be made. Properties of DPNR and commercial samples are given in Table Chem. 1.



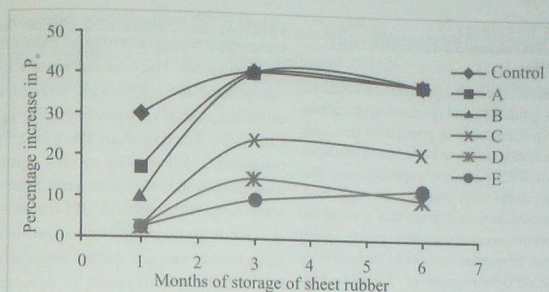


Fig. Chem. 1. Variation of initial plasticity ( $P_0$ ) with period of storage

Table Chem. 1. Raw rubber properties of different DPNR samples

Sample	Nitrogen (%)	$P_0$	PRI	Mooney viscosity, $M_1(1+4), 100^\circ\text{C}$
DPNR prepared by enzyme-cum-creaming method	0.1	41	54	83
Commercial sample 1	0.2	38	18	84
Commercial sample 2	0.1	31	9	59

production of DPNR have not been scaled up to industrial level mainly due to the long processing time and tedious methodologies which were not cost effective. Also the quality of the DPNR through these processes was found to be lower.

Conventionally DPNR has been prepared through a 48-72 hours proteolytic enzyme treatment or non-enzymatic process followed by either centrifugation or creaming process. The long processing time and complex processing techniques limit the high volume production and increase the production cost of DPNR.

A new method was developed to prepare high quality solid DPNR from field latex within one to four hour of treatment time with a deprotenisation mixture. The new method completely eliminates the creaming/centrifugation steps and hence facilitates high volume production at low cost. DPNR was prepared through direct acid coagulation after treating the fresh field latex with a deproteinizing mixture developed at RRII, based on a proteolytic enzyme. DPNR with N content in the range of 0.05 to 0.09 per cent could be obtained.

#### 1.2.2. Improved technique for preparation of solid deproteinized natural rubber (DPNR-S) without centrifugation/creaming

Solid natural rubber (TSR/sheet rubber) usually has a N content of about 0.5 per cent, whereas DPNR preferably should have a N content of less than 0.1 per cent. Solid DPNR is mainly used for engineering products and currently this is imported by the product manufacturers. The in-house technologies developed so far in India (RRII) for the

## 2. Latex technology

### 2.1. Radiation vulcanised natural rubber latex (RVNRL)

Radiation vulcanised NR latex (RVNRL) is good for sulphur free and nontoxic applications. Efforts have been made to popularize this latex for various applications in latex based products. Samples of RVNRL were supplied to a few manufacturers for their consumption and feedback. Manufacturers informed that though the basic properties were reasonably good, ageing and modulus properties are yet to be improved for large scale consumption of RVNRL. Also continuous supply RVNRL has to be ensured. A carpet backing unit showed specific interest for consumption of this latex. Steps were initiated for renewal of licence from Atomic Energy Regulatory Board (AERB) for the gamma irradiation source.

### 2.2. Cytotoxicity studies (residual accelerators and biocompatibility) of surgical gloves

The quantification of residual accelerators

present in NR surgical gloves belonging to six commercial brands available in Indian market was carried out. The total accelerators leached into acetonitrile from these glove samples were estimated using UV-Visible Spectroscopy (Fig. Chem. 2). Separation of the glove extract components, followed by specific accelerator identification and subsequent quantification was achieved by HPLC method. HPLC studies have shown that the major accelerator used in all the glove brands was zinc diethyl dithiocarbamate (ZDEC). The quantification by UV analysis showed that the total residual accelerators varied from 926 to 2725  $\mu\text{g g}^{-1}$  (Table Chem. 2). The quantification of the specific ZDEC peak by HPLC revealed that ZDEC content varied from 873 to 2616  $\mu\text{g g}^{-1}$  among the pre-powdered surgical gloves (Table Chem. 3). The biocompatibility studies of the gloves are under progress.

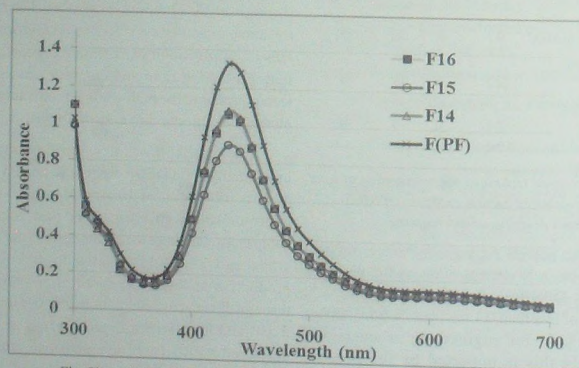


Fig. Chem. 2. UV-VIS absorbance spectra of copper complex of total residual accelerator of glove brands 'F 16', 'F 15', 'F 14' and 'F' (powder free)

Table Chem. 2. Total residual accelerator ( $\mu\text{g g}^{-1}$ ) from different glove brands by UV spectroscopy

Glove Brands	Absorbance at 430 nm	Concentration of ZDEC solution ( $\mu\text{g mL}^{-1}$ )	Total residual accelerator ( $\mu\text{g g}^{-1}$ )
A	1.050	26.9	2687
B	0.868	22.2	2203
C	0.378	09.4	926
D	0.820	20.9	2085
E	0.502	12.7	1259
F 14	1.069	27.4	2725
F 15	0.891	22.8	2276
F 16	1.056	27.1	2703
F (P F)	1.339	34.5	3298
B (P F)	0.355	08.8	880

Table Chem. 3. Residual ZDEC accelerator from different glove brands by HPLC analysis

Glove Brand	Retention time (minute)	Area of HPLC peak at 430 nm	Concentration of ZDEC solution ( $\mu\text{g mL}^{-1}$ )	Residual accelerator ( $\mu\text{g g}^{-1}$ )
A	7.510	767598	20.43	2038
B	7.545	690882	18.76	1862
C	7.495	237056	8.89	873
D	7.382	1009945	25.70	2558
E	7.474	498181	14.57	1449
F14	7.488	1039059	26.33	2616
F 15	7.441	890648	23.11	2307
F 16	7.463	997845	25.44	2538
F (Powder Free)	7.468	1049947	26.57	2543
B (Powder Free)	7.443	255359	9.29	925

### 3. Rubber Technology

#### 3.1. Reinforcement

##### 3.1.1. NR/ Polymeric filler system

A new polymeric system (PF2) was tried in a tyre tread formulation. In this study, 10 and 15 parts of polymeric filler (PF2) were added along with varying concentration of HAF carbon black. Formulation of the mixes and technological properties are given in Tables Chem. 4 and 5.

Technological properties such as tensile strength, modulus, tear strength, hardness and flex resistance of NR tread compound containing 10

parts polymeric filler (PF2) along with 30 parts HAF black and 15 parts PF2 along with 20 parts of HAF black were almost comparable to that of the control compounds. These compounds showed better elongation properties than the control tread compounds. PF2 modified composites showed very low heat build up compared to the control compounds. Payne effect studies of the samples with 10 parts PF2 along with 30 parts HAF black and 15 parts PF2 along with 20 parts of HAF black showed lower filler-filler interaction than the conventional control tread compounds.



Table Chem. 4. Formulation of the mixes

Ingredients	C1	C2	S1	S2	S3	S4	T1	T2	T3	T4
NR	60	100	100	100	100	100	100	100	100	100
Polybutadiene	40	0	0	0	0	0	0	0	0	0
PF2	0	0	10	10	10	10	15	15	15	15
Zinc oxide	5	5	5	5	5	5	5	5	5	5
Stearic acid	2	2	2	2	2	2	2	2	2	2
6PPD	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HSL	1	1	1	1	1	1	1	1	1	1
HAF	50	50	0	10	20	30	0	10	20	30
Naphthenic oil	6	6	0	1	2	3	0	1	2	3
TBBS	1.3	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Sulphur	1.2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Table Chem. 5. Technological properties of the vulcanizates

Properties	C1	C2	S1	S2	S3	S4	T1	T2	T3	T4
Tensile strength, MPa	26.2	21.2	27	28.2	31.4	29.6	29	31	33	32
Modulus, 100%, MPa	2.5	2.2	1	1.4	1.9	2.3	2.4	2.65	3.65	4.33
Modulus, 300%, MPa	9.9	8.6	2.9	4.5	6.3	7.4	6.2	8.4	10.9	12.3
EB, %	653	625	802	790	756	720	702	669	645	631
Tear strength, N mm <sup>-1</sup>	102	91	54	58	65	68	66	72	79	92
Heat build up, °C	26	24	6	7	10	12	7	10	12	14

### 3.1.2. NR Latex - carbon black masterbatch

Studies on the preparation of NR latex - carbon black master batch was continued as a collaborative project with M/s. Apollo Tyres Ltd. Masterbatch samples were prepared and sent to the collaborator for evaluation of the technical properties at their end. Based on the feedback from their end, the process was refined and the properties of the latex - carbon black master batches were evaluated at RRII. The results were presented at the collaborating industry and based on their comments further refinements are in progress.

### 3.1.3. Silica reinforcement of NR

#### 3.1.3.1. NR Latex-silica masterbatch

Precipitated silica has highly polar hydroxyl groups on its surface. This makes silica incompatible with non-polar polymers such as natural and synthetic rubber. Hence, mixing of silica in hydrocarbon rubbers in the dry state is difficult and is an energy intensive process. Dry mixing also causes pollution problems in the factory floor. Hence, as an alternative to dry mixing, NR latex - silica masterbatch was attempted. Eight latex based masterbatches of NR - silica were prepared from field latex preserved with different preservation systems.

Formulation of the mixes are given in Table Chem. 6 in which FL-P1 to FL-P8 denote latex masterbatches prepared from latex preserved with varying preservation systems, D-9 represents NR - silica dry mix and CB-10 corresponds to NR-carbon black mix. The tensile

and technological properties of the vulcanizates prepared found that NR latex-silica masterbatch based vulcanizates exhibited properties comparable to that of the corresponding dry mix. Heat build up of silica filled composites was much lower than the carbon black filled composites.

Table Chem. 6. NR Latex -silica masterbatch - formulation of mixes

Property	Compound number									
	FL-P1	FL-P2	FL-P3	FL-P4	FL-P5	FL-P6	FL-P7	FL-P8	D-9	CB-10
Natural rubber	100	100	100	100	100	100	100	100	100	100
Zinc oxide	4	4	4	4	4	4	4	4	4	4
Stearic acid	2	2	2	2	2	2	2	2	2	2
Anti oxidant (6 PPD)	2	2	2	2	2	2	2	2	2	2
Naphthenic oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5
Vegetable oil plasticizer	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	-
Silica (Ultrasil VN3)	48	48	48	48	48	48	48	48	48	-
ISAF Black	-	-	-	-	-	-	-	-	-	48
CBS	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DPG	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	-
Sulphur	2	2	2	2	2	2	2	2	2	2
Silane (TESPT)	3	3	3	3	3	3	3	3	3	-

### 3.13.2. Silica-carbon black combination as reinforcement in NR

Natural rubber-silica composites were prepared through latex masterbatch route. Silane (TESPT) treatment was given at six per cent of silica weight. Carbon black was further incorporated in to these composites in the mixing mill. Silica to black ratio in the composites varied from 50/0 to 0/50 as shown in Table Chem. 7. These composites were further compounded using the formulation given in Table Chem. 7. Vulcanizate samples were prepared and tested as per various standards. Combination of filler showed advantages in some properties such

as modulus, tear strength, abrasion resistance and heat build up.

### 3.1.4. Reinforcement of NR using modified silica

#### 3.1.4.1. NR-Silica composites with improved rubber-silica interactions

Papain was used as a surface modifier for silica. Papain coated silica was prepared. The silica to papain (w/w) ratio of 100:1, 100:3 and 100:5 were prepared and dried in an air oven. Rubber compounds were prepared with unmodified silica and papain modified silica. Rubber compounds with the following formulations were prepared on the two roll mill. ISNR5 (100), stearic acid (2phr), zinc oxide

Table Chem. 7. NR-silica-carbon black blend reinforcement-Formulation of mixes

Ingredients	Compound number						
	I	II A	II B	III	IV	V	VI
Natural Rubber	100	100	100	100	100	100	100
Silica (Ultrasil VN3)	50.2	38.0	36.7	28.1	19.4	10.2	0
Carbon black, ISAF	0	12.1	13.4	21.9	30.6	39.8	50
Zinc oxide	4	4	4	4	4	4	4
Stearic acid	2	2	2	2	2	2	2
Mernox 6C	2	2	2	2	2	2	2
Naphthenic oil	2.5	2.5	2.5	2.5	2.5	2.5	5
V. Plasticizer	2.5	2.5	2.5	2.5	2.5	2.5	0
CBS	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DPG	1.5	1.3	1.3	1	0.8	0.5	0
Sulphur	2	2	2	2	2	2	2

(4 phr), naphthenic oil (5 phr), sulphur (2.5 phr), silica modified with 1, 3 and 5 per cent papain content (w/w) for compounds P1, P3 and P5, CBS (0.7 phr), DPG(1.5 phr) and silica (50 phr).

The filler type was varied with 50 phr silica respectively. Cure characteristics of these VN3 for control compound and 50 phr each of composites are given in Table Chem. 8.

Table Chem. 8. NR - modified silica composites - cure characteristics

	Control	P1	P3	P5
Cure time, $T_{90}$ , min	7.48	6.55	6.05	6.07
$T_{50}$ , min	2.24	2.34	2.24	2.22
Maximum Torque, $M_H$ , dNm	33.78	31.63	31.61	32.13
Minimum Torque, $M_L$ , dNm	8.23	6.76	6.27	6.69
$M_H$ - $M_L$ , dNm	25.55	24.87	25.34	25.44
$\tan \delta$ at $M_H$	0.105	0.099	0.094	0.097

Results of the 'Payne effect' studies of the compounds are given in Fig. Chem. 3. Modulus at low strain was higher for the unmodified silica compound (control) and for the silica with papain coating of one per cent (P1), whereas the modulus decreased with increase in papain coating to three per cent (P3) and five per cent (P5) in the uncured sample. This indicates reduced filler-filler network between silica particles due to protein coating on the silica.



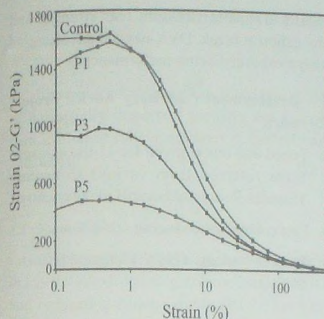


Fig. Chem. 3. Payne effect studies of uncured rubber-silica compounds

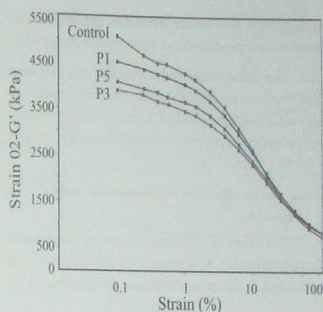


Fig. Chem. 4. Payne effect studies of cured rubber-silica vulcanizates

Protein coating reduces the filler-filler interaction making the processing of rubber compound energy efficient.

Compared to the control, about 20 per cent increase in Modulus @100 per cent, nine per cent in Modulus @300 per cent was achieved for the vulcanisate P3. Tear strength and elongation remains comparable.

Results of the Payne effect studies of the various cured rubber-silica vulcanizates is given in Fig. Chem. 4. In cured vulcanizates, the Payne effect was higher for the unmodified silica compound (control), whereas the Payne effect decreased with increase in the papain coating from one per cent (P1) to three per cent (P3). This indicates reduced filler-filler network between silica particles due to protein coating on the silica and shows better dynamic properties. At five per cent papain content on silica (P5) the Payne effect was slightly greater than the three per cent modification.

### 3.2. Recycling of rubber: Devulcanization

#### 3.2.1. Comparison of devulcanizing agent (DVA) assisted devulcanization with industrially practiced devulcanization processes

At present two commercially used mechano-chemical processes are in practice.

- i. Commercial process I, which uses a mixture of selected accelerators as devulcanizing agent during the mechanical shearing of vulcanized rubber powder (as given in patent) and
- ii. Commercial process II which uses a mixture of a dicarboxylic acid and urea as devulcanizing agent (as given in patent).

RRII has developed a simple mechanical process where shearing of crumb rubber powder in a two roll mill without the addition of any chemical can also devulcanize rubber.

A comparison of these devulcanization processes with the new devulcanization process

Table Chem. 7. NR-silica-carbon black blend reinforcement-Formulation of mixes

Ingredients	Compound number						
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Natural Rubber	100	100	100	100	100	100	100
Silica (Ultrasil VN3)	50.2	38.0	36.7	28.1	19.4	10.2	0
Carbon black, ISAF	0	12.1	13.4	21.9	30.6	39.8	50
Zinc oxide	4	4	4	4	4	4	4
Stearic acid	2	2	2	2	2	2	2
Mernox 6C	2	2	2	2	2	2	2
Naphthenic oil	2.5	2.5	2.5	2.5	2.5	2.5	5
V. Plasticizer	2.5	2.5	2.5	2.5	2.5	2.5	0
CBS	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DPG	1.5	1.3	1.3	1	0.8	0.5	0
Sulphur	2	2	2	2	2	2	2

(4 phr), naphthenic oil (5 phr), sulphur (2.5 phr), silica modified with 1, 3 and 5 per cent papain content (w/w) for compounds P1, P3 and P5, CBS (0.7 phr), DPG(1.5 phr) and silica (50 phr).

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Cure time, $T_{90}$ , min	7.48	6.55	6.05	6.07
$T_{80}$ , min	2.24	2.34	2.24	2.22
Maximum Torque, $M_H$ , dNm	33.78	31.63	31.61	32.13
Minimum Torque, $M_L$ , dNm	8.23	6.76	6.27	6.69
$M_H - M_L$ , dNm	25.55	24.87	25.34	25.44
Tan $\delta$ at $M_H$	0.105	0.099	0.094	0.097

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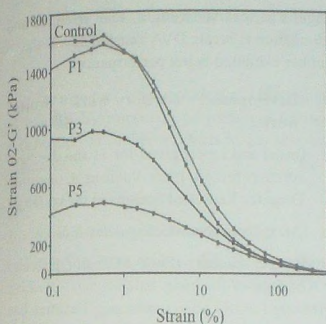


Fig. Chem. 3. Payne effect studies of uncured rubber-silica compounds

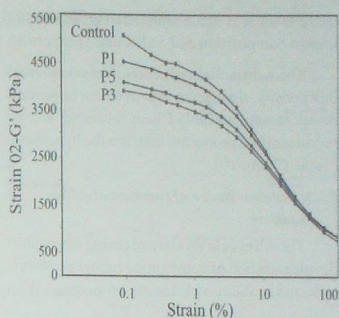


Fig. Chem. 4. Payne effect studies of cured rubber-silica vulcanizates

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- ii. Commercial process II which uses a mixture of a dicarboxylic acid and urea as devulcanizing agent (as given in patent).

RRII has developed a simple mechanical process where shearing of crumb rubber powder in a two roll mill without the addition of any chemical can also devulcanize rubber.

A comparison of these devulcanization processes with the new devulcanization process



using DVA was made from NR vulcanisates of known composition and vulcanisate properties.

The substantial reduction in residual cross link density, significant improvement in per cent devulcanisation (Table Chem. 9) and improved re-vulcanisate properties confirm the hypothesis (Table Chem. 10).

### 3.2.2. Devulcanisation of commercial rubber powders

The effect of DVA assisted during mechanical devulcanisation of commercial rubber powder is reflected in the re-vulcanisate properties. With

regard to tensile strength, tear strength and elongation at break, DVA assisted devulcanised rubber exhibited better performance.

### 4. Development / advisory work / project work

- a. Tested and report given for 11 the damaged tyres referred from various Consumer Disputes Redressal Forums in the country.
- b. Latex/ Dry rubber testing - 250 Nos
- c. Project students (PhD/ M.Tech / B.Tech / M.Sc.) - 7.

Table Chem. 9. Comparison of residual x-link density and percent devulcanization

Sample	X-link density/residual x-link density $\times 10^4$ (mol/cm <sup>3</sup> )	Percent devulcanization
Original NR vulcanizate	2.4	-
Mechanical devulcanization	1.5	37.5
Commercial process I	1.5	37.5
Commercial process II	1.6	33.5
DVA assisted devulcanization	0.5	79.2

Table Chem. 10. Comparison of re-vulcanizate properties

Sample	Tensile strength (MPa)	Elongation at break (%)	M100 (%)	M200 (%)	M300 (%)	Tear strength (N/mm <sup>1</sup> )
Original NR vulcanizate	26.1	605.5	2.1	4.8	8.9	114.0
Mechanical devulcanization	14.8	346	2.3	4.8	9.5	34.6
De-Link process	11.1	207.4	4.5	11.7	—	24.2
Lev-Gum process	9.3	284.9	2.3	5.3	10.5	30.7
DVA assisted process	22.2	480.8	2.3	5.1	9.5	51.4

## TECHNICAL CONSULTANCY DIVISION

The major activities include R and D activities of industrially important projects and testing/certification of rubber products as per relevant standards.

### 1. Research Projects

#### 1.1. Nano-dispersions for latex technology

Graphene related materials such as graphene oxide (GO)/exfoliated graphene oxide (XGO) and reduced graphene oxide (RGO) recently attracted much interest in nanocomposite research. In this study, the synthesis of RGO by a green route and its efficacy as potential filler for vulcanizates was explored. The synthesized XGO and RGO suspensions were characterized by different analytical and spectroscopic tools. The mechanical, morphological and electrical properties of the RVNRL-XGO/RGO nanocomposites were evaluated as a function of filler content. The percolation threshold of RVNRL-RGO composite was 0.1 weight per cent. Compared to control, significant improvement in conductivity (Fig. TC. 1) was obtained for latex-XGO nanocomposites at 1wt per cent XGO loading indicating increased polymer-filler interaction. The morphological results showed aggregation of filler particles at a concentration of 1.25 weight per cent.

#### 1.1. Peroxide vulcanization of rubbers-Thermal oxidative behaviour of peroxide cross-linked NR

Peroxide vulcanization of NR was carried out with four chemically different peroxides, namely 2,5-dimethyl-2,5-bis (*tert*-butylperoxy) hexane (DHP), 1,1-Di (*tert*-butylperoxy)-3,3,5-trimethylcyclohexane (TMCH), 1,3 1,4-bis (*tert*-

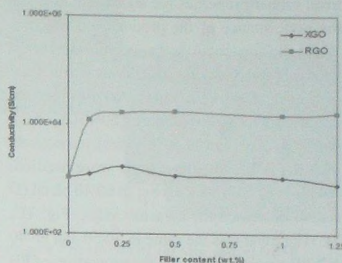


Fig. TC. 1. Electrical conductivity as a function of filler content for RVNRL nanocomposites containing XGO and RGO

butyl peroxy isopropyl) benzene (DIPP) and dicumyl peroxide (DCP). Fourier transform infrared spectroscopy (FTIR) and thermo gravimetric analysis (TGA) were used to characterize the by-products formed as a result of the thermal oxidative degradation. Tensile strength, elongation at break, modulus at different elongations were tested before and after ageing. The vulcanizates were also evaluated for hardness, crosslink density and compression set. The reactivity of different peroxides towards the crosslinking of NR was rated based on the properties of the respective vulcanizates.

#### 1.3. Design a cost effective tread formulations complying with Association of State Road Transport Undertaking specifications (ASRTU)

All possible blend proportions of NR/PB were formulated along with essential rubber chemicals and the testing was carried out as per the requirement. Mechanical properties of vulcanizates containing different proportions of

polybutadiene content were examined in order to produce tyre-treads having better mileage. The tread rubber properties are significant as far as the performance of the product is concerned. While sticking strictly to the specifications of the tread rubber it is also important to design the same in a cost effective manner. Accordingly the influence of reclaimed rubber in 60/40 (A series), 50/50 (B series) and 40/60 (C series), respectively of NR/PB blend proportions were studied. Addition of reclaimed rubber to 60/40 blend (D series) decreased the abrasion index (Fig. TC. 2). The trend was the same in 50/50 and 60/40 NR/PB combinations. As the proportion of PB increases the index values also increased.

Among the blend formulations, NR rich mixes fully comply with the ASRTU specifications.

#### 1.4. Cell structure and performance of expanded rubber

This project was initiated to identify the suitable grade of blowing agent for different applications in microcellular soles. Effect of different blowing agents on cell structure and its influence on technological properties were evaluated. Microcellular sheets from rubber mixes containing blowing agents DNPT, ADC and OBSH at varying dosages viz. 4, 6 and 8 phr were used for the study. The technological properties of the sheets prepared were tested in accordance with IS 10702.

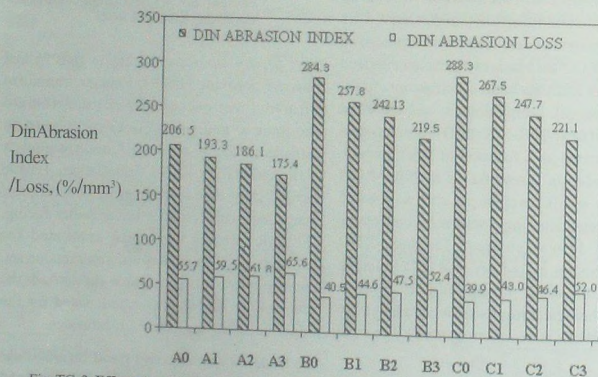


Fig. TC. 2. Effect of reclaimed rubber on abrasion indices of various NR/PB blend combinations

The results showed that blowing agent DNPT up to a loading of 6 phr was sufficient for producing microcellular sheets conforming to IS 10702, suitable for Hawaii sole applications. Blowing agents ADC and OBSH produced

microcellular sheets of relatively high hardness whereas DNPT produced cells were of uniform structure and maximum expansion as from evident the optical photographs of the specimens (Fig. TC. 3).



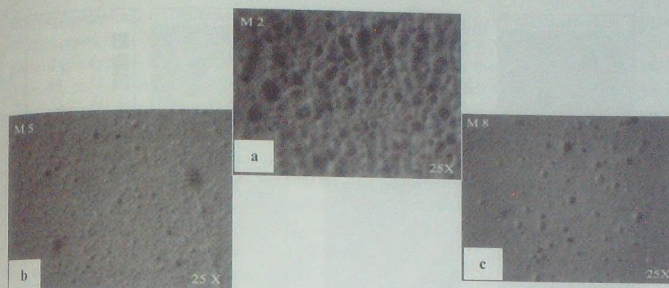


Fig. TC. 3. Optical photographs of expanded sheets with 6 phr (a) DNPT (b) ADC and (c) OBSh

#### 1.5. Optimization of amount of EPDM reclaim in EPDM vulcanizates

Ethylene-propylene rubbers (EPDM) continue to be one of the most widely used and fastest growing synthetic rubbers being used in specialty and general-purpose applications. In this study, EPDM-reclaim has been used as partial replacement for virgin EPDM, which eventually reduce the production cost, provided the material properties do not deteriorate significantly. A standard formulation was also used in the study. Mechanical properties such as tensile strength and modulus showed gradual deterioration while hardness increased to some extent and then decreased in correlation with the modulus values. Tear strength showed a drastic decrease while heat build-up increased to some extent and then tapered off. Overall results revealed that an addition of 10 phr of reclaimed EPDM in to EPDM compounds was acceptable when all the properties were taken in to consideration. Mixing large quantities (>10 phr) of reclaimed rubber with virgin EPDM resulted in poor dispersion.

#### 1.6. Peroxide vulcanization of natural rubber

A Face Centered Central Composite Design (FCCD) with four factors and three levels was used to obtain the relationship between vulcanizate properties and the doses of compounding ingredients. The four factors selected were ZnO, antioxidant, co-agent and filler/oil ratio. The filler/oil ratio was kept at a constant value of 5:1 throughout the experiment.

The mechanical properties of peroxide cured NR were optimized using FCCD. The properties selected as responses were tensile strength, elongation at break, modulus at 100 per cent elongation, tear strength, hardness, compression set (70 and 100°C) and crosslink density. Regression equations, interaction plots, response surface plot, contour plots and overlaid contour plots were generated using MINITAB software. Optimized the formulations based on the predictions of the mechanical properties and the same was verified by actual trail.

Interaction plots for tear strength, and compression set (70 and 100°C) showed

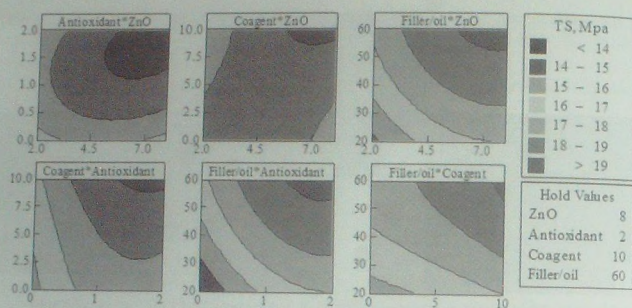


Fig. TC. 4. Contour plots for tensile strength

significant interaction between parameters. From the contour plots Fig. TC. 4, it is clear that high tensile values can be obtained from a number of combinations.

Comparison of predicted and observed values are shown in Table TC.1. These validations confirmed the suitability of the design chosen, method of sample preparation and property evaluation.

#### 1.7. Variation of particle size/extractable protein in different processing/product development techniques used in NR latices

This study was conducted based on three different sub topics, (1) extractable protein content and particle size of different types of NR latices in different production techniques (2) effect of vulcanization on protein content and particle size in sulphur (PVNRL) and radiation

Table TC. 1. Comparison of predicted and observed values

Properties	Predicted	Observed	% change
Tensile strength MPa	18.1	19.2	6.1
Elongation at break, %	327	324	-0.9
Modulus (100%), MPa	2.5	2.7	8.0
Hardness, Shore A	62	60	-3.2
Tear strength, kN/mm	24.8	24.1	-2.8
Crosslink density $\times 10^{-5}$ , g/cm <sup>3</sup>	25.5	25.0	-2.0
Compression set (70 °C)	18.3	18.1	-1.4
Compression set (100 °C)	48.0	46.3	3.4

(RVNRL) prevulcanized latices and conventional latex compounds (POVLC) and (3) effect of protein content on storage of field latex at different concentration of ammonia. Latex films were prepared by casting and coagulation techniques. It was found that prevulcanized latex film had lesser extractable protein content than the conventionally vulcanized film. Because of the easiness of extractability of the proteins from the films of conventional latex compound than the prevulcanized ones because of the partial vulcanization of prevulcanized latices films. In addition to this, it was also found that on storage the protein content of preserved latex increased with ammonia content.

#### 1.8. Particle size variation of NR latex with the addition of styrene butadiene latex, skim latex and precipitated calcium carbonate dispersion

Since adulteration of NR latex concentrates causes difficulties in manufacturing process, it was proposed to evolve analytical tools for the identification of adulterants. In this work particle size analysis was used as a quality control tool for various latex and latex composites. The work has been initiated by incorporating different proportions of SBR latex and calcium carbonate dispersions (major adulterants) in NR latex. Particle size measurements of the mixtures and colloidal stability were evaluated.

#### 1.9. NR field latex based adhesives

The effect of potassium hydroxide (KOH), ethylene diamine tetraacetic acid (EDTA), combination of KOH and EDTA, 2, 2, 4-trimethyl-1,2-dihydroquinoline (TDQ) and tackifiers such as wood rosin and phenolic resin in the adhesion strength of preserved field latex at various ageing conditions was studied. Peel strength was

determined in almost all cases. Dry rubber content (DRC) was varied in the experiments and it was found that peel strength and shear strength increased with DRC in all ageing conditions. Peel strength after thermal ageing showed a decreasing trend than other ageing conditions in the case of EDTA. Antioxidant TDQ increased the peel strength in all conditions. Tackifiers like wood rosin and phenolic resin had little effect on adhesion properties

## 2. Testing and certification

The major activity of the Division was testing and certification of rubber products as per national (IS and ASRTU) and international standards (ISO, ASTM, EN and BS). For the testing of raw materials and rubber compounds/products, consistent support was offered especially to small and medium entrepreneurs. Products coming for testing can be categorised into three categories namely, (i) tyre associated products (ii) non-tyre products and (iii) latex products. Total number of samples tested and the revenue collected during the reporting year are given in Table TC. 2.

Table TC. 2. Number of samples tested and revenue collected (2015-16)

No. of clients	489
No. of samples tested	1188
No. of parameters analyzed	6600
Consultancy letters	1546
Total revenue collected (Rs.)	21,54,221.00

## 3. Product development

Know - how for a large number of products were developed and transferred to clients as per their requirements Table TC. 3.



Table TC. 3. Know-how transfers to industries

Type of rubber products developed	Nos. transferred	Products
Rubber based engineering components for railway, defence, BSF, Kochi Metro <i>etc.</i>	16	Bridge bearing
Automobile components	21	O-rings, oil seals, washers beadings and channels
Pre-cured tread, bonding gum and tube valve (ASRTU specification)	11	PCTR, BG, butyl tube valve
Fire resistant sealing and mats	6	Channels and beadings
Rubber based agro-machinery components	5	Butter fly valve and neck rings
Pharmaceutical closures and agitator bush as per US FDA spec	2	Agitator bush and closures
Latex based dipped and foamed goods	14	Coir foam and coco basket
Adhesives	17	Latex and solution adhesives

Major clients who are availing the services of the division.

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

#### 4. Project profiles/Technical bulletins

As per the request of the entrepreneurs, three project profiles and three technical bulletins were prepared.

#### 5. Advisory services

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

#### 6. NABL Accreditation

In connection with NABL accreditation of the laboratory, the application for NABL accreditation was filed during the year. Also done, adequacy audit and based on adequacy report, completed closing of non-conformities.

## ECONOMICS DIVISION

The broad research areas of the Division comprise of farm management, primary processing and marketing, rubber products manufacturing industry and foreign trade and intercrops and by-products. In these areas inter-divisional collaborate projects are also undertaken for comprehensive understanding of the sector. Five projects were completed and reported during the period.

#### 1. Age-composition of mature area under natural rubber in India: A comparative analysis

The study revisited an earlier study undertaken in the Division in 2008 on the trends in age-composition of mature area under natural rubber cultivation in India and reviewed projected status for the period from 2008-09 to 2014-15. The projections of the earlier study showed that the share of area under the yield-declining phase would rise to touch 53.3 per cent by 2011-12 before falling to 46.0 per cent by 2014-15. The earlier study had also highlighted inconsistencies

in the official area statistics and recommended a national census of rubber area for fixing the same. However, the earlier study failed to capture postponement of replanting of rubber trees beyond 22 years of tapping and the resultant emergence of a senile group of trees having more than 22 years of tapping. Due to retention of aged trees, the gap between the projected figures of mature area and the corresponding official figures sharply widened from 2.2 per cent in 2008-09 to 25.3 per cent in 2014-15. The area occupied by trees that were tapped for more than 22 years steadily grew by 144.7 per cent during the period from 2008-09 to 2014-15. In absolute terms, the area under this age-group increased from 10,255 to 99,313 hectare between 2008-09 and 2014-15 seriously compromising total production (Table Eco. 1).

The differences in the age-composition of mature area based on the projections of earlier study and the official area statistics were also estimated.

Table Eco. 1. Tapping year-wise area (ha)

Year	Year of tapping				Total mature area*
	1-3	4-13	14-22	Above 22	
2008-09	37030 (8.0)	186977 (40.4)	228868 (49.4)	10255 (2.2)	463130
2009-10	39010 (8.3)	171248 (36.6)	231113 (49.3)	27109 (5.8)	468480
2010-11	39920 (8.4)	162371 (34.0)	231122 (48.4)	43817 (9.2)	477230
2011-12	47240 (9.6)	153730 (31.3)	227226 (46.3)	62774 (12.8)	490970
2012-13	58280 (11.6)	148810 (29.5)	220980 (43.8)	75970 (15.1)	504040
2013-14	73830 (14.2)	147940 (28.6)	208598 (40.3)	87732 (16.9)	518100
2014-15	85500 (16.0)	153070 (28.7)	196117 (36.7)	99313 (18.6)	534000
CARG (%)	21.8	-3.0	-2.4	144.7	2.6

\* Official data of Rubber Board on mature area; CARG: Compounded annual rate of growth; Figures in the parentheses are percentage shares of the total mature area.

According to the projections of the earlier study, the share of area under the tapping age group "14 years and above" would go up from 50.5 per cent in 2008-09 to 53.3 per cent in 2011-12 before gradually declining to 46.0 per cent in 2014-15. But, the estimates based on official area statistics revealed a worsening age-composition characterised by an increase in the share of area under the tapping age group "14 years and above" from 51.6 per cent in 2008-09 to 59.1 per cent during 2011-12. Though subsequently it marginally declined to 55.3 per cent during 2014-15, the emerging trends have serious policy implications in the context of challenges to the sustainability of NR production in the country. The results of the study reconfirmed the need for a national census of areas under rubber, as proposed in the earlier study.

## **2. Challenges of evolving a National Rubber Policy in India: Towards a conceptual framework**

The study provided a conceptual framework for the National Rubber Policy in India based on the inherited characteristics of the rubber sector and issues surfaced during the past two decades of trade policy reforms. Historically the inherited interdependence observed between a vibrant NR production segment and a highly developed rubber products manufacturing industry had been unique and nurtured for achieving the twin national objectives of self-sufficiency and import substitution. In practice during the pre-reforms period, the efficacy of the policy intervention had been prefixed by a very high degree of domestic market orientation and protection to both segments from external competition. Administered prices had been an integral component of the comprehensive institutional interventions employed to sustain the tempo of growth in NR production during the period. However, growing exposure to external

competition through multilateral and Regional Trade Agreements (RTA) routes during the past two decades left serious strains on the harmonious relationships prevailed in the rubber sector.

The strains of market integration are increasingly witnessed in the domestic market than in the export markets. A major casualty of these changes has been the interventionist policy approaches which guided the growth dynamics of the sector during the pre-market integration era in rubber sector (1947-91). Therefore, a comprehensive national rubber policy is expected not only to recognise the strategic importance of sustaining a self-reliant rubber sector but also to identify the inherent strengths and accumulated weaknesses of the embedded structure to capture synergies in the era of market integration. Hence, among competing priorities, the study elaborated seven policy components which deserve attention from a long-term policy perspective *viz.* i) construction of a reliable database, ii) agro-climatic zoning and enhancement of replanting subsidy, iii) comprehensive crop insurance scheme, iv) labour banks, v) group processing, vi) rejuvenation of non-tyre sector and vii) consolidation of the organizational network.

## **3. Trends in seasonality of natural rubber production in major producing countries: A disaggregate level analysis**

The study examined the seasonality in production of NR in major producing countries like Thailand, Indonesia, Malaysia and India. Using the monthly data on production of NR and the technique of Change-Point Analysis (CPA) the seasonality and its trends were examined at aggregate (across period) and disaggregate levels (within months) for the 23 years period from 1991 to 2013. The seasonality in the production of NR in the four countries is given in Table Eco. 2.



Table Eco. 2. Mean monthly production with the co-efficient of variation (CV) for the four NR producing countries and its statistically grouped peak, moderate and lean seasons

Month	Indonesia (%)	CV (%)	Thailand (%)	CV (%)	Malaysia (%)	CV (%)	India (%)	CV (%)
Jan	8.3 <sub>bcd</sub>	14.5	10.0 <sub>a</sub>	11.4	10.2 <sub>a</sub>	12.1	10.9 <sub>e</sub>	5.4
Feb	7.7 <sub>d</sub>	8.8	8.7 <sub>b</sub>	11.8	8.9 <sub>bcd</sub>	12.9	5.4 <sub>i</sub>	15.4
Mar	8.1 <sub>bcd</sub>	9.4	7.6 <sub>d</sub>	17.6	7.2 <sub>g</sub>	15.9	5.6 <sub>i</sub>	6.3
Apr	8.0 <sub>cd</sub>	7.9	6.2 <sub>e</sub>	15.3	6.0 <sub>b</sub>	9.2	6.6 <sub>h</sub>	6.1
May	8.3 <sub>bc</sub>	10.5	6.7 <sub>e</sub>	11.8	7.0 <sub>g</sub>	8.7	7.2 <sub>g</sub>	11.8
Jun	8.5 <sub>abc</sub>	15.3	7.9 <sub>cd</sub>	15.8	8.2 <sub>ef</sub>	6.2	6.3 <sub>h</sub>	9.6
Jul	9.0 <sub>a</sub>	9.3	9.1 <sub>b</sub>	10.9	9.4 <sub>b</sub>	4.6	6.6 <sub>h</sub>	9.9
Aug	8.5 <sub>abc</sub>	15.0	9.1 <sub>b</sub>	9.5	9.1 <sub>bc</sub>	6.6	8.0 <sub>f</sub>	5.5
Sep	8.6 <sub>ab</sub>	13.4	8.9 <sub>b</sub>	12.0	8.9 <sub>bcd</sub>	7.3	9.3 <sub>e</sub>	7.4
Oct	8.4 <sub>bc</sub>	10.3	8.7 <sub>b</sub>	12.1	8.6 <sub>de</sub>	11.7	10.2 <sub>d</sub>	5.1
Nov	8.1 <sub>bcd</sub>	10.4	8.8 <sub>b</sub>	15.6	7.8 <sub>f</sub>	9.8	11.6 <sub>b</sub>	5.8
Dec	8.6 <sub>ab</sub>	11.4	8.5 <sub>b</sub>	20.2	8.7 <sub>cde</sub>	13.1	12.4 <sub>a</sub>	4.8

□ Peak    ■ Moderate    ■ Lean Seasons

Note: Subscripts indicate Fisher's Least Significant Differences (LSD)

When Thailand and Malaysia have two seasons (peak and moderate) India has three seasons (peak, moderate and lean) and Indonesia has no seasonality at all i.e., production is almost stable throughout the year. The peak production season in Thailand and Malaysia extended from June to February (80%) while production was moderate during the period from March to May (20%). In India the peak season was from August to January (62%), lean season was during February to March (11%) while moderate production was observed during April to July (27%). Variability in production was higher during the peak period and lower in the lean/moderate season in India, Thailand, and Malaysia.

#### 4. Census of rubber tappers in Kerala

The first ever census of rubber tappers in the smallholders sector of Kerala was an attempt to generate a reliable database on the supply of rubber tappers in the sector.

The census conducted during 2013-14 enumerated 77207 rubber tappers engaged in the smallholder rubber sector in Kerala. Historically, rubber tapping in the smallholder sector in the state had been dominated by male tappers. Gender-wise regional distribution of rubber tappers as observed during the census is presented in Table Eco. 3.

Table Eco. 3. Gender-wise regional distribution of tappers

Region	Male	Female
South Kerala	14914 (84.5)	2744 (15.5)
Central Kerala	28641 (94.6)	1624 (5.4)
North-central Kerala	7347 (91.1)	717 (8.9)
North Kerala	20515 (96.7)	705 (3.3)
Kerala	71417 (92.5)	5790 (7.5)

Figures in the parentheses are per cent shares of the total

Despite the regional variations, the dominance of male tappers (92.5 %) was evident. The skewed distribution towards male tappers was highest in North Kerala (96.7%) and lowest in South Kerala (84.5%). Thus, female tappers' participation in the state varied from 3.3 per cent in North Kerala to 15.5 per cent in South Kerala.

The census revealed that the share of tappers in the productive age group of less than 60 years was 89.7 per cent and majority of them (65%) were in the age group of 40-60 years. The age composition of the tappers is presented in Table Eco. 4.

Table Eco. 4. Region-wise age-composition of tappers

Region	Age distribution (%)		
	<40 Years	40-60 Years	>60 Years
South Kerala	25.7	63.5	10.8
Central Kerala	15.6	70.6	13.8
North-central Kerala	40.3	54.5	05.2
North Kerala	31.7	61.5	06.8
Kerala	24.7	65.0	10.3

The share of younger tappers below 40 years is only 24.7 per cent. North-central Kerala (40.3%) had the highest share of young rubber

tappers and Central Kerala (15.6%) had the lowest. Conversely, Central Kerala had the highest share (13.8%) of tappers in the age group of above 60 years which is above the state average of 10.3 per cent.

An important organizational feature of the rubber tappers market has been single grower dependence embellished with the informal contractual arrangements. Table Eco. 5 shows that 75 per cent of the tappers still had single grower dependence.

Table Eco. 5. Region-wise grower dependence of tappers on the number of growers (%)

Region	No. of growers served		
	One	Two	Three
South Kerala	78.8	15.4	5.8
Central Kerala	65.0	30.8	4.2
North-central Kerala	88.3	10.6	1.1
North Kerala	77.0	20.2	2.8
Kerala	75.0	21.3	3.7

A closely related technical aspect of the pattern of labour dependence on growers is the tapping system-wise employment. Table Eco. 6 shows region-wise shares of tappers' employment under different tapping systems.

Table Eco. 6. Region-wise share of tappers' employment under different tapping systems (%)

Regions	Tapping system			
	S/2 d1	S/2 d2	S/2 d3	Others
South Kerala	30.6	65.6	2.7	1.1
Central Kerala	9.6	83.4	6.9	0.1
North-central Kerala	10.0	86.1	3.5	0.4
North Kerala	6.4	89.7	3.6	0.3
Kerala	13.7	81.2	4.7	0.4

Despite a significant growth in the share of the tappers with multiple grower dependence, 94.9 per cent of the holdings are under the high frequency tapping systems; S/2 d1 and S/2 d2. The fact that almost 95 per cent of the tappers are engaged in either daily or alternate daily tapping reveals the unacceptable predominance of high frequency tapping still happening in Kerala.

The census data revealed that only 19.8 per cent of the tappers in the state received formal training in tapping either through TSS or TISP. The share of tappers who had formal training in tapping is presented in Table Eco. 7.

Table Eco. 7. Share of rubber tappers who had formal training

Region	Share of trained tappers (%)			
	TSS	TISP	Total	Untrained
South Kerala	8.7	12.1	20.8	79.2
Central Kerala	5.7	11.7	17.4	82.6
North-central Kerala	3.7	17.4	21.1	78.9
North Kerala	12.1	9.3	21.4	78.6
Kerala	8.0	11.8	19.8	80.2

The share of trained tappers was the lowest in Central Kerala (17.4%) and the highest in North Kerala (21.4%), but the per cent of tappers

who had no formal training was unacceptably high throughout Kerala.

##### 5. India's tariff policy, trade liberalisation initiatives and trends in balance of trade under the Regional Trade Agreements (RTAs): The case of rubber and rubber products

The time series external trade data for the past 15 year period from 2000-01 to 2014-15 (Export Import Data Bank, Department of Commerce, Government of India) was used for the study. Results of the study revealed that India provided ample protection to its NR production segment under various RTAs except under Asia Pacific Trade Agreement (APTA) and to less developed countries under the South Asian Free Trade Area (SAFTA). However, the total value of imports of NR under the RTAs grew at the rate of 39.5 per cent and the negative balance of trade during 2014-15 was US \$ 785.35 million.

At the aggregate level, India's exports and imports of rubber and rubber products under the RTAs grew at the rates of 16.8 and 26.3 per cent respectively during the period under review. The magnitude of the emerging trends is evident from a higher negative balance of trade with the RTAs (US \$ 1469 million) than with the world during 2014-15. The results of the study underlined the need for a comprehensive policy package for India's rubber sector from a long-term policy perspective rather than short-term, segmented *ad-hoc* measures.



### CENTRAL EXPERIMENT STATION, CHETHACKAL, KERALA

The Central Experiment Station (CES), Chethackal situated at a distance of about 50 km from Kottayam was established to cater the research needs of the different divisions of the RRII. The station has a total land area of 254.8 ha which is planted for different research projects. The total tapping area is 167.6 ha, and immature area is 36.3 ha. Bud wood nursery and close

planting was established in 15.9 ha during the current year.

During the reporting period, the total crop realized was 1,18,982 kg. A total of 298 tapping days was obtained during the year and 63 tappers were engaged for tapping. The total man-days engaged were 38,941.5 days. The dispensary attached to the station caters to the medical needs of the workers.

### REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

#### 1. Crop Improvement

##### 1.1. Performance of clone RRII 429

Girth and yield in RRII 429 was higher than RRIM 600 in grower's field under the agroclimate of Goalpara, Assam. The increased yield in RRII 429 could be observed throughout the year and about 28 per cent average annual yield increase over RRIM 600 was recorded.

##### 1.2. On-farm evaluation of potential clones

Girth was found superior in RRII 417 followed by RRII 430 and RRIM 600 while it was low in RRII 422.

##### 1.3. On-farm evaluation of selected ortets of *Hevea*

An on-farm trial was initiated in Gopalkrishna Tea Estate, Morigaon, Assam to evaluate the performance of three selected primary ortets viz. RRSg 9, RRSg 3 and RRSg 1 along with two check clones RRIM 600 and RRII 429. Vacancy in RRSg 9 was significantly higher than the check clones.

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

Arunachal Pradesh being one of the potential areas for rubber cultivation in North East India, an on-farm evaluation trial with ortets identified from Nagrakatta, Agartala and Guwahati with 11 potential clones was initiated.

### REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The Research Station is actively involved in various research programs under Crop improvement, Crop management, Latex harvesting and Ecosystem study. Advisory services on scientific fertilizer recommendation

and DRC estimation of latex samples were also continued.

#### 1. Crop Improvement

The crop improvement programs in the station includes development of location specific

clones, evaluation of promising clones and the standardization of DUS norms. A germplasm garden having 213 wild *Hevea* accessions, source bush nurseries and a breeding orchard are maintained at the Station.

#### 1.1. Development and evaluation of clones

A total of 1293 locally recruited genotypes were evaluated in three seedling nurseries and based on their growth and yield performance, 153 genotypes (120 hybrids, 28 half-sibs and 5 open pollinated progenies) were selected for further evaluation. Among the 30 selected clones evaluated in two clonal nurseries, six hybrids (98/37, 98/38, 99/1/24, 99/5/9, RR11 403 and RR11 407) and two ortets (P 132 and P 152) were selected for further evaluation in Large Scale Trial (LST) and On Farm Trial (OFT) based on their growth and yield performance compared to RRIM 600. Ninety locally recruited clones of hybrids, open pollinated progenies and half-sib progenies are under evaluation in six clonal nurseries. First set of 13 pipeline clones from North East India was field planted for evaluation in Large Scale Trial 2015.

#### 1.2. Evaluation of clones for their adaptability and yield performance

Two On Farm Trials (OFT) and two Large Scale Trials (LST) are in progress. In LST (1995), RR11 105 ( $62.4 \text{ g t}^{-1} \text{ t}^{-1}$ ) and SCATC 88/13 ( $58.4 \text{ g t}^{-1} \text{ t}^{-1}$ ) were the high yielders compared to RRIM 600 ( $45.6 \text{ g t}^{-1} \text{ t}^{-1}$ ). In another LST (G x E) RR11 422 ( $76.1 \text{ g t}^{-1} \text{ t}^{-1}$ ) and RR11 429 ( $71.9 \text{ g t}^{-1} \text{ t}^{-1}$ ) were found high yielding compared to RRIM 600 ( $70.6 \text{ g t}^{-1} \text{ t}^{-1}$ ).

Promising clones are under evaluation in one mature and one immature OFT in which RR11 429 exhibited highest mean yield ( $38.1 \text{ g t}^{-1} \text{ t}^{-1}$ ) compared to RRIM 600 ( $32.6 \text{ g t}^{-1} \text{ t}^{-1}$ ) during

second year of tapping in OFT, Pathalia (2005). Clone RR11 429 had the highest girth (48.3 cm) in another OFT at Hirapur (2009).

In clonal nursery evaluation involving popular clones (2009), PB 255 ( $155 \text{ g t}^{-1} \text{ t}^{-10}$ ) and PB 314 ( $151.8 \text{ g t}^{-1} \text{ t}^{-10}$ ) had significantly higher test tap yield than RRIM 600 ( $92.5 \text{ g t}^{-1} \text{ t}^{-10}$ ). In clonal nursery evaluation (2009) for evaluating pipeline clones from traditional region, except P 060 and P 021, all the pipeline clones evaluated were found to be inferior to RRIM 600.

#### 1.3. Standardisation of DUS norms

Field trial involving 57 clones for the identification of reliable juvenile and mature characteristics is also in progress.

### 2. Crop management

#### 2.1. Soil nutrient mapping

Evaluation of soil fertility status and nutrient mapping of soils under rubber plantations in Tripura is under progress. During the reporting year 118 composite soil samples were collected based on GPS points. Analysis of the samples showed that soils are acidic with soil pH ranging between 3.52 - 5.25, medium in OC content (0.97%) and low in available P ( $0.53 \text{ mg } 100 \text{ g}^{-1}$ ) and available K ( $4.1 \text{ mg } 100 \text{ g}^{-1}$ ) status.

#### 2.2. Development of cropping systems and management practices

In the experiment on evaluation of cropping system models, after eight years the girth of rubber in the intercropping plots (51.1 and 52.6 cm, respectively for Model I and II) and in monocropped plots (50.9 and 54.5 cm, respectively for Model I and II) was statistically on par. Intercropping was carried out for entire immaturity period in Model I (Paired row system) and only for five years in Model II (usual

rectangular planting). Among all the crops cultivated under immature rubber, BCR for Colocasia (3.3) was the highest followed by Banana (2.7) and Cowpea (2.0) indicating the economic viability of growing these crops as intercroops during the unproductive phase of rubber.

For the zero tillage experiment, the girth data indicated that pits of larger dimensions had no advantage on growth of plants. The girth during fourth year were 32.6, 32.1 and 31.9 cm for conventional tillage, zero tillage (poly bag) and zero tillage (root trainer), respectively.

In the experiment on development of specific package of practices, the mean girth (cm) of rubber in vertical mulching (36.5 cm) and conventional mulching (35.8 cm) was significantly higher than control (32.8 cm) in fourth year of planting. Drying of branches due to summer stress was least in vertical mulching plots (0%) over conventional mulching (1.7%) and control (8%).

### 3. Latex harvest technology

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

Response of RRII 400 series clones (RRII 417, RRII 422, RRII 429, RRII 430) and RRIM 600 to yield stimulation in two systems of tapping viz. d2 and d3 was studied at Taranagar farm. Tapping was continued with two months rest in d2 and without rest in d3 with two, three and four stimulations per year. It was observed that d3 system of tapping with stimulation was comparable with d2 system of tapping. Clone

RRII 429 showed the highest yield among the clones.

The effect of planting density viz. 400, 445, 489, 544 and 699 trees ha<sup>-1</sup> on different tapping systems was studied in clone RRII 429. Trees were tapped in two systems viz. d2 and d3. Lower density had higher percentage of trees available for tapping. It was observed that d3 system was comparable with d2 system of tapping in all the densities.

In another experiment, clone RRIM 600 and RRII 429 were tapped in three systems of tapping viz. d2, d3 and d4 for the last seven years. Tapping was continued round the year with d3 and d4 systems with three and six stimulations respectively. However, d2 system was under rest for two months. Yield under d3 system of tapping with stimulation was comparable with d2 system of tapping for both the clones. The d4 system of tapping had lower yield.

### 4. Ecosystem study

Ecological niche modelling was used to analyse the present and potential future distribution of rubber trees (*Hevea brasiliensis*) in two biogeographically distinct regions of India i.e., the Western Ghats (WG) and North East (NE). The rubber tree is an economically important plantation species, and therefore factors other than climate may play a significant role in determining its cultivation. To assist in future planning, the Maximum Entropy (Maxent) model was used to predict plausible areas for the expansion of rubber cultivation under a changing climate scenario. Two ecological niche modelling approaches (Maxent and GARP) were employed to predict the present distribution of NR in two bio-geographical regions of India. GARP over-estimated NR area in NE compared to WG.



Maxent model predicted the rubber cultivation more accurately in both regions as it considers presence -only data, which is more accurate for this species which is not naturally existing in NE India.

## 5. Advisory work

Discriminatory fertilizer recommendation based on soil was offered to 171 rubber growers in the state of Tripura and 379 latex samples were analyzed for dry rubber content.

## REGIONAL RESEARCH STATION, TURA, MEGHALAYA

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

### 1. Crop Improvement

#### 1.1. Polycross progeny evaluations

From the poly-cross progeny evaluation trial (2008), potential seedlings were selected on the basis of test tap yield and were bud-grafted. The new potential ortets were put in to clonal nursery trial in 2014 at the Rubber Board campus, Dakopgre, Tura in two designs. In another poly-cross progeny trial (2011), a total of 34 top yielders were selected on the basis of growth performance and juvenile yield and are being maintained in the field for further evaluation.

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

Based on test tap yield and girth after 2<sup>nd</sup> year, promising seedlings were selected. The selected ortets were preserved in budwood nursery for further evaluation.

#### 1.3. Clonal nursery evaluation trial 2010

A clonal nursery trial was initiated with the best ortets collected from Tura, Agartala and Guwahati along with RRIM 600 as the check clone. The juvenile yield ranged from 14.0 to 132.8 g t<sup>-1</sup>t<sup>-10</sup> during the 2<sup>nd</sup> year of growth (Table Tura1). Promising ortets were maintained in nursery for further evaluation.

Table Tura 1. Girth and juvenile yield of primary ortets (2 yrs old)

Mother ortets	Girth (cm)	Juvenile yield (g t <sup>-1</sup> t <sup>-10</sup> )	Mother ortets	Girth (cm)	Juvenile yield (g t <sup>-1</sup> t <sup>-10</sup> )
T1X1	22.4	56.9	T8RRSA121	24.3	96.5
T2X2	28.0	76.9	T9RRSA315	24.5	14.0
T3X3	22.8	47.2	T10RRSA461	26.0	38.6
T4X9	26.5	125.4	T12RRIM600	28.0	51.4
T5RRST24	29.5	69.9	Mean	26.1	71.0
T6RRST37	30.3	132.8			
	SD	CV(%)			
Gmb	5.2	21.7			
Juvenile yield	16.2	18.0			

#### 1.4. On-farm evaluation of selected clones

Three on farm trials were started during 2009 and 2010 with 400 series clones in Mendipathar (North Garo Hills) and in Bolchugre, West Garo Hills. In the North Garo Hills, highest mean girth was recorded in RRII 429 (49.0 cm) while lowest girth was recorded in RRII 422 (43.2 cm) (Table Tura 2), while in West Garo Hills, highest mean girth and girth increment were recorded in RRII 417 followed by RRII 422 (Table Tura 3).

Table Tura 2. Growth of different clones in the On-farm trial at Mendipathar, North Garo Hills

Clones	Girth (cm)		Girth increment (cm yr <sup>-1</sup> )	
	Rabha	Momin	Rabha	Momin
PB 235	46.4	51.1	7.6	7.2
RRII 417	41.7	52.2	8.4	6.6
RRII 429	44.6	53.3	9.3	6.0
RRII 422	39.2	47.2	11.1	7.7
RRII 203	45.5	50.3	9.0	6.4
RRIM 600	43.0	48.4	7.4	6.1
SD	2.6	2.3	1.4	0.7

Table Tura 3. Growth of different clones at Bolchugre, West Garo Hills

Clones	Girth (cm)	Girth increment (cm yr <sup>-1</sup> )
RRII 417	35.0	7.9
RRII 422	30.1	7.4
RRII 429	26.2	7.0
RRIM 600	26.5	7.3
SD	4.1	0.4
CV (%)	13.9	5.0

#### 1.5. Studies on the nature of wintering, flowering and seed germination in *Hevea* clones in Garo Hills

Clonal variation was observed in wintering, refoliation and flowering pattern. During the reporting year, none of the clones produced significant quantity of seeds due to heavy rainfall and thunderstorm in the months of March-April, 2015. The clones RRII 105, RRII 118, PB 5/51, PB 86, PB 235, RRII 600 and RRII 605 exhibited early defoliation and refoliation compared to the other clones. All clones refoliated by the fourth week of February 2015, except RRII 105 and RRII 118 which showed refoliation by third week of February 2015.

#### 1.6. Nursery evaluation of poly cross seedlings (2013 & 2014 trials)

The polyclonal seeds collected from Polyclonal Seed Garden, Mizoram were planted in the field in the year 2013 at two locations of the RRS, Ganolgre farm and one location at R.B. campus, Dakopgre, Tura. After two years, the growth data was recorded and test tapping was completed and data were analyzed. The growth data of polycross seedling trial 2013 and 2014 exhibited an average height of 4.1 m (1.8 to 4.8 m), number of leaves 46.5 (12.0 to 64.0), number of whorls 5.5 (3.0 to 8.0), girth of about 10.4 cm (4.0 to 16.0 cm) and mean test tap yield of 3.0 g t<sup>-1</sup>t<sup>-10</sup> (0.2 to 13.4 g t<sup>-1</sup>t<sup>-10</sup>). In another location of Ganolgre farm where 54 plants had a mean plant height of 3.6 m and a girth of 9.8 cm, the test tap data showed an average yield of 2.2 g t<sup>-1</sup>t<sup>-10</sup> (range of 0.1-7.9 g t<sup>-1</sup>t<sup>-10</sup>). Since the survival of plants was very low during 2013, a new set of 400 poly clonal seeds were planted in the field during 2014 in Ganolgre farm in which 180 plants survived.

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

In 2013, seeds selected on the basis of seed weight were planted in the field. Two years after planting, the growth data were recorded and analyzed. The plants had an average height of 3.2 m (1.2 to 3.6 m) girth of 6.4 cm. and an average test tap yield of  $0.9 \text{ g t}^{-1} \text{ t}^{-10}$  (0.1 to  $4.3 \text{ g t}^{-1} \text{ t}^{-10}$ ).

### 1.8. Germplasm arboretum at Teksragre farm

In order to maintain the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> sets of Germplasm Arboretum under the agro-climatic condition of Garo Hills of Meghalaya at Teksragre farm near Anogre, 1848 budded stumps belonging to 222 accessions and 11 control clones were planted in the polybag nursery. The plants are ready for field planting during May/June, 2016.

### 2. Crop Physiology and latex harvesting Technology

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

Severe low winter temperature is one of the main factors for depression of yield and per cent dry rubber content in *Hevea* under the agro-climatic condition of Garo Hills. The annual mean yield was  $51.3 \text{ g t}^{-1} \text{ t}^{-1}$  and DRC was 33.7 per cent during the reporting year. Low temperature adversely affected the yield and DRC. During winter, DRC ranged from 28.5 to 29.5 per cent. Lowest soil moisture content was recorded in the months of February and March.

#### 2.2. Study on controlled upward tapping

Treatment-wise monthly yield was recorded and the data showed that maximum annual average yield ( $74.8 \text{ g t}^{-1} \text{ t}^{-1}$ ) was recorded in T1

(S/4U d/25 % ET 21 days interval- S/2 d/2 2.5 % ET) followed by T2 ( $72.2 \text{ g t}^{-1} \text{ t}^{-1}$ ) (S/4U d/2 5 % ET Monthly interval- S/2 d/2 2.5 % ET) and T3 ( $68.0 \text{ g t}^{-1} \text{ t}^{-1}$ ) (S/3U d/2 5 % ET monthly interval- S/2 d/2 2.5 % ET). Lowest yield ( $62.6 \text{ g t}^{-1} \text{ t}^{-1}$ ) was recorded in T4 (S/3U d/2 5 % ET 45 days interval- S/2 d/2 2.5 % ET). TPD (%) was highest in T1 (7.3 %) while lowest was in T4 (5.8 %). Under CUT 33-60 per cent yield increase was observed over normal tapping.

#### 2.3. Shallow tapping—an option 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 ( $42.4 \text{ g t}^{-1} \text{ t}^{-1}$ ) followed by normal continuous tapping ( $40.9 \text{ g t}^{-1} \text{ t}^{-1}$ ) and LFT + normal tapping ( $38.6 \text{ g t}^{-1} \text{ t}^{-1}$ ) and lowest was in shallow + normal tapping system ( $33.7 \text{ g t}^{-1} \text{ t}^{-1}$ ). DRC was low in normal continuous tapping and was high in shallow + normal tapping system. Normal continuous tapping system showed higher TPD (8.6 %) followed by the shallow + normal tapping system (7.2 %) and LFT + normal tapping (5.8 %) and minimum was in normal tapping system (5.3 %).

#### 2.4. Location specific stimulant application

Ethylene induced stress response in the tapping panel of the *Hevea* trees was initiated with the aim to reduce the stress in tissues in the tapping panel. In RRIM 600 six treatments were adopted with bark applications of five per cent ethephon. Results showed that maximum yield ( $50.3 \text{ g t}^{-1} \text{ t}^{-1}$ ) and low DRC (33.5%) were recorded in T3 (bark application of 5% ethephon at 150 cm height from bud union and near bud union) with minimum yield ( $27.5 \text{ g t}^{-1} \text{ t}^{-1}$ ) and high DRC (34.4 %) 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 that the highest girth and yield were obtained in the treatment combination,  $N_{60}P_{30}K_{45}$  kg ha<sup>-1</sup> compared to other treatments. Positive linear response to N, P and K fertilizer application on dry rubber yield was recorded. Continuous application of N, P and K fertilizer was found to improve the fertility status of the soil. Hence, an annual fertilizer dose of 60:30:45 N: P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O kg ha<sup>-1</sup> may be considered for mature rubber in Central Brahmaputra Valley Zone of Assam.

#### 3.2. Soil moisture retention characteristics in the rubber growing areas of Meghalaya

Soil samples were collected every month at the depth of 0-15, 15-30 and 30-60 cm and assessed for soil moisture. Soil moisture content increased with increase in depth. Highest soil moisture status was recorded in September and lowest in January / February. Annual mean was 23.6, 24.5 and 25.2 per cent, respectively for 0-15, 15-30 and 30-45 cm depth.

#### 3.3. Analytical/ Advisory work

During the year, took classes to 128 trainees in 10 batches at DDC, Jengitchekgre on nutrient management, disease management in rubber and also demonstrated method of soil and leaf sample collection from the field. Twenty four soil samples received from the growers were analyzed and fertilizer recommendations were issued.

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

Initiated the work and collected 24 soil samples from the rubber growing areas of Garo hills of Meghalaya (West, North, East and South-West district) using GPS system with the help of extension officers from the Rubber Board Regional Office at Tura. Soil samples were air dried and processed and kept ready for analysis at RRS, Agartala.

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

The experiment initiated to assess the performance of the seedlings raised in root trainers consisting of eight different combinations of potting media. One year after sowing the seeds in the individual root trainer cups, the growth observations *viz.* plant height and girth data was recorded and then brown budding was carried out in all seedlings. It was found that top soil + Arecanut shell + Dry Fish @ 1:1:1 attained mean height of 107.5 cm and girth of 4.4 cm followed by FYM mixed with top soil @ 1:2 ratio which showed average height of 104 cm and girth of 3.9 cm. The lowest height was recorded in Top soil + Aracanut shell @ 1:1 whereas the lowest girth was recorded in Poly bag seedling plants.

#### 3.6. Weed flora studies

During the year weed samples were collected from the immature rubber fields in the West and North Garo Hills of Meghalaya using GPS system and some weeds were identified. But, all the weeds could not be identified due to the limited availability of literature and lack of expertise. In general, there was not much variability in the weed composition.

## REGIONAL EXPERIMENT STATION, NAGRAKATTA, BENGAL

## 1. Crop Improvement

## 1.1. Evaluation of clones

In trial I and trial II of multidisciplinary evaluation of clones initiated in 1990 in the Sub-Himalayan Bengal region, RRIM 612 had higher girth followed by RR11 118 and SCATC 93-114. Out of the 18 clones, 10 showed significantly higher girth than RR11 105. In trial III, girth of all the clones was at par with RRIM 600 (check clone) and SCATC 93-114 showed highest girth among the clones. Similarly, in clone trial IV, none of the clones recorded superior girth over check clone RRIM 600.

Five clones *viz.* PB 235, SCATC 88/13, PB 311, GT 1 and RRIM 703, showed significantly higher yield over RR11 105 in trial I and II. However, in trial III none of the clones was significantly superior to the check clone in terms of yield. PB 235 and RR11 208 were found to be the high yielder among the clones. Yield of RRIC 102 and PB 260 were significantly lower than the check clone. In trial IV while yield in PB 280 and RR11 208 was significantly higher than check clone RRIM 600, rest of the clones were at par with RRIM 600 (Table Nag. 1).

Table Nag. 1. Pattern of yield in different clone trials

Trial I and II	Yield (g t <sup>-1</sup> t <sup>-1</sup> )	Trial III	Yield (g t <sup>-1</sup> t <sup>-1</sup> )	Trial IV	Yield (g t <sup>-1</sup> t <sup>-1</sup> )
RRIM 612	17.7	RRIC 102	21.1	SCATC 93-114	22.8
GI 1	23.8	PB 260	23.0	RR11 105	27.3
PB 86	29.3	RRIM 612	24.6	RRIC 104	28.3
Haiken 1	30.2	Haiken 1	31.3	PR 261	29.8
RRIM 605	32.4	SCATC 93-114	33.0	RR11 300	30.1
PB 5/51	34.4	PR 107	34.1	PB 235	33.0
PR 107	35.0	PB 86	35.3	Haiken 1	33.8
RR11 203	36.2	PB 310	36.3	RR11 308	33.8
SCATC 93-114	37.0	RR11 208	40.4	RR11 208	37.7*
RR11 118	39.7	PB 235	43.0	PB 280	43.8**
RR11 300	40.4	<b>RRIM 600</b>	<b>39.2</b>	<b>RRIM 600</b>	<b>28.1</b>
RR11 208	42.5	CD (P=0.05)	NS	CD (P=0.05)	9.6
RRIM 703	43.6*				
GT 1	46.7*				
PB 311	51.1**				
SCATC 88-13	52.4**				
PB 235	58.7**				
<b>RR11 105</b>	<b>26.9</b>				
CD (P=0.05)	16.4				

\* Significant at 0.05% level;

\*\* Significant at 0.01% level

### 1.2. Evaluation of germplasm

Twenty one germplasm accessions were evaluated for cold response in the hot-spot area of Nagraakatta, West Bengal in 1998 with a check clone RRII 105. Among the wild accessions, highest girth was recorded in RO 3172 followed by RO 2890 and RO 5348. Among the accessions,

six accessions viz. RO 3172, RO 2890, RO 5348, RO 2635, AC 619 and RO 5557 showed significantly higher girth than RRII 105. In terms of yield, only RO 5363 showed significantly higher yield than check clone. In general, the performance of Rondonia was better than the Acre and Mato Grosso accessions (Table Nag. 2).

Table Nag. 2. Growth and yield in different accessions of *Hevea*

Accessions	Girth (cm)	Yield (g t <sup>-1</sup> t <sup>-1</sup> )	Accessions	Girth (cm)	Yield (g t <sup>-1</sup> t <sup>-1</sup> )
AC 1950	68.4	12.2	RO 2629	75.0	5.3
AC 607	44.1	8.8	RO 2635	81.9*	6.0
AC 619	81.7*	4.8	RO 2890	85.8**	6.5
AC 623	54.2	7.1	RO 3172	88.7**	8.1
AC 68	72.4	8.0	RO 5329	66.5	9.2
AC 763	61.6	27.4	RO 5348	84.4**	4.3
			RO 5363	72.7	37.6*
MT 196	75.3	31.4	RO 5408	67.2	5.5
MT 2229	74.1	10.0	RO 5430	75.0	19.8
MT 2594	65.1	9.0	RO 5557	80.6*	10.1
MT 44	75.2	8.6	RO 6139	62.9	11.8
<b>RRII 105</b>	<b>67.6</b>	<b>25.8</b>	CD (P=0.05)	11.7	10.6

\* Significant at 0.05% level;

\*\* Significant at 0.01% level

### 1.3. Performance of polyclonal seedlings

Polycross seeds collected from seed garden of Kanyakumari, Tamil Nadu were raised in Bengal during 1990's with 240 trees in CRD (single tree single plot) at normal distance (5x 5 m). Mean girth of the population after 25 years of planting was 74.5 cm and the average yield was 34.6 g t<sup>-1</sup>t<sup>-1</sup> where 33.2 per cent plants showed above average yield. Selected ortets were maintained at the nursery for further evaluation.

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

Half sib progenies of seven different clones were raised in polybags (2012) followed by field planting in August, 2013. Half sibs raised from seeds of RRII 429 attained highest average girth followed by RRII 208 and RRII 422. The coefficient of variation was more in half-sib progeny of RRII 422, SCATC 88/13 and RRII 429 than the others.



### 1.5. On farm evaluation of promising clones

In the experiment laid out in 2015, to study the performance of selected promising clones under the agroclimate of Sub-Himalayan Bengal, RRII 417 (30.2 cm) had superior girth followed by RRII 600 (28.5 cm). However, no significant difference was observed among the clones.

### 1.6. Intracultural variability studies

In the experiment on tree to tree variability in yield and yield components (volume, DRC and yield) in three clones intracultural variation in volume of latex was more in RRII 105 followed by RRII 600 and PB 311. In terms of yield, variation in RRII 105 was more followed by PB 311 and RRII 600. Intracultural variation was more in PB 311 when DRC was considered and it was more uniform between trees in RRII 600. The data indicated that consistency in yield, volume and DRC was more in RRII 600 compared to RRII 105 and PB 311.

## 2. Crop Management

### 2.1. Inter-planting trial

The inter-planting trial on rubber under tea was continued since 1999. Four different planting combinations of tea-rubber along with monoculture of tea and rubber as control were evaluated. Green tea leaf yield in inter-planted plots was significantly lower than that of the pure plot due to heavy shade imposed by the mature rubber trees and also due to severe pest attack in inter-planted plots. Rubber yield in pure plot was higher 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

A study was initiated on performance of seedlings, raised from seeds developed in varied agroclimate, under the climatic condition of sub-Himalayan Bengal. Seeds were collected from

the research stations at Kanyakumari in Tamil Nadu (South India), Tura of Meghalaya, Kamrup of Assam (North East India) along with that of Jalpaiguri (Sub-Himalayan Bengal). At seventh year, annual average girth and tappable plants (%) of seedlings from the four different sources was similar. Girth as well as number of tappable plants from Kanyakumari source were high whereas it was low among the different sources in Nagrakatta. Mean girth of tappable plants was similar for all the sources.

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

A few promising rubber clones were planted in the high pH soil of Sarugaon Tea Estate, Birpapar under Dooars area of Sub-Himalayan Bengal in around 1.25 ha area with a control block with a pH of 5.5 at the experiment station of RRII, Nagrakatta. Girth at 150 cm height was measured at 5<sup>th</sup> year of planting. Though the growth of plants in high pH soil was lower than the normal soil no significant difference was observed between girth in six clones studied in both the soil types. In soil with pH 5.5 girth of RRII 208 and RRII 429 was better than the rest of the clones whereas, in high pH soil, RRII 208 showed better growth.

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

In order to study the field performance of ortets selected from cold and drought stress prone (non-traditional) areas, three field trials were initiated under the agroclimate of sub-Himalayan Bengal (Nagrakatta), Konkan region of Maharashtra (Dapchhari) and Traditional region (Kottayam). The girth data of plants at Nagrakatta showed that the clone RRII 417 attained significantly higher girth (39.3 cm) followed by RRII 429 (32.6 cm) compared to the check clone

(30.6 cm). Ortets namely, RRSg 1, RRSg 9 (from Guwahati), RRST 37 (from Tura) were found comparable among the clone/ortets followed by RRII 105 and RRSg 3 (from Guwahati). Significant difference was observed

among the clones/ortets in terms of yield. RRII 600 was found superior ( $80.0 \text{ g t}^{-1} \text{ t}^{-1}$ ) among the clones/ortets, although the ortets RRSg 1 ( $79.2 \text{ g t}^{-1} \text{ t}^{-1}$ ) and RRST 37 ( $77.1 \text{ g t}^{-1} \text{ t}^{-1}$ ) were found comparable to check clone.

## REGIONAL RESEARCH STATION, DAPCHARI, MAHARASHTRA

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

### 1. Environmental Physiology

#### 1.1. Drip and basin method of irrigation

The experiment started during 1987 with different levels of ETc based irrigation scheduling with basin and drip methods was continued. Basin irrigation at 1.0 ETc treatment registered the highest girth of 80.0 cm followed by 76.2 cm and 73.8 cm in basin 0.5 ETc and 0.25 ETc, respectively (modified reduced irrigation). It was found that trees under different levels of basin irrigation showed higher girth as compared to drip system and control (rainfed). Higher yield ( $29.5 \text{ g t}^{-1} \text{ t}^{-1}$ ) was recorded with basin irrigation scheduling at 0.5 ETc compared to control ( $21.7 \text{ g t}^{-1} \text{ t}^{-1}$ ). Reduced irrigation in both cases recorded sustainable yield without decline. Plant

girth and yield was not affected due to reduced level of irrigation in both irrigation methods (Table Dap. 1).

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

Treatment	Girth ( $\text{g t}^{-1} \text{ t}^{-1}$ ) (cm)	Yield ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ )	
Control	67.6	21.7	819
Basin 1.00 ETc	80.0	24.1	912
0.25 ETc*	76.0	29.4	1113
0.50 ETc	76.2	29.5	1115
Drip 0.75 ETc	55.6	23.5	889
0.25 ETc**	73.8	22.8	861
0.25 ETc	71.5	23.6	894
CD (P=0.05)		NS	NS

\* Changed from 0.75 ETc to 0.25 ETc;

\*\* Changed from 0.50 ETc to 0.25 ETc

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

The trial was started in 2011 to evaluate the mechanism of adaptation and elucidate common mechanisms, if any, involved in cold and drought tolerance using molecular physiology/biochemical tools for the selection of ortets from cold and drought prone agro-climatic regions and to study the G x E interaction for growth and yield.

branching height, bark thickness, branch number and total chlorophyll content were recorded.

Significant differences in girth and branch height were recorded among the ortets. The RRSA 98 continued to attain higher girth of 16.9 cm while the lowest girth was recorded in RRST 24 (8.6 cm). RRII 105 recorded the lowest girth (9.9 cm) while highest girth was noticed in RRII 414 (12.3 cm) followed by RRII 430 (16.0 cm). In general, RRII 400 series clones showed higher girth than RRII 105 and RRII 600 (9.9, 12.0 cm). In general, ortet RRSA was found to be superior for all growth characters studied.

## 2. Crop Improvement

### 2.1. Ortet selection

The trial started during 2008 to evaluate the growth and yield performance of ortets selected from polycross seedling was planted at this station with control clones. OS 35 recorded highest girth (30.5 cm) compared to RRII 105 (23.9 cm). The lowest girth was recorded in OS 8 (19.7 cm). Among the control clones, RRII 430 recorded highest girth (31.3 cm). It was also noticed that all ortets were superior in girth than clone RRII 105 except ortet OS 8.

### 2.2. Germplasm screening

In this trial on screening for wild *Hevea* accession (130) for drought (2003) with RRII 105, RRII 600 and Tjir 1, Mato Grosso accessions were found superior in all growth characters than those from Rondonia and Acre provenances. Based on field performance, 25 potential drought tolerant accessions were identified for further detailed studies.

### 2.3. Further evaluation of selected wild *Hevea* accessions

FET was laid out in July 2007 using 25 selected drought tolerant wild *Hevea* accessions for drought along with 5 HP clones and RRII 105, RRII 600, Tjir 1, RRII 430 and RRII 208 in Rectangular Lattice Design. The accessions

showed a wide variability for all characters studied.

A clonal nursery experiment with clones selected from half sib progeny of prepotent clones was initiated in 2010 with the objective to evaluate the clones in a clonal nursery and advance the potential ones showing drought tolerance with good rubber yield to LST and PCE to reduce the breeding cycle trial is in initial stage.

### 2.4. Clonal nursery evaluation

Evaluation of half sib progeny of 15 clones in nursery field was started in 2011 for selection of primary ortets. Trial for evaluation of pipeline clones for drought tolerance was started in 2011 using 50 pipeline clones and two check clones in Rectangular Lattice Experiment with the objective to identify drought tolerant clones for their adaptability and suitability to the agro climatic condition of Maharashtra.

The experiment on identification of reliable juvenile and mature characteristics for clone identification, standardization of DUS testing norms for evolving specific guidelines for varietal registration in rubber was laid out in 2011 with 50 divergent clones in RBD. Morphological data on individual plants in the juvenile stage covering 40 traits have been recorded from all three locations at the age of one year. Data was also recorded for a set of traits from mature plants of the popular RRII clones (RRII 5, RRII 105, RRII 118, RRII 203, RRII 208 and RRII 400 series). Quantitative data on girth and height have also been recorded to measure G x E effects for these clones.

## 3. Crop Management

### 3.1. Drought management and soil moisture conservation

Study was initiated in 2008 with the view to find out the effect of vertical mulching and Kaolin spray (6.0%) on growth and yield of rubber. The results showed that, there was no significant difference among the treatments.



## REGIONAL RESEARCH STATION, DHENKANAL, ODISHA

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

### 1. Crop Improvement

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

#### 1.1. Clone evaluation

In trial 1 (1987), clone RRII 105 recorded highest mean yield of 50.5 g t<sup>-1</sup> t<sup>-1</sup>. GT 1 recorded the lowest yield (37.4 g t<sup>-1</sup> t<sup>-1</sup>) while exhibiting significantly higher mean girth over RRII 105 and RRII 600. In terms of growth all three clones performed well in the region (Table Odi. 1).

Table Odi. 1. Growth and yield performance of clones

Clone	Yield (g t <sup>-1</sup> t <sup>-1</sup> )	Girth (cm)
RRII 105	50.5	74.2
RRIM 600	47.6	78.3
GT 1	37.4	81.7
CD(P=0.05)	8.2	1.9

In clone trial 1990, RRII 208 (73.7 g t<sup>-1</sup> t<sup>-1</sup>) and SCATC 88/13 were found superior in yield followed by RRIM 600 (62.1 g t<sup>-1</sup> t<sup>-1</sup>) while SCATC 93-114 recorded lower yield (42.3 g t<sup>-1</sup> t<sup>-1</sup>). However, SCATC 93-114 recorded better girth.

In the 1991 experiment, clones differed significantly in mean yield, RRII 208 (81.7 g t<sup>-1</sup> t<sup>-1</sup>) and PR 255 (77.5 g t<sup>-1</sup> t<sup>-1</sup>) recorded better yield among the clones. Polyclonal seedling population

(53.8 g t<sup>-1</sup> t<sup>-1</sup>) recorded low yield in spite of better growth and adaptability to the stress conditions.

In the 2000 clone trial, the higher mean yield was observed in RRIM 600 (66.0 g t<sup>-1</sup> t<sup>-1</sup>) and PB 28/59 (62.7 g t<sup>-1</sup> t<sup>-1</sup>) while RRII 51 (42.2 g t<sup>-1</sup> t<sup>-1</sup>) had the least. RRII 300 exhibited superior girth (66.5 cm).

#### 1.2. Polyclonal ortet evaluation

Under test tapping, ortet clones OR 3 and OR 4 recorded comparatively higher yield and almost at par with high yielding clone RRII 208. The ortets OR 8 (40.8 cm) followed by OR 4 (38.4 cm) exhibited better girth (Fig. Odi. 1).

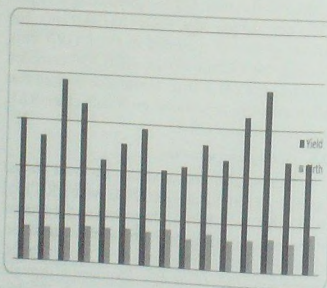


Fig. Odi. 1. Yield and girth performance of Ortet clones

### 2. Latex harvest technology

#### 2.1. Controlled upward tapping experiment

Controlled upward tapping (CUT) with ethephone application in clone RRIM 600 showed increase in rubber yield.

## REGIONAL RESEARCH STATION, PADIYOOR, KERALA

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

### 1. Crop Management

#### 1.1. Response to application of 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 three clones (RRII 105, RRII 414 and RRII 429) and four levels of fertilizers viz. recommended dose (D1), twice (D2) and thrice (D3) the recommended dose and a zero fertilizer control. Clonal differences in yield were not significant (Table Pad. 1).

Table Pad. 1. Effect of fertilizer treatments on yield

Treatment	Yield (g t <sup>-1</sup> t <sup>-1</sup> )		
	RRII 105	RRII 414	RRII 429
D1	50.9	40.0	57.5
D2	43.3	50.1	32.1
D3	42.3	63.9	72.1
Control	58.6	64.8	61.3
CD (P=0.05)	NS		

### 2. Crop Improvement

#### 2.1. Large scale evaluation of clones

Mean annual yield of IRCA 130 (Table Pad. 2) was the highest at 65.8 g t<sup>-1</sup> t<sup>-1</sup> and was on par with clones RRII 105 (56.3 g t<sup>-1</sup> t<sup>-1</sup>) and PB 255

(54.9 g t<sup>-1</sup> t<sup>-1</sup>). Summer yield of IRCA 130 was the highest at 61.1 g t<sup>-1</sup> t<sup>-1</sup> and significantly superior to all the other clones tested. IRCA 18, IRCA 130 and PB 330 exhibited significant superiority with respect to girth over other clones.

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

Clones	Mean annual yield (g t <sup>-1</sup> t <sup>-1</sup> )	Summer yield (g t <sup>-1</sup> t <sup>-1</sup> )
PB 255	54.9	44.2
PB 314	37.9	30.8
PB 330	35.7	28.6
PB 28/59	23.9	17.9
RRIM 703	26.6	20.1
IRCA 18	32.7	21.2
IRCA 109	35.4	33.3
IRCA 111	31.7	26.7
IRCA 130	65.8	61.1
IRCA 230	28.9	22.2
RRII 105	56.3	38.6
CD (P=0.05)	11.9	10.5

#### 2.1. Evaluation of rubber clones/selections at high altitude

The average annual yield of clones RRII 203, RRIC 100, PB 86 and the ortets P 213 and P 270 were on par and this was significantly superior to that of RRII 105 and other clones/ortets tested. RRII 203 recorded the highest summer yield (35.9 g t<sup>-1</sup> t<sup>-1</sup>) and was significantly superior to RRII 105 (18.4 g t<sup>-1</sup> t<sup>-1</sup>) (Table Pad. 3).

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

Clones	Mean yield (g t <sup>-1</sup> t <sup>-1</sup> )	
	Annual yield	Summer yield
RRII 105	22.8	18.4
RRII 203	47.3	35.9
RRIC 100	56.4	28.7
RRIC 102	19.8	15.3
PB 86	56.9	33.4
P 1	24.1	21.2
P 2	25.3	20.3
P 90	26.0	20.9
P 121	27.7	22.3
P 155	20.7	17.4
P 213	48.7	32.9
P 270	52.0	25.4
P 280	23.2	22.8
P 296	22.7	26.0
Irrity	47.1	31.5
CD (P=0.05)	28.9	15.8

### HEVEA BREEDING SUB-STATION, KADABA, KARNATAKA

Hevea Breeding Sub-station (HBSS) with a research farm at Nettana aims at evaluation of clones for commercial cultivation in Karnataka region. The objective of crop improvement programmes in the station is to identify high yielding clones with tolerance to drought and leaf diseases caused by *Phytophthora* and *Corynespora*, which are the major constraints in this region. The farm has a source bush nursery for generating nucleus planting material and a well-established Class B Agro-meteorological Observatory.

#### 1. Large scale clone trial (1989)

Clones RRII 203 (49.2 g t<sup>-1</sup> t<sup>-1</sup>) and PB 255 (42.3 g t<sup>-1</sup> t<sup>-1</sup>) had higher yield than the check clone RRII 105 in 2015 (Table Kad. 1). Clone RRII 203 (62.9 g t<sup>-1</sup> t<sup>-1</sup>) also had significantly higher yield over 13 years of tapping compared to check clone RRII 105 (44.9 g t<sup>-1</sup> t<sup>-1</sup>). Clones KRS 163, PR 261 and SCATC 93-114 recorded significantly lower yield compared to RRII 105 in 2015 while SCATC 93-114 and PR 261 recorded significantly lower yield compared to RRII 105 in 2015 and over 13 years of tapping as well.



Table Kad. 1. Yield and growth performance of clones in LST (1989)

Clone	Mean yield (g t <sup>-1</sup> t <sup>-1</sup> )	
	During 2015	Over 13 years
Haiken 1	26.1	36.0
KRS 128	22.7	44.3
KRS 163	19.8 <sup>#</sup>	42.2
KRS 25	34.4	51.8
PB 255	42.3	53.1
PR 255	25.9	38.5
PR 261	19.8 <sup>#</sup>	30.4 <sup>#</sup>
RRII 105	38.9	44.9
RRII 203	49.2	62.9 <sup>*</sup>
RRII 300	27.4	31.0 <sup>#</sup>
RRII 308	30.5	44.6
RRIM 600	27.7	35.1
SCATC 88/13	28.7	38.7
SCATC 93-114	15.3 <sup>#</sup>	15.9 <sup>#</sup>
CD (P=0.05)	18.6	13.4

# Significantly lower than RRII 105

\* Significantly higher than RRII 105

### 2. Large scale clone trial (1990 A)

Among the 15 clones tested in Large Scale Trial (LST) planted in 1990, HP 223 (58.4 g t<sup>-1</sup> t<sup>-1</sup>), HP 372 (68.4 g t<sup>-1</sup> t<sup>-1</sup>) and PB 235 (65.5 g t<sup>-1</sup> t<sup>-1</sup>) had significantly higher yield than RRII 105 in 2015 (Table Kad. 2). Clones HP 223, HP 372, PB 235 and PB 260 recorded higher yield than RRII 105 in mean over 12 years of tapping as well.

### 3. Trial on estimation of genetic parameters (1990 B)

In this trial, 12 parent clones and their half-sib progenies were evaluated. Among the parent clones, PB 235 (93.8 g t<sup>-1</sup> t<sup>-1</sup>) was the highest yielder followed by RRII 203 (57.8 g t<sup>-1</sup> t<sup>-1</sup>). Mean yield of progenies was the highest for RRII 203 (38.0 g t<sup>-1</sup> t<sup>-1</sup>) followed by IAN 45/873 (33.6

g t<sup>-1</sup> t<sup>-1</sup>). Based on growth and yield performance, 98 preliminary selections were made for further multiplication and evaluation.

Table Kad. 2. Yield and growth performance of clones in LST (1990 A)

Clone	Mean yield (g t <sup>-1</sup> t <sup>-1</sup> )	
	During 2015	Over 12 years
PB 260	46.9	63.4 <sup>*</sup>
HP 223	58.4 <sup>*</sup>	62.2 <sup>*</sup>
Mil 3/2	45.2	38.0 <sup>#</sup>
HP 204	21.5 <sup>#</sup>	27.7 <sup>#</sup>
HP 185	42.9	39.9 <sup>#</sup>
PB 217	46.0	56.9
RRII 105	38.9	51.9
HP 372	68.4 <sup>*</sup>	65.1 <sup>*</sup>
PB 311	50.9	55.4
Tjir 1	23.7 <sup>#</sup>	25.7 <sup>#</sup>
GI 1	19.3 <sup>#</sup>	31.5 <sup>#</sup>
Hil 28	29.1	31.3 <sup>#</sup>
GT 1	37.4	49.8
PB 235	65.5 <sup>*</sup>	64.3 <sup>*</sup>
HP 187	25.6	31.9 <sup>#</sup>
CD (P=0.05)	14.3	7.8

# Significantly lower than RRII 105

\* Significantly higher than RRII 105

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

Three Small Scale Trials (SST) viz. 1991 A (36 clones), 1991 B (13 clones) and 1991 C (13 clones) were planted in 1991. In trial 1991 A, RRII 300 (82.6 g t<sup>-1</sup> t<sup>-1</sup>) recorded highest yield in 2015 followed by PB 235 (78.6 g t<sup>-1</sup> t<sup>-1</sup>) and PB 280 (73.3 g t<sup>-1</sup> t<sup>-1</sup>) and were significantly higher than RRII 105 (45.8 g t<sup>-1</sup> t<sup>-1</sup>). Compared to the check clone RRII 105 (51.6 g t<sup>-1</sup> t<sup>-1</sup>), mean yield over 11 years was significantly higher for clones PB 314, PB 312, PB 235, RRII 203, RRII 300 and PB 280.

In trial 1991 B, RRII 5 ( $74.1 \text{ g t}^{-1} \text{ t}^{-1}$ ) recorded highest mean yield and was significantly higher than RRII 105 ( $45.63 \text{ g t}^{-1} \text{ t}^{-1}$ ). There was no significant difference in mean yield among the 13 clones in trial 1991 C and PB 28/59 recorded the highest yield ( $57.0 \text{ g t}^{-1} \text{ t}^{-1}$ ) among the clones tested.

#### 5. Large scale trial (2000)

The LST planted in the year 2000 consists of hybrids RRII 403, RRII 407, RRII 414, RRII 422, RRII 429 and RRII 430 and their parents viz. RRIC 100 and RRII 105. Analysis of yield data in 2015 revealed that there was no significant difference between the clones tested and the highest mean yield was recorded for the clone RRII 430 ( $66.4 \text{ g t}^{-1} \text{ t}^{-1}$ ) while check clone RRII 105 recorded  $41.9 \text{ g t}^{-1} \text{ t}^{-1}$  (Table Kad. 3).

Mean yield over seven years was highest for RRII 430 ( $70.5 \text{ g t}^{-1} \text{ t}^{-1}$ ) and was significantly superior to RRII 105 ( $49.0 \text{ g t}^{-1} \text{ t}^{-1}$ ). Clones RRII 414, RRII 422 and RRII 429 also recorded significantly superior mean yield than RRII 105. The data revealed highest girth (cm) for clones RRII 414 (82.6) and RRII 430 (79.0) and were significantly superior to RRII 105 (67.5). But mean girth of RRII 422 was significantly lower than RRII 105.

#### 6. Polycross garden (1995)

The station maintains a polyclonal seed garden with 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 for seed collection.

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

Clone	Girth (cm)	Mean yield ( $\text{g t}^{-1} \text{ t}^{-1}$ )	Mean yield over seven years ( $\text{g t}^{-1} \text{ t}^{-1}$ )
RRII 100	75.0*	46.1	47.5
RRII 105	67.5	41.9	49.0
RRII 403	63.1	53.0	50.3
RRII 407	69.4	33.4	46.0
RRII 414	82.6*	45.4	69.0*
RRII 422	59.0#	64.0	67.3*
RRII 429	69.7	63.7	64.8*
RRII 430	79.0*	66.4	70.5*
CD ( $p=0.05$ )	6.9	NS	15.4

\*Significantly higher than RRII 105

# Significantly lower than RRII 105

## HEVEA BREEDING SUB-STATION, THADIKKARANKONAM, TAMIL NADU

### 1. Genetic Improvement

#### 1.1. Conventional breeding

During the period under report, four projects were pursued, viz. clone evaluation, hybridization and clonal selection, new generation polyclonal seed garden and participatory clone evaluation.

##### 1.1.1. Clone evaluation

Field performance of the modern high yielding clones in these trials was studied during the period under report. The block trials initiated in Palazhi and Bethany estates were under second year of tapping. Girth of the trial clones along with RR11 105 after nine years of growth indicated highest growth in clones RR11 429, RR11 414 and RR11 422. The commercial yield data over two years indicated clones RR11 422 and RR11 430 as top yielders with 56.9 and 51.6, respectively in the block trial at Bethany estate.

In the block trial at Palazhi estate, clone RR11 430 was top yielding with 92.0 g t<sup>-1</sup> t<sup>-1</sup>. During the same period, the yield of RR11 105 had 50.5 and 80.6 g t<sup>-1</sup> t<sup>-1</sup>, respectively at Bethany and Palazhi estates.

Based on the results of the concluded large scale experiments conducted in the region, a block trial for evaluating the commercial yield performance of the clones PB 255, PB 314, IRCA 109, IRCA 111, RR11 203, RR11 414, RR11 417, RR11 422, RR11 430 and RR11 105 was initiated.

#### 1.1.2. Hybridization and clonal selection

The breeding orchards consisting of 51 parental clones were properly maintained and around 2486 hand pollinations were attempted with six different parental combinations. The hybrids obtained during 2015 were raised in seedling nursery for preliminary evaluation. The hybrids of 2014 were test tapped to evaluate the performance and secondary characters.

##### 1.1.3. New generation polyclonal seed garden

The seed garden at New Ambady estate was maintained well. The polyclonal seedlings raised out of polycross seeds collected during 2014 were test tapped and the promising ones were pollarded for multiplication and further evaluation. Around 1800 healthy polyclonal seeds were collected and a seedling nursery was raised for evaluation.

### 2. Participatory clone evaluation experiments

The annual growth of 11 pipeline clones and the three check clones planted at Tharuvaiyar estate were recorded. The clones P 10 (50.6 cm) and P 21 (50.2 cm) were more vigorous based on eight years of growth. However, the average tappareability was only around 57 per cent. In the On-Farm Trial (OFT) at Bethany estate (2010), of the 14 trial clones assessed, clones P 44 (47.6 cm) and P 27 (46.5 cm) exhibited better growth. Similarly, the assessment of the annual girth of the 11 trial clones in the OFT planted at Bethany estate in 2012 indicated superior performance of clones P 102 (30.0 cm), P 104 (28.6 cm) and P 49 (28.3 cm).



### LIBRARY AND DOCUMENTATION CENTRE

Rubber Research Institute of India has a well maintained Library and Documentation Centre with a collection of 22920 books, 23800 bound volumes of periodicals, 6002 standards, 1563 reprints, 173 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 two books were added and 22 books written off to the stock. Received and registered 552 issues of journals as subscription/exchange.

Compiled and disseminated the information bulletins viz. Documentation List (1-4) 2015, New Additions List 2015 and Staff Publications list 2014-2015. Databases were updated with the details of 82 books and 210 articles. Classified/reclassified and catalogued 636 books, circulated 1402 books, filed 2955 press clippings of relevant articles, issued 1030 SDI bulletins and provided 10200 photocopies. Library membership were issued to 51 users and library services were extended to 871 outsiders.

As a part of sales promotion of RRII publications, library organized the sale and distribution of 604 copies of the journal *Rubber Science* and 270 other publications including RRII Annual Reports.

### SCIENTIFIC ADVISORY COMMITTEE RECOMMENDATIONS

- Upgrading the clone RRII 208 from Category II to Category I for North Eastern Region.
- Include stimulation schedules under d3, d4 and d6 frequencies in 7d/7 systems in addition to existing recommendation of yield stimulation under d3, d4 and d6 in 6d/7 systems.
- Polypropylene woven fabric as an alternative mulch for soil moisture conservation and weed control in young rubber especially in dry areas.
- Practice surface tillage of plant basin (0.75 m<sup>2</sup> around basin) at the end of the rainy season as additional soil moisture conservation measure to tide over the drought situations in young rubber plants.

# ANNUAL EXPENDITURE

Expenditure at a glance (2015-16)

Head of Account	Expenditure (Rs. in lakhs)
<b>Non-Plan</b>	
RRII HQ	6,17.58
CES, Chethackal	484.47
<b>Total</b>	<b>1,102.05</b>
<b>Plan</b>	
Research other than NERDS	365.59
North East Rubber Development Scheme	2,001.79
<b>Total</b>	<b>2,367.38</b>
<b>Grand Total</b>	<b>3,469.43</b>

## LIST OF MAJOR EQUIPMENTS

## RRII HEAD QUARTERS

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°C & -80°C)  
 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  
 Hardness tester (shore A,D,M,0)  
 High speed microcentrifuge  
 High speed Table Top Centrifuges  
 High Voltage Power Pack  
 High-Speed Centrifuge  
 Histo Embedder  
 HPLC system  
 Hybridization oven – Incubator shaker  
 Hydraulic press (14" × 14")  
 Image processing and Analysis System  
 Incubator & Accessories  
 Incubator shaker with cooling  
 Infrared thermometer  
 Inverted 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  
 Measuring mixer (80cc)  
 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  
 Oxygen electrode  
 Ozone chamber  
 P700 chlorophyll fluorescence measurement system  
 PAM-2000 portable flurometer  
 Particle size analysers  
 PCR machines  
 Phase contrast Microscope & accessories  
 Phosphor Image Analyser  
 Phosphor imager  
 Plant growth chamber  
 Plate reader  
 Polarizing Microscope & accessories  
 Projection microscope with accessories  
 Protein separating systems - 2D  
 Real time PCR machines  
 Recirculating Cooler  
 Refrigerated and Heating Circulator  
 Refrigerated high speed micro centrifuge  
 Refrigerated shaker  
 Refrigerated Table Top Centrifuge  
 Remote Sensing and Geographical Information System (RS & GIS)  
 Research microscope and image analyser  
 Ross flexing Machine  
 Rotary Evaporator  
 Rubber Process Analyser  
 Sap flow meter  
 Sequencing gel electrophoresis unit  
 Sequi-Gen GT system (sequencing system)  
 Soil Respiration Analyser

Sonicator  
 Specific gravity balance  
 Spectro radiometer 90  
 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 system  
 Water purification system  
 Wet sieving apparatus  
 Zeta potential analyser

#### NORTH EAST RESEARCH STATIONS

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

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- GENBANK SEQUENCE SUBMISSION**
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- Best Research Paper Award
- Singh, R.P., Dey, S.K., Satisha, G.C., Singh, R.S. and Jacob, J. (2015). Growth and yield performance of seven popular *Hevea* clones and soil properties in sub-tropical areas of Mizoram. *International Conference on Frontier of Plant Sciences & Developing Technologies*, 7<sup>th</sup> November 2015, Banarus Hindu University, Varanasi, India.
- TRAINING/WORKSHOP ATTENDED**
- Ray, D. *Workshop on Rubber Plantation, Raising Nursery and Rubber Tapping*, 21<sup>st</sup> November 2015, Principal Office building, Tripura Tribal Areas Autonomous District Council, Khumulwng, Tripura.



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*Continued from inside front cover*

#### Research divisions and functions

The major research divisions are Agronomy/ Soils, Biotechnology, Botany, Climate Change & Ecosystem Studies, Germplasm, Latex Harvest Technology, Plant Pathology, Plant Physiology, Rubber Technology, Technical Consultancy and Economics. Studies on Clone Evaluation, Genome Analysis and DRIS Fertilisation are dealt separately.

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

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

#### Central Experiment Station

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

#### Regional Research Stations

RRII has established a North-Eastern Research Complex with headquarters at Agartala having regional research stations at Agartala in Tripura, Guwahati in Assam and Tura in Meghalaya. The RRII has also set up regional research establishments at Dapchhari (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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