

RUBBER
RESEARCH
INSTITUTE OF INDIA



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Rubber Research Institute of India

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

Location

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

Organization

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

Continued on page 109

ANNUAL REPORT 2017-2018



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CONTENTS

The Rubber Board	iv
Director's Review	vii
Agronomy and Soils Division	1
Fertilizer Advisory Group	4
Climate Change and Ecosystem Studies	5
Botany Division	9
Germplasm Division	23
Biotechnology Division	29
Genome Analysis Laboratory	35
Plant Pathology Division	42
Plant Physiology Division	50
Latex Harvest Technology Division	57
Rubber Technology Division	61
Technical Consultancy Division	68
Economics Division	71
Quality Control Division	74
Central Experiment Station, Chethackal, Kerala	77
Regional Research Station, Guwahati, Assam	77
Regional Research Station, Agartala, Tripura	78
Regional Research Station, Tura, Meghalaya	81
Regional Experiment Station, Nagrakata, West Bengal	82
Regional Research Station, Dapchari, Maharashtra	84
Regional Research Station, Dhenkanal, Odisha	88
Regional Research Station, Padiyoor, Kerala	89
Hevea Breeding Sub-Station, Kadaba, Karnataka	91
Hevea Breeding Sub-Station, Thadikkaranonam, Tamil Nadu	93
Library and Documentation Centre	94
Scientific Advisory Committee Recommendations	95
Annual Expenditure	97
Publications	98
Scientific and Senior Supporting Personnel	104
Research Establishments	108

THE RUBBER BOARD



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

Organization

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

Chairman/Executive Director

Dr. A. Ajithkumar IAS (upto 23.11.17)

Dr. Shanmuga Sundaram IAS (w.e.f. 24.11.17)

Rubber Research Institute of India

Dr. James Jacob

Director

Crop Improvement

Dr. Kavitha K. Mydin

Joint Director

Botany

Deputy Director (vacant)

Biotechnology

Joint Director (vacant)

Genome Analysis Laboratory

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Principal Scientist

Germplasm

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Principal Scientist (Officer-in-charge)

Crop Management

Joint Director (vacant)

Agronomy and Soils

Dr. M.D. Jessy

Joint Director

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Crop Physiology

Climate Change and Ecosystem
Studies

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Plant Physiology

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Mr. Sabu P. Idicula
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Plant Pathology

Joint Director (vacant)

Economics Research

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Joint Director (vacant)

Latex Harvest Technology

Dr.R.Rajagopal
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Rubber Technology

Joint Director(vacant)

Rubber Technology

Deputy Director(vacant)
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Technical Consultancy

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Deputy Director(vacant)
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Regional Research Station, Agartala

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Joint Director

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Regional Research Station, Tura

Deputy Director (vacant)

Regional Research Station, Dapchari

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Regional Research Station, Dhenkanal

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Senior Scientist (Officer –in-charge)

Regional Research Station, Padiyoor

Dr.RadhaLakshmanan
Principal Scientist (Officer –in-charge)

Hevea Breeding Substation, Kadaba

Dr.P. Deepthy Antony
Scientist (Officer –in-charge)

Hevea Breeding Substation,**Thadikarankonam**

Dr.M.Suryakumar
Scientist (Officer –in-charge)

Administration

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Deputy Secretary

Finance & Accounts

CA. Zachariah Kurian
Joint Director

Instrumentation

Deputy Director(vacant)
M.R.Anilkumar
Instrumentation Officer (Officer –in-charge)

Library & Documentation Centre

N. Latha
Documentation Officer

Statistics & Computer

B. Biju
Assistant Director (Systems)



DIRECTOR'S REVIEW

The year 2017-18 witnessed several important achievements in R&D. The Online Fertilizer Recommendation System or Rubber Soil Information System (RubSIS) originally launched for the district of Kottayam during 2016 was extended to cover the entire rubber plantations in South India. RubSIS is now available in three different languages namely Malayalam, Tamil and Kannada. A mobile App version of RubSIS was also developed which is far easier to use than the online version. Efforts were made to popularize RubSIS by conducting group meetings in different parts of South India in association with the Rubber Production Department of Rubber Board. Work has been extended to develop RubSIS for rubber growing regions of North East India.

Another major R&D outcome was recommending region-specific clones for the different agro-climatic regions where rubber is cultivated in India. Region-specific clone

recommendation has been made up to the district level in the traditional regions and state level in the non-traditional regions based on long years' of field observation. During the reporting year two promising clones were added to the list of pipeline clones, making the total number of pipeline clones to 283. Out of these, 90 pipeline clones are grown in 44 trials in different locations as part of the farmer participatory clone evaluation programme.

It is interesting to note that girth of a segregating polycross progeny of *Hevea* seedlings exhibited normal distribution of *Hevea*, but test tap yield had a highly skewed distribution towards the left. The genetic implications of this differential distribution in growth and yield need to be further examined.

Among the wild germplasm collection, RO 2629, AC 4149 and AC 716 gave yields close to some Wickham clones such as RR11 208, RR11 105 and RRIM 600. A total of 442 wild

accessions are maintained in the field arboretum in Meghalaya. Several introduced clones, both wild accessions and promising lines imported from different countries were also evaluated for their intrinsic tolerance to abiotic and biotic stresses.

A system for producing anti-biotic marker-free GM plants was successfully tested in a model plant. Several new lines of GM rubber plants were developed incorporating agronomically important genes.

We could successfully use 16 SSR markers for genotyping the international clones recently introduced through the IRRDB multilateral clone exchange programme. The SALB tolerant clones developed by Michellin-CIRAD and supplied to India from Cambodia and Ghana were markedly different from the Asiatic imported clones, clearly demonstrating the non-Wicham origins of the SALB tolerant clones evolved in Brazil as evident from the percentage of the imported clones. SNP markers of HMGS and CPT genes showed an association with rubber yield, a finding with potential implications for marker assisted selection of high yielding clones. Another interesting finding from root transcriptome studies was the presence of arbuscular mycorrhizal fungus (AMF), possibly inside the cortical cells of rubber roots. The ecological and agronomic relevance of AMF association with roots of rubber plants needs to be better understood. A collaborative project to

understand microbial dynamics of rubber growing soils of Kerala under different cropping system was initiated with CSIR-NEERI, Nagpur.

Studies on high density genetic linkage maps of different *Hevea* species and QTLs for disease tolerance and yield were in progress with good results. Validation of QTL markers and fine mapping of the QTL regions for disease tolerance were carried out using KASP marker technology. In the case of a heterozygous tree species like *Hevea*, association mapping may be more beneficial than QTL mapping for crop improvement research.

PB 260 trees crown-budded (in the field) with FX 516 (highly tolerant to ALF) continued to show excellent tolerance to ALF even as their girth was better than the normal PB 260 trees. The amount of latex produced by crown-budded trees and its properties were comparable to latex produced by normal PB 230 trees. Efforts were continued to develop crown-budded plants in large root trainer cups in the nursery itself.

Twelve isolates of *Corynespora* were tested for their virulence. All of them were equally virulent and had *CasI* gene (responsible for producing the toxin, cassiicolin) present in them. This toxin was successfully employed for *in vitro* screening of large number of *Hevea* genotypes for *Corynespora* tolerance.

The role of ethylene receptor genes in stimulating latex yield during ethephon stimulation

is interesting and this deserves to be further probed. Are these genes also responsible for high yield in elite clones even without ethephon stimulation? Are these genes less expressed in wild germplasm lines that produce little latex? Answers to these questions will open a new window of opportunity in crop improvement research.

Satellite-based mapping of existing rubber plantations and locating potential areas for expanding NR cultivation was continued. Nearly 910 ha of mature area was located and mapped in Andaman's & Nicobar Islands, Meghalaya, Manipur and Mizoram had 10730, 1062 and 874 ha of natural rubber, respectively. Nearly 5300 ha of rubber in Assam was cultivated below an altitude of 50 m MSL making it higher flood-prone with Brahmaputra valley. A study was undertaken to identify agroclimatically suitable area for natural rubber cultivation in left wing extremism (LWE) affected areas based on long-time climate data of the region and working out "climate tolerance limits".

A new project was initiated to work out the carbon footprint of the Indian rubber industry. Based on life cycle analysis it was estimated that one ha of rubber plantation can sequester as much as 26.7 M Ton of CO₂ during a plantation cycle for 28 years. A study on impact of El nino/ *La nina* episodes in rubber growing regions of India was initiated. Irrespective of such episodes climate warming persisted.

Field trials continued to prove the benefits of low frequency tapping (to reduce cost of production) and controlled upward tapping (to increase productivity of old/senile trees). But these innovations were not adopted by growers in full earnest due to logistic reasons (eg. tapper availability, lack of skill for doing CUT) and possibly low level of awareness among growers.

Our studies showed that natural rubber cultivation has successfully improved household income and standard of living of native communities in Mayurbhanj district of Odisha. A study was undertaken to examine the effectiveness of functioning of Rubber Producers Societies (RPSs). It was found that in general there was a decline in the involvement of members in the activities of RPSs. It was felt that RPSs should evolve into a complete farm service provider. A quick analysis of the draft agreement of RCEP showed that further concessions as demanded by member countries will have major negative implications on the domestic natural rubber producing and rubber products manufacturing countries. The social and economic risk of over-reliance of the Indian rubber industry on huge imports of natural rubber was analyzed. It was concluded that unless domestic natural rubber production is boosted, the country will lose huge amounts of foreign exchange to import raw natural rubber even as its seamless and cheap international availability cannot be taken for granted. Many natural rubber

exporting countries are increasing their consumption of natural rubber for making value added products which these countries could export natural rubber in large volumes in future instead of raw rubber.

An Indian patent for "Stable free radical assisted mechanical devulcanization of rubber" was filed. Using the devulcanized rubber, different formulations were prepared which can be used for making cycle tyre, rubber mats *etc.* An attempt was made to minimize the quantity of detergent and the enzyme concentration in the deprotenization mixture developed last year. Pilot scale evaluation of a new process to recover rubber from skim latex was successfully carried out at four centrifuging units (10,000L).

Better abrasion resistance and flex resistance were achieved in the natural rubber polymeric filler system using blends of polymers. Latex carbon black master-batches with varying carbon black content were prepared and these samples were sent to the collaborator for evaluation at their end. Our studies have established the possibility of substituting NR/PB blend (50/50) in tyre side wall with NR/EPDM blends formulate peroxide vulcanized NR/EPDM

blend (60/40) vulcanizates with good mechanical properties and high flex crack growth resistance.

During the reporting year, more than 362 entrepreneurs from the MSME sector were given advisory services. In addition, through letters and emails another 1450 clients were given advises. More than 530 test reports were issued based on testing about 780 samples. Technological knowhow for 10 rubber products were given to prospective entrepreneurs. During the reporting year NOC for importing a total of 4.65 lakh tonnes of natural rubber was issued by the Quality Control Division of RRH. Out of this, more than 82 per cent was block rubber and nearly 17 per cent was sheet rubber. Other forms (crepe, latex *etc.*) contributed just 0.5 per cent.

Vacancies in the scientist cadre continued to increase as no new recruitments were made during the reporting year. Three issues of *Rubber Science* were published with 26 articles during 2017-18. Additionally 25 conference papers, 20 popular articles, three book chapters, three reports, two PhD theses and four MSc dissertations were also published and six scientific recommendations were approved.

Dr. James Jacob

AGRONOMY / SOILS DIVISION

Developing sustainable farm practices, reducing cost of cultivation, generating additional income and improving biodiversity are the major thrust areas of research of the Division. Various experiments with these objectives were continued. Evaluation of low input strategies for improving soil health was also initiated. Rubber Soil Information System (RubSIS), the powerful Information and Communication Technology (ICT) tool which brings soil fertility status and fertilizer recommendation to the finger-tips of rubber growers was released for the entire rubber growing regions of South India. RubSIS was translated to Malayalam, Tamil and Kannada and a Mobile App version was developed for easy access of soil fertility status and fertilizer recommendation by rubber growers. RubSIS was demonstrated in group meetings held in different parts of South India and is being popularised through various extension programmes. Soil fertility mapping of rubber growing regions of NE India was initiated.

1. Soil fertility mapping, soil health monitoring and development of online fertilizer recommendation system for rubber growing regions of South India (Collaborative project of RRII with NBSS & LUP, ICAR, IIITM-K, Thiruvananthapuram)

Rubber Soil Information System (RubSIS) was developed for the entire rubber growing regions of South India. RubSIS was demonstrated in 23 group meetings held in different parts of South India and feedback was collected from 1134 growers and communicated to Ministry. RubSIS was translated to Malayalam, Tamil and Kannada and a Mobile App was developed. RubSIS was popularised through popular articles, post cards,

letters, SMS and training programmes. A project was developed for soil fertility mapping of rubber growing regions of NE India. Soil sample collection was initiated in Tripura, Assam and Meghalaya.

2. Nutrient Management

Nursery trial on nutrient management, initiated to revise the current fertilizer recommendation for root trainer rubber plants was continued. A second experiment was conducted with revised treatments based on the first trial. The results indicated the superiority of integrated management and foliar nutrition for the growth of root trainer rubber seedlings.

The field experiment at CES, Chethackal to study the effect of long term use of inorganic and organic manures on growth and yield of rubber and soil properties was continued. The residual effect of treatments was studied without applying fertilizer and farm yard manure (FYM). Superiority of the treatment, 25 per cent fertilizer + 75 per cent FYM on growth of rubber was maintained. Recommended practice (30:30:30 kg NPK ha⁻¹ year⁻¹) and the treatments with 25 per cent from fertilizer + 75 per cent from FYM and 50 per cent from fertilizer + 50 per cent from FYM were comparable in yield.

Field trials on secondary and micronutrient nutrition, initiated during 2011 at Thamarasserry, Palappilly and Cheruvally estates were continued. The residual effect of Ca, S, Zn and B applied during the first year of planting on soil test basis was continued for 5-6 years. Hence, one time application of these nutrients is sufficient during immature phase, while annual application of Mg is necessary to maintain the soil status. An incubation study on supplementing B and Zn

through coir pith indicated that soil available B was higher in coir pith applied soils compared to control.

The field trial initiated at Chithalvetty estate, State Farming Corporation of Kerala to study the effect of the organic manure 'Geo green' on growth of young rubber was continued. No significant difference between treatments was noticed in growth. In the study on performance of rubber in a coastal sandy soil of Thanneermukkom, Cherthala, normal growth and yield of rubber was observed with cover crop establishment, organic manures and need based irrigation during the initial years.

Initiated a field trial on targeted application of soluble fertilizers into the root zone of young rubber plants. Water soluble fertilizers were applied into the root zone of the plants using disposable plastic bottles with wicks inserted.

A glass-house experiment to establish critical level of soil Mg was initiated. No significant difference was observed between the different levels of Mg application on girth of plants after six months. The study on leaf nutrient status of low and high yielding rubber trees was continued. In the rhizosphere study, variations in available N and organic P was observed between rhizosphere and bulk soil samples among locations. There was no significant difference in growth and dry biomass of seedlings up to 30 days irrespective of variations in pH and base status. The incubation study on lime application in two soils varying in pH (extremely acidic soil and strongly acidic soil) indicated changes in soil properties in both soils after incorporation of lime. In the seedling response study, reduction in soil Mg and K was observed with lime incorporation.

Two block trials were initiated at Thevervelil Estate, Perunadu, one in immature and the other in mature rubber to study the effect of liming on growth and yield of rubber and soil properties.

Treatments were imposed and pre-treatment soil samples were collected.

3. Soil and water conservation

The experiment on evaluation of biological/vegetative hedges for soil and water conservation in rubber plantation was continued. The establishment of hedges had no significant effect on growth of rubber over a period of eight years compared to plots without hedges. Among the vegetative hedges vetiver and guniea grass perished by the seventh year and the roots were also completely decayed.

4. Intercropping and cropping systems

The field experiment on development of a multispecies rubber-based cropping system for Tamil Nadu region was in progress. The growth of rubber continued to be significantly higher in all intercropped plots except cocoa. In the intercropped area, more than 70 per cent of rubber plants attained tappable girth at the age of seven years as against 62 per cent in the control plots.

Experiment on interplanting of rubber with timber trees viz. teak, wild jack and mahogany was continued. Growth and yield of rubber was not significantly influenced by row spacing and type of timber intercrops. The experiments on intercropping perennial crops with rubber initiated at CES, Chethackal in 2001 was maintained and the growth and yield of rubber continued to be not influenced by cultivating coffee as intercrop.

Block trials initiated to study the effect of retaining pineapple after four years on growth and yield of rubber and soil chemical properties at Paliyar estate was continued. Significant improvement in soil organic carbon status was noticed in the block where pineapple was retained than in the block where pineapple was removed. Another block trial was initiated in Malankara estate with the same objective.

5. Ground cover management

The field experiment to study the effect of legume covers and natural flora on growth of rubber, soil physico-chemical and biological properties, biomass and nutrient turnover, laid out during 2012 at CES, Chethackal was continued. Growth of rubber plants was affected significantly by allowing natural flora with life-saving weeding. Growth of rubber plants in plots where *Pueraria* was established was significantly superior to all others. The dry matter production was the highest in *Mucuna* established plot followed by natural cover with one m² weeding.

Conducted an incubation study with various intercrop residues/litter in rubber plantations viz. rubber leaves, weeds, cover crops (*Pueraria* and *Mucuna*), pineapple, banana, cocoa, coffee, nutmeg, teak and anjili @ 20 kg ha⁻¹ in soils with varying initial pH (<4.5, 5.0 and >6.0). Ninety days after incubation, it was found that addition of these intercrop residues/litters increased pH of rubber soil. Significantly higher K availability was noticed in the soil when incubated with residues of natural weeds.

An observational trial to find out the feasibility of establishing *Calapogonium caeruleum* under partial shade initiated at Kaliyar estate was continued. It was observed that *C. caeruleum* established well under partial shade.

A pot culture study was initiated with different flora viz. grass, soft weeds and *Pueraria* to study the differential effect of flora on soil nutrient dynamics. A field trial was also initiated with *Mucuna*, grasses, soft weeds and natural flora (control) with this objective. Pre-treatment soil samples were collected and selective weeding is being carried out for maintaining the selected weed population.

The feeler experiment on efficacy of latex coated mulch materials showed that low cost

mulch materials such as latex coated newspaper and gunny are effective in preventing weed growth and conserving soil moisture. Cost of mulch material for one m² area was Rs. 20/- for newspaper and Rs. 55/- for gunny (both coated with 400 ml latex each), and Rs. 25/- for biodegradable plastic.

6. Planting techniques

The field experiment initiated to study the impact of mechanized land preparation on soil erosion and physico-chemical properties of rubber growing soils was continued. Significant difference in rate of soil erosion and growth of rubber was not observed in plots with different land preparation operations. The experiment to evaluate different planting designs was in progress at Cheruvally estate. In the modified planting designs, the canopies of rubber trees continued to be asymmetrical in growth pattern. Girth of plants in the twin planting system was comparable with control. In the paired row planting system, an area with width around two meter is available between two paired rows without significant shading for cultivation of intercrops even eight years after planting. Yield recording was commenced in the experiment area.

7. Development of agromanagement technique for reducing the gestation period

The field experiment initiated at CES, Chethackal during 2008 to evolve an agronomic package to reduce the immaturity period of rubber 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. Preliminary yield data indicated the superiority of direct-seeded plants.

The field experiment investigating the comparative field performance of one-whorl, two-whorl and three-whorl polybag and root trainer rubber plants initiated at CES, Cherthackal during 2008 was continued. Growth of three-whorl polybag plants was significantly superior to all other types of planting material. Yield recording was initiated in the experimental area. Preliminary yield data did not indicate any significant difference among different types of planting material.

8. Rubber growing soils

The experiment to assess the contribution of root respiration towards soil respiration was continued. In the second year also, higher soil respiration rate was recorded in rubber-banana system compared to mature rubber and rubber-pineapple systems.

9. Stress management

In the drought mitigation studies, accumulation of K in drought tolerant rubber genotypes was studied. Higher K content was observed in the root zone and leaves of RRII 430

(a relatively drought tolerant clone) than RRII 105 (a relatively drought susceptible clone).

10. Environmental aspects of rubber cultivation

In the study on assessment of water quality in watersheds dominated by rubber, tea and cardamom plantations, water sample collection (surface and ground) for the second year was completed and analysis for different physico-chemical parameters is in progress.

11. Level of adoption of agromanagement practices

Data from the survey conducted in rubber growing regions of South India were digitized and information on agromanagement practices in rubber plantations were retrieved. Majority of rubber growers practiced integrated nutrient management, and preferred mixtures and complex fertilizers compared to straight fertilizers. For mature rubber, about 25 to 65 per cent farmers applied fertilizers in small pits which is not a recommended practice and needs intervention.

FERTILIZER ADVISORY GROUP

The group operates through the central soil and leaf testing laboratory at RRII and the seven regional laboratories in Kerala and offers recommendations on fertilizer application to large estates as well as small growers on the basis of analyses of soil and leaf samples. Advices on fertilizer use were also provided during the visit of the growers to the laboratory or as clarifications on telephonic enquiries or email queries. The major activity of the regional laboratories was dry rubber content estimation of the latex samples.

- 70,965 latex samples were tested for dry rubber content.
- Offered site-specific fertilizer recommendations to individual fields from large estates on the basis of analyses of 495 leaf samples and 1460 soil samples.
- Six leaf samples and 314 soil samples from small holdings were analyzed and offered site-specific fertilizer recommendations.
- Advices to small holders were provided through telephone and during the visit of the farmers to RRII.

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

Sample details	Number	Revenue (Rs.)
Soil	1774	3,63,086.00
Leaf	501	1,76,574.00
DRC of latex samples	70965	41,74,690.00
Total revenue		47,14,350.00

CLIMATE CHANGE AND ECOSYSTEM STUDIES DIVISION

One of the major thrust areas of research in the Division is development of information system on rubber cultivation using satellite-based remote sensing (RS) to estimate area under rubber cultivation and identify suitable areas where rubber cultivation can be extended in the country. Agromet data base in the traditional and non-traditional rubber growing regions is regularly updated to assess climate change. Studies were undertaken on vegetation dynamics of rubber plantation ecosystems, impact of land use on ground water recharge and the influence of El-Nino/La-Nina events on regional climate of the rubber growing areas. Studies were completed to identify climate suitability for rubber cultivation in the left wing extremism affected (LWE) areas.

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

Mapping and estimation of natural rubber plantations in Andaman and Nicobar Islands was completed. Satellite-derived NR holdings (age three years and above) in Andaman and Nicobar island for the year 2015 was 910 ha. Natural rubber holdings were distributed in South Andaman, Middle Andaman, North Andaman, Katchal islands and Great Nicobar islands.

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

Mapping and estimation of extent of rubber cultivation in Meghalaya, Nagaland, Manipur and Mizoram states was accomplished as of the year 2015. Landsat satellite images (of spatial resolution 30m) were used for identification and estimation of spatial extent of NR cultivation.

2.1. Meghalaya

Satellite-derived area under rubber cultivation in Meghalaya (age three years and above) was around 7950 ha. Area under rubber was the highest in West Garo Hills (2791 ha) and North Garo Hills districts (2714 ha). Considerable extent of NR plantations was present in the districts of Ri-Bhoi, South Garo Hills, East Garo Hills and South West Garo Hills also.

2.2. Nagaland

Satellite-derived extent of rubber plantations (age three years and above) in Nagaland was around 10,730 ha. Area under NR was the highest in Wokha district (6773 ha) followed by Mokokchung (2435 ha), Kohima (790 ha) and

Dimapur (614 ha) districts. Extent of rubber cultivation was found less in Tuensang, Mon and Zunheboto districts.

2.3. Manipur

Remote sensing based acreage of NR in Manipur (age three years and above) was 1062 ha. NR cultivation was the highest in Imphal East district (1042 ha). Very few patches of NR holding were present in Churachandpur and Tamenglong districts. NR is cultivated mainly in Jiribam, Jarolpokpi, Mullargao, Kashimpur, Uchathol, Bakhal and Aglapur areas of Imphal East district.

2.4. Mizoram

Rubber plantations were sparsely distributed in different districts of Mizoram such as Mamit, Kolasib, Aizawl, Lunglei and Serchhip. Satellite-derived extent of rubber cultivation in the state was 874 ha (age three years and above). Largest area was planted in Mamit district (437 ha) followed by Aizawl (198 ha), Kolasib (115 ha) and Lunglei (115 ha). Actual area under rubber cultivation in the State is likely to be more than this estimate because of coarse resolution of the satellite data (30m).

2.5. Identification of NR areas vulnerable to flood in Assam

Assam in general and the Brahmaputra valley in particular is vulnerable to flood and it affects vegetation, food crops and life of people

and livestock almost every year. To assess the risk of flooding in rubber cultivated areas of Assam geospatial analysis was done. Using DEM, based on the elevation classes of less than 50m, 50-100m, 100-500m and above 500m MSL, area under rubber plantations was estimated. As of 2009, about 5280 ha of rubber was cultivated below 50m MSL, 4449 ha between 50-100m, 6353 ha between 100-500m and 789 ha of rubber grown above 500m MSL in Assam. A total of about 9729 ha of rubber area was cultivated below 100m MSL. Areas below 50m MSL are vulnerable to flooding in Brahmaputra valley.

2.6. Terrain characterization according to NR distribution in Meghalaya and Nagaland

About 34 per cent of NR holdings in Meghalaya were distributed below 100m elevation and 54 per cent in 100-300m and remaining 12 per cent of NR was distributed >300m elevation (above MSL). In Nagaland there was no rubber plantation found below 100m elevation. About 47 per cent of rubber cultivation in the state was distributed between 100 to 300m MSL and 53 per cent of rubber area was identified above 300m MSL. Results may be useful to planners for selection of optimum set of terrain classes for rubber cultivation/expansion in Meghalaya and Nagaland. NR holding distribution according to elevation in Meghalaya and Nagaland was given in Table CCES. 1.

Table CCES. 1. Rubber plantation distribution according to elevation in Meghalaya and Nagaland

Elevation (above MSL in m)	NR area (ha) in Meghalaya	NR area (ha) in Nagaland
<100	2710 (34)	-
100-300	4255 (54)	5050 (47)
>300	986 (12)	5680 (33)

2.7. Calculation of potential net savings by supposed skipping of chemical fertilizers in rubber plantations based on soil organic carbon status and soil depth

The extent of mature rubber area having high organic carbon status and soil depth above one meter in Kerala and Kanyakumari district of Tamil Nadu was estimated using geo-spatial overlay analysis. Mature NR holdings with high organic matter content and soils deeper than one meter are not adversely affected if chemical fertilizers are not applied for a few years as per reported literature. Extent of mature NR area qualifying for fertilizer skipping was estimated from RubSIS data base. The approximate net savings by skipping application of chemical fertilizers in mature rubber plantations in different districts (including the cost of chemical fertilizers and labour charge) were calculated. A total of 1,61,911 ha of mature rubber area in Kerala and Tamil Nadu was identified suitable for skipping chemical fertilizers resulting in a potential net savings of about Rs. 87 crores year⁻¹.

3. Identification of agroclimatically suitable areas for natural rubber cultivation in the Left Wing Extremism (LWE) affected States

The study was undertaken to assess the agroclimatic suitability of ten Left Wing Extremism (LWE) affected states of India for cultivating NR. Monthly mean climatic parameters affecting the growth and yield of rubber viz. maximum temperature (T_{max}), amount of rainfall, number of rainy days, potential evapotranspiration, hot degree days, vapour pressure deficit and aridity index (A_p) were chosen to assess the suitability of NR cultivation initially for the districts of Odisha. Area-Under-Curve (AUC) from constructed graphical plots was utilized for the analysis. Parameter wise

AUCs for all districts in Odisha were compared separately with those of Kottayam and Kanyakumari districts (traditional region), considering optimum climatic conditions for rubber growth and yield. Individual AUC variation of a district from the reference curves of Kottayam and Kanyakumari (in percentage) was compared. Considering a tolerable variability limit of up to 20 per cent AUC as a thumb rule for determining suitability, no districts in Odisha were found suitable from the analysis. Selecting the A_p as a single parameter, the analysis was further carried out for all other LWE states in India. The study revealed that only Ratnagiri district in Maharashtra was at par with Kanyakumari and four districts in West Bengal up to the 20 per cent variability limit. A_p based AUC differences with Kottayam for corresponding two long term periods (1901-1955 and 1956-2002) were tested to find out whether there was any significant variation due to climate change. Independent T-tests showed no change between the two periods.

Another attempt was made to analyses of suitability of LWE districts based on the Climate Tolerance Limits (CTL) or the tolerance limits of important climatic parameters beyond which detrimental effects are noticed in terms of growth and yield of rubber trees. Five important climatic parameters were considered with normal CTL, d"10 and d"20 per cent variability levels from the normal CTL. Results showed that a total of 10 districts could be selected from the LWE districts within the normal CTL. By relaxing the CTL favourability by 10 per cent from the normal, a total of 26 districts could be identified. Further relaxing it to 20 per cent from the normal, a total of 76 districts in 8 states emerged as suitable under this category. Maximum number of districts below the 10 and 20 per cent CTL levels were found in Odisha and West Bengal.

Identification of suitable districts using the above CTL approachs should be taken with adequate caution since it is not known how stressful it would be to grow NR commercially at a location with 10 per cent variability from the traditional areas. Moreover, the east coast states, particularly Odisha are prone to frequent cyclonic storms almost every year. Results from this study could be used as a general indication and further studies would be required for locating with greater confidence, areas conducive for NR cultivation in the LWE affected states.

4. Climate resource characterisation of rubber growing areas

4.1. High temperature can be a constraint during the SW monsoon in NE region of India

Gridded data on T_{max} , T_{min} and T_{mean} was documented to prove the already indicated result of the prevalence of high temperature during June to September in NE compared to traditional region during SW monsoon.

4.2. "Ockhi" cyclone – Significant impact on natural rubber cultivation

The event time line, reports, forecasts and inferences related to the Ockhi cyclone was documented. It was observed that first warning of depression maturing to cyclonic storm was issued by the web site well ahead of 30th morning, hours before the causality occurred. It was noted that wind speed which caused the uprooting of the natural rubber in vast areas of Kanyakumari district in the early morning hours of 30.11.2017 were in the range of 74-100 km hr⁻¹ with a gusting speed of 93 km hr⁻¹ according to the event time line. The reason for the causality must be the rapid intensification of wind speed from 65 km hr⁻¹ to 111 km hr⁻¹ within a time span of 24 hours. Annual average track forecast skill of IMD before 12h was 60 per cent whereas before

24 hit was 96 per cent which might be a contributing reason for the increased causality.

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

Characterized the El nino events from 1950-51 to 2015-16 in terms of initiation, duration, maturity and intensity. Association of El nino indicators to the seasonal rainfall of Kerala state was found out using correlation matrix. Change point analysis revealed that annual frequency (1891-2017) of cyclonic disturbances, cyclones and severe cyclons which has a bearing to the seasonal rainfall exhibited significant changes overtime. Frequency of occurrence of cyclonic disturbances exhibited two significant changes i.e. one in 1922 where frequency increased from 10-15 and the other in 1983, where frequency decreased from 15-9. Based on Oceanic Nino Index (ONI) as indicator it was difficult to explain the devastating effect of El nino. 1991-92 was the severely affected El nino year as per the agricultural point of view though ONI was high for 1997-98 El nino.

6. Vegetation dynamics in rubber plantation ecosystems

An initial survey of existing vegetation was conducted and more than 50 species of plants including trees, shrubs, herbs, climbers and ferns were identified from the location. *Ixora johnsonii*, a Southern Western Ghats endemic and critically endangered species is reported from the experimental plots. High frequency of invasive species such as *Clidemia hirta* and *Alteranthera brasiliiana* in rubber plantations pose threat and may have influence on the community structure of the natural flora of rubber growing regions.

7. Impacts of land use on ground water recharge and stream flow pattern of rubber dominant watersheds

There is a disputable argument that water resources in the traditional rubber cultivating regions are depleting in a rapid pace, resulting in water scarcity and setting of drought during summer. Degradation of the water resources of

traditional rubber regions of Kerala are evident by lower flow rate or total drying up and salt water intrusion during summer seasons. Ground water level data from Kottayam and Pathanamthitta districts collected from Central Ground Water Board (2007 to 2017) showed that there is a decrease in ground water level in many regions of the districts, including rubber growing regions.

BOTANY DIVISION

Bridging the escalating gap between demand and production of natural rubber is expected to drive greater expansion of plantations in to the non-traditional regions of the country. Since this would require movement of clones to newer environs, there was urgent need for identification of locally adaptive region-specific genotypes. Envisioning of this vital necessity led to a significant breakthrough in region-specific clone recommendation based on long years data from the field. Clones imported under international clone exchange during 2014-17 were screened for tolerance to biotic and abiotic stress parameters. Conventional genetic improvement including hybridization, polycross breeding and ortet selection were continued with an aim of developing clones with high rubber yield and important secondary traits. Ninety superior pipeline clones were evaluated in Central Large Scale Trials and satellite on-farm trials spread over 44 locations in traditional and non-traditional regions for selecting superior clones through participatory plant breeding approach. Pipeline clones were also evaluated for their response to Ethephon stimulation.

1. Region-specific clone recommendation

The formulation of region specific clone recommendations based on agro-climatic zoning was a long felt need of the rubber plantation sector, so as to enable maximisation of rubber yield from each region and thereby improving rubber production in the country. Based on results of 29 field evaluations of rubber clones in the traditional region as well as studies on disease reaction, genotype x environment reaction studies and yield from several large scale and on farm evaluations of these clones in Tripura, Assam and Northern part of West Bengal in North East India, region specificity in performance of the clones was determined. A new region specific advisory of clones suited to each of the nine agro climatically distinct zones in the traditional and non-traditional regions of rubber cultivation in India was formulated and approved in the reporting year for communicating to the rubber plantation sector (Table Bot.1).

Table Bot. 1. Region specific clone advisory for different rubber growing regions of India

Region	States/ Districts	Clones recommended * @ #
North East India	Tripura, Assam, Northern West Bengal, Meghalaya	RRII 208, RRII 429, RRII 600
East and West Central India	Maharashtra, Odisha	RRII 429, RRII 208, RRII 430, RRII 600
South-West Karnataka and Goa	Goa and Dakshin Kannada	RRII 430, RRII 414, GT 1, RRII 203
North Kerala	Kozhikode, Malappuram, Kannur, Kasargode	RRII 430, RRII 417, RRII 105
North Central Kerala	Trichur, Palghat	RRII 417, RRII 429, RRII 430
Central Kerala	Ernakulam, Kottayam, Pathanamthitta, Alleppey	RRII 430, RRII 414, RRII 417
South Kerala	Kollam, Trivandrum	RRII 422, RRII 430, RRII 417
Tropical high altitude regions	Wynad, Idukki, Kulathupuzha, Vithura	RRII 422, RRII 429, RRII 417
South Tamil Nadu	Kanyakumari	RRII 430, RRII 105, RRII 429

*RRII 429 has proved to be high yielding and can be considered for planting in the traditional tract subject to effective management of pink disease. Clone RRII 430 is not suitable for high altitude regions and NE India where high severity of *Oidium* infection is experienced.

@Recommended plant protection measures are advised in all rubber growing regions.

The system of tapping recommended for the above high yielding clones is 1/2Sd3. Clone RRII 105 can also be tapped at lower frequencies as recommended by the Rubber Board.

2. Evolving high yielding clones for the traditional area

2.1. Hybridization and clonal selection

While newer hybridization programs were taken up during the year, hybrids evolved from earlier HPs were evaluated over the long term in 15 small scale trials.

2.1.1. Small Scale Trials (SST)

Selections of hybrid clones from three SSTs (1995 A, B and C) were evaluated for their response to stimulation. In the 1995 A trial comprising of 34 experimental clones, all the high yielding selections showed good response with

increase in yield ranging from 46 (89/95) to 90 per cent (89/124). Selection 89/27 was the top yielder under un-stimulated ($78 \text{ g t}^{-1} \text{ t}^{-1}$) and stimulated conditions although response to stimulation was comparatively less (53%). Clone 89/309, which was the second best yielder under un-stimulated condition ($64 \text{ g t}^{-1} \text{ t}^{-1}$) showed good response to stimulation (72%) while check clone RRII 105 showed 81 per cent response to stimulation.

In the 1995 B trial consisting of 26 WxA hybrids, selections showed significant variation in response to stimulation. Among selections, the

highest yielding hybrid 90/10 exhibited maximum response (82%) compared to check clone RR11 105 (85%). The top selections also maintained high yield performance in comparison with check clone.

In SST 1995 C trial which is under 14th year of tapping, 17 W x A hybrids were evaluated for their response to stimulation. Among the selected high yielders, 90/109 (148 g t⁻¹ t⁻¹), 90/129 (134 g t⁻¹ t⁻¹) and 90/274 (115 g t⁻¹ t⁻¹) were toppers on stimulation. Based on overall yield performance over 13 years, hybrids viz. 90/109, 90/55 and 90/274 showed high yield performance.

In the 1999 A trial, hybrid progeny 95/131 (55 g t⁻¹ t⁻¹) followed by 95/7 (54 g t⁻¹ t⁻¹), 95/95 (52 g t⁻¹ t⁻¹) and 95/242 (51 g t⁻¹ t⁻¹) showed better yield performance on the basis of mean yield of 17 hybrid clones over 10 years of tapping. Based on response to stimulation, hybrids 95/131 and 95/242 showed high yield performance. In SST 1999 B trial, there was no significant variation for the trait among the hybrids in the 12th year of tapping. Top selection 95/306 maintained superior growth and yield trend (yield - 77 g t⁻¹ t⁻¹; girth - 96 cm) compared to check clone RR11 105 (yield - 48 g t⁻¹ t⁻¹; girth - 75 cm).

In another SST (1999) comprised of progenies from 1994 hybridization programme, there are five superior selections viz. 94/87 (RR11 105 x RR11 703), 92 (RR11 105 x Mil 3/2), 296 (RR11 105 x RR11 118) and 567 (RR11 600 x RR11 203) identified based on superiority in growth and yield. A study was initiated for assessing the yield response of above selections under stimulation conditions. In the 2001 A trial, top yielding clones viz. 95/413, 95/425, 95/121,

95/304 and 95/410 maintained their superiority in terms of growth and yield performance with dry rubber yield ranging from 50 to 65 g t⁻¹ t⁻¹ as compared to check clone RR11 105. Among the 36 clones under field testing in 2003 trial, two selections viz. 96/417 and 96/459 maintained high yield performance during 14th year of evaluation. Among 21 hybrid progenies under clonal nursery evaluation (2008), six selections (02/688, 02/638, 02/690, 02/844, 02/514 and 02/335) registered superior growth and yield performance compared to check clone RR11 414. The above selections have been identified for evaluation under next phase of Farmer Participatory Clone Evaluations.

2.1.2. New generation hybrids

With an aim to develop high-yielding clones through transgressive introgression, hybridizations were carried out during 2011-14 using high-yielding Wickham clones (RR11 105, RR11 414, RR11 429 and RR11 430) as male parents and superior Wickham / Amazonian hybrids (95/10, 95/34 and 95/274) as female parents. Presently, hybrid seedlings (353 nos.) from 14703 hand pollinations and more than a thousand half-sibs of above parents are being evaluated in seedling nurseries. Based on mean test tap yield of two years, 22 per cent of hybrids and 23 per cent of half-sibs were selected for next stage of evaluation under clonal nursery trials. Details are given in Table Bot. 2. Hybridisation was continued in 2017 in the breeding orchard at RR11, Kottayam, using selected parental combinations. Hybrid progenies (100) from above hybridization programme as well as half-sibs (620) were planted in seedling nursery for further evaluation.

Table Bot. 2. Details of selections made from hybrids and half-sibs

	Year			Total
	2012	2013	2014	
Total no. of full sibs	92	139	54	285
No. of full sibs selected	14	30	19	63
Per cent recovery	15	22	35	22
Total no. of half sibs	227	240	306	773
No. of half sibs selected	69	59	50	178
Per cent recovery	31	25	16	23

2.1.3. Phenological studies

In order to document characters of different clones with regard to flowering attributes, observations were made in thirty clones in a breeding orchard at RRII, Kottayam, during normal flowering season within a window period from February to March. Clones with synchrony in flowering were identified for potential use as parents in various hybridization programmes. Observations regarding wintering pattern, off-seasonal flowering and flower initiation, maturation of male and female flowers and duration of fruit maturity were recorded for preparing a database that could be used in the future breeding programmes.

2.2. Ortet selection

Ortet selection was restricted to clonal nursery evaluation of selected ortets from the Andamans and large scale evaluation of ortet clones selected from large estates and small holdings.

2.2.1. Clonal Nursery Evaluation

In clonal nursery trial (2012) of Andaman ortets at Chethackal, ortets selected from the oldest surviving seedling populations (source GG1 and GG2 seedlings of PBIG, Malaysia) located at south Andamans, were assessed in the 4th year of planting. Yield of ortets ranged from 21 (And-

Or 103/8) to 111 g t⁻¹ t⁻¹⁵ (And-Or 104/10) compared to check clone RRII 105 (75 g t⁻¹ t⁻¹⁰) and RRII 430 (150 g t⁻¹ t⁻¹⁰). Girth of superior ortet 104/10 was 22 cm compared to 18 cm and 23 cm for RRII 105 and RRII 430, respectively.

2.2.2. Small Scale Trial

In the evaluation trial of ortets from small holdings (1995), ortets were assessed for growth and timber yield in the 22nd year of planting. Among the ortets, O 81 showed highest vigour in terms of both girth and clear bole volume (CBV). Selections of ortets with high yield viz. O72 and O 73 also exhibited high vigour and timber yield which is comparable to the vigorous check clone RRII 203 (Table Bot. 3).

Table Bot. 3. Girth and bole volume of ortets

Clone	Girth*	CBV*
OK 49	95.833 ^{abcd}	0.257 ^{ab}
O 77	73.943 ^{ef}	0.147 ^{cd}
O 74	92.143 ^{abcde}	0.197 ^{abcde}
O 73	97.807 ^{abc}	0.26 ^{ab}
O 75	83.427 ^{bcdef}	0.187 ^{abcde}
O 76	77.157 ^{cdef}	0.147 ^{cd}
O 21	88.693 ^{abcdef}	0.19 ^{abcde}
O 72	99.167 ^{ab}	0.247 ^{abc}

O 36	92.193 ^{abcde}	0.207 ^{abcd}
O 81	105.333 ^a	0.287 ^a
O 79	82.917 ^{bcdef}	0.2 ^{abcd}
O 80	73.06 ^{ef}	0.12 ^d
O 78	83.45 ^{cdef}	0.177 ^{bcd}
OK 66	77.667 ^{cdef}	0.113 ^d
RRII 203	94.86 ^{abcde}	0.257 ^{ab}
RRII 105	76.717 ^{bcf}	0.123 ^d
RRIM600	68.75 ^f	0.11 ^d

* Values followed by same letters are not significantly different at 95 per cent confidence level by DMRT

In the ortet evaluation programme at HBSS, Nettana, 16 ortets (Ayr 1, Arl 53, Ert 40, Ces 150, Ert 38, Arl 54, Pkm 16, Pov 1, Kan 46, Pkm 14, Edm 37, Ces 151, Mkm 23, Mkm 22, Tly 57 and Ces 9) were evaluated along with three check clones (GT 1, RRIM 600 and RRII 105). Ortet Ayr 1 followed by GT 1, Arl 53, RRII 105 and RRIM 600 maintained high growth and yield performance. Ortet Arl 53 followed by Ert 40 and Arl 54 continued to exhibit better tolerance to *Phytophthora* ALF disease in terms of high leaf retention as compared to other clones.

3. Evaluation of clones

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

3.1. Large Scale Trial (LST)

In the two LSTs of RRII 400 series clones at CES (LST I and LST II), significant clonal variation in yield under stimulation during the 15th and 16th year was detected. In LST I, RRII 417 (79 g t⁻¹ t⁻¹) outyielded RRII 105 (69 g t⁻¹ t⁻¹) under stimulation. In LST II, PB 330 (91 g t⁻¹ t⁻¹) was found superior to RRII 105. RRII 430 also performed well with >70 g t⁻¹ t⁻¹ in this period.

The study proved the superiority of RRII 430, RRII 417 and RRII 414 in terms of long-term yield also. Yield components (total latex volume); dry rubber content, were evaluated in the renewed bark during summer and peak yielding seasons. Significant variation was observed for the above parameters. Clones RRII 414 and RRII 430 showed highest latex volume as well as dry rubber yield in both summer as well as peak yielding seasons. While clones responded differently to summer stress, there was uniform reduction in latex volume during stress period. The drop in total latex volume during stress period ranged from 39.5 to 64 per cent in LST I and 36.6 to 60.9 per cent in LST II. Highest drop in summer latex volume was recorded in clone RRII 430. When yield performance in virgin and renewed panels was compared, the total volume of latex was less in renewed panel compared to virgin panel in both the LSTs with few exceptions.

In the 1994 trial, where 12 indigenous and exotic clones are under evaluation, average yield over 13 years of tapping showed that clones 86/44 (73 g t⁻¹ t⁻¹), RRIM 722 (70 g t⁻¹ t⁻¹) and 86/120 (70 g t⁻¹ t⁻¹) had yield performance comparable with that of RRII 105 (71 g t⁻¹ t⁻¹). Clones RRIM 712 (133 g t⁻¹ t⁻¹), 86/120 (119 g t⁻¹ t⁻¹) and RRIM 722 (118 g t⁻¹ t⁻¹) exhibited superior yield under stimulation.

In the 1999 A trial, among the nine clones, clone 4 (77 cm) and 12 (74 cm) showed superior growth compared to check clone RRII 105 (65.5 cm). On the basis of average yield over eight years, clones 12 (45 g t⁻¹ t⁻¹) and 4 (42 g t⁻¹ t⁻¹) showed yield on par with RRII 105 (47 g t⁻¹ t⁻¹). Under stimulation, highest yield was found in RRIM 600 (132 g t⁻¹ t⁻¹) followed by RRII 105 (131 g t⁻¹ t⁻¹) and clone 12 (117 g t⁻¹ t⁻¹).

In the LST of nine ortets from Cheruvally estate, five ortets showed significantly superior

girth compared to the check clone RRII 105 (Table Bot. 4). Mean yield of the ortets in the initial five years of tapping ranged from 15 to 48 g t⁻¹ (RRII 105, 41 g t⁻¹). Mean yield of five ortets over five years of tapping was comparable with the check clone RRII 105. Although ortet Cyo 72 found superior in terms of yield as well as girth, it was found highly susceptible to

Phytophthora (ALF disease). Ortets Cyo 41 (P 126), Cyo 35 (P 133), Cyo 48 (P 129) are under evaluation in PCE trials in various locations. Ortet Cyo 41 showed significantly superior girth and very low incidence of ALF disease while its yield was comparable with RRII 105. In terms of yield in the 5th year of tapping Cyo 35 closely followed Cyo 43.

Table Bot. 4. Girth (cm), yield (g t⁻¹) and ALF incidence in ortet clones

Clone	Girth	ALF incidence	Mean yield*	Overall mean yield**
Cyo 72	58.2 ^c	Very high	58.9 ^{ab}	47.6 ^a
Cyo 30	63.4 ^b	Low	59.0 ^{ab}	46.0 ^a
Cyo 43	60.1 ^{bc}	Medium	67.3 ^a	45.1 ^{ab}
RRII 105	57.6 ^c	Medium	60.5 ^{ab}	40.9 ^{ab}
Cyo 41	72.4 ^a	Very low	50.8 ^{abc}	40.5 ^{ab}
Cyo 35	64.0 ^b	High	66.7 ^{ab}	40.0 ^{abc}
Cyo 18	63.1 ^b	Medium	62.5 ^{ab}	36.9 ^{bcd}
Cyo 48	74.5 ^a	Low	49.6 ^{bc}	31.2 ^{cd}
Cyo 68	52.2 ^d	Medium	35.8 ^c	28.2 ^d
Cyo 31	60.1 ^{bc}	Medium	16.9 ^d	14.5 ^e

*5th year of tapping; **Mean of initial 5 years; *Values followed by same letters are not significantly different based on DMRT

In the LST of ortets selected from Mundakkayam and Koney estates at CES, check clones RRII 105 (53 g t⁻¹) and RRII 430 (46 g t⁻¹) were found superior in terms of yield performance. Among the ortets, MO 7 (44 g t⁻¹) and KO 9 (42 g t⁻¹) exhibited better yield performance.

3.2. On-farm evaluation

To evaluate the performance of pipeline clones in the South Karnataka region, an on-farm trial was laid out at KFDC, Karnataka, with fourteen high-yielding pipeline clones and seven control clones. The above pipeline clones are being evaluated under PCE Phases I (2008),

II (2010) and III (2012), in various locations. Six pipeline clones showed better growth compared to check clone RRII 105. Clone P 102 had maximum girth followed by RRII 414 and RRII 430.

4. Polycross progeny evaluation

Evaluation of progenies of nine prepotent clones over a period of 24 years for growth, yield and secondary (attributes in two field trials planted in 1993) was completed. Timber volume was also estimated. Wood samples were collected for laboratory studies on quality parameters. The 35 selections made from these progenies were classified as dual purpose latex-timber clones and

timber-latex clones as well as latex clones and timber clones. Fifteen clones exhibited stability in performance over 12 years of tapping. The most promising 12 selections in the pipeline, out yielded the check clone by over 20 per cent. Response to stimulation was studied in the 13th to 15th years of tapping. The selections in the pipeline are now under participatory evaluation trials in various locations.

Performance of polyclonal seedlings (Nettana seed garden origin) and check clone

RRII 105 was assessed in 5th year of tapping. Mean yield of polyclonal seedlings was 59.6 g t⁻¹ t⁻¹ compared to check clone, RRII 105 (42 g t⁻¹ t⁻¹). Six polyclonal seedlings had more than 120 g t⁻¹ t⁻¹ and 24 had yielded more than 90 g t⁻¹ t⁻¹ with potential for further selection. Polyclonal seedlings were also better in terms of girth. Girth of polyclonal seedlings (Fig. Bot. 1) ranged from 45-112 cm (mean girth, 73.6 cm) while RRII 105 had 58.5 cm.

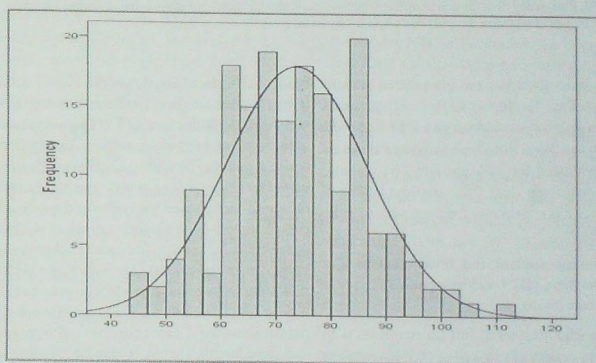


Fig Bot. 1. Distribution pattern of polycross progenies of Nettana origin based on girth at 12 year planting (n=172) (x-axis, girth in cm)

Under the experiment on estimation of out-crossing rate in polyclonal seed garden of rubber at Kanyakumari, second set of progenies were evaluated for growth and yield performance at CES, Chethackal. Test-tap yield of progenies ranged from three to 258 g t⁻¹ t⁻¹ in the third year after planting (Fig. Bot.2). Twenty four progenies had more than 90 g t⁻¹ t⁻¹, nine progenies

with more than 150 g t⁻¹ t⁻¹ and three progenies were out-standing with more than 200 g t⁻¹ t⁻¹. Overall, forty nine progenies performed well above the population mean of 58.4 g t⁻¹ t⁻¹. There is tremendous scope for selection of considerable number of progenies for advancement to further stages of clonal selection.

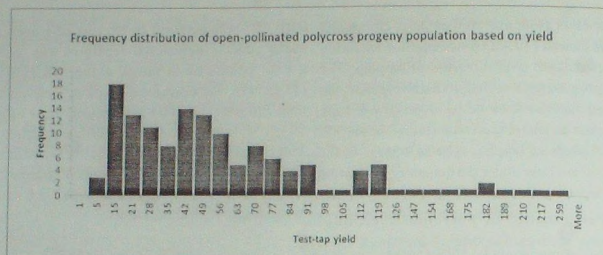


Fig Bot.2. Frequency distribution of open-pollinated polycross progeny population based on test-tap yield performance (n=312) (x-axis, test-tap yield, g t⁻¹ t¹⁰)

In order to utilize the pre-potency trait of PB clones in developing high yielding clones through half-sib evaluation and selection, 337 half-sib seedlings from high yielding PB clones were evaluated for yield through test tapping. Based on test tap yield, four seedlings exhibited yield over 25g t⁻¹⁰ while 40 seedlings yielded more than 10g t⁻¹⁰.

5. Genetic studies and investigations on genotype (G) x environment (E) interactions

Genetic analysis of full sib progenies with regard to wood traits as well as GxE interactions in popular clones and clones in the pipeline were studied.

5.1. Genetic parameters

A study was carried out to assess genetic variation and heritability of growth and wood traits including specific gravity, fibre and vessel characteristics in *Hevea* and to analyze their interactions, using a full-sib progeny population consisting of 11 families of full-sib progenies and their parent clones. Specific gravity of wood was

found to be under moderate genetic control. Fibre traits viz. fibre length (FL), fibre diameter (FD) and fibre wall thickness (FWT) were under moderate to strong genetic control. Among fibre derived indices, flexibility coefficient showed moderate to very strong genetic control. Runkel ratio and slenderness ratio showed moderate genetic control. Girth showed very strong positive genetic correlation with fibre wall thickness and strong positive correlation with fibre width indicating scope of indirect selection potential for these traits (Table Bot. 6). Wood specific gravity did not show significant correlation with growth and fibre length. Hence, it may be possible to exert independent control on these traits. Due to positive genetic relationships, selection for growth would possibly lead to concomitant improvement in fibre diameter and fibre wall thickness. Since there is negative relationship between growth and fibre length, it may not be possible to simultaneously attain vigorous growth and longer fibres which may be required for various end uses, either for furniture or paper industry. Moderate to very high estimates of heritability for fibre traits, girth and specific gravity indicated

the possibility of realizing genetic gain for these traits in *Hevea*.

Table Bot. 6. Genetic correlation between growth and fibre traits

	FL	FD	FWT
Girth	-0.48	0.456	0.781
FL	1.00	-1.936	-0.25
FD		1.00	0.793

5.2. Genotype x environment interaction studies

Growth and yield of pipeline clones were assessed under cold and drought conditions at Agartala and Dapchari, respectively. Under drought conditions at Dapchari, P 102 (26 cm) and P 26 (22 cm) performed better than RR11 208. Fourteen pipeline clones were found better than RR11 430. In terms of test tap yield, P 20 topped yield along with RR11 430 ($50 \text{ g t}^{-1} \text{ t}^{1/2}$) while eleven clones performed better than RR11 105. At Agartala, under cold condition, pipeline clones P 107, P 102, RR11 429 and P 21 (34 cm) showed maximum girth while 10 clones performed better than RR11 105 with regard to test tap yield, P 21 and RR11 430 ($138 \text{ g t}^{-1} \text{ t}^{1/2}$)

topped with maximum yield. The above observations indicated local adaptive nature of pipeline clones under contrasting environs.

In the GxE interaction studies (1996) with popular clones across five locations, collection of yield data was concluded from three LSTs in Kanyakumari, Agartala and Nagrakata. Among the other two locations, in the LST at RRS, Padiyoor, where commencement of tapping was delayed, yield data collection continued in the renewed bark. Yield of the RR11 400 series clones except RR11 429 and RR11 414, showed a rising yield trend and was found significantly superior than RR11 105 in the 10th year of tapping. Clone RR11 430 showed highest yield ($65 \text{ g t}^{-1} \text{ t}^{1/2}$) followed by RR11 417 and RR11 422 ($64\text{--}60 \text{ g t}^{-1} \text{ t}^{1/2}$) when compared to RR11 105 ($45 \text{ g t}^{-1} \text{ t}^{1/2}$). GEI analysis for long term yield across four diverse locations was attempted using different models.

In the LST at Odisha, where the trial was affected by cyclone, long term data on growth, yield, DRC and structural parameters was summarised (Table Bot. 7). Clones RR11 429, RR11 430 and RR11 422 showed significantly superior yield than the check clone RRIM 600.

Table Bot. 7. Growth, yield and secondary traits of 12 clones in Odisha

Clone	Tappability (%) [*]	Girth at opening [*]	Yield ^{*,**}	DRC [*]	Bark thickness [*]	LVR [*]
PB 217	55.0 ^{bcd}	49.7 ^f	63.19 ^{abc}	34.62 ^{cd}	6.73 ^{bc}	7.26 ^{bcd}
RRIC 100	74.0 ^b	60.5 ^{ab}	62.20 ^{abc}	36.35 ^{abcd}	7.62 ^{ab}	7.72 ^{bcd}
RRII 51	52.0 ^{cd}	50.4 ^f	31.80 ^d	35.57 ^{bcd}	7.64 ^{ab}	6.66 ^d
RRII 105	55.0 ^{bcd}	51.0 ^f	31.70 ^d	37.10 ^{abc}	6.74 ^{bc}	8.64 ^{abc}
RRII 176	51.0 ^d	50.8 ^f	36.30 ^d	37.20 ^{abc}	6.55 ^{bc}	7.80 ^{bcd}
RRII 203	64.0 ^{bcd}	59.1 ^b	57.47 ^{bc}	34.49 ^{cd}	7.91 ^a	6.99 ^{cd}
RRII 414	72.0 ^{bc}	55.4 ^{cd}	55.17 ^c	33.03 ^d	7.42 ^{ab}	7.92 ^{bcd}
RRII 417	73.0 ^b	59.5 ^{ab}	63.08 ^{abc}	39.22 ^a	7.59 ^{ab}	10.00 ^a
RRII 422	70.0 ^{bcd}	57.7 ^{bc}	74.00 ^{ab}	36.40 ^{abcd}	6.98 ^{ab}	7.28 ^{bcd}
RRII 429	52.0 ^{cd}	54.1 ^{de}	78.70 ^a	36.23 ^{abcd}	5.70 ^c	9.11 ^{ab}
RRII 430	89.0 ^a	62.0 ^a	75.00 ^{ab}	38.33 ^{ab}	6.88 ^{ab}	9.90 ^a
RRIM 600	59.0 ^{bcd}	51.8 ^{cd}	60.32 ^{bc}	34.81 ^{cd}	7.10 ^{ab}	7.41 ^{bcd}
SE	3.4	1.3	3.59	1.58	0.78	0.82
CV (%)	18.6	8.1	13.36	5.36	13.57	12.50

*Values followed by a common letter are not significantly different at 95 per cent confidence level by DMRT; **12 year after planting; *mean over 4 years.

The adaptive plasticity of RRII 430 and RRII 422 was reconfirmed from the GEI analysis based on temporal yield variation from the region. Clone RRII 429 was specifically adapted for the Odisha region while RRII 414 showed high GEI (Fig. Bot.3). Based on the findings, seven clones including RRII 417, RRII 203, PB 217 and RRIC 100 were found suitable for further large scale evaluation in Eastern India region.

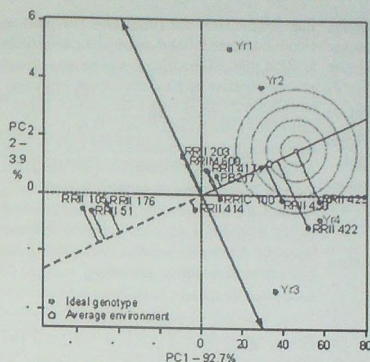


Fig. Bot. 3. GGE biplot of 'clone x year' interaction for dry rubber yield among 12 clones. Clones at the right side the line perpendicular to the average environment axis are highly suitable for growing in the Eastern Indian region. Genotype ranks fall as they fall back from the ideal genotype

6. Participatory evaluation of rubber clones in the pipeline

6.1. Source Bush Nurseries of pipeline clones

At the Central Experiment Station, 267 pipeline clones were maintained in 16 source bush nurseries. Fourteen clones selected from clonal nursery evaluations of ortets and half sib selections were added to the pipeline, thus making a total of 281.

6.2. PCE trials under mature phase

A total of six Central Large Scale Trials at CES and RR11 Farms and 39 satellite On Farm Trials were in progress under five phases of Farmer Participatory Clone Evaluation.

6.2.1. Phase I (2008)

In the Central LSTs which were in the first year of tapping, in terms of girth in LST I, clones P 21, P 61 and P 74 along with the check clone, RR11 414 and RR11 430 were found vigorous. Clones, P 021, P 066 and P 073 gave higher yield than RR11 414, the highest yielding check clone

(with 60 g t⁻¹ t⁻¹). Seven clones were found on par with RR11 414. In LST II, Clones RR11 414, P 44, P 26 and RR11 430 were found vigorous in terms of girth. Three clones had higher DRC than RR11 105. While P 044 was the highest yielding, seven clones were found comparable to RR11 414, the highest yielding among the check clones.

In the OFT at Vithura, among the pipeline clones, highest girth was recorded for P 61 (65 cm) followed by P 21 (63 cm), P 84 (63 cm), P 88 (62 cm) and P 68 (62 cm). Lowest girth was recorded for P 60 (57 cm). In the OFT at Kanjirappally, P 26 recorded 89.4 per cent tappareability. Even though the clones generally showed poor growth due to local conditions, seven pipeline clones showed better growth compared to RR11 430, while none were superior to RR11 414. In the OFT at PCK, Athirapilly estate, trees were assessed for tapping during 3rd year with d/3 system of tapping. Maximum mean yield was recorded in P 067 (77 g t⁻¹ t⁻¹) when compared to check clone RR11 430 (49 g t⁻¹ t⁻¹). Pipeline clone P 074 (36%) and

P084 (35%) recorded maximum DRC. In the OFT at Calicut Estate, during 3rd year of tapping, maximum yield was recorded by RRII 414 (36 g t⁻¹ t⁻¹) followed by P 067 (28 g t⁻¹ t⁻¹).

6.3. PCE trials under immature (Phases II to V)

The Phase two trials laid out in 2010 were gaining tappability while the trials laid out in 2012, 2014 and 2016 phases were at various stages of immaturity.

6.3.1. Phase II (2010)

In the Central LST at CES, pipeline clones P 44, P 70, P 80 and check clone RRII 430 and RRII 414 were found vigorous in the 8th year. In OFT at Pudukkad estate, Thrissur, under Phase II of PCE, pipeline clones P 44 (54 cm) and P 64 (50 cm) were found better in terms of growth compared to RRII 430 (54 cm) and RRII 105 (46 cm). The trial was also opened for tapping during 7th year after planting. Clone P 064 exhibited better yield performance (mean yield, 66.6 g t⁻¹ t⁻¹) compared to check clone RRII 430 (64.7 g t⁻¹ t⁻¹). Three pipeline clones performed better than RRII 105 (43.5 g t⁻¹ t⁻¹). In the OFT at Vaniampara estate (Trichur) laid down under phase II, seven clones registered more than 60 percent tappability in the 7th year. Girth ranged from 45 cm (P 094) to 61 cm (RRII 430). RRII 105 recorded vigorous growth in the region with a mean girth of 52 to 60 cm in all the covariant plots. Pipeline clone P 080 showed comparatively better tolerance to disease incidences.

5.3.2. Phase III (2012)

The OFT at Chemoni Estate was assessed for growth performance over five years after planting. Clone P 110 showed highest girth (36 cm) compared to RRII 417 (37 cm) and RRII 430 (36 cm). Five pipeline clones performed better than RRII 105 (32 cm). At Kumbazha estate (Pathanamthitta), girth of pipeline clones ranged

from 37 (P112) to 47 cm (RRII 430). Two pipeline clones P 110 and P 104 showed girth comparable to RRII 430. In the trial at Cheruvally estate, among pipeline clones, maximum girth was recorded in P 110 (38 cm), followed by P 101 (37 cm) P 104 (36 cm) and P 142 (34 cm), and the minimum girth was recorded for P 158 (26 cm). At Calicut Estate, Kozhikode, two pipeline clones showed better growth compared to RRII 105. Most of the experimental clones were found better than RRII 105 in terms of disease tolerance. Among the pipeline clones, P 102 and P 110 showed maximum growth.

6.3.2. Phase IV (2014)

In the 2014 LST at Kottayam, three years after planting, pipeline clones P 129 and P 177 (23 cm) were found on par with RRII 430 (23 cm) and RRII 417 (24 cm). At Chemoni, P 48 (28 cm) and RRII 414 (26 cm) performed well. At Shomur, P 129 topped with 22 cm followed by RRII 414 in terms of girth. At Bethany estate, Kanyakumari, the 5-year-old plants showed leaning after the incidence of Okhi cyclonic storm. Twisting of trunk, trunk snap and cracking was sporadic. Uprooting was observed only in four plants in one plot. All trees were salvaged by proper staking. A maximum girth of 30 cm was recorded during the 3rd year of planting. In the trial at Manikal estate, Mundakkayam, highest girth was observed in P 129 (29.1 cm) followed by P 133 (26.7 cm), P 168 (24.4 cm), P 71 (21.5 cm), and the lowest girth was recorded in P 177 (17.8 cm). At Thirupampady Estate, Kozhikode, four pipeline clones showed better growth compared to RRII 105. Among the pipeline clones, Cheruvally oriets P 126 and P 133 showed maximum growth.

5.3.2. Phase V (2016)

In the Phase five of PCE trial at State Farming Corporation, Punalur, vacancy

enumeration was done after fire outbreak and 450 buddings done for casualty replacement.

7. Breeding for other specific objectives

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

7.1. Breeding for drought tolerance

In the SST 1998 A, four selections with high un-stimulated yield were subjected to second round of ethephon stimulation. Ortets 272 and 58 showed better response than RRII 105 with 50 per cent improvement in yield. Among the ortets selected from Dapchhari, Dap 111 responded better to stimulation with 40 per cent yield improvement than Dap 236 which showed 27 per cent increase in the second year of stimulation. In SST 1999 A, analysis of average yield over nine years showed maximum yield in 94/50 ($70 \text{ g t}^{-1} \text{ t}^{-1}$) followed by 94/23 and 94/44 ($69 \text{ g t}^{-1} \text{ t}^{-1}$) and 94/101 ($64 \text{ g t}^{-1} \text{ t}^{-1}$). The clones were assessed for response to stimulation. Maximum yield after stimulation was recorded in 94/50 ($108 \text{ g t}^{-1} \text{ t}^{-1}$) followed by 94/44 ($108 \text{ g t}^{-1} \text{ t}^{-1}$).

In an attempt to develop drought tolerant clones for the non-traditional areas, the drought tolerance capacity of the selected progenies, developed through hybridization between high yielding clone (RRII 105; female parent) and a drought tolerant clone (PB 280; male parent) and the reciprocal crossing (PB 280 x RRII 105), were evaluated in a clonal nursery trial in the drought prone area (RRS, Dapchhari). Forty trial clones along with nine control clones were planted in the trial. Sixteen clones in terms of girth and five clones in terms of test tap yield were found superior to the check clones RRII 422 and RRII 417. Selection 69 showed superior growth and yield performance.

Growth and yield performance of selections was monitored in 13th year of tapping among 15

hybrids and check clones in 1998 A SST at CES. Selections, which are also under evaluation in Phase 5 of PCE viz. 93/214 ($69 \text{ g t}^{-1} \text{ t}^{-1}$) and 93/216 ($57 \text{ g t}^{-1} \text{ t}^{-1}$), maintained superior yield compared to RRII 105 ($45 \text{ g t}^{-1} \text{ t}^{-1}$).

6.2. Breeding for disease tolerance

Hybrid progenies developed under breeding for disease resistance programme showed better test tap yield up to $63 \text{ g t}^{-1} \text{ t}^{-10}$ in Fx 516 x RRII 414 and $34 \text{ g t}^{-1} \text{ t}^{-10}$ in RRII 105 x Fx 516. First ever set of hybrids generated through hybridization between RRII 400 series and *Oidium* tolerant germplasm accession was recovered and progenies were planted in seedling nursery for further evaluation. Second set of Fx 516 OPs was assessed for yield and screened for tolerance to *Corynespora*. It was possible to recover very high-yielding half-sib selections from Fx 516 with test-tap yield up to $93 \text{ g t}^{-1} \text{ t}^{-15}$. The Fx 516 half-sibs as well as hybrids were screened for disease resistance in collaboration with Crop Protection Division. Half-sibs and hybrids, tolerant to *Corynespora* were also identified.

8. Anatomical investigations

During hardening process, root trainer plants developed callus tissue at the root apex through air pruning. It is presumed that structural organisation and metabolic status of this starved tissue may have immense potential to be used as explant in tissue culture experiments. Callus regeneration from above tissue is underway. Preliminary study with the leaves of five clones (RRII 105, RRII 414, RRII 417, RRII 422 and RRII 430) revealed the taxonomic significance of petiole anatomy.

9. Studies on propagation

A study aimed at developing drought tolerant root stocks for the non-traditional area was

carried out in a nursery trial at RRS, Dapchhari. Seeds were collected from three drought prone non-traditional rubber growing areas, namely Maharashtra (RRS, Dapchhari), Orissa (RRS, Dhenkanal) and Karnataka (HBSS, Nettana) and from traditional areas, namely Kerala (CES, Chethackal) and Tamil Nadu (HBSS, Paraliar). Assorted seeds as well as seeds from polyclonal seed gardens and drought tolerant clone RRIM 600 and drought susceptible clone RRH 105 were collected from each of above locations. Seeds were also collected from drought tolerant clone RRH 203 growing in Orissa. The seedlings were maintained under rain-fed condition at Dapchhari. Bud-grafting of those seedlings which survived drought condition at Dapchhari was carried out using drought susceptible clone RRH 105 as scion. Bud-grafting of the unselected seedlings at Central Nursery Karikkattoor was also done using RRH 105 as scion. Field planting of plants which survived drought as well as the control plants was carried out at RRS, Dapchhari for further evaluation.

10. International clone exchange

Replenishment of domesticated germplasm with clones from other rubber growing countries via bilateral and multilateral exchanges was successfully accomplished. A total of 44 high yielding and disease resistant clones were imported from 10 countries during 2014-2018.

10.1. Bilateral clone exchange

During 2017, five CMS SALB -resistant clones viz. FDR 5597, FDR 5802, CDC 56, MDX 624 and CD 1174 were imported from Ghana and two CMS SALB-resistant clones viz. FDR 4575 and FDR 5665 were imported from Cambodia. The above exotic clones were successfully recovered through budding and were subsequently planted in source bush nurseries at Central Experimental Station, Chethackal.

10.2. Multilateral clone exchange

Four clones viz. IRCA 41, IRCA 733, IRCA 347 and IRCA 825 were re-imported from Côte d'Ivoire. While there was very low budding success for three of the clones, one clone did not show sprouting. In the SBN at CES, no major disease incidences were observed in IRCA 331, two ARCPC clones from Myanmar and clones imported from other countries, including Vietnam, China and Cambodia. Three clones (USM1, Philippines; RRISL 203 and RRISL 219, Sri Lanka) were also imported. The above clones were bud-grafted and polybag nursery was established at CES, Chethackal. Clones imported from other countries, including those from Vietnam (8), China (5), Cambodia (5), Thailand (5), Indonesia (3) and Sri Lanka (3) are also being maintained in the SBNs at CES, Chethackal.

10.3. Studies on imported clones

The imported clones in the International *Hevea* Clone Museum (2017) were screened for tolerance to *Corynespora* through toxin-bioassay in collaboration with Pathologists. One Vietnam clone was found to be highly tolerant to *Corynespora* toxin. Two clones from Vietnam showed better drought tolerance capacity in terms of physiological parameters.

10.4. International clone museum

An international *Hevea* clone museum, consisting of high-yielding as well as SALB-resistant clones imported from various countries, was established and maintained at RRII Main Campus, Kottayam. As of this year, there are a total of 23 international clones in the museum.

11. Arboreta of *Hevea* clones and forest species

An arboretum of 55 popular *Hevea* clones and another arboretum of 63 assorted forest plant species along with *Hevea*, (planted in 2014) are being maintained at RRII Main Campus.

GERMPLASM DIVISION

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

1. Introduction, conservation and documentation

1.1. Domesticated gene pool (Wickham collection) from secondary centres

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

The five IRCA clones introduced in 1992 are being conserved and evaluated in Germplasm garden 92. Among the clones, IRCA 130 (85.2 cm), and IRCA 111 (84.5 cm) recorded the highest girth. IRCA 130 (56.4 g t⁻¹ t⁻¹), was superior to all other clones for yield. In the Germplasm garden 94 comprising 20 Wickham clones, RRIM 600, RRIC 100 and RRII 23 continued to show superiority over the remaining clones for yield (94-71 g t⁻¹ t⁻¹) compared to 66.9 g t⁻¹ t⁻¹ for the

control. The most vigorous clones were RRII 23, PB 255 and RRIC 100 (109-97 cm), compared to the popular clone RRII 105 (79.3 cm). Along with high yield and girth, RRII 23 also had a high number of latex vessel rows and W x A hybridization was attempted this year using it as a parent.

1.2. 1981 IRRDB wild germplasm

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

1.2.1 Conservation nurseries

3576 accessions have been re-established in compact new conservation-cum-source bush nurseries (SBNs) from 2003 to 2008, while the older nurseries continue to serve as a source of flowers for hybridization programmes. RO 2871, identified as *Oidium* tolerant earlier, is being used in a W x A HP with RRII 105, and the progeny generated last year are being evaluated in the seedling nursery. Profiling of bark anatomical traits in a set of 651 wild accessions in SBN 2005 has identified six wild accessions with a high number of latex vessel rows, which will be included in the breeding pool for yield improvement. The large number of potentially useful accessions identified during the characterization and preliminary evaluation in the juvenile stage for yield, yield contributing traits like latex vessels, disease and drought tolerance traits, are being put into Further Evaluation Trials. All these accessions are also being established in a separate working collection. The next set of 61 wild accessions was planted in the conservation nursery in 2017, bringing the total

to 119 wild accessions in the Germplasm Working Collection so far.

1.2.2 Arboretum

All the available genetic resources are being established in a separate arboretum at Teksragre farm, Tura, Meghalaya, with the primary intention of ensuring an insurance collection, as well as facilitating free cross pollination and genetic mixing between the different gene pools. So far, a total of 442 wild and Wickham accessions have been established here. For the fourth set to be planted, due to winter effect, sprouting success of budded stumps in the polybag nursery at Teksragre farm, Tura was very less. Polybag plants survived from 20 wild accessions and four Wickham clones (out of 82 wild accessions and 10 Wickham clones) are maintained at Tura farm for field planting in 2018. Another arboretum established earlier and comprising of 120 accessions, is being maintained at Central Experiment Station, Chethackal.

1.3. Other *Hevea* species

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

2. Characterization and preliminary evaluation

The last three trials in the Preliminary Evaluation Trial format were planted at RRS, Padiyoor in 2000 (A&B) and 2002. Nine relatively high yielding accessions AC 3131, AC 552, RO 2136, RO 1313, AC 567, AC 1964, AC 341, MT 4351 and RO 210 and vigorous

accessions for timber traits MT 4219, AC 4140, MT 387, AC 647 and RO 2883 are being conserved as male parents for future W x A hybridization programmes at RRS, Padiyoor.

3. Further evaluation and selection

Detailed evaluation of selections from preliminary evaluations are carried out in clonal nurseries (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 two rounds of test tapping. Out of 15 wild accessions evaluated, identified three accessions (AC 2199, MT 1056, AC 2027) with good growth and test tap yield higher than RRII 105.

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.

Growth and yield were evaluated in FET 2003 comprising 22 wild accessions and three control clones. RO 2629 recorded the highest girth followed by MT 2233 and AC 626 compared to the check clone RRII 105, while RO 2629, AC 4149 and AC 716 recorded the highest yield. Girth increment over four years was also high in RO 2629. In the eleventh year after planting, highest bole height was recorded in MT 2233 (3.1m) compared to check clone RRII 105 (2.5 m), while highest bole volume was recorded in RO 2629 (0.10 m³) as compared to check clone RRII 105 (0.05 m³) (Table Germ.1).

Table Germ.1. Variability for yield and growth related characters in wild *Hevea* germplasm

Characters	Wild accessions		Control clones			
	Minimum	Maximum	General mean	RRII 105	RRII 208	RRIM 600
Mean yield (g t ⁻¹ t ⁻¹) over first 2 years	5.7 (AC 605)	29.4 (RO 2629)	13.6	31.9	21.6	27.6
Girth (cm) - 7 th year	26.2 (MT 4529)	47.8 (RO 2629)	34.8	36.2	32.7	34.3
Girth (cm) - 11 th year	42.4 (MT 4529)	73.2 (RO 2629)	53.5	54.8	44.7	50.9
Girth increment (cm year ⁻¹) over 4 years	3.6 (RO 3804)	6.4 (RO 2629)	4.7	4.6	3.0	4.2
Bole height (m)	2.2 (RO 3804)	3.1 (MT 2233)	2.5	2.5	2.2	2.9
Bole volume(m ³)	0.03 (RO 3804, MT 4529, MT1009, RO 287)	0.10 (RO 2629)	0.05	0.05	0.03	0.05

Note: Figures in parenthesis denotes the name of accession

Fifteen clones in FET 2005 comprising 22 wild accessions and three controls showed more than 70 per cent tappareability. Among these, clone averages were highest for MT 4788 with 47.4 g t⁻¹ t⁻¹ followed by AC 2004 with 32.8 g t⁻¹ t⁻¹, while RRII 105, PB 260 and RRIM 600 gave 59.2, 34.9 and 37.5 g t⁻¹ t⁻¹, respectively. Clonal differences for girth were statistically significant, with AC 2004 recording the highest girth (74.4 cm), followed by the control PB 260 (71.3 cm) and MT 43 (70.7 cm). Visual assessment of ALF showed that five wild accessions including two of the high yielding ones, had less than 20 per cent leaf fall.

In FET 2008, RO 2846 (68.7 cm), AC 176 (66.4 cm) and MT 200 (64.8 cm) recorded the highest girth in the tenth year of growth as compared to the check clone RRII 105 (55.1 cm).

Among the 13 accessions evaluated in FET 2010 at CES, Chethackal, there were seven

accessions with girth higher than clone RRII105 and two accessions with girth on par with clones RRII 430 and RRII 414. Another set of 22 selected wild accessions along with three control clones are in the fourth year of growth in FET 2013 at CES. Among the wild accessions, AC 167 (30.3 cm) followed by AC 5280 (26.7 cm) and RO 2784 (24.2 cm) recorded the highest girth as compared to the check clone RRII 105 (21.5 cm). A set of 12 wild accessions and three controls were monitored for growth in the field conditions in a further evaluation trial 2014 at RRS, Dapchhari.

3.3. On-farm trials

Selections from FETs are subjected to multi location evaluation in On-Farm Trials (OFT) 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 eighth year of growth. Girth of the clones was recorded in all the five locations. At Mooply estate, among the four test clones, wild accession AC 166 recorded the highest girth followed by IRCA 130. Among the check clones, RRII 430 and RRII 414 were superior to RRII 105. The trees were opened for tapping. Monthly yield recording will be started from August 2018 onwards. At Malankara estate, among the four test clones, clone IRCA 111 recorded the highest girth followed by IRCA 130. Among the check clones, RRII 414 was superior to RRII 430 and RRII 105. Panel marking was done and the trees will be opened for tapping during May 2018. The highest girth was recorded in the clone AC 166 IRCA 130, followed by IRCA 111 and as compared to the check clone RRII 105 at BC Cheruvally estate, Erumely. At Calicut estate, also similar growth trend was observed for these clones. The fifth OFT at Bethany estate was severely damaged in the cycle of Ockhi.

4. Screening for stress tolerance

4.1. Screening for biotic stress tolerance

In a 'hotspot' trial at Ulickal nursery, Iritty, a set of 41 shortlisted wild *Hevea* accessions along with two control clones were under evaluation for confirmation of field tolerance to *Corynespora* disease.

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

In the clonal nursery of 40 potential half-sibs of nine clones at RRS, Dapchhari, 26 half-sib progenies from eight pre-potent clones were selected on the basis of test tap yield and growth

under drought stress. These selections will be advanced to a large scale trial (LST) at RRS, Dapchhari for developing drought tolerant clones. For multiplication of these selections, a seedling nursery was raised using seeds brought from Kanyakumari. In the clonal nursery at RRS, Padiyoor with 31 potential half-sibs of eight clones, six half-sib progenies from three pre-potent clones were selected on the basis of test tap yield and growth under drought stress. These selections will be advanced to LST.

In the further field evaluation of selected *Hevea* clones at RRS, Dapchhari in collaboration with Botany Division, growth during the summer and peak periods of growth in the 34 selected *Hevea* clones planted in 2007 comprising 23 wild accessions, five HP clones and six check clones viz. RRII 430, RRII 414, RRII 105, RRIM 600, RRII 208 and Tjir 1 was assessed. Out of 34 clones in this trial, four wild accessions showed girth superior to drought tolerant clone RRIM 600. Conducted test tapping in trees with girth 40 cm and above at 60 cm height from collar region to assess their yield potential in peak season and test tapping is being continued in the summer season also in order to get an idea on yield performance of these accessions under drought stress condition before subjecting the trees for opening for regular tapping. Around 200 OP seeds were collected from this field trial and raised a seedling nursery. The growth and yield performance of these seedlings will be studied for selecting potential recombinants of wild and Wickham clones.

135 wild germplasm accessions planted in 2003 at RRS, Dapchhari for field screening were subjected to test tapping in peak season and being continued in current summer season also in order to assess their yield potential after 14 years under

drought stress. Selections will be advanced to large scale trial at drought prone region.

4.2.2. Cold tolerance

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

5. Screening for timber characteristics

5.1. Field screening

Twenty five genotypes were evaluated for annual girth and branching height in a timber evaluation trial at RRS, Padiyoor. MT 941, MT 1032 and AC 650 recorded the highest girth and timber potential as compared to the control clone RR11 118.

6. Utilisation of *Hevea* germplasm

6.1. Hand pollination programmes

At CES, Chethackal, six promising hybrids from the 2009 hand pollination programme involving three wild accessions and six cultivated

Wickham clones, were multiplied for further evaluation. At RRS, Padiyoor, 29 seedling progenies derived from two cross combinations in 2009, along with 25 OP seedlings of RR11 105, are under evaluation in a seedling nursery. Out of 10 hybrid progenies of parental combination RR11 105 x AC 675, four hybrids and among 19 hybrid progenies of parental combination RR11 105 x RO 368, ten hybrids were found to be promising on the basis of test tap yield and were multiplied along with five promising OP seedlings. A bud wood nursery of these selections was raised at RR11 main campus which will be advanced to a clonal nursery evaluation.

At RR11, the seedling nursery of the 2013 W x A HP involving two cross combinations was cut back for budwood generation. Nursery evaluation was completed. Of the 36 seedlings testtapped, 8 and 11 accessions were finally selected for further evaluation in FET and CN, respectively based on test tap yield levels. In HP 2014, growth and yield were monitored in the 25 progenies of the three surviving W x A combinations (Table Germ.2). Six and five accessions were selected for FET and CN evaluation, respectively. Progenies of AC 4833 had the highest proportion of high yielders as well as the highest mean yield.

Table Germ. 2. Performance of HP2014 W x A progeny in the 3rd year of growth

	AC 4833 x RR11 105		RR11 105 x MT 182		RR11 105 x MT 4788	
	Girth (cm)	TTY 2 ($\text{g t}^{-1} \text{t}^{-1}$)	Girth (cm)	TTY 2 ($\text{g t}^{-1} \text{t}^{-1}$)	Girth (cm)	TTY 2 ($\text{g t}^{-1} \text{t}^{-1}$)
Number	8	8	3	3	14	12
Average	19.5	4.0	19.3	0.6	16.8	1.0
Min	13	0.4	15	0.4	8	0.2
Max	27	6.0	27	0.7	22.5	2.0
SD	4.0	2.0	5.4	0.2	4.0	0.6

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

In the 2016 HP, of the 100 WxA hybrids planted in the seedling nursery, 74 survived in the first year of growth. Girth ranged from 3-10 cm of the three WxA combinations, progenies of the cross with the *Oidium* tolerant parent RO 2871 showed the highest vigour and survival percentage.

6.2. Open pollinated progeny evaluation

A total of 482 open pollinated seeds were collected from the further evaluation trials FETs 2003 and 2005, which comprised preliminary selections from the wild germplasm, interspersed with high yielding Wickham control clones and planted for evaluation in a seedling nursery.

6.3. Phenotyping of mapping population for QTL identification

The total number of seedlings planted in the nursery from all the interspecific RRII 105 x F 4542 HP programs (HP 2009, 2010, 2013 and 2014) was 306, of which 255 are now surviving. 86 progenies of HP 2009 were phenotyped for leaf pubescence as the parents show extreme phenotypes. No transgressive segregation was seen. Progeny tended to resemble female parent RRII 105 with scanty pubescence, indicative of maternal inheritance, epistasis or recessive gene

action governing the trait. Leaf samples were collected from HP 2013 and 2014 for phenotyping for disease tolerance for QTL analysis (results reported elsewhere). HP 2013 was cut back as nursery phenotyping was complete. Growth and juvenile yield of the HP 2014 interspecific progenies was monitored (Table Germ. 3). In HP 2013, of the 62 seedlings testtapped 5 and 14 were selected respectively for further evaluation in FET and CN based on their testtap yield levels. In the 74 tappable seedlings of the HP 2014 population, in the second round of testtapping, 10 progenies had yield ranging from 3.0-7.4 g t⁻¹, while 13 progenies had yield ranging from 1.5-3.0 g t⁻¹ in the third year of growth.

Table Germ. 3. Juvenile performance of interspecific hybrids in HP 2014

	Girth (cm)	TTY2 (gr ⁻¹ t ⁻¹)
Number	79	72
Average	19.1	1.4
Minimum	8.5	0.0
Maximum	28.0	7.4
SD	4.6	1.3

7. Other studies

7.1. Studies on alternative sources of natural rubber yielding plants

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

ADVANCED CENTRE FOR MOLECULAR BIOLOGY AND BIOTECHNOLOGY (ACMBB)

The Advanced Centre for Molecular Biology and Biotechnology (ACMBB) set up during the XIth Plan period is a functional grouping of scientists working in the areas of Molecular Biology, Biotechnology, Genome Analysis, Molecular Physiology and Molecular Pathology. This was mainly done to functionally merge different labs working in similar areas for better efficiency and saving of resources. ACMBB together constitutes about 15 per cent of RRII

research. The ACMBB conducts research on various projects which includes studies on the molecular basis of genetic improvement of natural rubber trees and biotechnological interventions for developing Genetically Modified (GM) rubber. Studies at ACMBB would help to speed up crop improvement, aiding in developing new high-yielding, climate resilient and disease tolerant clones faster.

I. BIOTECHNOLOGY DIVISION

The main focus of the research activities in the Biotechnology Division was on developing *Hevea* transgenics integrated with genes harbouring desirable traits. Genetic transformation experiments were fine tuned for producing transgenics with multiple gene integration as well as transgenic plants having enhanced biotic and abiotic stress tolerance and improved latex yield. Development of antibiotic marker free transgenics is now a priority area since this could bypass major biosafety concerns regarding the commercial cultivation of GM plants. Other research programmes carried out in the division are: somatic embryogenesis systems from different elite *Hevea* clones, *in vitro* regeneration of ploidy variant plants and *in vitro* approaches to complement conventional breeding programmes.

1. Development of transgenic plants
- 1.1. Genetic transformation of *Hevea brasiliensis* for stress tolerance

Agrobacterium mediated genetic transformation experiments were carried out with

HbMnSOD gene construct using embryogenic calli derived from anther, ovule and leaf explants of RRII 105. Several antibiotic resistant cell lines that emerged from the infected target tissues were selected after performing GUS assay and the cell lines were proliferated and tried for embryo induction. The transgenic lines obtained last year failed to produce embryos and therefore attempts were also made to develop new transgenic lines integrated with two genes namely *hmgr1* and *MnSOD*. Transformation experiment was done with the *HbSOD* gene construct using *hmgr1* transgenic cell line as the initial explant. The new transgenic cell lines emerging in the selection medium were transferred for proliferation. Genetic transformation work with two high yielding clones RRII 414 and RRII 430 was also initiated for increasing abiotic stress tolerance. *Agrobacterium* mediated genetic transformation was carried out with proliferated friable callus obtained from immature anthers for incorporation of S-6-PDH and SOD genes. Viable tissues obtained after

bacterial infection were selected and cultured on selection medium for the production of transformed cell lines.

1.1.1. Confined field trial of MnSOD transgenic plants at RRS Guwahati

As an initial step towards the confined field trial of the transgenic plants, the MnSOD Transgenic lines L_1 and L_2 were multiplied along with the control and check clones, transported to RRS, Guwahati and maintained in poly bags.

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

1.2.1. Genetic transformation with the *hmgR1* gene

Transgenic plants integrated with *hmgR1* were developed and hardened earlier. DNA was extracted from the leaves of these plants. PCR analysis was carried out to monitor the presence of the integrated *hmgR1* gene in the plants. Positive amplification for the *hmgR1* transgene was observed in twenty out of 36 plants tested and 13 plants were *hpt* positive. Plants attained the girth of 15 to 20 cm were tapped and the rubber yield was recorded under containment facility. The transgenic plants showed a range of latex yield. Some GM plants produced more latex than the control plant indicating possible regulatory role of *hmgR1* gene in the latex biosynthetic pathway.

Transgenic cell lines were produced from the embryogenic callus derived from the integument tissue. Embryogenesis was achieved from four transgenic cell lines. The embryos were separated and cultured for maturation and germination. Twenty five plantlets were developed *in vitro* from four different transgenic cell lines (Fig. Biotech. 1). Plantlets were transferred for hardening. Four more cell lines were made embryogenic and cultured for embryo induction.

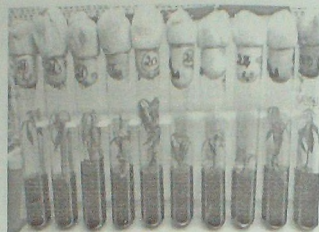


Fig. Biotech. 1. Plantlets developed *in vitro*

1.3. Genetic transformation with osmotin gene

Agrobacterium infections were carried out with clonal as well as zygotic explants for developing new transgenic events integrated with osmotin gene. A few transgenic lines could be developed and were proliferated. Mature embryos were transferred to new media combinations for improving the regeneration percentage as well as hardening. About 10 transgenic plants were regenerated in the new media combination, and transferred for hardening. Out of them, three plants survived the initial phase of hardening and survived for three months. However, they failed to develop further. Experiments are continuing for developing new plants.

For the evaluation of drought tolerance in the already developed GM plants, membrane stability was assessed using the standard protocol. The membrane stability was compared between transgenic and control plants. Transgenic plants showed better membrane stability, compared to control, which is an indication of the positive expression of inserted gene towards stress tolerance. DNA isolation and PCR with gene specific primers were carried out and all the plants tested were PCR positive.

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

The plant regeneration system from leaf explants was utilized for *Agrobacterium* mediated genetic transformation for incorporation of iso-pentenyltransferase (*ipt*) gene. Tissue infection were carried out thrice with *ipt* gene using proliferated friable fresh callus and embryogenic callus obtained from leaf explants of clone RRII 105 and RRII 414 as target tissues. Bacterial overgrowth in infected tissues was prevented by routine subculture in fresh selection medium every month. Several putatively transgenic lines were obtained. Few lines from these were proliferated and somatic embryo induction obtained from two lines incorporated with *ipt* gene.

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

1.5.1. Synthesis of a binary vector (PNS 15) harbouring osmotin gene

For developing marker free transgenics, attempts were initiated for synthesizing a new gene construct (PNS15) containing osmotin gene in PNS14 vector with marker free technology. Plasmid DNA was isolated from *E.coli* cells with osmotin gene using standard protocol. Restriction sites: *MluI* and *NorI* were introduced in the flanking regions of the osmotin gene through PCR amplification and the amplicon was gel purified. PNS 14 plasmid DNA was also isolated for cloning of osmotin gene in the same restriction sites present in the vector.

1.5.2. Development of antibiotic marker-free transgenic *Hevea brasiliensis* plants

One event from the previous transformation experiments, using PNS 15 gene construct harbouring SOD gene was proliferated. DNA was isolated from the proliferated callus and subjected to PCR using SOD specific and marker specific primers. However, no positive

amplification was obtained with the gene specific and marker specific primers. The line was assumed to be a false positive and discarded.

2. Propagation of *Hevea* elite clones

2.1. Somatic embryogenesis from immature anther of RRII 400 series clones

In *Hevea*, somatic embryogenesis is strictly genotype-dependent and thus it is necessary to optimize the system for each clone for further utilization in transformation. Somatic embryogenesis from immature anthers of 400 series clones was initiated. Callus induction from immature anthers of 400 series clones (RRII 414, RRII 417, RRII 422 & RRII 430) was attempted. Experiments on the effect of BA on callus induction were repeated and four concentrations of BA (0.5, 1.0, 2.0, 3.0 mg L⁻¹) was tested. Inclusion of BA showed a positive response on callus induction. At all four combinations tried, 100 per cent callus induction efficiency was obtained, but callus growth was varying. From the experiment, 2.0 mg L⁻¹ BA was identified as the optimum and this combination was used for inducing callus for other clones viz. RRII 417, RRII 422, RRII 430. Proliferated callus was made friable and cultured for embryogenic callus production.

White cotyledonary embryos induced from the earlier experiments for clone RRII 414 were separated and cultured for maturation. Matured embryos were germinated and transferred for full plant development. Well rooted plants were transplanted for hardening into earthen pots containing potting mixture. During hardening, healthy plants survived and plants established in big polybags were growing well.

2.1.1. Hardening and acclimatization of somatic derived plants

Since hardening of *in vitro* plants is still difficult in *Hevea*, somatic plants were developed

with clone RRII 105. Somatic embryo induction was obtained from callus induced in immature anthers. Cotyledonary embryos after embryo maturation were transferred for germination and further for full plant development. A large number of plants were regenerated. For improving hardening, different methods were adopted. Different potting mixtures like, autoclaved sand and soil, sand, soil and cow dung and non-sterile potting mixtures were tried. Different potting vessel like, earthen pots, polybags, small cups were also tried. Experiments on inclusion of PEG, varying agar concentrations were also tested. Hardening of plant under *in vitro* condition was also attempted by slightly lifting the cotton plugs and finally full removal of plugs for two weeks. Good plant recovery as well as healthy plants was regenerated in medium with 3.0 mg L⁻¹ agar. With the inclusion of PEG in germination medium, healthy plants were obtained. *In vitro* hardening of plantlets enhanced survival. Compared with polybags, plants survived better in earthen pots. In all experiments, plant survival was more in the initial phase and after three weeks, leaves gradually got dried up. More experiments are needed in this direction and experiments are being continued.

2.2. Somatic embryogenesis from leaf explants

Somatic embryogenesis from leaf explants of clone RRII 105 and 400 series clones was attempted from cultures initiated during the year. Callus was induced in explants collected from glass house grown bud grafted plants as well as from *in vitro* developed somatic plants. Callus proliferation was attempted in earlier standardized medium by repeated subculture. Silver nitrate/picloram was also supplemented in the medium for proliferation. Silver nitrate (1 mg L⁻¹) and picloram (3 mg L⁻¹) helped in texture improvement and friable callus formation.

In another experiment, the effect of 2-chloroethyl phosphonic acid (CEPA) on embryogenic callus induction was studied. Here, enhanced cytokinin (2ip/BA) along with auxins was added to the callus induction medium. Compact callus obtained was maintained in the same medium for two to three months. Callus proliferation medium was supplemented with different concentrations (0-5 mg L⁻¹) of an ethylene inducer, 2-chloro ethyl phosphonic acid (CEPA). The compact callus when transferred to medium with CEPA (2mg L⁻¹), embryogenic callus induction was obtained after one month, in clones RRII105 and RRII 414. Somatic embryo induction from the proliferated embryogenic callus was obtained in earlier standardized medium. Embryos after initial one week culture in maturation medium germinated after slow desiccation. Desiccated embryos enlarged with simultaneous germination. *In vitro* plant regeneration was obtained in earlier standardized medium.

Experiments were continued with medium modifications and different culture conditions for direct embryo induction. Cultures were initiated with leaf explants from *in vitro* cultures in MS, WPM and ChuN6 medium after auxin shock pretreatment. Globular structures and proembryos were induced in ½ MS and ChuN6 basal medium containing picloram/silver nitrate in presence of phytohormones BA/TDZ. Auxin shock pretreatment triggered swelling and formation of globular structures. Ethylene inhibitors such as silver nitrate and amino ethoxy vinyl glycine (AVG) were tried for proembryo to embryo conversion. In presence of AVG/ silver nitrate (1 mg L⁻¹) size increment of the embryos was observed these are tried for maturation. Experiments are being continued for their further development.

2.2.1 Effect of anti-gibberellins on *in vitro* developed roots

Experiments in inducing adventitious root both *in vitro* and *ex vitro* in cultured shoots were repeated. Cultures were initiated with zygotic embryos, axillary buds, shoot tips of seedlings and clones were cultured. Both *in vitro* and *ex vitro* rooting of seedling/clonal shoots were attempted. Rooted plants and *in vitro* regenerated somatic plants were cultured in medium supplemented with paclobutrazol. In presence of paclobutrazol, the plants exhibited shoot dwarfing and root thickening along with branching.

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

3.1. Gynogenic haploids in *Hevea brasiliensis*

The embryogenic callus and embryos obtained from the embryo sac cells of the unfertilized ovules was sub cultured for embryo induction, maturation and plant regeneration. Fifteen plantlets were developed *in vitro* from the embryo sac callus. The plantlets were transferred to small polybags for hardening. Cytological investigations were done in selected plants which confirmed the haploid nature of the plants.

Embryo sac derived calli of clones RRII 105 and RRII 414, generated in the previous year, were proliferated after which they were transferred to embryo induction media. Embryogenic callus could be induced in clone RRII 414 which later gave rise to several embryos. In RRII 105 embryogenesis was achieved at a low frequency. These embryos were sub cultured to maturation medium. Some of the matured embryos of the clone RRII 414 are in the germination phase.

Embryo sac explants cultured for direct embryo induction did not respond positively,

instead they all dried up. Isolation and culturing of embryo sacs of all the four clones RRII 105, RRII 414, RRII 430 and RRII 422 in the callus induction medium was carried out during the flowering season in the reporting period. New media combinations for direct embryogenesis were also attempted and the results are awaited.

3.2. Development of polyploids

Fresh embryogenic callus emerging from colchicine treated callus of clone RRII 105 were proliferated and transferred to embryo induction medium. Embryogenesis from these cultures is awaited. In an earlier standardisation experiment, calli derived from zygotic explant were subjected to colchicine treatment and were maintained by sub culturing to appropriate media combinations. Embryo induction and maturation followed by embryo germination could be obtained. Many of the germinating embryos have been transferred to the plant regeneration medium. Regenerated plantlets are now in the acclimatisation phase.

4. *In vitro* screening for drought tolerance

An *in vitro* method was attempted for screening of drought tolerant clones. Three clones RRII 430, RRII 414 and RRII 105 were selected for the study and PEG -6000 was used to create *in vitro* water stress at five concentrations (0, 5, 10, 15 and 20%). A liquid PEG supplemented medium was used. Proliferated callus induced from immature anthers of three clones was used. For RRII 414, proliferated callus was cultured in liquid PEG medium for five different incubation periods (0h, 1h, 2h, 3h & 4h). After each time period, callus clumps were cultured on proliferation medium for further callus growth. Each treatment consisted of five replications and the experiment was repeated twice and results are awaited. For the other two clones, RRII 105 and RRII 430, callus proliferation is attempted and the experiments are continued.

5. *In vitro* screening of rubber clones against *Corynespora* pathogen

5.1. Standardization work on *Corynespora* leaf fall disease in *Hevea brasiliensis*

Clones susceptible and tolerant to *Corynespora cassiicola* were identified and four prominent clones were selected and included in each group for further study.

5.2. Measurement of chitinase activity

Chitinase activity in *Hevea* leaves under normal conditions was assayed using the leaves from field grown clones belonging to both susceptible and tolerant groups. In order to study the impact of *Corynespora* infection on chitinase activity in *Hevea*, leaves of the plants belonging to the selected clones mentioned above were exposed to cassiicolin toxin for 12 hrs and the induced chitinase activity was measured. Under normal conditions, clones belonging to each group showed considerable difference in chitinase activity and tolerant clones had more activity than the susceptible ones. Upon exposure to cassiicolin, there was significant increase in chitinase activity in the tolerant clones as compared to the susceptible ones.

5.3. Identification of chitinase gene from *Hevea brasiliensis*

Genomic DNA was isolated from RRII 105 using CTAB method. Chitinase gene specific primers were designed based on the available mRNA sequences with accession numbers AJ010397.1, DQ873889.2 & AJ007701.1

Forward primer F -
5'CTTGCAATAATGGCAAAAG 3'

Reverse primer R -
5'GGGTGCACCGAATAATTCT 3'

PCR amplification of the full-length chitinase gene was carried out using the designed primers. An amplicon of 4 kb size (Fig. Biotech.2) was obtained.



Fig.Biotech.2. PCR amplification of chitinase gene

Lane1 – DNA marker; Lane2- PCR amplified product

In order to confirm the identity of amplicon, it was purified from the agarose gel using QIAEX II gel extraction kit and cloned into pGEM-T Easy vector. After ligation, the products were transformed into competent DH5α *E.coli* cells. Recombinant colonies were selected through blue white screening in X-gal + IPTG medium and were subjected to colony PCR for confirmation of the cloned gene. Two colonies with chitinase gene were confirmed whereas in the other two colonies there was no amplification (Fig. Biotech.3).

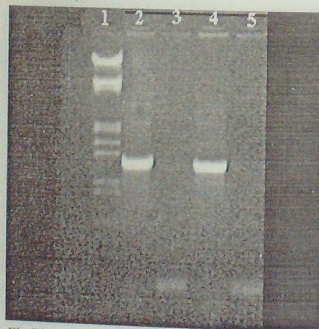


Fig.Biotech.3. Colony PCR of chitinase gene
1 - Marker; 2-5 Recombinant colonies with chitinase gene

Plasmids P1 & P2, harbouring the gene of interest, were isolated from the positive colonies and were sequenced. The sequences obtained were trimmed to remove the vector sequences

and were subjected to BLASTx analyses. Sequence analysis of P1 plasmid revealed 99 per cent homology with *Hevea brasiliensis* chitinase mRNA (DQ873889.2).

II. GENOME ANALYSIS LABORATORY

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

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

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

Forty one SSR marker loci based on NBS-LRR transcripts (R genes) generated from disease transcriptome were assessed for polymorphisms using three cultivated clones: RR11 105, GT1, RRIM 600 and *H. benthamiana* for

marker development. Out of 41 SSR loci, 19 were found polymorphic. Segregation analysis of these SSR markers was performed in a mapping population derived from the hybridization between RR11 105 and *H. benthamiana* for their incorporation in a linkage map.

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

RR11 could successfully establish 40 best clones imported from almost all major rubber growing countries: Indonesia, Cambodia, Thailand, Philippines, Vietnam, Sri Lanka, Myanmar, Ghana and Cote D'Ivoire through international clone exchange program. It is necessary to do the genetic assessment of these clones along with the high yielding clones existing in RR11, especially the RR11 400 series clones and RR11 105 using molecular markers. This will help in understanding the extent of genetic diversity existing among these clones compared to RR11 clones and also to develop clone specific SSR profiles for their identity. So far 16 SSR markers were used in genotyping of these clones. Allelic profiles obtained through different SSR markers are common to the allelic profiles of RR11 collections reflecting that all Southeast Asian rubber clones had their common origin from the 'Wickham collections'. The four rubber clones received from Cambodia showed more variations.

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

1.3.1. Identification of SNPs in latex biosynthesis genes from *H. benthamiana*

Identification of species level nucleotide variation in latex biosynthesis genes has been attempted. Entire genomic region of HMGR and GGDPS from *H. benthamiana* was amplified to identify more polymorphic SNPs between *H. benthamiana* and RRII 105 to be used in linkage map construction. The complete genomic region of REF gene from *H. benthamiana* was amplified using three primer combinations. Repeated amplification using the primer combination REF2 - F & R gave three distinct bands in *H. benthamiana* contrary to a single band observed in the other clones belonging to *H. brasiliensis*. The additional bands amplified may be either isoforms or alleles of REF gene, which could be established through sequencing of the cloned fragments.

1.3.2. CPT gene isoforms associated with rubber biosynthesis

Extensive sequence analysis of CPT genes from whole genome, transcriptome and nucleotide databases revealed that three major groups of isoforms of the gene existing in *Hevea* are mainly involved in the synthesis of extra-long chain backbone of natural rubber. The rest of the sequences designated as CPT Isoforms were mostly dehydrodoliehyl diphosphate synthases associated with shorter chain isoprenoids biosynthesis.

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

A mapping population derived from an interspecific cross between *H. brasiliensis* clone

RRII 105 (high yielder with low disease resistance potential) and *H. benthamiana* F4542 (low yielder with high level of disease resistance) was used for the construction of high density genetic linkage maps of *H. brasiliensis* and *H. benthamiana*. Previously, linkage map was generated using proprietary software developed by Diversity Arrays Technology, which is not available in the public domain. Therefore, in order to integrate other markers, R package 'ASMap' was used to develop high density linkage map using DArT SNPs and presence/absence variants (PAVs) markers. Refinement of the linkage maps was carried out by eliminating single-dose markers and markers showing excessive segregation distortion. Finally 5187 and 8082 good quality markers for *H. brasiliensis* and *H. benthamiana* respectively were used and linkage map was constructed for each parent. All DArTseq tags that mapped to parental maps (*H. brasiliensis* and *H. benthamiana*) were blasted against the *Hevea* draft genome (AJJZ000000000.1). Correct LGs in those two parental maps were assigned using scaffold information of the draft genome.

Linkage maps of *H. brasiliensis* and *H. benthamiana* assembled into 18 linkage groups, in agreement with the known number of haploid chromosomes in *Hevea* ($n = 18$). Total length of the map was 6666.2 and 8779.9 cM for *H. brasiliensis* and *H. benthamiana* respectively. The length of each linkage group in *H. brasiliensis* ranged from 175.5 cM (LG 13) to 580.7 cM (LG 10), the distance between two markers was on an average 1.28 cM and the maximum space between two markers was 25.2 cM. Percentage of markers participated in mapping was 85 per cent. In case of *H. benthamiana*, length of each linkage group ranged from 304 cM (LG 17) to 685 cM (LG 5), distance between two markers was on an average

1.09 cM with a maximum space between two markers 16.2 cM. Percentage of markers that were able to map was 71 per cent.

The RAPD (96) and SSR (46) markers that were identified earlier were used for mapping along with the SNP and PAV data generated from DArTsequencing. In the *H. brasiliensis* map a total of 16 (12 RAPD and 4 SSR) markers got integrated, whereas in the *H. benthamiana* map, only seven (6 RAPD and 1 SSR) markers got integrated into the map.

Phenotypic assessment for disease response to three major pathogens: *Phytophthora meadii*, *Corynespora cassiicola* and *Colletotrichum acutatum* was carried out in controlled condition in the laboratory. Parental maps were used to detect QTLs using 'qtl' software in R package. QTLs for resistance to these three major diseases were identified from the paternal parent *H. benthamiana*, which is the disease resistant parent. Validation of QTL markers and fine mapping of the QTL regions for disease resistance was carried out using KASP marker technology.

1.5. Segregation analysis of the SNP markers derived from latex biosynthesis genes

Genotyping of the interspecific mapping population was carried out using SNP markers for segregation analysis and also to establish correlation between genotyping data with the test tap yield of the progenies.

SNP markers developed from the genes *MYK* (MYK2628A/G), *PMVK* (PMVK1786C/T), *HMGS* (HMGS3059A/G) and *CPT* (cis-pre1438C/T) were used in genotyping of a mapping population (86 progenies) derived from the cross between RRII 105 x F4542. Segregation ratio of MYK2628A/G was found to be approximately 1:1 as expected (48 progenies were heterozygous with A/G type and 38 were

homozygous with A/A type). For PMVK marker the segregation ratio was highly skewed with 56 progenies of C/T type and 30 progenies of C/C type. It is interesting to note that similar segregation trend was observed with 3 SNP markers from *PMVK* gene in another progeny population (RRII 105 x RRII 118). SNP markers generated from *HMGS* (HMGS3059A/G) and *CPT* (cis-pre1438C/T) genes gave a segregation ratio of 1:1 in this progeny.

Simple correlation analysis with yield data was carried out using the SNP genotyping data from the genes: MYK, PMVK, HMGS and CPT. Correlation with test tap yield was noted for the markers from the genes HMGS and CPT.

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

2.1. HSP sequence analysis (Stress responsive genes)

Heat Shock Proteins (HSPs) are important chaperones having potential role in abiotic stress tolerance. Genes encoding 17.5 kDa, 18.2 kDa and 15.7 kDa HSP family proteins in rubber were amplified and cloned. Two heat shock proteins, 18.2 kDa and 15.7 kDa were confirmed through sequencing. Development of a binary vector cassette with 15.7 kDa for *Agrobacterium*-mediated transformation is in progress.

2.2. Methylation dynamics of *Hevea brasiliensis* genome

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

A partial promoter region of REF gene was amplified from 24 DNA samples derived from

the clone RR11 105, RR11 600 and RR11 100 before imparting the stress, after two weeks of giving stress and one month post stress maintained in growth chamber. Cloning of bisulfite PCR products of this gene from 16 plants was completed and plasmid DNA was isolated from multiple colonies and kept ready for sequencing. Simultaneously, cloning of bisulfite converted HMGS gene promoter was completed from all the 24 plants and plasmids from 4-5 colonies from each sample were purified for sequencing.

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

Bisulfite sequencing of the polymorphic bands derived from the MSAP analysis of bud grafted plants was performed to identify the effect of rootstock on DNA methylation in the scion. Genomic DNA from the five plants was treated with bisulfite and PCR was performed to amplify the region containing the three bands/fragments (EC8TAG-7, C19TAC-2 and C19TAC-3) from these five plants. The amplicons obtained were purified and cloned for sequencing to confirm the methylation status. Bisulfite sequencing of the three fragments revealed the presence of CHH and CG type of methylation in the C19TAC-2 and EC8TAG-7 fragments whereas CHH, CHG and CG patterns of methylations were detected in the C19TAC-3 fragment. Unique methylation patterns as well as common patterns were observed in the case of all the three fragments apart from some random methylation variations.

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

Both genomic and cDNA sequences encoding coniferaldehyde-5-hydroxylase

(*Cald5H*) were cloned and characterized from RR11 105, which is known to play a key role in regulating S/G ratio by channeling the intermediate compounds of guaiacyl precursor to syringyl lignin pathway without any influence on total lignin content. Multiple sequence alignment of *HbCald5H* with reported *Cald5H* sequences from other tree species revealed 70-80 per cent homology. The amino acids sequence showed 82 per cent sequence identity with *Cald5H1* gene of *Populus trichocarpa*. The *Cald5H* protein sequence displays all the characteristic features of a plant P450 protein, including Heme-binding ligands (PFGSGRR) at the C terminus, stop transfer sequence *etc.* The notable stretch between Pro-440 to Gly 450 region contains eight residues that comprise the Heme-binding domains and are highly conserved among plant P450s. Though *HbCald5H* has high similarity with P450 genes such as F5H, the N-terminal 34 amino acid sequence of *HbCald5H* is highly divergent from the F5H as it contains a hydrophobic region typical of the uncleavable signal peptide for anchoring P450 protein to the endoplasmic reticulum membrane.

3.1.1. Bacterial expression of recombinant protein

The initial experiments with different culture conditions like variable temperature for expression of full-length *Cald5H* cDNA using pET 32a expression in *E. coli* BL21 (DE3) cells did not yield any detectable levels of recombinant protein on SDS-PAGE. This might be due to lack of internal membrane system in *E. coli* to direct the plant P450 protein through their hydrophilic N-terminus resulting decreasing solubility and increased formation of inclusion bodies and loss of functional protein. An effort was made to induce the heterologous protein expression with the use of chemicals such as α -aminolevulinic acid (ALA) and 0.5 mM Ferric chloride (FeCl_3)

to the growth medium (Terrific Broth) which was reported to increase the expression level of some p450 family proteins as the addition of ALA to the culture medium stimulates heme biosynthesis to match the increase in P450 polypeptide synthesis. However, the chemical stimulation failed to produce any detectable levels of CALD5H protein expression in *E. coli*. Therefore, the PCR amplified *HbCALD5H* cDNA region devoid of N-terminal signal peptide sequences was cloned into pET32 vector and mobilized into BL21 (DE3) cells. Expression of partial recombinant protein was observed in *E. coli*.

3.1.2. Sense and antisense constructs of CALD5H in binary vector for plant transformation

Sense and antisense constructs of *Cald5H* were cloned into pCAMBIA 1301 vector. For the construction of antisense cassette, three constructs were made; reverse complement of the gene having lengths of 473 bp, 842 bp and a hairpin RNA (hpRNA) construct of 1200 bp. The hpRNA construct constitutes 500 bp of coding region in forward direction, a 200 bp linker DNA from intronic region and a reverse complement of the first 500 bp fragment. They were mobilized into the *Agrobacterium tumefaciens* (LBA4404). The *Agrobacterium* strain carrying the sense and antisense constructs of *HbCALD5H* in pCAMBIA1301 were used in genetic transformation of tobacco plant. The transgenic lines developed are being maintained *in vitro*.

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

4.1. *Hevea* genome sequencing

Genome assembly couldn't be performed due to non-availability of high performance computing server. However, we could do benchmarking for *de novo* assembly of rubber genome using two assemblers on IBM POWER

processor to assess the performance of the server after getting remote access to the same. Both benchmarking results helped us to understand the behaviour of these assemblers with our whole genome sequencing data and also helped us to roughly estimate the runtime for assembling the reads.

IBM High Performance Computing server with following specification was used for benchmarking: P8 S822LC Compute Node (Minsky), 20 cores, 2.06 GHz (up to 4.02 GHz with performance CPU governor), 512 GB memory, 2-Infiniband 100Gbps (EDR) ports and 4 – NVIDIA P100 GPUs.

4.1.1. Benchmarking I – MaSuRCA assembly

Illumina trial data of 180 GB were tested with MaSuRCA assembly. The run was completed in 129 hrs (5.3 days). Genome size of 1.3 Gb was assembled into 425043 scaffolds and 1.2 Gb was assembled into 448770 contigs with 1 Kb minimum length cut-off.

4.1.2 Benchmarking II – SOAPde novo assembly

In total, 270 GB of paired-end and mate-paired trial data were tested with SOAP *de novo* assembly. Genome size of 1.1 Gb was assembled into 972396 scaffolds and 1.0 Gb was assembled into 1041828 contigs with 500 bp cut off. Genome size of 706 Mb was assembled into 276557 scaffolds and 578 Mb was assembled into 331500 contigs with 1 Kb cut off. The benchmarking results helped us to roughly estimate the runtime for assembling the reads.

4.1.3. Mate-Pair (MP) sequencing data insert size analysis

Mate-pair sequencing data were subjected to read mapping and insert size analysis using BWA, PICARD and R tools. Latest RRIM 600 whole genome sequences (total scaffolds: 189316) were used as a reference for read

mapping and insert size peaks were obtained for 2, 4, 6 and 8 Kb MP Libraries.

4.2. Transcriptome sequencing

Bioinformatic analyses of 15 transcriptomes derived from leaf, root, bark and latex of rubber plant are in progress. Protein domain detection was carried out for all 15 RNA-Seq assemblies with Pfam database, which would help in functional annotation of the transcripts. Highest Pfam hit was obtained with bark transcriptome RRI-BA (34398) and lowest hit with TPD affected bark transcriptome T1 (7990). Identification of tissue specific transcripts, further annotation and screening of top 50 differentially expressed genes from RNA-Seq projects have been initiated.

4.2.1. Root specific transcripts from root transcriptome of rubber

To identify root specific transcript sequences using RNA-Seq assembly, leaf, latex and bark transcript sequences were clustered with 90 per cent sequence identity. A total of 38047 root transcript sequences were subjected to BLAST and FASTA alignment search against the clustered leaf, latex and bark sequences. A total of 344 root transcripts, which are not having any significant alignment with other tissues were extracted using in-house PERL scripts. Further, these 344 sequences were subjected to Open Reading Frames (ORFs) search, which resulted in 38 transcript sequences with full-length ORFs.

Further, these transcript sequences were subjected to gene annotation. Interestingly, five transcript sequences were aligning with "*Rhizophagus irregularis*". *Rhizophagus irregularis* is an arbuscular mycorrhizal fungus (AMF), which penetrates the cortical cells of the roots of vascular plants. Based on these results and literature evidences, we believe that there may be beneficial interactions between *Hevea*

root at their earlier stages of development and *Rhizophagus irregularis*.

4.2.2. Differential Gene Expression (DGE) screening

Top 50 differentially expressed genes were screened from following RNA-Seq data using in-house PERL scripts:

- N - Bark sample from healthy plant
- T - Bark sample from TPD plant (20%, 50% and 80% TPD samples pooled together)
- YA- Latex from high yielding germplasm accession
- YB- Latex from low yielding germplasm accession
- YC- Latex from low yielding clone
- PHY-600-CO: Control sample (susceptible)
- PHY-600-TP: Phytophthora challenged sample
- PHY-FX-CO: Control sample (tolerant)
- PHY-FX-TP: Phytophthora challenged sample
- COL-105-CO: Control sample
- COL-260-CO: Control sample
- COL-105-TP: Colletotrichum challenged sample
- COL-260-TP: Colletotrichum challenged sample

4.2.3. Re-annotation of "hypothetical proteins" identified in latex transcriptome

Many differentially expressed proteins from RNA-Seq projects were annotated as "hypothetical proteins" using NCBI "nr" database. To cross check the annotation, a total of 10515 transcript sequences, which were annotated as "hypothetical protein" from

transcriptome data of YA- Latex from high yielding germplasm accession were screened. These transcripts (10515) were subjected to in-house blast search against *Ricinus communis* protein data set. In total 6173 sequences were annotated from blast search. Among these, more than 2000 transcripts were annotated as "Putative uncharacterized protein". A good number of Pentatricopeptide repeat-containing protein, DNA binding protein, ATP binding protein, Transcription factors etc. were also found in the alignment. Due to computational limitation, we could not screen the entire sequences against NCBI nr database and only *Ricinus communis* protein data was used for initial screening.

A total of 7904 transcript sequences, which were annotated as "hypothetical protein" from transcriptome data of YB - Latex from low yielding germplasm accession were screened. These 7904 transcripts were subjected to in-house blast search against *Ricinus communis* protein data set. In total 3504 sequences were annotated from blast search. Among these, more than 1000 transcripts were annotated as "Putative uncharacterized protein".

A total of 7227 transcript sequences, which were annotated as "hypothetical protein" from transcriptome data of YC - Latex from low yielding clone were screened. These 7227 transcripts were subjected to in-house blast search against *Ricinus communis* protein data set. In total 4974 sequences were annotated from blast search. Ubiquitin carboxyl-terminal hydrolase was the top hit in blast results. A total of nine transcript sequences which were re-annotated as Ubiquitin carboxyl-terminal hydrolase was extracted and subjected to blast search against NCBI nr database and it was

reconfirmed that all nine sequences were aligning with Ubiquitin carboxyl-terminal hydrolase.

5. Metagenomics and microbe identification in rubber ecosystems

The project on metagenomics of soil microbes from rubber growing areas with different cropping systems has been initiated in collaboration with CSIR-NEERI, Nagpur. Soil samples were collected based on a nested design from 20 sites from a plot. In total 130 soil samples were collected from rubber grown under different management practices like, only rubber, rubber and weed, rubber and cover crop, rubber and inter crop (cocoa) and native forest. Total DNA was isolated from samples using soil DNA isolation kits and the DNA yield was 2.0–3.5 µg from 500 mg of soil samples. DNA samples were sent to NEERI for amplicon sequencing using NGS platform and sequencing is in progress.

6. Advisory work

Clonal identity of the rubber trees in a holding of RR11 105 showing better performance compared to the DNA of RR11 105 after isolating DNA from the leaf sample of tapped, untapped and source bush nursery. Isolated genomic DNA was used in SSR profiling along with two RR11 105 samples as references/controls using 14 informative SSR marker loci (heterozygous in RR11 105). SSR marker profiles of six samples (BWN, Tapped, Untapped1, Untapped2 and two RR11 105 standard) with all the marker loci clearly revealed that the samples taken from the holding as well as from authentic source bush were very similar to the reference sample i.e., the clone RR11 105. Thus it was established that the trees giving high yield were also RR11 105 and not a different clone.

PLANT PATHOLOGY DIVISION

Plant Pathology Division focuses on monitoring the occurrence and development of pests and diseases, their management through chemical and biological means, evaluation of new clones for disease tolerance, identification of genes involved in disease tolerance, identification of quantitative trait loci for disease tolerance, understanding biotic etiology of tapping panel dryness and use of beneficial microorganisms for plant growth.

In addition to research, the Division also takes up testing of spraying equipments, plant protection chemicals and water samples for bacterial contamination. Training on disease management, maintenance of spray equipment, mushroom culture and apiculture are the other activities undertaken by the Division. Advisory work on disease management is also undertaken through field visits, WhatsApp and the Online Rubber Clinic. About 2400 cases were attended through WhatsApp and 432 water samples were

analysed during the reporting year.

1. Leaf diseases

1.1. Abnormal Leaf Fall disease

Studies to assess the impact of abnormal leaf fall (ALF) on growth and yield of rubber in four modern clones *viz.* RRII 414, RRII 422, RRII 429 and PB 260 were in progress in BO2 panel at Central Experiment Station, Chethackal. Highest girth was noticed in PB 260 and the lowest in RRII 422. The growth of trees in sprayed block was significantly higher than unsprayed in RRII 414 and RRII 429. The girth increment and bark thickness did not give any trend irrespective of clones and treatments (Table Path. 1). The severity of Abnormal Leaf Fall (ALF) disease, in general, during 2017 disease season was less. Among the clones, leaf fall of 30 to 40 per cent was recorded in unsprayed block of RRII 414, whereas in other test clones it was mild and ranged between 20-30 per cent.

Table Path. 1. Comparison of growth parameters in experimental clones under sprayed and unsprayed conditions

Clone	Girth (cm)	Girth increment (cm)	Bark thickness (mm)
RRII 414			
Sprayed	71.7**	4.4	11.9
Unsprayed	69.6	5.1	13.0
RRII 429			
Sprayed	71.2**	4.17	11.8
Unsprayed	69.3	5.07	12.1
RRII 422			
Sprayed	64.3	3.9	10.6
Unsprayed	63.2	3.6	12.5
PB 260			
Sprayed	73.5	6.2	11.2
Unsprayed	72.3	6.2	13.0

**t test significant

Impact of ALF was consistently more in clone RR11 414 which recorded yield drop in the current year also. Clones RR11 429 and RR11 422 did not register yield drop in unprotected blocks due to less disease during the season. DRC did not seem to get influenced by ALF. Tapping panel dryness (TPD) was more in PB 260 and in other test clones, no difference could be seen. Between sprayed and unsprayed, no definite trend in occurrence of TPD was observed.

Experiment to evaluate the effectiveness of nursery crown budding in clone PB 260 at Central Experiment Station, was in progress in BO2 panel. The girth and girth increment were found significantly higher in crown-budded than control (Table Path. 2). The ALF and Phytophthora shoot rot were mild in crown-budded trees where as both diseases were moderate in control.

Table Path.2. Growth of PB 260 crown-budded trees

Treatment/crown	Girth (cm)	Girth increment (cm)	Bark thickness (mm)
Crown-budded/ Fx 516	7	4.8	13.7
Control/ PB 260	72.7	5.9*	12.2

The crown-budded trees recorded significantly higher yield, whereas DRC did not show significant variation. The tapping panel

dryness (TPD) was found more in crown-budded trees (Table Path. 3).

Table Path.3. Yield, DRC and TPD in crown-budded PB 260

Treatment/Crown	Yield (g t ⁻¹ t ⁻¹)	Dry rubber content (%)	TPD in BO-2 Panel (%)
Crown-budded/Fx 516	42.1**	38	14
Control/PB 260	39.2	40	8
Yield increase over control (%) 7			

The effect of crown modification in clone PB 260 on raw rubber properties was studied. The Mooney Viscosity was found less in crown budded trees whereas Plasticity (Po) and Plasticity Retention Index (PRI) were on par.

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

Impact of leaving trees of clones RR11 105, RRIM 600, PB 235 and PB 5/51 unprotected from ALF and powdery mildew diseases is being assessed in B11 panel at RRS Padiyoor. Powdery mildew disease was mild in RR11 105 and RRIM 600, but moderate in PB 235 and severe in PB 5/51. Abnormal Leaf Fall disease was moderate in RR11 105, PB 235 and PB 5/51, but severe in RRIM 600. The leaf retention was 70 per cent in the RR11 105 unsprayed area and it was 30, 50 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 RR11 105, RRIM 600, PB 235 and PB 5/51 for the year was worked out to be 6, 54, 29 and 46 per cent,

respectively (Table Path. 4). Tapping panel dryness was lesser in RRIM 600 than other test clones. Growth difference was not significant between protected and unprotected trees in all test clones.

Table Path. 4. Percentage leaf fall and yield ($\text{g t}^{-1} \text{t}^{-1}$) in experimental blocks at RRS, Padiyoor

Clone	leaf fall (%)		Yield ($\text{g t}^{-1} \text{t}^{-1}$) 2017-18		
	Protected	Unprotected	Protected	Unprotected	Crop loss (%)
RRII 105	20	30	58.3	47.00	6
RRIM 600	25	70	39.8**	19.58	54
PB 235	25	50	36.7*	24.91	29
PB 5/51	30	50	33.2*	23.09	46

Efficiency of half-dose (20 kg ha^{-1}) application of oil-based COC against Abnormal Leaf Fall disease in clone RRII 105 and PB 260 was carried out in a hot spot area. The treatments were 4 kg COC: 20 L spray oil, 8 kg COC: 40 L spray oil and untreated control. Spraying was undertaken during the second fortnight of May and leaf retention was recorded periodically. The results showed that leaf retention in half-dose applied plots of RRII 105 and PB 260 was comparable to full dose application of COC.

1.2. *Corynespora* leaf disease

The compatibility of antagonist microbes with recommended fungicides was tested *in vitro*. The antagonist endophytic bacteria 8LK showed tolerance to the recommended fungicide carbendazim (0.05% a.i.). Evaluation of bioagents (endophytic bacteria) and efficacy of these organisms in integrated control against *Corynespora* leaf fall disease on RRII 105 was

carried out at Ulickal nursery. The results showed that the integrated control with carbendazim (0.025%) and antagonistic endophytic bacteria was effective and on par with recommended fungicide (Table Path. 5; Fig. Path. 1).

Table Path. 5. Effectiveness of integrated treatments in *Corynespora* disease control

Chemical	Concen- tration	Disease intensity (%)
Carbendazim	0.05%	2.4
Antagonistic endophytic bacteria	$1 \times 10^9/\text{ml}$	2.3
Carbendazim+		
Antagonistic endophytic bacteria	0.025%+	
	$1 \times 10^9/\text{ml}$	1.3
Control (unprotected)		4.7
CD ($P=0.05$)		0.2



Fig. Path.1. *Corynespora* disease severity between integrated and unprotected treatments

Evaluation of new generation fungicide, in Karnataka during 2017 disease season. Among the treatments, thiophanate methyl 0.07 per cent along with recommended fungicides and untreated control was carried out at two locations treatments in controlling the disease (Table Path.6).

Table Path.6. Effect of fungicides on *Corynespora* disease control

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

In order to study the virulence of the pathogen, *C. cassiicola* infected leaves were collected from different rubber growing areas of Kerala and Karnataka during January to March 2017. The virulence of isolates was studied by leaf wilt bioassay using crude toxin and spore inoculation method. The isolates showed high and faster wilting in susceptible clones during *in vitro* leaf wilt bioassay. The twelve tested

Corynespora isolates were found equally aggressive. *Cas1*, the cassiicolin gene was amplified from the genomic DNA of all isolates tested.

The clone Fx 516 showed high tolerance to *Corynespora* in spore inoculation/toxin bioassay method and no infection was observed in Fx 516 plants at Uliekal nursery during peak disease season. The half sibs of Fx 516 were screened

for their tolerance to *Corynespora* using crude toxin.

Assessment of level of disease tolerance among 202 Wickham clones of rubber as well as six species of *Hevea* (*H. brasiliensis*, *H. benthamiana*, *H. nitida*, *H. camargoana*, *H. spruceana* and *H. pauciflora*) to *C. cassicola* was carried out using crude toxin derived from *C. cassicola* under controlled *in vitro* laboratory conditions. Clones were ranked based on their disease response reaction. Clones showing high levels of tolerance and high level of susceptibility were shortlisted for genotyping to identify putative markers linked to resistance loci. Second round of screening is in progress to confirm their levels of tolerance. Following another round of screening, genotyping of the selected set of clones (tolerant and susceptible) using molecular markers will be initiated to identify putative markers linked to the tolerance loci. 117 clones were genotyped using DArT sequencing for association mapping studies.

Assessment of disease tolerance to *C. cassicola* among the progeny population (*H. brasiliensis* x *H. benthamiana*) was also carried out. Although susceptible progenies were identified, progenies processing greater tolerance than the tolerant parent *H. benthamiana* were also identified, which could be directly used in resistance breeding program.

Next Generation Sequencing technology (RNA-Seq) was adopted to gain insight on differentially regulated genes involved in host tolerance to *Corynespora cassicola*, through transcriptome analysis of control (healthy) and challenged (infected) susceptible (RRII 105) and tolerant (GT 1) cultivated rubber clones. A few key genes involved in disease resistance were identified in tolerant clone. Studies on over

expression of selected genes and analysing the cellular changes following pathogen infection is in progress through real-time PCR analysis. Preliminary studies on virus-induced gene silencing (VIGS) have been initiated to identify genes and knock down expression of selected genes.

1.3. Powdery mildew disease

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

1.4. Colletotrichum leaf disease

Evaluation of spray schedule for the management of Colletotrichum leaf disease (CLD) of rubber was undertaken at TR&T estate, Mundakayam. Among the four schedules evaluated, the alternate spraying with Carbendazim+Mancozeb (2g L⁻¹) and Troloxystrobin + Tebuconazole (1g L⁻¹) at weekly interval recorded lowest disease intensity.

The level of disease tolerance in 202 Wickham clones to *Colletotrichum* spp. was assessed by using crude toxin derived from *C. acutatum* under controlled *in vitro* laboratory conditions. Consolidation of the results on inherent tolerance of these clones revealed that only a few clones possessed over 70 per cent tolerance and most of the clones fell into the moderate level of resistance category or were highly susceptible. Second round of screening is in progress to confirm the level of resistance. Genotyping of the selected set of clones (resistant and

susceptible) will be initiated following confirmation using molecular markers to identify putative markers linked to the resistance loci.¹¹⁷ Wickham clones were genotyped using DArT sequencing, which provided both SNP and PAV markers, which would be used for association mapping studies.

Assessment of disease resistance to *Colletotrichum acutatum* among the progeny population (*H. brasiliensis* x *H. benthamiana*) was also carried out. Progenies processing greater resistance than the resistant parent *H. benthamiana* and greater susceptibility than the susceptible parent *H. brasiliensis* were identified.

1.5. Thread blight disease

A survey on the incidence and severity of thread blight disease was undertaken in the Thodupuzha and Kothamanalam regions. The incidence and severity at Thodupuzha and Kothamangalam region were 30 per cent and 15 per cent, respectively. About 10 alternate hosts were identified during the survey.

2. Stem diseases - Pink disease

Two new generation fungicides were evaluated for the curative effect at TR&T Estate, Mundakayam on 3 year old plants of the clone RRII 105. Curative application was carried out by single man operated power sprayer. Observation on the recovery of plants showed that both the fungicide trifloxystrobin + tebuconazole (1g L⁻¹) and tebuconazole (1ml L⁻¹) recorded better protection in checking the pink disease when applied during the cob-web stage of infection.

3. Tapping Panel Dryness

Recent investigation on TPD - affected trees showed typical bark necrosis spreading downwards from tapping cut to root stock. DAPI

(nuclear stain) stained TPD and healthy bark when examined under laser scanning microscope showed presence of living organisms in phloem vessels and their absence in healthy samples. Phytoplasma-enriched DNA was isolated from TPD and a few healthy bark samples. Nested-PCR assays using two universal primer pairs, R16mF2/R1 and R16F2n/R2 yielded an amplification of ~1300bp in all TPD- affected plants. PCR amplification was obtained in certain healthy plants also. Cloning and sequencing of amplified PCR product were carried out and the sequence on optional blasting showed similarity to other reported Phytoplasmas. The healthy plants which were positive in PCR reaction later showed TPD symptoms.

Scanning Electron Microscopic studies of Phytoplasma-positive bark samples showed round pleomorphic bodies similar to Phytoplasma in the sieve tubes. The size of the Phytoplasma-like bodies ranged between 400-1800 nm.

4. Microbial inoculants for growth improvement

4.1. Effect of PGPR on growth of young rubber plants

Four PGPR isolates and two consortia were evaluated in root trainer plants at 25 and 50 per cent recommended levels of fertilizer. Uninoculated plants applied with 25, 50 per cent and full fertilizer were kept as controls. Inoculated plants showed better girth and height of plants than fertilizer applied plants. The highest girth of plants was recorded in those plants applied with RH 104 at 25 per cent fertilizer application. Plants inoculated with 25 and 50 per cent fertilizer application did not show much difference in growth and was higher than uninoculated plants at the same levels and with full level of fertiliser applied plants. Root growth was more in inoculated plants.

5. Construction of high density genetic linkage map and mapping of Quantitative Trait Loci (QTLs) for disease resistance

A mapping population derived from an interspecific cross between *H. brasiliensis* clone RRII 105 (high yielder with low disease resistance potential) and *H. benthamiana* F4542 (low yielder with high level of disease resistance) was used for the construction of high density genetic linkage maps of *H. brasiliensis* and *H. benthamiana*. Polymorphic markers (heterozygous in one parent and homozygous in the other parent) generated from DArT sequencing [both single nucleotide polymorphisms (SNPs) and presence/absence variants (PAVs)] were used from the segregating progeny population. Refinement of the linkage maps was carried out eliminating single-dose markers and markers showing excessive segregation distortion. Finally 5187 and 8082 good quality markers for *H. brasiliensis* and *H. benthamiana*, respectively were used and linkage map was constructed for each parent. Mapping was done using ASMap package in R statistical environment. All DArTseq tags that mapped to parental maps (*H. brasiliensis* and *H. benthamiana*) were blasted against the *Hevea* draft genome (AJJZ000000000.1). Correct LGs in those two parental maps were assigned using scaffold information of the draft genome.

Linkage maps of *H. brasiliensis* and *H. benthamiana* assembled into 18 linkage groups, in agreement with the known number of haploid chromosomes in *Hevea* ($n = 18$). Total length of the map was 6666.2 and 8779.9 cM for *H. brasiliensis* and *H. benthamiana*, respectively. The length of each linkage group in *H. brasiliensis* ranged from 175.5 cM (LG 13) to

580.7 cM (LG 10), the distance between two markers was on an average 1.28 cM and the maximum space between two markers was 25.2 cM. In case of *H. benthamiana*, length of each linkage group ranged from 304 cM (LG 17) to 685 cM (LG 5), distance between two markers was on an average 1.09 cM with a maximum space between two markers 16.2 cM.

Phenotypic assessment for disease response to three major pathogens: *Phytophthora meadii*, *Corynespora cassiicola* and *Colletotrichum acutatum* was carried out in controlled condition in the laboratory. Parental maps were used to detect QTLs using 'qtl' software in R package. QTLs for resistance to these three major diseases were identified from the paternal parent *H. benthamiana*, which is the disease resistant parent. Validation of QTL markers and fine mapping of the QTL regions for disease resistance was carried out using KASP marker technology.

Of the 84 F₁ progeny that were assessed for tolerance against *Phytophthora*, four (4.8%) were highly tolerant, 27 (32.1%) were tolerant, 33 (39.3%) were moderately tolerant/susceptible, 11 (13.1%) were susceptible and nine (10.7%) were highly susceptible (Fig. Path. 2). With assessment using a crude extract of toxin from *Corynespora cassiicola*, the *H. benthamiana* parent was confirmed to be highly tolerant and the *H. brasiliensis* parent highly susceptible. Of 85 progeny tested with this extract, nine (10.6%) were highly resistant, 18 (21.2%) were highly susceptible and 58 were intermediate (Fig. Path.3 A). Phenotyping for resistance to *Colletotrichum acutatum* using a crude extract of the toxin, the *H. benthamiana* parent was confirmed to be highly resistant and the *H. brasiliensis* parent to be highly susceptible. Of 85 progeny tested with C.

acutatum toxin extract, 17 were highly resistant (20%), six were highly susceptible (7.1%) and 62 (72.9%) were intermediate (Fig. Path.3B).

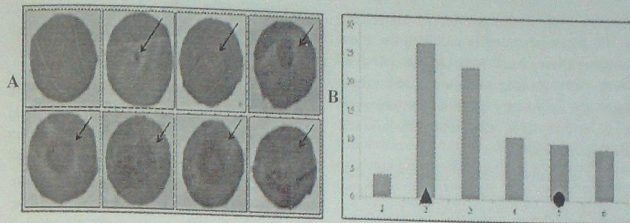


Fig. Path. 2 Assessment for *Phytophthora* disease response based on size of lesion produced on leaf following infection with zoospore suspension after 96 h of infection (A). *Phytophthora* disease severity assessed with 84 progeny. Disease severities of parents are indicated by a circle (*H. brasiliensis*) and a triangle (*H. benthamiana*) (B)

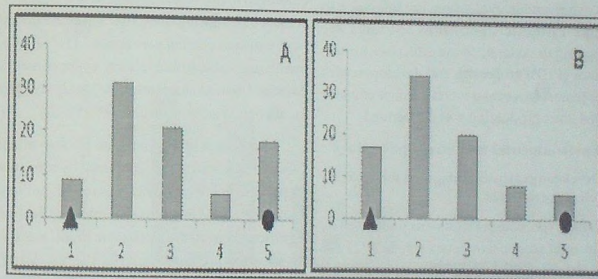


Fig. Path. 3. *Corynespora* leaf disease (A) and *Colletotrichum* leaf disease (B) assessed with 85 progeny (*H. brasiliensis* x *H. benthamiana*). Disease severities of parents are indicated by a circle (*H. brasiliensis*) and a triangle (*H. benthamiana*)

5.1. QTLs detected for disease resistance

For *Phytophthora* resistance, three QTLs were mapped in *H. benthamiana*: two on LG 8 and one on LG 1. For *Corynespora* resistance, three were mapped in *H. benthamiana*: one on each of LG 1, LG 12 and LG 13. For

Colletotrichum resistance, three were mapped on LG 10, LG 12 and LG 18 in *H. benthamiana* and on LG 5, LG 6 and LG 11 in *H. brasiliensis*. Validation of QTL markers and fine mapping of the QTL regions for disease resistance is being carried out using KASP marker technology.

PLANT PHYSIOLOGY DIVISION

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

Around twenty introduced clones from other rubber growing countries were evaluated for their drought tolerance potential by assessing physiological parameters. Clonal responses were studied in *Hevea* under high and low temperature stresses and a few stress tolerant clones were identified. Many wild germplasm accessions and ortets were under evaluation for tolerance to extreme climatic conditions. Studies are progressing to understand the effect of a growth regulator (PBZ) on growth and development of rubber plants. Molecular mechanism of ethylene induced latex production was deliberated.

1. Environmental and stress physiology

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

Gene expression analysis was performed for two drought responsive genes viz. sHSP23 (23 k Da chloroplast protein coding gene) and HbRab1 (stress induced cytoplasmic RAB protein). GAPDH gene was used as endogenous control for normalization and quantitative expression of these genes. Quantitative gene expression analysis indicated up-regulation of chloroplast sHSP23 gene in drought exposed plants. Drought tolerant clones, RRIM 600 and RRH 430 recorded around two fold over expression when compared to susceptible clones, RRH 105 and RRH 414. In case of RAB gene

there was no definite trend for gene expression pattern for drought responses.

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

1.2.1. Evaluation of exotic clones for drought stress tolerance

Twenty introduced clones from four countries (Vietnam - 8 (V1 - V8), China - 5 (C1 - C5), Thailand - 5 (T1 - T5) and Cambodia - 2 (K1-K2) were tested for their response to drought stress, both *in vitro* and in the field. The parameters tested were photosynthesis rate, chlorophyll fluorescence rate and chlorophyll content index during stress free and stress periods (soil moisture content was around 11.7%). Cell membrane stability and relative water content in detached leaf discs were also analysed after incubating in an osmoticum (40% PEG).

Except in a few clones, the photosynthetic CO_2 assimilation rate (P_n) declined by more than 70 per cent in most of the clones tested. Stomatal conductance and transpiration rate also declined significantly in all the clones due to soil moisture deficit stress. Clones viz. T2, T1, V4, V1 and V2 recorded relatively better gas exchange parameters under drought conditions in the field, while C2, C4, V5 and V6 were found poor. Though the rate of reduction in Fv/Fm and $\dot{O}PS$ II was small under stress, clones V4, T1, T2, V2, V1 and T4 recorded better photo chemical efficiency under dark as well as light adapted conditions, whereas V6, V5, V7, C4 and V8 were showing a low rate of photochemical efficiency. Clones T1, V3, V4, T3, C4, V2 and V1 had lower

membrane damage under PEG stress while V7, V8, K2, T5 and K1 had inflicted with higher level of membrane damage. Clones, T5, T1, K1, K2, C4, V7 and V5 showed better leaf chlorophyll content under stress conditions. Relative water content (RWC) of leaf discs were analysed during the stress period and it was found that clone T5 maintained more than 90 per cent RWC in leaf and V7, V3, V6, T3, V7, V1 and V4 are the other clones having higher RWC.

Variations were more among the clones for each parameter, and very few clones showed stability in tolerance potential for most of the parameters tested. One clone each from Vietnam (V4) and Thailand (T1) showed consistently better performance under stress situation, compared to other clones.

1.2.2. Effect of high temperature stress on *Hevea*

The effect of high temperature stress in young plants of *Hevea* and their tolerant capacity was studied in four popular clones (RRII 105, RRII 414, RRII 430 and Tjir 1) under controlled conditions in a walk-in growth chamber. The photochemical efficiency and ETR across PS I and PS II declined in leaf discs exposed to high temperature conditions compared to control. Gas exchange parameters declined significantly in all the clones on exposure to high temperature for 5 days (Fig. 1). Antioxidant enzyme activity and lipid peroxidation rate initially increased and then declined, indicating the process of acclimation upon exposure to high temperature stress. Among the clones tested, RRII 430 showed consistently better adaptation to high temperature stress with respect to most of the parameters studied.

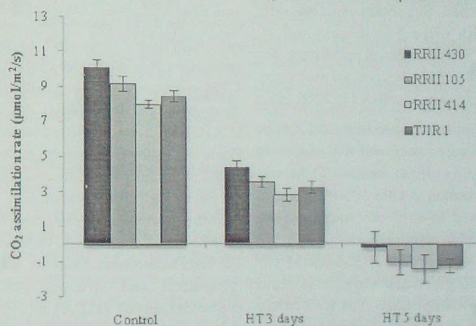


Fig. Phy. 1. CO₂ assimilation rate in rubber clones subjected to high temperature stress under controlled conditions

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

The experiment was initiated with nine *Hevea* clones viz. RRII 105, RRII 208, RRII 414, RRII

422, RRII 429, RRII 430, RRII 600, RRII 100 and SCATC 88/13. Three sets of experiments were carried out using polybag grown young plants. After 10 days under controlled condition

PLANT PHYSIOLOGY DIVISION

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

Around twenty introduced clones from other rubber growing countries were evaluated for their drought tolerance potential by assessing physiological parameters. Clonal responses were studied in *Hevea* under high and low temperature stresses and a few stress tolerant clones were identified. Many wild germplasm accessions and ortets were under evaluation for tolerance to extreme climatic conditions. Studies are progressing to understand the effect of a growth regulator (PBZ) on growth and development of rubber plants. Molecular mechanism of ethylene induced latex production was deliberated.

1. Environmental and stress physiology

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

Gene expression analysis was performed for two drought responsive genes viz. sHSP23 (23 k Da chloroplast protein coding gene) and HbRab1 (stress induced cytoplasmic RAB protein). GAPDH gene was used as endogenous control for normalization and quantitative expression of these genes. Quantitative gene expression analysis indicated up-regulation of chloroplast sHSP23 gene in drought exposed plants. Drought tolerant clones, RRIM 600 and RRII 430 recorded around two fold over expression when compared to susceptible clones, RRII 105 and RRII 414. In case of RAB gene

there was no definite trend for gene expression pattern for drought responses.

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

1.2.1. Evaluation of exotic clones for drought stress tolerance

Twenty introduced clones from four countries (Vietnam - 8 (V1 - V8), China - 5 (C1 - C5), Thailand - 5 (T1 - T5) and Cambodia - 2 (K1-K2) were tested for their response to drought stress, both *in vitro* and in the field. The parameters tested were photosynthesis rate, chlorophyll fluorescence rate and chlorophyll content index during stress free and stress periods (soil moisture content was around 11.7%). Cell membrane stability and relative water content in detached leaf discs were also analysed after incubating in an osmoticum (40% PEG).

Except in a few clones, the photosynthetic CO_2 assimilation rate (P_n) declined by more than 70 per cent in most of the clones tested. Stomatal conductance and transpiration rate also declined significantly in all the clones due to soil moisture deficit stress. Clones viz. T2, T1, V4, V1 and V2 recorded relatively better gas exchange parameters under drought conditions in the field, while C2, C4, V5 and V6 were found poor. Though the rate of reduction in Fv/Fm and ÖPS II was small under stress, clones V4, T1, T2, V2, V1 and T4 recorded better photo chemical efficiency under dark as well as light adapted conditions, whereas V6, V5, V7, C4 and V8 were showing a low rate of photochemical efficiency. Clones T1, V3, V4, T3, C4, V2 and V1 had lower

membrane damage under PEG stress while V7, V8, K2, T5 and K1 had inflicted with higher level of membrane damage. Clones, T5, T1, K1, K2, C4, V7 and V5 showed better leaf chlorophyll content under stress conditions. Relative water content (RWC) of leaf discs were analysed during the stress period and it was found that clone T5 maintained more than 90 per cent RWC in leaf and V7, V3, V6, T3, V7, V1 and V4 are the other clones having higher RWC.

Variations were more among the clones for each parameter, and very few clones showed stability in tolerance potential for most of the parameters tested. One clone each from Vietnam (V4) and Thailand (T1) showed consistently better performance under stress situation, compared to other clones.

1.2.2. Effect of high temperature stress on Hevea

The effect of high temperature stress in young plants of *Hevea* and their tolerant capacity was studied in four popular clones (RRII 105, RRII 414, RRII 430 and Tjir 1) under controlled conditions in a walk-in growth chamber. The photochemical efficiency and ETR across PS I and PS II declined in leaf discs exposed to high temperature conditions compared to control. Gas exchange parameters declined significantly in all the clones on exposure to high temperature for 5 days (Fig. Phy.1) Antioxidant enzyme activity and lipid peroxidation rate initially increased and then declined, indicating the process of acclimation upon exposure to high temperature stress. Among the clones tested, RRII 430 showed consistently better adaptation to high temperature stress with respect to most of the parameters studied.

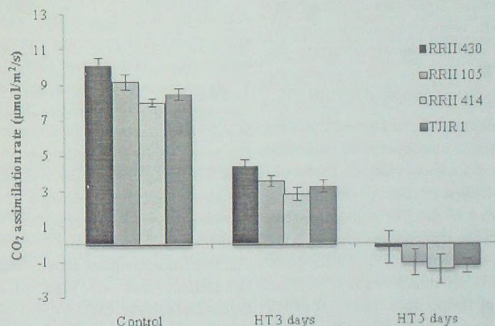


Fig. Phy. 1. CO_2 assimilation rate in rubber clones subjected to high temperature stress under controlled conditions

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

The experiment was initiated with nine *Hevea* clones viz. RRII 105, RRII 208, RRII 414, RRII

422, RRII 429, RRII 430, RRII 600, RRII 100 and SCATC 88/13. Three sets of experiments were carried out using polybag grown young plants. After 10 days under controlled condition

(30°C/22°C day/night, 13h photoperiod), these plants were subjected to low temperature condition by gradually lowering the temperature to 15°C/7°C (day/night). Drought stress was imposed in another set of plants under controlled condition by withholding irrigation for ten days. Membrane damage on exposure to low temperature stress was found relatively less in RRIM 600, RRII 208 and SCATC 88/13. Leaf mass per area (LMA) and specific leaf area (SLA) were higher in RRII 208 and RRII 430. The level of antioxidant enzyme activity was increased after two weeks exposure to low temperature. Among the clones, high rate of activity was observed in SCATC 88/13 and low rate in RRII 105. Genotypic variations to low temperature responses were worked out in one cold tolerant (SCATC 88/13) and susceptible (RRII 105) clones. Significant variations were observed with respect to morphological, physiological and biochemical parameters. The clone SCATC 88/13 showed fast rate of recovery from cold stress when compared to RRII 105.

Cell viability test indicated that RRII 208 and SCATC 88/13 were functioning well under cold stress among the clones studied. There was significant reduction in chlorophyll content of leaves exposed to cold or drought stress. Xanthophyll cycle pigments analysed in *Hevea* clones, RRII 422, RRII 105 and RRIM 600 revealed significant clonal variation in plants exposed to low temperature. The level of UV absorbing compound such as anthocyanin was increased under low temperature condition. RRIM 600 and RRII 208 showed relatively high cellular metabolic activity whereas RRII 430 and SCATC 88/13 were on par with each other for the rate of metabolic activity under low temperature condition. Under drought stress

condition also similar pattern of metabolic activity was observed in these clones.

Dormancy was noticed during the period of low temperature exposure. New whirl was started appearing after allowing a recovery period to tropical conditions, but the rate of growth recovery varies with clones. Under drought stress such a condition was not observed but during the stress recovery period the formation of new whirl was very fast when compared to recovery from cold stress. Biochemical analysis of leaf samples collected from drought and cold imposed plants is in progress.

1.4. Evaluation of modern *Hevea* clones for drought tolerance: Studies on drought effects on *Hevea* in relation to oxidative stress and antioxidant responses

The present study was undertaken to evaluate the drought associated oxidative stress and antioxidant responses in germplasm accessions. Wild accessions (MT 5100 and MT 4788) were assessed along with elite *Hevea* clones such as RRII 105, RRII 208, RRIM 600, SCATC 88/13 and RRII 400 series at RRII farm. Plants were subjected to drought stress for ten days. Leaf samples were collected after assessing the physiological performance of the plants. Growth performance of germplasm lines was on par with *Hevea* clones under drought condition. The level of antioxidant activity was estimated in control and stressed plants. Both malondialdehyde (MDA) and glutathione contents increased in stress exposed plants and decreased in control ones. Their quantity increased further when the drought stress extended for more than one week. Cell viability test reflected that the germplasm accession MT 5100 was on par with RRII 208 and SCATC 88/13. Analysis of crucial biochemical parameters

relevant to oxidative stress responses and antioxidant metabolism are in progress.

1.5. Evaluation of environmental stress tolerance and physiological adaptation of cold and drought tolerant ortets under varying agro-climates in India

A multi-location trial with sixteen ortets and seven check clones were planted at three different locations in 2012, in which CES, Chethackal is considered as control location. Plant girth at 50 cm height from the bud union and yield recording by test tapping was done every February and September, since 2015 at CES, Chethackal. Mean girth during February 2018 ranged from 25.0 cm for ortet GH 1 to 43.7 cm for RRSA 98, which is higher than that of check clone, RRI 430. Cumulative yield of ten test tappings was recorded in September 2017 (peak yielding period). The highest yield was recorded in clone RRII 430 followed by clone RRII 417 among the clones and RRSA 98 followed by RRSA 585 and DAPI recorded higher yield whereas RRST 24, NGK 69 and DAP 34 recorded the lowest yield among the ortets.

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

Five *Hevea* clones have been subjected to cold treatment inside a growth chamber. Among these clones, RRIC 100 and RRIM 600 were found to be the most cold susceptible and tolerant clones, respectively based on chlorophyll fluorescence (Fv/Fm) and net photosynthesis (A) measurements. Total proteins were extracted from leaves of these two clones for comparative studies on stress related proteins through LC-MS/MS. Up-regulation of several proteins viz. superoxide dismutase [Cu-Zn] – chloroplastic isoform X3, beta-glucosidase 24-like protein, peroxidase 12-like and triosephosphate isomerase

and down-regulation of several proteins like acyl carrier protein 1 – chloroplastic-like isoform X2, glycine cleavage system H protein were found in both the clones after cold exposure.

1.7. Growth regulation and high density planting (HDP) in natural rubber for productivity enhancement

Field planting of clone RRII 430 was carried out in June-July 2015 with three densities using two types of planting material viz. polybag and root-trainer plants. First application of the growth regulator, paclobutrazol (PBZ) was carried out in the month of January 2017. Data on girth and plant height have been recorded for successive three years. Phenotypic observations indicated that profuse branching and compact canopy in plants treated with PBZ. Expression analysis of genes encoding two enzymes GA₃ oxidase and alkaline invertase in *Hevea brasiliensis* was carried out. The growth regulator, PBZ blocks the biosynthesis pathway of gibberellins in plant system. To ascertain this in *Hevea*, quantitative expression analyses of two genes viz. GA₃-oxidase, involved in gibberellin biosynthetic pathway and alkaline invertase gene, responsible for sucrose catabolism in rubber-producing laticifers in *Hevea* were made by qPCR technique. GA₃-oxidase gene was found to be significantly down-regulated (17.4%) in the PBZ-treated young field plants than the control plants. Similarly, reduced expression levels were noticed in case of alkaline invertase gene by 16.5 per cent in PBZ-treated plants.

1. Physiology of growth and yield

1.1. Yielding pattern in double budded *Hevea* clones

Early wintering clones (RRIM 600 and PR 107) and late wintering clones (RRII 105 and GT 1) were budded on a single stock to study the communication between early and late

wintering scions and to check whether one scion influences the other in the combination for wintering pattern. After 17 years of growth, the scions of double budded plants did not show any different pattern of wintering from their single budded counterparts, indicating that there is no marked communication between scions in terms of wintering behaviour. Early wintering clones took more time for refoliation than late wintering clones. Yield data showed that early wintering clones had lesser rubber yield compared to late wintering clones in both single and double budded plots. Double budded trees are practically not viable for commercial purposes. Therefore, the project was concluded during the reporting year.

2.2. Inter-cropping with tree crops in rubber

The growth and yield parameters of rubber planted with tree intercrops were monitored by measuring the annual trunk girth and cup lump yield fortnightly. The mean girth of rubber trees was 71.5 cm for control, 70.2 cm and 69.3 cm for rubber with three rows of Mahogany and rubber with one row of Mahogany trees and one row of Pathimugom trees, respectively. The trees were continuously tapped for six years under S/2 d3 system of tapping and from April 2017 onwards the tapping system was converted to low frequency (LFT) i.e., S/2 d7 and monthly stimulation given since September 2017 onwards. Yield between rubber plants alone and rubber intercropped with tree species exhibited significant variation with 69.3, 56.6 and 58.4 g t⁻¹ for control, with three rows of Mahogany and two rows of Mahogany trees, respectively. Among the intercrops, the Pathimugom stand was poor with many trees drying and poor growth due to shading effect of mature rubber trees, while Mahogany trees (52.8 cm) were found growing better as intercrop in this trial at CES, Chethackal.

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

Experiment on stimulation induced changes in latex regeneration mechanism was continued at CES Chethackal in clones RRII 105, PB 217, PB 260, RRIM 600, Tjir 1, RRII 33 and RRII 38. The trees were under d3 system of tapping with recommended stimulation schedule. Latex samples were collected from control and stimulated trees (with 2.5% ethephon) after each stimulation. Biochemical parameters related to rubber biosynthesis (sucrose, inorganic phosphorous, thiols and ATP in latex), oxidative stress indicators (peroxidase, glutathione reductase, thiols in C-serum, proline and protein content) were analysed. Dry rubber yield and dry rubber content (drc) were also recorded. Latex ATP, thiol metabolism and antioxidant enzymes activities increased after stimulation in clones RRII 105 and PB 217 compared to PB 260 and RRIM 600. These parameters were not altered in low yielding clones (RRII 33 and RRII 38) after stimulation and there was no significant increase in yield also.

2.4. Relationship of ATP status of latex with rubber yield

Field experiment with five polyclonal selections based on high latex ATP and three control elite clones (RRII 105, RRII 417 and RRII 430) were continued at RRII farm to evaluate the yield and stress tolerance potentials of these selections compared to control clones. Biochemical parameters related to antioxidant defense mechanism were studied in these clones during stress and stress free seasons. RRII 430 and selection 63 showed better antioxidant capacity (based on stress indicators) than the other clones/selections for successive three years.

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

With the objective of understanding the molecular mechanism of ethylene induced latex production, quantitative gene expression analysis of ethylene receptors, ETR1 and ETR2 was carried out in different clones; RRII 105, PB 217, RRIM 600, Tjir 1, RRII 33 and RRII 38. Comparison of expression level of ETR1 and ETR2 genes among six clones indicated that the expression of these genes were induced in clones RRII 105 and PB 217 immediately after stimulation and closely related to its yield increasing trend. ETR2 expression was higher in RRIM 600 after stimulation however, not reflected in yield increase. In low yielding clones, the expression of these genes were not significant between control and stimulated trees (Table Phy.1).

Table Phy.1. Relative quantification of expression of ETR1 and ETR2 in ethylene stimulated samples of different clones compared to unstimulated control

Clones	ETR1	ETR2
RRII 105	1.88±0.12	1.59±0.16
PB 217	2.95±0.17	2.05±0.27
RRIM 600	0.98±0.08	3.2±0.097
Tjir 1	0.84±0.09	0.91±0.24
RRII 33	0.85±0.06	0.52±0.06
RRII 38	0.69±0.14	0.43±0.09
Un-stimulated control	1.0	1.0

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

Yield and biochemical parameters (in latex and bark) related to stress effects caused by tapping and ethephon stimulation at different positions and different concentrations in the tapping panel were compiled and analyzed. Over stimulation with 5 per cent ethephon above and below regions of tapping panel led to higher utilization of sucrose combined with high metabolic rate (high inorganic phosphate) in latex and increased level of MDA, proline and cyanide content and low level of β -cyanoalanine synthase activity (β -CAS) in the bark. Stimulation with high dose of ethephon leads to more stress effects in the tree and high incidence of TPD compared to recommended practice (2.5% ethephon panel application) in clone RRII 105 under S2 d3 system of tapping. Though higher yield was obtained during initial years when stimulated with 5 per cent ethephon above and below regions of tapping panel, TPD incidence was severe in this treatment. Ethephon application (5%) at the bottom region alone (above bud union) showed less stress effects (based on stress indicators) and sustainable yield increase (Table Phy. 2&3).

Table Phy.2. Rubber yield and biochemical indicators after stimulation (mean of three years)

Treatments	Yield ($\text{g t}^{-1} \text{t}^{-1}$)	Sucrose (mM)	Pi (mM)
T1 (5% ethephon 150 cm above the bud union)	62.05 ^b	5.42 ^b	16.05 ^b
T2 (5% ethephon just above the bud union)	82.2 ^a	4.89 ^b	15.03 ^b
T3 (5% ethephon both T1 & T2 position)	73.1 ^{ab}	3.68 ^c	17.91 ^a
T4 (2.5% ethephon panel application)	69.6 ^{ab}	5.57 ^c	15.03 ^b
T5 (unstimulated control)	59.2 ^b	7.13 ^a	11.88 ^c

Table Phy.3. Level of biochemical parameters in bark samples after stimulation in different treatments

Treatments	MDA (μ M)	Proline (mM)	Phenol (mM)	Cyanide (μ M/g)	β -CAS (units)	Peroxidase (units)	H ₂ O ₂ (μ M/g)
T1 (5% ethephon 150 cm above from the bud union)	4.84 ^{ab}	0.09 ^b	11.9	2.29 ^c	50.07 ^{ab}	3.81 ^a	3.57
T2 (5% ethephon just above the bud union)	3.52 ^c	0.11 ^b	10.15	2.97 ^{bc}	56.57 ^a	3.87 ^a	3.53
T3 (5% ethephon both T1 & T2 position)	5.59 ^a	0.17 ^a	10.85	4.76 ^a	45.58 ^{bc}	2.10 ^b	4.18
T4 (2.5% ethephon panel application)	4.04 ^{bc}	0.09 ^b	10.05	3.77 ^{ab}	58.42 ^{ab}	1.77 ^b	4.27
T5 (unstimulated control)	3.62 ^c	0.09 ^b	10.17	3.97 ^{ab}	40.47 ^c	1.73 ^b	3.24
			NS				NS

3. Ecological impact of natural rubber cultivation

3.1. Estimating carbon footprint of rubber production

Various potential sources of CO₂ emission in rubber plantation activities starting from nursery practices, land clearance, field establishment to production of fresh latex was accounted. Almost all farm activities are emitting considerable level of GHGs. In rubber plantations primary farm management practices are emitting significant amount CO₂. Mechanization of farm management practices like land clearance, felling of old trees, transport of wood logs, pit making for new plantation, transportation of planting materials and fertilizers, spraying activities *etc.*

consumes carbon intensive fossil fuels like diesel and petrol.

Fertilizer application and fungicides spray are the major sources of emission from rubber plantations. Assuming most of the rubber growers in the traditional region follow the recommended package of practices prescribed by the Rubber Board, India, all the recommended doses of fertilizers, weedicides and fungicides and their potential CO₂ emission equivalents are accounted. Total possible emission due to various farm activities was worked out to be around 26.7 MT CO₂ ha⁻¹ in a life cycle analysis. Further accounting of emission from primary latex processing unit is progressing.

LATEX HARVEST TECHNOLOGY DIVISION

Division continued research activities on applied aspects of crop harvesting in rubber. The programme on popularizing weekly tapping among small and medium holdings was continued and progressed well during the period under report with promising results. New experiments on Low Frequency Tapping (LFT) under d10 were laid out in two locations in clone RRH 105. Other than these the Division was also actively involved in testing and evaluation of various products, farm mechanization, advisory and training on all aspects of crop harvesting in rubber.

1. Low Frequency Tapping

1.1. Programme on popularizing weekly tapping

During the period, holdings that have followed weekly tapping under various regional offices showed promising results. However, tapping days realized showed considerable variation among locations due to practices followed.

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

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

Once in 20 days stimulation was imposed in 10 tapping blocks under d10 frequency of tapping. Mean dry rubber yield of 1858 kg ha⁻¹ could be obtained under d10 frequency of at

Kanthimathy Estate, Kulasekharam, and Tamil Nadu during 2017-18 as against estate average (global mean) of 1940 kg ha⁻¹.

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

In this trial, trees were tapped under d7 frequency of tapping with monthly stimulation in BO-2 panel during 2002-2004. From April 2004 onwards the tapping frequency was changed to d10 frequency of tapping with once in 20 day's stimulation. The exploratory trial at CES under d10 frequency of tapping continued to give promising yield during 2017-18 also. CUT was practiced during November onwards. Yield of 2272 kg 400⁻¹ trees was obtained in the seventh year of B1-1 panel. Low yield was obtained during the month of June and February due to missing of one tapping day.

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

A large scale experiment on d10 frequency of tapping from opening onwards (Panel BO-1) was laid out at Kanthimathy Estate, Kulasekharam, Tamil Nadu with five treatments and different levels of stimulation. Dry rubber yield was observed to be higher under weekly tapping compared to d10 frequency of tapping in the first year of tapping (Table LHT. 1)

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

Treatment	Yield	
	(g t ⁻¹ t ⁻¹)	(kg t ⁻¹ year ⁻¹)
T1- S/2 d6 ET 2.5% 24/y (2w)	106.5 a	4.8 a
T2- S/2 d6 ET 5% 12/y (m)	102.5 a	4.6 a
T3- S/2 d10 ET 2.5% 18/y	89.1 b	2.5 b
T4- S/2 d10 ET 5% 18/y	86.1 b	2.3 b
T5- S/2 d10 ET 2.5%, 5% 18/y	91.5 b	2.5 b

Values followed by common letters are not significantly different

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

Another large scale RBD with 4 replication experiment on Low Frequency Tapping (d10) in clone RRII 105 at CES, Chethackal was initiated during July 2017. There were six treatments comprising d7 (with monthly stimulation as control) and d10 frequency of levels tapping with different stimulation. Nine months yield data under weekly tapping with monthly stimulation

(ET.2.5%) showed significantly higher yield than once in 10 days tapping. Yield of 2326 kg 400⁻¹ trees was obtained in the 1st year of BO-2 panel (Table LHT. 2). Only 39 tapping were realized under d7 tapping. Frequency under d10 with once in 10 days stimulation showed higher yield than monthly stimulation. Mean drc was higher under d10 frequency with monthly stimulation (43%) than weekly tapping (38.7%).

Table LHT2. Yield response of Low Frequency Tapping (S/2 d10) in clone RRII 105 (Panel BO-2)

Treatment	Yield (kg 400 ⁻¹ trees)	DRC (%)
T1- S/2 d7 ET 2.5% 12/y (m)	2326 a	38.7
T2- S/2 d10 ET 2.5% 36/y	1854 b	40.0
T3- S/2 d10 ET 2.5% 18/y	1500 cd	41.0
T4- S/2 d10 ET 5% 18/y	1776 bc	40.9
T5- S/2 d10 ET 2.5% 12/y (m)	1190 e	43.0
T6 - S/2 d10 ET 5% 12/y (m)	1389 de	41.4

Values followed by a common letter are not significantly different

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

During 2016, two more blocks of demonstration plot on weekly tapping in clone RRII 105 were initiated in field 2000 Agro at Central Experiment Station (CES). Both blocks were under weekly tapping from July 2016 onwards. During 2017-18, twelve rounds of yield with stimulation were imposed at monthly interval 2.5 per cent ethephon. Yield of both blocks gave sustainable yield of 1967 (block 1) and 2352 kg 400trees⁻¹ respectively during 2017-18. It was 4.9 and 5.9 kg tree⁻¹ respectively during twelve months of tapping. Higher yield was obtained in block 2 when compared to block 1. During North-

East monsoon, sustainable yield was obtained due to fixing of mini rainguarding during the period. There is no difference in dry rubber content of both blocks (42.1%). The average yield of both blocks was 5.4 kg t⁻¹ year⁻¹

In another demonstration trial in field 93C under weekly tapping at CES, Chethackal during the period the yield was 1506 kg 400trees⁻¹. Low yield was due to missing of eleven tapping days due to absence of regular tapper and unavailability of reserve tapper (Khali). Introduction of CUT during November onwards gave good yield response.

2. Controlled Upward Tapping (CUT)

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

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

The large scale on farm trial on LFCUT with periodic panel change under weekly tapping was continued at Kanthimathy estate in 12 tapping blocks (8 blocks mixed clone and 4 blocks seedling population). The Low Frequency Controlled Upward Tapping (LFCT) under weekly tapping seems to be promising even for mixed clone and seedling population. Yield ranged from 64 to 139 g t⁻¹ in mixed clone whereas as it ranged from 82 to 191 g t⁻¹ in seedling population.

3. Other Experiments

3.1. Response of RR11 400 series clones to yield stimulation

At CES, Chethackal, RR11 400 series clones (RR11 414, RR11 422 and RR11 429) were identified for the experiment. The statistical design was completely randomized single tree single plot consisting of more than 100 trees of each clone. Tapping system adopted in this trial S/2 d3 6d/7. Three rounds of stimulation (ET 2.5% Pa) was given during 2017-18 in comparison with the unstimulated control trees (50% trees and 50% trees unstimulated, for each stimulated clone).

Significantly higher yield was recorded in stimulated trees of clone RR11 422 than unstimulated trees. No significant yield increase was noticed in stimulated trees of clones RR11 414 and RR11 429 (Fig. LHT. 1). Panel change effect was in clone RR11 429 than other two clones viz. RR11 414 and RR11 422 in BO-2 (2) panel.

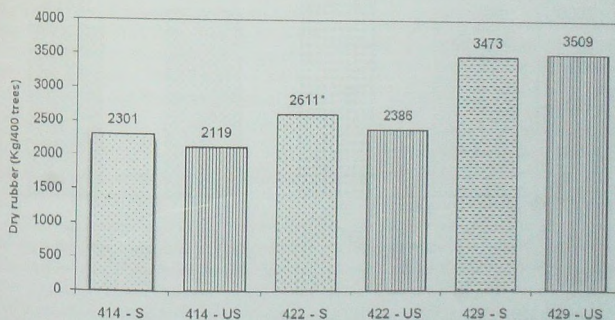


Fig.LHT.1. Cumulative yield performance of RR11 400 series

There is no difference in annual mean dre of clone RRII 429 both under stimulated and unstimulated situations (36 %). But in clone RRII 414 and RRII 422, annual mean dre was 2 per cent higher in unstimulated trees than stimulated trees. TPD percentage was higher in stimulated trees of clone RRII 414 than other two clones.

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

In another experiment at HML, Palapilly Estate, response of RRII 430 clone to yield stimulation under Low Frequency of Tapping (d3, d4 and d7) was commenced from June 2017. The statistical design was RBD with four treatments and five replications. Each replication consists more than 400 trees. The four treatments comprised d3 (without stimulation as control), d3 (2/y), d4 (4/y) and d7 (12/y)

frequencies of tapping. During the period yield was recorded from April 2017 to March 2018.

Yield was not significantly higher under d3 frequency of tapping with two round of stimulation than unstimulated d3 and d4 frequencies of tapping with four rounds of stimulation (Fig. LHT.2). Only 5 per cent yield could be increased in stimulated d3 tapping system when compared to control (d3 without stimulation) during twelve months of tapping. Breakeven yield of 87 per cent was obtained under weekly tapping when compared to d3 frequency of tapping with stimulation. Low yield under d7 frequencies of tapping was due to failure of proper thickness of bark shaving and leaking of trees. The dre values of trees tapped under low frequency tapping system (d7) are higher than the d3 frequency of tapping.

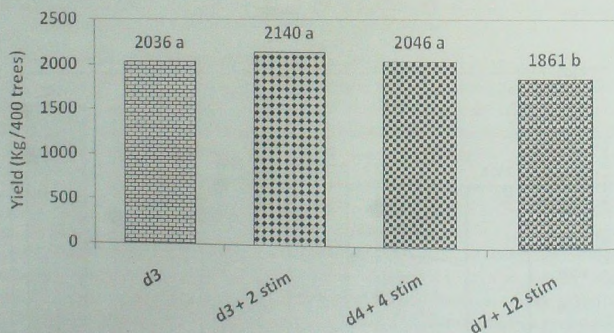


Fig. LHT.2. Yield performance of clone RRII 430 under LFT systems

Values followed by a common alphabet are not significantly different

ADVANCED CENTRE FOR RUBBER TECHNOLOGY

Advanced Centre for Rubber Technology (ACRT) established during the 10th plan period by integrating different units in rubber technology/ processing disciplines under Rubber Board started functioning in the present form from 2006 onwards and a new building commissioned in February 2009. Now ACRT comprises of two Divisions namely, Rubber Technology Division (RT) and Technical Consultancy Division (TC). The Centre mainly focusses research on the downstream activities

of rubber sector like primary processing, rubber technology research, quality control of rubber products, product development etc. The Centre also conducts research work on long-term projects which has industrial importance. The Centre is also engaged in the preparation of project reports, defect analysis and trouble shootings of rubber products/factory processes. Being a recognized research centre of Universities, ACRT has registered scholars for Ph.D programmes.

I. RUBBER TECHNOLOGY DIVISION

In the current year, activities of the Division were focused mainly on evolving improved techniques in processing of rubber, production of solid deproteinised natural rubber (DPNR), recovery of quality rubber from skim latex, latex stage incorporation of fillers such as silica and carbon black in natural rubber, reinforcement of NR using polymeric filler, blends of NR and EPDM rubbers and devulcanisation of used rubber products.

1. Processing

1.1. Optimization of solid deproteinised natural rubber process

The deproteination was carried out with the deproteinisation mixture developed at RRIL. The attempt was to minimise the detergent and the enzyme concentration within the deproteinisation mixture developed last year. The Mg content in the field latex was removed by DAHP addition. Effect of high ammonia (HA)

and LATZ preservation on deproteination was also tried.

1.3. Solid DPNR preparation using a new detergent and combination of detergents

Last year we have reported the development of a deproteinisation mixture at RRIL for effective deproteinisation of field latex with detergent 2 (Methods A to F). The detergent content within the deproteination mixture was on higher side. Hence, a new detergent 1 and detergent 2 combination (two type) was added to the deproteination mixture.

Detergent combination reduced the nitrogen content significantly than detergent 1 alone. The effective amount of detergent within the deproteinisation mixture could be reduced to half that of the previous year report by using the combination (50% reduction in detergent). Raw rubber properties of the DPNR sample is given in Table Chem. I.

Table Chem.1. Raw rubber properties of the DPNR samples evaluated

Sample by preparation method	Deproteinisation time (h)	Nitrogen content (%)	P ₀	PRI	Mooney Viscosity (M ₁ (1+4) 100°C)
D) DPNR with Detergent 2	24	0.12	42.5	19	77.6
C) DPNR with Detergent 1 and 2	24	0.082	46	16	78.8
B) Control with Detergent 1 and 2	2	0.448	45.5	05	80.4

1.4. Evaluation of cure behaviour of the newly developed DPNR

The DPNR prepared by two methods (C and D), was compared with the control rubber (A) prepared from HA preserved latex without deproteination and without the detergent additives and control (B) prepared from HA preserved latex without deproteination and with the detergent additives. A semi efficient

vulcanisation system with ISAF as the black filler was employed. The formulation of both DPNR (C and D) and control rubber samples (A and B) - 100 phr with stearic acid - 2, zinc oxide - 5, mernox 6C-2, ISAF - 50, naphthenic oil-5, sulphur -2.1 and CBS -2.1 phr was used in the study. Table Chem.2 shows the mechanical properties of the DPNR.

Table Chem.2. Comparison of mechanical properties of DPNR vulcanizates with control

Sample	N ₂ %	Modulus 100% MPa	Modulus 200% MPa	Modulus 300 % MPa	Elongation at Break, % MPa	Tensile strength MPa	Tear strength N/mm
A	0.43	3.4	7.7	13.1	584.9	27.1	95.9
B	0.44	3.0	6.9	12.1	523.0	24.4	104.8
C	0.08	2.6	5.8	10.2	592.5	25.5	104.5
D	0.12	2.5	5.8	10.4	568.0	26.3	103.9

2. Latex technology

2.1. Recovery of rubber from skim latex

A new simple, efficient and cost effective process was developed for the recovery of rubber from skim latex. The skim latex after treatment with a chemical is acidified. Skim rubber gets coagulated as lump with complete recovery. The pilot scale evaluation of the new process carried out at four centrifuging units for nearly 10,000L.

2.2. Studies on blends of skim rubber recovered by the new process with rubber from field latex

Blends of skim rubber with rubber from field latex at varying ratios of 3:97, 6:94, 10:90, 20:80, 30:70 prepared at the dry rubber stage and compared with rubber from fresh field latex as control. Blend prepared at the dry stage of the modified skim rubber and the rubber from field latex in a two roll mill.

2.3. Preparation of rubber compounds with skim rubber

Two roll mill was used for compounding. The formulation with blends of rubber recovered from skim latex through new method and rubber from fresh field latex -100 phr each with, stearic acid- 2, zinc oxide - 4, ISAF - 50, naphthenic oil-5, sulphur -1.5 and CBS -1.5 phr was used

in the study. This is also compared with a rubber coagulated from fresh field latex with same formulation.

2.4. Technological properties of the blends of skim rubber with rubber from fresh field latex

The incorporation of skim decreases the tear strength significantly, but other properties are almost comparable (Table Chem.3).

Table Chem. 3. Technological properties of the blends of skim rubber and fresh field latex

Sample	Modulus 100% (MPa)	Modulus 200% (MPa)	Modulus 300% (MPa)	Elongation at Break, (%)	Tensile strength (MPa)	Tear strength (Nmm ⁻¹)
Control(Field latex)	2.52	5.89	10.51	593.25	27.1	136.9
10:90	2.37	5.36	9.71	600.7	25.63	111.4
20:80	2.46	5.56	9.87	585.16	25.22	104.6
30:70	2.67	5.75	10.09	567.9	24.39	104.4

2. Rubber Technology

3.1. Rubber reinforcement

3.1.1. NR/Polymeric filler system

NR polymeric filler system (PF) tried in tyre tread application showed comparatively low abrasion and flex resistance compared to conventional tread compound. Conventional

tread formulation consists of 60:40 NR/ Polybutadiene blend. Hence, trials were conducted using NR/PF along with polybutadiene. Polybutadiene used for this study was master batch of polybutadiene with carbon black. The formulation of the mixes is given in Table Chem. 4.

Table Chem. 4. Formulation of the mixes

Ingredients	Control	100/10 NR/PF	80/10/20 NR/PF/PB	70/10/30 NR/PF/PB	60/10/40 NR/PF/PB	50/10/50 NR/PF/PB
NR	60	100	80	70	60	50
PF	0	10	10	10	10	10
PB	40	0	20	30	40	50
ISAF	50	0	6	9.9	13.2	16

Other common ingredients: ZnO-5, starci acid-2, TDQ-1, IPPD-1.5, CBS-1.5, sulphur-1.5

Cure characteristics of the compounds were almost comparable to that of control compound. Maximum torque and minimum torque slightly

increased with increasing the polybutadiene concentration. Technological properties are given in Table Chem. 5.

Table Chem. 5. Technological properties of NR/polymeric filler system

Properties	Control	100/10 NR/PF	80/10/20 NR/PF/PB	70/10/30 NR/PF/PB	60/10/40 NR/PF/PB	50/10/50 NR/PF/PB
Tensile strength, MPa	27.2	26.8	26.2	26.0	26.0	25.8
Modulus, 100%, MPa	2.14	4.2	4.0	3.8	3.7	3.7
Modulus, 300%, MPa	10.2	11.2	10.7	10.7	10.4	10.2
Tear strength, N/mm	78	76	76	75	76	75
Hardness, Shore A	62	60	60	60	60	62
Heat build-up, ΔT °C	24	8	8	9	10	12
DIN Abrasion loss, mm ³	52	120	102	87	73	56
Demattia						
Crack initiation, Cys	55000	24000	27000	38000	42000	48000

3.1.2. NR Latex - carbon black masterbatch

Studies on the preparation of NR latex - carbon black master-batch was continued as a collaborative project with a tyre industry. Master-batch samples were prepared and sent to the collaborator for evaluation of the technical properties at their end. Based on the feedback from the collaborator, we have prepared latex-carbon black master-batches

from fresh latex instead of preserved field to reduce degradation, in presence of surfactants S1 and S2. The formulation used was ZnO-4.5, Stearic acid-3, TMQ-1, 6PPD-2.75, TBBS-1.6, sulphur-1.2 for 100 parts rubber. Cure characteristics of control (dry mix) and master-batches with surfactants S1 and S2 at 150°C are also shown below (Table Chem. 6).

Table Chem. 6. Cure characteristics of the compounds

Sample	T _s (mint)	T _c 90 (mint)	T _c 95 (mint)	M _h (dN-m)	M _i (dN-m)	M _h -M _i
Control	4.03	7.41	8.48	17.56	2.62	14.95
S1	3.11	6.41	7.58	16.99	2.21	14.78
S2	3.03	5.95	6.90	17.70	2.34	15.36

It can be seen that the cure pattern is same for all the master-batches and are similar to that of the dry mix (control). Mechanical properties of master-batches and control are shown in Table Chem.7. Tensile strengths of master-batches prepared with the surfactant S1 has better tensile strength, elongation at break and

modulus compared to control. However tear strength was lower for both S1 and S2 compared to control. Hardness, heat buildup and abrasion resistance were comparable with control. Compression set was slightly higher for both samples compared to control.

Table Chem.7. Mechanical properties of master batches and control

Tests	S1	S2	Control
Tensile strength, MPa	25.3	24.7	24.3
Elongation at break, %	548	538	526
Modulus, 100%, MPa	2.72	2.38	2.65
Modulus, 200%, MPa	6.48	6.03	6.55
Modulus, 300, MPa	12.01	11.7	11.90
Tear strength, N/mm	123	136	142
Hardness, Shore A	66	65	63
Compression Set, %	38	39	34
Heat build-up, DT °C	24	26	21
DIN Abrasion loss, mm ³	116	116	114

Cut growth resistance

Latex based master-batches showed better cut growth resistance and flex resistance compared to the dry mix.

Industrial evaluation

Latex-carbon black masterbatches of filler content 10 phr, 20 phr, 30 phr, 40 phr, 50 phr, 60 phr and 70 phr were prepared in five batches each and were sent to the industrial collaborator for evaluation. The results are awaited.

3.1.3. Silica reinforcement of NR**3.1.3.1. NR Latex-silica masterbatch**

Compared to carbon black, silica has poor interaction and dispersion in non-polar rubbers. The surface of precipitated silica is highly polar and hydrophilic due to the presence of silanol groups. In order to improve the compatibility of silica with non-polar rubber and to ensure its good bonding to the rubber matrix, coupling agents are used. In the present study incorporation of silica into natural rubber was done through latex stage master-batch method (Table Chem. 8).

Table Chem. 8. NR Latex-silica master-batch Formulation of mixes

Ingredients	Compound identification	
	S40 (Ultrasil VN3)	SS40 (Coupsil 8113)
NR	100	100
Silica	40	40
ZnO / Stearic acid / Mernox 6C/ Naphthenic oil	4 / 2/ 2/ 5	4 / 2/ 2/ 5
CBS / DPG/ Sulphur	1.5/1.5/2	1.5/1.5/2

Table Chem. 9. NR latex-silica master-batch compounds – Cure characteristics

Property	Compound identification	
	S40 (Ultrasil VN3)	SS40 (Coupsil 8113)
Maximum torque(M_H) - dNm	16.7	20.2
Minimum torque(M_L) - dNm	0.3	1.2
Scorch time, ts2, (min)	1.8	1.0
Optimum Cure time t90, (min)	4.8	4.0

Table Chem. 10. Technological properties

Property	Compound identification	
	S40 (Ultrasil VN3)	SS40 (Coupsil 8113)
100 % Modulus, MPa	2.17	3.1
300 % modulus, MPa	10.32	11.2
Tensile strength, MPa	26.4	30
Elongation at break, %	586	606
Hardness, Shore A	61	66
Abrasion loss, mm ³	116	131
Tear Strength, N/mm	78	128
Heat build-up, Δ °C	11	20
Compression set, %	30	42

Two latex based master-batches of NR - silica were prepared from preserved field latex. In one of the master-batch NR-Latex was mixed with dispersions of silica 'Ultrasil VN3' was used as the filler at a loading of 40 phr. In the second one, silanised silica 'Coupsil 8113- an Evonik product' was used as filler at a loading of 40 phr. In both the cases dispersions of silica were prepared and incorporated in to the preserved NR Latex. Latex and silica dispersions were mixed well, coagulated and dried at 60 °C. These composites are marked as S40 (With Ultrasil VN3 at 40 phr loading) and SS40 (With Coupsil 8113 at 40 phr loading). With Ultrasil VN3, heat treatment was given in a Rheocord internal mixer. With regard to the pre-treated silica no heat treatment was given. Master-batches were further compounded with new formulation. Cure

characteristics of these compounds are given in Table Chem. 9 and the technological properties are given in Table Chem. 10. Silanised silica showed some processing difficulty in making of the dispersion. From the vulcanizate technological properties it is observed that silanised silica based samples exhibited improved properties such as modulus, tensile and tear strength. However, heat build-up and compression set were higher than the unmodified silica.

3.2. Recycling of Rubber: Devulcanisation

3.2.1. Patent filing

The application process of the Indian patent on "stable free radical assisted devulcanisation of carbon black filled rubber vulcanisate" is completed and an India patent application number 201741017633 dated 19/05/2017 has been

assigned to the patent application.

3.2.2. Developing cycle tyre formulation using the devulcanised tread rubber buffing dust and whole tyre or ground tyre powder (GTR)

With the objective of industrial use of the new devulcanisation technology, devulcanised rubber prepared both from retread buffing dust and GTR were used for preparing different formulations and determining the vulcanisate properties to show the feasibility of using the new technology. Accordingly, various compounds using the devulcanised rubber were prepared and compared with the properties of a standard cycle tyre vulcanisates. It was observed that it is possible to prepare various cycle tyre formulations having different range of properties using the devulcanised rubber prepared using the new technology. It may be noted that the per cent of regenerated rubber is only 35 in typical cycle tread formulation while it can be increased to 60-80 per cent using the new technology

achieving comparable vulcanisate properties.

3.3 NR/EPDM blends

The broad objective is to study the possibility of substituting NR/PB blend (50/50) in tyre side wall with NR/EPDM blends. Since sulphur vulcanization of NR/EPDM blends leads to vulcanisate with poor mechanical properties due to the cure mismatch of NR and EPDM, peroxide vulcanization is utilized. But peroxide vulcanisates have lower flex cracking resistance and these compounds have low scorch safety, ionic crosslinks will be introduced with the addition of zinc dimethacrylate co-agents and stable free radicals for scorch control. NR/EPDM at 60/40 ratio is reported to have good ageing resistance and excellent ozone resistance. DCP is the peroxide used for vulcanization. The effect of introducing the ionic cross link on vulcanisate properties are given in Table Chem. 11.

Table Chem.11. Effect of introducing ionic crosslinks in NR/EPDM blends - Vulcanisate properties

Property	NR/EPDM, GPF,2DCP	NR/EPDM,GPF 2DCP, 5 Zinc dimethacrylate	NR/EPDM,HAF 2DCP, 5 Zinc dimethacrylate	NR/PB* 50/50), (GPF
Hardness, Shore A	50	55	59	58
Tensile strength, MPa	13.5	15.6	17.7	17.3
M100, MPa	1.58	1.32	1.88	1.7
M200, MPa	3.74	2.64	3.86	3.2
M300,MPa	6.63	4.75	6.68	5.1
EB, %	527	724	663	895
Tear Strength, N/mm	24.6	30.2	36.7	40.7
Heat buildup, Δ°C	21	25	30	12
Compression set, %	19.8	38.6	32.7	56.6

2. Collaborative project

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

3. Development/advisory work/project work

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

II. TECHNICAL CONSULTANCY DIVISION

Technical Consultancy (TC) Division of ACRT was set up at Rubber Research Institute of India in the year 1986, to cater the rubber based industrial growth of our country. The activities of the Division are designed in such a way that the rubber based units of our country amounting to over 5000 in number will be able to tap the benefits of applied research and developments being conducted in its laboratory. The major activities of the TC Division are testing/certification of rubber products as per relevant standards *i.e.* ISO, BIS, ASTM, EN, ASRTU *etc.* in addition to R and D activities. Technical Consultancy division is an NABL accredited laboratory (CAB No.3032) and due to acceptance of test reports, clients can very well compete in the export market. The Division is conducting research projects for the benefits of industries in the micro, small and medium Enterprises (MSME) category. These projects were undertaken on industrially important areas. The highlights of the projects are given below.

1. Research Projects

1.1. Latex based adhesives

Effect of various fillers on the viscosity and adhesion strength of natural rubber latex (NRL) and centrifuged latex of 60% DRC) based adhesives were examined. Both the viscosity and adhesion strength of latex adhesives were affected by the filler addition. The results indicated that addition of filler to a particular concentration can improve the adhesion strength. Adhesive properties of centrifuged latex and creamed latex adhesives were compared. It has been found that viscosity of creamed latex adhesive was higher than that of centrifuged latex-based adhesives. Centrifuged latex-based adhesive showed better peel strength than

creamed latex-based adhesives.

The role of different fillers on the adhesive properties of creamed NR latex based adhesives showed that the viscosity and peel strength was found to increase with increase in the concentration of the filler. Role of ZnO on the adhesive properties of NR field latex and blends of NR field latex-methyl methacrylate grafted NR latex blend was also studied. Thermal resistance of NR field latex adhesive was decreased by the addition ZnO.

Synthetic rexin substrates were bonded with different clay incorporated NRL based adhesives. It showed that at high clay loadings, failure in the rexin adherents observed which indicates the high adhesion strength of NRL-clay adhesive system. NR creamed latex adhesives were used to bond synthetic rexin substrates and the peel strength was lower compared to NR centrifuged latex adhesive. Similar to NR centrifuged latex adhesives, incorporation of clay fillers into creamed latex slightly improved the adhesion peel strength on rexin joints. Effect of adhesion promoter polyvinyl alcohol (PVA) on NR creamed latex/ clay adhesive was noted using synthetic rexin adherents. Incorporation of PVA greatly enhanced the viscosity of the adhesive whereas the spreadability decreased.

The characterization of NR latex/clay adhesive was studied using Fourier Transform Infrared spectroscopy (FTIR)(Fig.TC.1) and thermo gravimetric analysis (TGA). NR-based solution adhesives for tyre re-treading application were prepared and various physical properties of the adhesive were tested as per ASRTU specifications. The effect of different cure systems on the physical properties of NR based solution adhesive was studied. Studies revealed

that the properties greatly depend on the curative system used.

1.2. Binary blend for tread application

Six formulations based on natural rubber (NR) and polybutadiene (BR) for tire tread compounds were designed. The effect of blend-ratio on tensile strength, abrasion resistance and hardness were studied according to ASTM standards. The abrasion resistance and hardness were found to increase with BR content and the increase in the tensile strength was attributed to the increase in the NR content.

1.3. Prevulcanisation of natural rubber latex

A study on the effect of different radiation doses on the properties of radiation vulcanised natural rubber latex (RVNRL) was conducted. Results revealed that the order of addition of antioxidant has influence on the properties of RVNRL.

1.4. Finger printing of natural rubber latex

Effect of adulterants on NR latex was studied in detail. The adulterants used were SBR

latex, precipitated calcium carbonate (PCC) dispersion and skim latex. Study was conducted on various doses of adulterants like high (0 to 10 phr), medium (0 to 2 phr) very small (0.1 to 1 phr) dose. In all procedures total solid content of the latex was kept at 50 per cent. Effect of SBR latex (0.0693 micron size) on blending with centrifuged latex (0.555 micron size) at high dosages was investigated. The trend showed that as the amount of SBR increases the particle size decreases and mean time specific surface area get increased. This trend is just the opposite of the trend shown by precipitated calcium carbonate (PCC-NR) blend. The trend in variation in viscosity and MST showed that as the amount of adulterant increases above 1phr the viscosity increases from 25cP to 85cP. MST was also increased from 1300s to 1786s when the latex was adulterated in high dosage of SBR. This trend is just the opposite of the behavior shown by PCC dispersion. This implied that different adulterant blending with natural rubber latex and accordingly they can be identified.

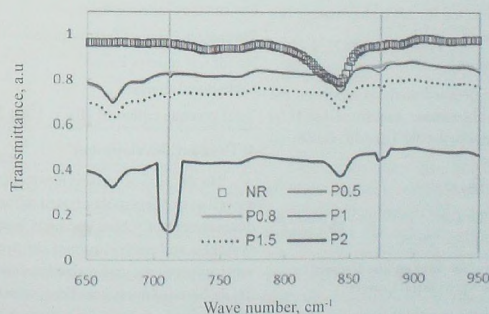


Fig.TC.1. FTIR spectra of latex films casted with NR-PCC blend

1. Rubber Industrial Promotion and Externally Funded Projects

2.1. Technology Business Incubation Unit

Officials of the division discussed with Kerala State Industrial Development Corporation (KSIDC) about the establishment of Technology Business Incubation (TBI) unit at RRII for rubber products. A proposal was submitted for the commissioning of a TBI unit at RRII, for promoting the innovation and business opportunities among young entrepreneurs in rubber products. An area of 10,000 sq. feet was earmarked for the proposed TBI unit.

2.2. Project report on surgical gloves

A project report was prepared for setting up a medical gloves manufacturing unit for M/s. SPSM Pharmaceuticals, Gujarat and collected Rs.50,000/- towards the consultancy fee.

2.3. Development of rubber product- Flower pots and rubber fly wheel

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

2.4. Analysis report

Prepared a detailed analysis report on the defects of rubber mats exported by M/s. Kerafibertex International (P) Ltd, Kochi on consultancy basis.

2.5. Shelf-life study of gloves

Study on the shelf-life of gloves based on specifications is in progress.

2.6. Evaluation of the smoke house designed by Tamil Nadu Agricultural University

As an experimental design, Tamil Nadu Agricultural University in consultation with RRII

designed and installed a smoke house at Elavampadam Rubber Producers Society at Vadakkacherry, Palghat. The smoke house utilises solar energy, bio gas and wood gasifier unit for drying of rubber sheets. Preliminary data on the operation of the smoke house were collected. It is observed that further trials are required to get quantifiable data pertaining to operation and efficiency of the smoke house and economics of the operation of the particulate smoke house system.

2. Testing and certification

For the testing of rubber compounds/products and raw materials, consistent support was offered especially to small and medium level entrepreneurs. Total number of samples tested and the revenue collected during the reporting period are given in Table TC. 1.

Table TC. 1. Number of samples tested and the revenue collected (2017-18)

No. of clients	362
No. of samples tested	782
No. of parameters analysed	3033
Consultancy letters/e-mail	1451
Trouble shooting of factory process	20
No. of test reports issued	536
Component analysis	61
Total revenue collected (Rs.)	21,14,406

4. Product development

The division offers services to entrepreneurs as well as existing rubber based industries in the development of rubber products based on both synthetic and natural rubber. In this area, the services given are categorized as follows (Table TC.2). Some of the major clients who are availing the services of the division are VSSC, BSF, BEML, BPCL, Kochi Metro *etc.*

Table TC.2. Know-how transfers to industries

Type of rubber products developed	Number
Adhesives	2
Automobile components	6
Fire resistant mats	1
Latex based dipped and foamed goods	3
Expanded rubber sheets and soles	3
Rubber molds	2
Pre-cured tread, bonding gum and tube valve (ASRTU specification)	5
Rubber based agro-machinery components	1
Rubber based engineering components for railway, defence, BSF, Kochi Metro <i>etc.</i>	1

5. Project profiles/Technical bulletins

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

6. 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 matter of concern among the clients. Division has given appropriate guidance in all these aspects.

7. Training programme imparted

Training classes on rubber technology were provided to students in B.Tech and M.Tech programmes and hands-on-training and consultancy services were provided to manufacturers of specific products.

8. Academic collaborations

Based on the academic collaboration between the Tripura University and RRII, the Division has conducted two weeks training cum practical classes for the BVoc students.

9. Awards/Appreciation

Division received the NABL Accreditation certificate as per ISO/IEC 17025: 2005.

ECONOMICS DIVISION

The Division continued its research activities in the broad areas comprising of farm management, primary processing and marketing, rubber products manufacturing industry and foreign trade, and intercropping and by-products. In these areas inter-divisional collaborate projects were also undertaken for comprehensive understanding of the rubber sector. Five projects were completed and reported during the period. The summaries of the results are given in the following sections.

1. Study on block rubber plantations in Mayurbhanj District of Odisha

A study was taken up in Mayurbhanj district in Odisha where the Rubber Board in association with the Government of Odisha has implemented Rubber Block Plantation Projects for the socio-economically marginalized scheduled tribes. The study revealed that the average annual income of the tribal households with income from rubber (Rs. 1,00,195/-) was three times higher than the tribal households without income from rubber.

The average expenditure of households with income from rubber was 65.8 per cent higher than their counterparts without income from rubber.

A transformation was noticed in the type of houses constructed from the traditional mud huts to *pakka* houses having modern amenities to enhance the standard of living, as income from rubber started to flow. The study revealed that additional income earned from rubber laid the foundation for a discernible change in the socio-economic status of the tribal communities and overall development of the region.

2. India's export potential under the Regional Comprehensive Economic Partnership (RCEP) Agreement: The case of rubber and rubber products

Negotiations leading to a larger trade agreement with ASEAN and its six FTA partners viz. India, China, Japan, Australia, New Zealand and South Korea called Regional Comprehensive Economic Partnership (RCEP) agreement are fast advancing. The agreement is expected to have far reaching impacts on the world rubber industry as it covers the major producers and exporters of raw rubber and rubber products in the world. The challenges of RCEP on the domestic rubber and rubber products industry of India are evident from the higher rate of growth in imports (20%) than exports (11%) with these countries. The present study analysed the challenges and scope of rubber and rubber products of India in the RCEP region. The study found that among the major export items of India, only new pneumatic tyres (HS 4011) and reclaimed rubbers (HS 4003) showed export potential in the region and the products classified under other articles of vulcanized rubber other than hard rubber (HS 4016) had comparative disadvantage in the RCEP market. Moreover, the benefits due to trade creation and trade diversion

under the RCEP are limited for export of rubber and rubber products from India. Therefore, it is argued that the prospects of market access to major export items of India, such as products under HS 4011 and HS 4016 are largely dependent on creation of trade competitiveness rather than market access due to tariff liberalisation. The present study observed that further tariff liberalisation under the proposed RCEP agreement may widen the trade deficit of India in rubber and rubber products. This will have far reaching implications on the domestic rubber and rubber products manufacturing industry.

3. Over-dependence of Indian rubber industry on imported natural rubber: the question of long-term sustainability

Natural rubber (NR) has a dominant role in the Indian rubber industry as it constitutes 66 per cent of the total amount of rubber the industry consumes. In recent years, NR production has been declining in the country as a result of growers abstaining from tapping the trees because of non-remunerative price. Despite its declining domestic production, NR consumption and the rubber industry continued to grow, albeit at lower rates, with substantial imports of NR. There are clear indications that Indian rubber industry is losing its momentum and its contributions to the economy are on the decline in recent years even as the industry started to become overly dependent on imported NR. There are structural changes happening to the rubber industries of major NR exporting countries and availability of cheap NR in the international market cannot be taken for granted indefinitely. Indian rubber industry is too important for the economy to be left to the uncertainties and vagaries of NR supply in the global market for long. Table Eco.1 shows the growth in NR consumption in major NR producing countries vis-à-vis world consumption.

Table Eco. 1. Domestic consumption of NR in major NR exporting countries ('000 tonnes)

Year	Indonesia	Thailand	Vietnam	Malaysia	Total	World consumption
2005	221(9.7)	335(11.4)	60(12.5)	387(34.4)	1003(14.7)	9206
2006	352(13.4)	321(10.2)	65(11.7)	383(29.8)	1121(14.7)	9688
2007	383(13.9)	374(12.2)	80(13.2)	450(37.5)	1287(16.9)	10175
2008	412(15.0)	398(12.9)	100(15.2)	469(43.8)	1379(18.2)	10187
2009	352(14.4)	399(12.6)	120(16.9)	470(54.8)	1341(18.7)	9289
2010	421(15.4)	459(14.1)	140(18.6)	458(48.8)	1478(19.3)	10764
Growth (%)	9.9	6.8	19.7	4.4	7.5	1.9
2011	474(15.9)	487(14.4)	145(17.9)	402(40.4)	1508(18.4)	10997
2012	548(18.2)	505(14.4)	150(17.4)	459(49.7)	1662(20.0)	11048
2013	579(17.9)	521(12.5)	154(16.2)	447(54.1)	1702(18.5)	11386
2014	580(18.4)	541(12.5)	157(16.5)	459(68.5)	1737(19.1)	12136
2015	541(17.2)	601(13.4)	176(17.4)	484(67)	1801(19.3)	12140
2016	590(18.4)	620(13.9)	207(20.1)	482(71.5)	1899(20.2)	12589
Growth (%)	3.1	5.2	6.7	3.2	4.1	3.0
Overall growth (%)	7.5	6.2	11.1	1.5	5.4	2.8

Source: Natural Rubber Trends and Statistics, ANRPC (various issues); Rubber Statistical Bulletin, IRSG (various issues).

Figures in parentheses show domestic NR consumption as a percentage of domestic production

With the current per capita NR consumption in India being one of the lowest in similar economies, the country will require unhindered access to this critical raw material for the continued growth of the industry. Over-dependence of the Indian rubber industry on imported NR may pose serious challenges to its sustainable growth and competitiveness. The longer the present decline in domestic NR production continues, the more difficult it will be to reverse the trend because of the perennial nature of this crop. Therefore, proactive steps to sustain the domestic NR production base with adequate public investment are urgently required to ensure sustained domestic supply of NR to the Indian rubber industry.

4. Comparative advantage and export performance of India's rubber sector: An exploratory analysis

Despite India's positive balance of trade in rubber and rubber products since the 1970's there has been considerable deterioration in the balance of trade over time. As a result of higher growth in imports and lower growth in exports in the last ten years ending in 2016-17, rubber and rubber products showed negative balance of trade consistently. In 2016-17, the country had a negative balance of trade of US\$ 414.76 million dollars. The study showed that despite a comparative disadvantage of the rubber sector at the aggregate level in the world market, the three product groups, viz. reclaimed rubber in

primary forms or in plates, sheets or strip (HS 4003), inner tubes of rubber (HS 4013) and hygienic or pharmaceutical articles (including teats) of vulcanised rubber other than hard rubber, with or without fittings of hard rubber (HS 4014) exhibited comparative advantage consistently throughout the twenty one year period. The two major product groups viz. new pneumatic tyres of rubber (HS 4011) and other articles of vulcanised rubber other than hard rubber (HS 4016) which accounted for 68.7 per cent of the total value of exports from the sector showed varied trends. The study indicated the limitations of Indian rubber sector which was nurtured under a larger domestic market to emerge as a major player in the world market.

QUALITY CONTROL DIVISION

The Central Quality Control Laboratory, established in 1980, is undertaking various tests for raw natural rubber, chemicals, fertilizers, water, effluent water *etc.* which is beneficial to the growers, processors and the general public.

The laboratory is ISO:17025 certified and accredited by NABL for performing various tests in natural rubber and water. Quality Control Division jointly with Bureau of Indian Standards (BIS) implements and monitors ISI marking process for the processed rubber in the country and conducts inspections to such units. The Division plays a major role in the quality enforcement of processed natural rubber, quality

of imported and exported rubber and the issue of NOC for import

1. Commercial Testing Section

The Division has conducted commercial testing of preserved field latex, concentrated latex (Cenex and Creamed), dry rubber (ISNR, RSS, EBC, skim crepe) chemicals used in rubber processing, rubber product manufacturing and plant protection, fertilizers and organic manures, effluent water from rubber processing units and drinking water and water for civil construction. Details of samples tested during 2017-18 are presented (Table QC. 1). An amount of Rs. 33,51,679/- was collected as testing fee during the year.

Table QC. 1. Details of samples tested during 2017-18

Sl. No.	Type of samples	Testing details during the FY 2017-18	
		No. of samples	No. of parameters
1	Field latex	5793	6100
2	Dry rubber	3193	10765
3	Concentrated latex	236	1620
4	Water	512	5663
5	Effluent	106	700
6	Chemicals	295	594
	Total	10135	25442

2. Specification Section

2.1. BIS scheme of testing and inspection

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

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

For implementation of the scheme of testing and inspection (STI), BIS has appointed Rubber Board as their agent and 66.67 per cent of the marketing fee collected is paid to the Rubber Board. The scheme covers the natural rubber processing units in the four states, viz. Kerala, Tamil Nadu, Karnataka and Tripura.

Inspections were conducted during the period under BIS scheme and an amount of Rs. 26,13,732/- was received as share of marketing fee from BIS.

2.2. Preliminary inspection to issue BIS certification license

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

of License by the BIS. One such inspection was conducted during the year.

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

Random surprise inspections are conducted at the rubber processing units for checking the quality of processed block rubber/concentrated latex. During the reporting period 72 quality control inspections were carried out covering 41 dry rubber and 31 latex processing units.

2.4. Quality enforcement for TSR

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

For the enforcement of the above rule in the processing sector, the Rubber Board decided to introduce compounding of offences for violation of quality norms by the processors with effect from 1st January 2013. The compounding amount is Rs. 15,000/- for the first offence and will increase in arithmetic progression for repetition of offences till the 6th offence before revocation of the license of the processor. Eight processing units were compounded for violation of quality norms during the financial year and an amount of Rs. 2,25,000/- was collected from these units.

2.5. Import of natural rubber

It is mandatory that natural rubber imported to India shall conform to Indian Standard Specifications or any other National or International Standard Specifications. NOC for import of natural rubber for own consumption by manufacturers having valid Manufacturers'

License (M license) is issued by Rubber Board, provided all the documents viz. bill of entry, Invoice, test certificate and the request from the importer are in order. Inspections were carried out at random to ensure conformity with these standards. However, in the case of first time import, inspection of the consignment/collection of samples for quality check is always carried out by an authorized Officer of the Board prior to the issue of NOC (Table QC. 2). A total of 73 inspections were conducted during the year and this includes inspections by officers from other offices also.

Table QC. 2. Details of NOC issued for import of NR during 2017-18

Sl.No	Type of NR	Quantity(MT)
1	Block Rubber	384332.27
2*	Sheet Rubber	78662.97
3	Crepe Rubber	126.417
4	Latex	2243.4
5	Others	18.5
	Total	465383.6
No. of consignments of import		:4685

2.6. Export of natural rubber

Rubber Board conducts quality checking to ensure the quality of rubber exported from the country. NR can be exported either under the brand name "Indian Natural Rubber" with logo or as an individual export without logo. Consignments for export are inspected and

samples drawn by the officers of Market Promotion Department in the case of export with logo. All samples are analyzed at the Central Testing Laboratory for conformity with BIS Specifications. Quality certificates are issued based on test results. This procedure is applicable to both block rubber and concentrated latex. Thirty four latex samples and two TSR samples were tested at the Central Testing Laboratory during the reporting period.

3. Other activities

Officers of the Division functioned as resource personnel in various training programs organized by the Rubber Training Institute. (i) Imparted training to M.Tech (Polymer Technology) & B.Tech (Rubber Technology) students of CUSAT and conducted practical examination for them. (ii) Imparted practical training to students of Madras Institute of Technology, Chennai, B.Tech students of M.G. University, Government Engineering College, Thodupuzha, M.Sc. Students of S.B. College, Changanacherry and entrepreneurs from various rubber goods manufacturing sector.

4. Advisory work

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

CENTRAL EXPERIMENT STATION, CHETHACKAL

The Central Experiment Station, Chethackal is situated at a distance of about 56 km from Kottayam. The station has a total land area of 254.76 ha, of which 24.58 ha is under immature rubber, 169.30 ha under mature rubber and 20.35 ha area is under bud wood nursery and close planting.

Field experiments and evaluations of various Divisions are conducted at CES. There are 85 field experiments in the Station. Breeding for high yield and other beneficial secondary characters, evaluation of clones, intercropping, planting systems, good agricultural practices, low frequency tapping systems, disease management *etc.* are the major areas of research undertaken

in the Station. Considerable area is also under Germplasm evaluation, bud wood nurseries and nursery experiments. The Eddy covariance tower installed in the Station continues to give micro environmental data. The station also functions as a centre for training growers in various aspects of farming operations.

During the reporting period, the total crop realized was 183.1 MT. There were 298 tapping days and 73 tappers were engaged for tapping. The total man-days engaged in the year was 43,238. The dispensary attached to the Station caters to the medical needs of the workers and 4,103 patients were given medical care during the period under report.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

I. Crop improvement

I.1. Performance of clone RR11 429 in grower's field in Assam

Girth of RR11 429 was similar to that of RRIM 600. Yield over four years for RR11 429 was significantly higher than RRIM 600 in Goalpara, Assam. The increase in yield was maintained by RR11 429 throughout the year and the increase in average yield was around 27 per cent higher compared to RRIM 600.

I.2. On-Farm evaluation of selected ortets of *Hevea* in Assam

The best three selected ortets from Assam were cultivated in Morigaon, Assam during to evaluate the performance of these primary ortets viz. RRSg 9, RRSg 3 and RRSg 1, RRIM 600

and RR11 429 were the check clones. Girth of ortets at 30 cm height was on par with RRIM 600 after 20 months of planting. Girth in RRSg 3 ranked first followed by RRSg 1.

I.3. On-farm evaluation of potential clones / ortets under the agroclimate of Arunachal Pradesh

Continued the two trials initiated during 2015-16. In trial I, planting of 11 promising clones viz. RRIM 600, RR11 208, RR11 429, RR11 417, RR11 430, RR11 422, RR11 105, PB 235, PB 260, SCATC 88/13 and Haiken 1 was completed. In trial II, budding of RRSg 1, RRSg 9, RRSg 8, RRSN 1, RRSN 47, RRSN 69, RRSA 114, RRSA 585, RRSA 315, RRSA 114, RRSA 585 and RRSA 315 along with check clones RRIM 600 and RR11 429 completed and polybag planting finished.

2. Crop management

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

Fifty soil samples were collected in the initial phase. Soil sample collection from Kamrup (M&R) district of Assam was completed and started collection from Goalpara district of Assam.

2.2. Ground cover management

The study on spread of *Mikania micrantha* from the rubber plantations of Assam indicated

that rubber plantations are an ideal place for growth and spread of *Mikania*. The intensity of infestation was found to be more within and localities closer to the plantations and reduced when moving away outwards. The population load of *Mikania* was high in rubber plantation and resulted in its spread to the nearby areas though the intensity of spread reduced as the distance increased from the plantation.

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The research programs of the Station are mainly focused on evaluation of clones, crop management, latex harvesting and ecosystem studies. Advisory services on discriminatory fertilization to growers and latex analysis for industries are being continued.

1. Crop improvement

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

1.1. Development and evaluation of clones

The crop improvement programmes were undertaken for development and evaluation of location specific clones to identify the promising ones for the region. To develop clones for this region, 131 locally recruited selected clones viz 20 hybrids, 21 OP progenies and 90 half-sib progenies are under evaluation in six clonal and seedling nurseries.

Evaluation of clones for their adaptability and yield performance undertaken in two On Farm Trials (OFT) and two Large Scale Trials (LST) are in progress. In the first LST, DD/6/5 showed highest girth (17.7 cm) during third year of planting compared to control RRIM 600 (13.1 cm). In the other LST (GxE trial), highest yield was observed in clone RRII 429 (74.3 g t⁻¹ t⁻¹) followed by RRII 422 (64.3 g t⁻¹ t⁻¹) and control RRIM 600 (62.2 g t⁻¹ t⁻¹).

High yielding clones are under evaluation in one mature and one immature OFT and RRII 429 recorded the highest mean yield (37.7 g t⁻¹ t⁻¹) in OFT, Pathalia, which was higher than RRIM 600 (33.6 g t⁻¹ t⁻¹) in the fourth year of tapping. Clone RRII 429 had the highest girth (60.7 cm) in another OFT, Hirapur.

In another GxE trial with 45 pipeline clones and four check clones, high yield performance was observed in P60 (107 g t⁻¹ t⁻¹) followed by P44 (97 g t⁻¹ t⁻¹). Twenty six clones had better performance than RRIM 600 (check clone) while

nine clones were better than RR11 430 (another check clone).

The field trial involving 57 clones for the identification of reliable juvenile and mature characteristics for clone identification is in progress. A germplasm garden having 213 wild *Hevea* accessions, source bush nurseries and a breeding orchard are being maintained in the Station.

2. Crop management

2.1. Soil fertility mapping

To prepare a soil fertility map for rubber growing regions of North East India and to develop an on-line fertiliser recommendation, collection of composite soil sample per 50 ha of area was initiated in the state of Tripura. The details of the sampling location like clone, age of plantation, planting history, cultivation practice and location co-ordinates are being recorded using GPS. Total 677 composite soil samples at the depth of 0-30 cm were collected during the year, covering three districts of Tripura viz. West, Sepaijhala and Kowhai. Analysis of soil samples showed that 56 per cent of these samples were medium in OC content, 85 per cent was low in available P and 60 per cent low in available K.

2.2. Development of cropping system and management practices

In the cropping system model, after nine years, the girth of rubber in the intercropping plots (57.8 cm 58.4 cm, respectively, for Model I and II) and in monocropped plots (59.2 cm and 59.5 cm respectively for Model I and II) is 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). In this trial, pineapple was retained and yield was 567 kg ha⁻¹ in the ninth year.

Growth of rubber plants showed that pits of larger dimensions have no advantage compared to smaller dimension pits six years after planting. The mean girths were 40.2 cm for normal pit and 39.4 and 37.8 cm for two reduced size pits, respectively. For confirmation of this results an on farm trial was initiated on reduced pit size at Twaisaplang-I block plantation unit. Girth recorded after second year of planting showed no difference between normal and reduced pit size. The mean girth was 22.4 cm for normal and 21.8 cm for reduced pit size.

Results of the experiment on specific package of practices, show that the mean girth of rubber in vertical and conventional mulching is statistically on par but it is superior to control with a girth of 47.4, 48.1 and 42.5cm for conventional mulching, vertical mulching and control, respectively. Apart from that, the percentage of plants less affected by summer stress is least in vertical mulching plots. Moreover, moisture per cent in 0-60cm layer of soil was higher in vertically mulched pits than in conventional mulching and control i.e. 15.3, 14.5 and 12.3 per cent, respectively in the sixth year.

An observational trial was initiated during the period under report with a view to study the influence of silt pits on growth and yield of rubber grown in the hillocks (slope of 5-7%) of Tripura. No significant improvement in girth of the plants was recorded. However, some increase in yield was observed due to imposition of silt pits. Plants under silt pit registered annual yield of 1063 kg ha⁻¹ and without pit registered an 916 kg ha⁻¹ during 1st year of tapping. An amount of 3.56 tonnes of soil ha⁻¹ was recovered in one year.

3. Latex harvesting

The low frequency tapping in clone PB 235 has been continuing in three systems of tapping viz. d3, d4 and d6. Higher yield was observed in d3 compared to d4 and d7 system.

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

Study on the effect of planting density on d3 tapping system is being continued with clone RRII 429. Higher yield was observed in high density planting compared to lower density. Another experiment on block trial on d3 and d7 system of tapping was initiated with clone RRII 600.

4. Ecosystem studies

Invasive potential of the cultivated tree species (*Hevea*) and three weed species differing in habit and bio-geographic origin were assessed through ecological niche modelling in the 2000 and 2050 climate scenarios in North-East India. It was observed that *Ageratum conyzoides* and *Hevea brasiliensis* with South American origin exhibited higher potential to invade/distribute in North eastern region of India by 2050. On the other hand, though *Urena lobata* and *Imperata cylindrica* are of South Asian origin, their potential to invade further in North East India by 2050 is limited compared to its distribution in 2000.

In another study, challenges for predicting distribution of *Hevea brasiliensis* in India based on its native range was analysed. Evidence of shifting niche in case of *Hevea* species through assisted migration was obtained. Other findings

indicated that the realized niches of *Hevea* trees in its native range and introduced range are different. Hence, the concept of niche conservatism across the different introduced range is observed to be challenged, and the proposition of niche shifting is evidenced in case of *Hevea* trees in India.

Maxent modelling of *Hevea* suitability in LWE region was attempted. Bioclimatic data of this region (Andrapradesh, Bihar, Chattisgarh, Maharashtra, Madhyapradesh, Odisha, Telengana, Uttarpradesh and West Bengal) in respect to 2050 climate scenario were used for predicting suitability of *Hevea* in LWE region. The existing rubber plantation of Kerala and some locations in Odisha, Andra Pradesh and West Bengal were taken as reference climate of rubber while interpolating the modelling results of LWE region.

An ecological study was initiated in 20 year old rubber-tea intercropping trial at Taranagar farm. It was observed that eight weed species were found in the tea rows and 14 weed species in rubber rows over two seasons. In another 30 year old pure rubber plantation, 23 species of weeds were observed when the plantation was unweeded for 20 years.

5. Advisory work

Discriminatory fertilizer recommendation based on soil analysis was offered to 147 rubber growers in the state of Tripura. A total of 96 latex samples were analyzed for drc and other latex parameters. Total 3080 m of bud wood of high yielding clones were supplied to the growers.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

The Station continued its research activities on evaluation of clones, polyclonal population, crop physiology, latex harvest technology and crop management practices suitable for this region.

1. Crop Improvement

1.1. Poly-cross progeny evaluations

Thirty per cent of the promising polycross progenies from 2008 and 2011 trials were maintained in nursery. A nursery trial was initiated from 2008 selections.

1.2. On-farm evaluation of selected clones

Three on farm trials were started during 2009 and 2010. Trial I includes blocks of six clones, viz. RRII 417, RRII 422, RRII 429, PB 235, RRII 203 and RRIM 600, in Mendipathar (North Garo Hills) and Trial-II includes four clones viz. RRII 417, RRII 422, RRII 429 and RRIM 600 in Bolchugre, West Garo Hills. In North Garo Hills 2nd year tapping was started during August 2017 onwards in both the trials. The highest mean annual yield was recorded in RRII 429 ($35.0 \text{ g t}^{-1} \text{ t}^{-1}$) followed by RRIM 600 ($32.9 \text{ g t}^{-1} \text{ t}^{-1}$) and RRII 422 ($32.0 \text{ g t}^{-1} \text{ t}^{-1}$) and minimum yield was recorded in PB 235 ($22.1 \text{ g t}^{-1} \text{ t}^{-1}$)

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

The poly clonal seeds collected from Poly Clonal Seed Garden, Mizoram were planted in the field during 2013 at two locations viz. Ganolgre farm of RRS and Rubber Board campus, Dakopgre, Tura. On the basis of test tap yield, top 25 best performing progenies were selected and maintained in the budwood nursery at Rubber Board campus, Tura.

1.4. Germplasm arboretum at Teksragre farm

To maintain the Germplasm Arboretum under the agro-climatic condition of Garo Hills of Meghalaya 534 poly bag plants (belonging to 178 Accessions) were planted in the field at Teksragre farm near Anogre and maintained. From a total of 692 budded stumps belonging to 82 accessions and 10 control clones sprouting success was only 24.3 per cent. Field planting will be carried out during May/June, 2018.

2. Crop Physiology/Latex Harvesting Technology

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

Severe low winter temperature is one of the major reasons for yield depression and low dry rubber content in *Hevea* under the agro-climatic condition of Garo Hills. Low temperature adversely affected the yield and DRC. The annual mean yield ($34.6 \text{ g t}^{-1} \text{ t}^{-1}$) and DRC (33.8 %) was recorded for the year. Early defoliation and refoliation was observed and DRC ranged from 28.5-29.2 per cent during winter season. Soil moisture content was the lowest in the months of February and March.

2.2. Location specific stimulant application

The study on ethylene induced stress responses in tapping panel of rubber trees was continued. Six treatments were adopted with bark applications of ethaphon (5 %) in RRIM 600. Results showed that highest yield ($46.9 \text{ g t}^{-1} \text{ t}^{-1}$) and low DRC (33.5 %) was recorded in T3 (Bark application of 5 per cent ethaphon at 150 cm above the bud union and near bud union and lowest yield ($32.0 \text{ g t}^{-1} \text{ t}^{-1}$) and high DRC (34.5 %) was recorded in T6 (unstimulated trees). There was no significant difference in DRC between treatments.

3. Crop Management

3.1. Analytical/advisory work for fertilizer recommendation

A total of 24 soil samples from the rubber growing areas indicated that organic carbon (OC) content was at medium range (0.85 to 1.24 %) in the surface soil (0-30 cm), available phosphorus was at low range (2.8 to 7.1 mg kg⁻¹) and available potassium was at medium range (72.0-94.5 mg kg⁻¹). The soil is generally acidic in nature with pH ranging from 4.2-5.8 and fertilizer recommendation was given accordingly.

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

The collaborative project with Crop Management group of RRII, Kottayam was

continued and 111 composite soil samples (0-30 cm depth) were collected during the year from the rubber growing areas of Garo hills of Meghalaya (West, North, East and South-west district) using GPS system, with the help of Rubber Board, Regional Office, Tura. So far around 194 soil samples were collected. Analysis of soil samples will be completed within two years. All the soil samples were air dried and processed and are ready for analysis at Soil Testing Laboratory, RRII, Kottayam.

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

The experiment is repeated with all the treatments for further confirmation of the best performing potting media/treatments.

REGIONAL EXPERIMENT STATION, NAGRAKATA, BENGAL

1. Crop Improvement

1.1. Evaluation of clone

Twenty six promising clones were evaluated under the agro-climatic conditions of sub-Himalayan West Bengal. At 26th - 27th year of

planting, trunk girth of clones viz. PR 107, SCATC 93/114, RRIM 703, RRIM 605, RRII 118 and RRIM 612 was significantly higher than RRIM 600. Clones, PB 310 and PB 280 recorded significantly higher rubber yield than check clone RRIM 600 (Table Nag. 1).

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

Trial I and II	Yield (g t ⁻¹ t ⁻¹)	Trial III	Yield (g t ⁻¹ t ⁻¹)	Trial IV	Yield (g t ⁻¹ t ⁻¹)
RRIM 612	21.7	PB 86	36.6	RRII 118	41.7
GL 1	23.8	RRII 203	36.9	PB 235	42.6
RRII 105	26.7	PB 5/51	37.2	RRIM 703	42.8
RRIC 104	27.3	GT 1	37.2	RRII 208	43.5
RRIC 102	31.9	RRII 308	38.0	SCATC 88/13	45.4
RRIM 605	32.4	PR 107	38.2	PB 280	47.0*
PB 260	33.8	HK 1	38.3	PB 310	48.1**
SCATC 93/114	34.6	RRII 300	40.5	RRIM 600	38.9
PR 261	35.8	PB 311	40.9	CD (P=0.05)	6.79

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

1.2. Evaluation of germplasm

A total of 21 germplasm accessions were evaluated under the agroclimate of Nagrakata, Bengal. Maximum girth was recorded in RO 2629, MT 44, RO 5430, RO 2635, MT 196, MT 2229, AC 619, RO 5557, RO 2890, RO 3172 and RO 5348. Dry rubber yield of AC 763 was significantly higher than check clone, RR11 105. In general, the growth and yield performance of Mato Grosso accessions were found better than Acre and Rondonia accessions in this station.

1.3. Performance of polyclonal seedlings

Since 1990, a study on performance of polyclonal seedlings has been under progress in Nagrakata in a block of 240 trees in CRD. After 27 years of planting, mean girth of the population was 79.3 cm. The average block yield of the population was 28.5 g t⁻¹ where 36 per cent plants showed above average yield. Selected Orlets are maintained at the nursery for further evaluation.

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

Half sib progenies were raised from seven different clones in 2014. The juvenile yield of progenies raised from seeds of SCATC 88/13 during non-winter period showed the highest yield followed by RO 5363. Number of plants having above average juvenile yield also showed same trend. During winter period, the average juvenile yield of SCATC 88/13 progenies was higher than that of RRIM 600, followed by RR11 417 and RR11 429. Number of seedling plants showing above average juvenile yield was also higher in SCATC 88/13. The potential of half-sib progenies of SCATC 88/13 was shown to be prominent from this study.

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

Evaluation of five promising new generation clones under the cold agroclimate of sub-Himalayan West Bengal showed that girth and yield of all the clones were on par with RRIM 600. However, yield of RR11 422 was found high followed by RR11 429 and RR11 417 during this year. Comparatively RR11 414 recorded lower yield than other clones in this region.

2. Crop Physiology

2.1. Performance of polycross progenies raised from seeds of locally adapted mature rubber plantation

Mean rubber yield of the population was 34.6 g t⁻¹ and average yield of seedlings raised from seeds collected from four different places were similar ranging from 2.5 g t⁻¹ to 117.6 g t⁻¹. Twenty promising ortet mother plants recorded an yield potential of around 60 g t⁻¹ on 2nd year of tapping.

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

A total of five clones were under evaluation in high pH soil. Growth of all five clones was at par with that of RRIM 600 in high pH soil except RR11 422 which showed significantly lower growth. In normal soil, growth of all the five clones was at par with RRIM 600 indicating high pH sensitiveness of clone RR11 422.

2.3. Evaluation of Orlets for abiotic stress tolerance in different agro-climatic regions

Juvenile yield of the check clone RRIM 600 was high in both non-winter and winter periods followed by RRST 24, RRSg 1 and RRST 37 during non-winter and (Table Nag. 2) during winter period RRST 37, RRSg 1 and RRSN 69 recorded better yield following the check clone RRIM 600.

Table Nag.2. Girth and yield of ortets/clones

Ortet/Clone	Juvenile yield (g t ⁻¹ t ⁻¹⁰)		Ortet/Clone	Juvenile yield (g t ⁻¹ t ⁻¹⁰)	
	Non-winter	Winter		Non-winter	Winter
RRSD 1	51.8	142.0	RRST 24	62.6	146.1
RRSD 34	16.6	95.3	RRST 37	59.0	281.3
RRSD 35	43.6	124.0	RRST 39	41.6	85.4
RRSD 36	27.5	121.9	RRII 414	44.4	203.2
RRSG 1	60.7	276.6	RRII 417	38.9	238.9
RRSG 3	47.0	165.4	RRII 422	62.4	190.6
RRSG 9	28.6	210.1	RRII 429	55.1	256.8
RRSN 1	33.7	141.3	RRII 430	23.5	126.3
RRSN 47	44.9	165.3	RRII 105	28.5	230.5
RRSN 69	44.0	236.5	RRII 414	44.4	203.2
RRSA 315	28.9	104.5	CD (P=0.05)	NS	NS
RRSA 585	39.5	147.1			
RRSA 98	30.8	95.6			

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

REGIONAL RESEARCH STATION, DAPCHARI, MAHARASHTRA

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

1. Crop Improvement

A total of 12 experiments are being conducted to evaluate the growth and yield performance of clones, pipeline clones, hybrid

clones, ortets, wild *Hevea* accessions, half sib progeny, polycross seedlings, different root stock plants and root trainer plants.

1.1. Large scale trials

1.1.1. Evaluation of selected ortets

A trial started during 2008 to evaluate the growth and yield performance of ortets selected from polycross seedling planted at this station with control clones. Significant difference was noticed in girth. Girth among the ortets and check clones ranged from 26.7 cm (OS 42) to 40.2 cm (OS 236). Among the check clones, RRII 430 recorded highest girth of 37.7 cm while lowest girth was noticed in clone RRII 105 (33.0 cm). All ortets are at par with OS 236 except OS 42

and RRII 105. It was also noticed that all ortets were superior in girth than clone RRII 105 except ortet OS 8 (28.7 cm). Significant difference was noticed in branch height and it ranged from 198.0 cm (OS 136) to 288.6 cm (RRIM 600). All clones are at par with OS 136 except OS 8, OS 30, OS 42, OS 135, OS 173 and RRII 208. Significant difference was noticed in test tapping yield and ranged from 11.7 g (OS 42) to 34.7 g (OS 136). Among the check clone, RRII 430 gave the highest test tap yield of 31.2 g and clone RRII 105 recorded 16.9 g only. More than 25 g was recorded in OS 1, OS 35, OS 37, OS 136, OS 173, OS 230 and RRII 430.

1.2. Further field evaluation trial

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

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

The experiment was laid out using 25 potential drought tolerant wild *Hevea* accessions for drought under Dapchhari condition along with five HP clones and RRII 105, RRIM 600, Tjir 1, RRII 430 and RRII 208 as check clones in Augmented RBD. In order to confirming the drought tolerance potential of selected wild accessions from various preliminary field screenings and five HP clones they were grown at normal spacing at drought prone region and subjected to detailed studies and mature yield was recorded.

- The accessions showed wide variability for all the characters studied, after experimenting nine summer periods from 2008 to 2016. Four wild accessions recorded

girth higher than the proven drought tolerant clone RRIM 600.

- MT 40 recorded highest girth at 9th year under drought. RRII 430 and RRII 414 showed significantly better growth than RRIM 600 under the prevailing drought. Hybrid 93/270 recorded the highest girth.

1.2.2. Small scale further field evaluation trial of selected wild accession for drought tolerance (2010)

Experiment was initiated with 47 selection from wild *Hevea* accessions along with four check clones (RRII 105, RRII 208, RRII 430 and RRIM 600) to confirm the drought tolerance potential of selected seven wild accessions from preliminary field screening in 2003, by growing them at normal spacing at drought prone region and subjecting them to detailed studies along with recording mature yield.

1.3. Clonal nursery evaluation

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

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

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

1.3.3. *Clonal nursery evaluation of pipeline clones (2011)*

Trial laid out in 2011 in rectangular lattice experiment with pipeline clones (50 clones with 2 check clones RRII 105 and RRIM 600) to identify drought tolerant clones for their adaptability and stability to this agro climatic condition of Maharashtra is in progress. Clonal responses for field establishment were assessed. Standard cultural practice for establishment and early growth will be carried out. Recording of all growth, biochemical and physiological parameters are being carried out.

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

A half sib progeny of 15 clones planted in 2011 in the Station for selection of primary ortets to study the growth and yield performance of polyclonal seedling at early stage in the nursery and to examine the scope for the early selection based on dependable juvenile traits under rainfed condition is also in progress. The evaluation of poly cross progeny through nursery screening of the seedlings obtained from open pollinated polyclone evaluation trial is a step towards the nursery selection and identification of few superior locally adapted clones. Test tapping in peak winter and peak summer season were done.

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

Trials aimed at evaluation of progenies for promising individuals, selection and multiplication of superior individuals, study of yield, growth and other secondary characters of selections in clonal nurseries and evolving locally adaptable clones are in progress. Seeds were collected from multiclinal trials, polycross seeds and polyclonal

seed garden of Mizoram, Karnataka and Tamil Nadu.

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

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

1.4. *Germplasm screening*

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

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

A screening for wild *Hevea* accession (130) for drought under Dapchhari condition which was laid out in 2003 with RRII 105, RRIM 600 and Tjir 1 in augmented block design. Mato Grosso accessions were found superior for all growth characters studied than those from Rondonia and Acre provenances. Twenty five potential drought tolerant accessions were identified based on three to four years of field performance and further studies are in progress.

2. *Environmental Physiology*

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

The trial started in 2011 to evaluate the physiological and biochemical adaptation potential and common mechanisms involved in cold and drought tolerant traits using molecular physiology/

biochemical tools for ortet selections from cold and drought by interchanging the clones to different agro-climatic regions and to study the GxE interaction for growth and yield under varying agro-climates.

Significant difference in girth was recorded. Girth ranged from 11.2 cm in RRST 24 to 20.7 cm in RRII 414. Among the ortets RRSA 315 recorded higher girth of 20.6 cm in September 2017 and continued to attain higher girth in March 2018 (26.4 cm) and clones/ortets *i.e.*, RRIM 600, RRII 417, RRII 422, RRII 429, RRII 430, DAP 35, all ortets from RRSA and NGK, RRST 37 are at par with RRII 414. In general, ortet RRSA was found superior in girth (19.6, 24.2 cm during September 2016 and March 2018 (Table Dap. 1) with NGK occupying IInd rank and Dap ortets occupying IIIrd ranks. GH ortets were poor performer under this agro-climatic condition. A significant difference was noticed in branch height and the highest branch height was noticed in RRST 37 (262.6 cm) and lowest in GH 9 (183.3 cm). RRII 414, RRII 422, RRII 430, DAP 35, RRSA 98, RRSA 315, RRSA 585 and RRSD 39 are at par with RRST 37. In general RRSA recorded better growth performance (238.4 cm) while GH ortets recorded lowest (194.0 cm).

Significant difference in test tapping yield was also observed and it ranged from 8.7 g in GH3 to 96.2 g in RRII 417. In general, RRST recorded the highest test tap yield (63.1 g) and NGK recorded the lowest test tap yield of 35.8 g.

Table Dap. 1. Growth parameters in various ortets selected from various location of India (2017-18)

Clones/ortets	Girth (cm)		Branch ht. (cm)		Test tapping (g)
	Sep-17	Mar-18	Mar-18	Nov-17	
RRII 105	15.1	21.7	169.8	61.8	
RRIM 600	16.4	19.7	221.7	52.5	
RRII 414	20.7	25.6	231.0	63.1	
RRII 417	19.9	24.5	209.3	96.2	
RRII 422	17.9	21.1	254.0	56.7	
RRII 429	20.6	24.7	207.7	48.2	
RRII 430	18.2	22.1	240.8	73.7	
DAP 1	13.2	21.0	196.3	56.3	
DAP 34	13.8	17.0	226.3	22.1	
DAP 35	15.8	19.5	256.3	47.8	
DAP 36	11.7	15.8	197.8	17.5	
RRSA 98	19.6	23.2	256.8	40.2	
RRSA 315	20.6	26.4	227.5	65.4	
RRSA 585	18.6	23.1	230.8	69.6	
NGK 1	16.4	19.7	199.9	27.3	
NGK 47	14.2	17.2	188.3	41.7	
NGK 69	14.8	17.5	215.8	38.3	
GH 1	14.9	17.4	206.8	41.2	
GH 3	12.2	15.9	192.0	8.7	
GH 9	12.0	16.8	183.3	91.3	
RRST 24	11.2	19.0	169.5	41.0	
RRST 37	15.8	21.2	262.6	71.2	
RRST 39	13.1	21.7	238.8	77.0	
DAP	13.7	18.3	219.2	35.9	
RRSA	19.6	24.2	238.4	58.4	
NGK	15.2	18.1	201.4	35.8	
GH	13.0	16.7	194.0	47.0	
RRST	13.4	20.6	223.6	63.1	
Mean	15.9	20.5	216.7	52.5	
CD (P=0.05)	5.1	5.9	42.9	37.3	

REGIONAL RESEARCH STATION, DHENKANAL, ODISHA

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

1. Crop Improvement

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

1.1. Clone evaluation

In trial 1 (1987), the elite clone RR11 105 recorded highest mean yield of 42.2 g t⁻¹ and GT 1 recorded the lowest yield (33.4 g t⁻¹). However, GT 1 recorded significantly higher mean girth (82.7 cm) over RR11 105 (75.8 cm) and RR11 600. In terms of growth all three clones performed well in the region (Table Odi.1).

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

Clone	Yield (g t ⁻¹)	Girth (cm)
RR11 105	42.2	75.8
RR11 600	36.6	79.9
GT 1	33.4	82.7
CD(P=0.05)	---	4.6

In another clone trial (1990), SCATC 88-13 (75.5 g t⁻¹), RR11 208 (71.1 g t⁻¹) and RR11 600 (71.8 g t⁻¹) were found to be the most high yielding clones. Other popular clones also

performed well in the region. SCATC 93-14 recorded comparatively lower yield (41.8 g t⁻¹). However, SCATC 93-14 recorded highest growth in terms of girth, followed by SCATC 88-13 and RR11 208 (Table Odi. 2).

Table Odi. 2. Growth and yield performance of clones

Clone	Yield (g t ⁻¹)	Girth (cm)
Haiken 1	54.5	88.5
RR11 600	71.8	86.2
RR11 701	55.8	88.7
RR11 5	55.2	88.9
SCATC 88-13	75.5	94.5
SCATC 93-14	41.8	95.1
PB 310	56.0	89.1
RR11 208	71.1	89.3
PCK 1	58.3	89.0
RR11 300	55.2	89.1
CD (P=0.05)	11.9	—

In the 1991 experiment, clones differ significantly in mean yield. RR11 208 (85.9 g t⁻¹) and RR11 105 (78.8 g t⁻¹) and RR11 102 (75.0 g t⁻¹) recorded highest yield among the clones. Polyclonal seedling population (56.4 g t⁻¹) yielded low though having better growth and adaptability under the prevailed stress conditions (Table Odi. 3).

Table Odi. 3. Performance of different clones in the region

Clone	Yield (g t ⁻¹ t ⁻¹)	Girth (cm)
GT 1	65.1	100.1
RRII 105	78.8	87.1
RRII 208	85.9	97.0
RRII 5	69.1	90.3
RRII 300	68.0	95.1
PR 261	63.6	88.2
PR 255	65.9	95.4
RRIC 102	75.0	96.6
RRIM 600	71.7	88.1
Polyclonal	56.4	113.2
CD (P= 0.05)	NS	8.93

In the modern clone trial of 2000, the highest mean yield was observed in RRIM 600 (65.7 g t⁻¹ t⁻¹) and IRCA 111 (63.3 g t⁻¹ t⁻¹) and the lowest mean yield was recorded in RRII 51 (27.1 g t⁻¹ t⁻¹). Highest growth in terms of girth was observed in RRII 300 (68.8 cm) (Table Odi. 4).

Table Odi. 4. Yield and growth performance of clones

Clone	Yield (g t ⁻¹ t ⁻¹)	Girth (cm)
RRII 300	47.9	68.8
RRII 208	53.1	59.2
RRII 357	42.5	58.9
RRII 352	55.1	58.2
PB 28/59	59.0	61.1
RRIM 600	65.7	62.3
RRII 351	57.4	57.6
RCA 109	47.6	49.0
RRII 105	42.4	55.1
RRII 51I	27.1	58.0
RCA 111	63.3	63.1
CD(P=0.05)	18.6	NS

1.2. Polyclonal ortet evaluation

Ortet clones DNKL 3 and DNKL 4 recorded comparatively higher yield and were almost at par with high yielding clone RRII 208 in the region. The ortet DNKL 4 (41.0 cm), followed by OR 5 (40.5 cm) exhibited comparatively good girth attainment.

REGIONAL RESEARCH STATION, PADIYOOR

Identification of locally adaptable clones suited for commercial cultivation in 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 in the Station. A source bush nursery of promising clones/selections is established in the Station for further breeding programs.

1. Crop Management

1.1. Response to applied fertilizers in high yielding clones

The treatments comprised of three clones (RRII 105, RRII 414 and RRII 429) and four levels of fertilizers applied viz. recommended dose (D1), twice (D2) and thrice (D3) the recommended dose and a zero fertilizer (control). Treatment differences in yield were not significant (Table Pad.1).

Table Pad.1. Effect of applied fertilizer on yield

Treatment	Yield (g t ⁻¹ t ⁻¹)		
	RRII 105	RRII 414	RRII 429
D1	78.8	77.4	82.1
D2	84.7	84.3	75.4
D3	113.3	92.5	94.7
Control	83.1	89.1	99.6
CD (P=0.05)	NS		

2. Crop Improvement

2.1. Large scale evaluation of clones

Mean annual yield in the renewed bark of PB 330 (Table Pad.2) was the highest (63.4 g t⁻¹ t⁻¹) and was on par with IRCA 130 (59.2 g t⁻¹ t⁻¹). Summer yield of PB 255 was the highest at 36.8 g t⁻¹ t⁻¹ and was on par with IRCA 130 (32.8 g t⁻¹ t⁻¹), PB 330 (31.9 g t⁻¹ t⁻¹) and RRII 105 (30.1 g t⁻¹ t⁻¹).

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

Clones	Mean annual yield (g t ⁻¹ t ⁻¹)	Summer yield (g t ⁻¹ t ⁻¹)
PB 255	55.6	36.8
PB 314	55.4	17.7
PB 330	63.4	31.9
PB 28/59	51.8	24.5
RRIM 703	28.9	17.8
IRCA 18	39.7	22.6
IRCA 109	41.5	27.0
IRCA 111	35.0	21.8
IRCA 130	59.2	32.8
IRCA 230	50.6	19.9
RRII 105	44.4	30.1
CD (P=0.05)	11.2	9.5

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

The average annual yield of clones RRII 203, RRIC 100 and PB 86 and the ortets P 213, P 270 and Irrity were on par and significantly superior to that of RRII 105 and other clones/ortets tested. Summer yield of clones RRII 203, RRIC 100 and PB 86 and the ortets P213, P270 and Irrity were on par and significantly superior to RRII 105 (Table Pad. 3).

Table Pad.3. Yield performance in high altitude region Ambalavayal

Clones	Mean yield (g t ⁻¹ t ⁻¹)	
	Annual yield	Summer yield
RRII 105	28.0	20.5
RRII 203	58.0	39.4
RRIC 100	63.3	45.6
RRIC 102	10.5	9.0
PB 86	69.9	48.8
P 1	30.9	24.9
P 2	26.0	19.8
P 90	37.2	26.4
P 121	31.5	22.5
P 155	36.1	22.9
P 213	59.0	51.9
P 270	52.7	38.5
P 280	31.2	24.5
P 296	33.6	20.9
Irrity	60.9	38.7
CD (P=0.05)	33.9	24.5

HEVEA BREEDING SUB-STATION KADABA, KARNATAKA

South coastal Karnataka, a non-traditional region for rubber cultivation in India, experiences climate similar to that of traditional area except for prolonged drought and severe incidence of diseases viz. pink disease, *Phytophthora* and *Corynespora* leaf fall diseases. The Station was established in 1986 to develop suitable clones for the region with a research farm having an area of 47.6 ha at Nettana. Crop improvement programmes in the Station are aimed at developing and screening clones having high growth and yield along with tolerance to abiotic and biotic stresses. Identification of high yielding vigorous clones achieved through breeding strategies like hybridization, polycross breeding and ortet selection, will help in releasing clones suited for commercial cultivation in this region.

1. Large scale clone trial (1990)

In the large scale clone trial (1990A), 14 clones were evaluated along with check clone RR11 105. Clone HP 372 recorded the highest girth (109.8 cm) and was significantly superior to check clone RR11 105 (77.9 cm). Clones HP 223, PB 235, PB 260, Mil 3/2, HP 185, PB 217, GT 1 and PB 311 also recorded significantly higher girth than RR11 105. In clone trial 2017, HP 372 ($100.1 \text{ g t}^{-1} \text{ t}^{-1}$) had significantly higher mean yield than RR11 105 ($67.1 \text{ g t}^{-1} \text{ t}^{-1}$) under S/2 d6 6d/7 system of tapping with stimulation. Mean yield over 14 years was significantly higher for clone HP 372 ($69.4 \text{ g t}^{-1} \text{ t}^{-1}$), PB 235 ($66.4 \text{ g t}^{-1} \text{ t}^{-1}$), HP 223 ($65.8 \text{ g t}^{-1} \text{ t}^{-1}$) and PB 260 ($63.0 \text{ g t}^{-1} \text{ t}^{-1}$) compared to RR11 105 ($53.6 \text{ g t}^{-1} \text{ t}^{-1}$).

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

In the trial 1991A with 36 clones, PB 280 recorded the highest yield ($119.3 \text{ g t}^{-1} \text{ t}^{-1}$) in 2017, under S/2 d6 6d/7 system of tapping with stimulation, followed by PB 235 and RR11 203 which were significantly superior to RR11 105 ($43.1 \text{ g t}^{-1} \text{ t}^{-1}$). Clone PB 280 recorded the highest mean yield over 13 years of tapping ($84.0 \text{ g t}^{-1} \text{ t}^{-1}$) followed by PB 235 ($78.3 \text{ g t}^{-1} \text{ t}^{-1}$), and RR11 203, PB 314, PB 312 and RR11 300 which also recorded significantly superior yield than RR11 105 ($49.8 \text{ g t}^{-1} \text{ t}^{-1}$). Among the 10 clones which recorded significantly higher girth than RR11 105 (70 cm), clones RR11 203 (101.1 cm), PB 235 (97.3 cm) and PB 217 (94.1 cm) recorded highest girth.

Thirteen clones were evaluated in 1991 B, of which RR11 5 ($102.0 \text{ g t}^{-1} \text{ t}^{-1}$) followed by RR11 3 ($74.9 \text{ g t}^{-1} \text{ t}^{-1}$) recorded the highest yield, while check clones RR11 105 and GT 1 had a mean yield of $42.3 \text{ g t}^{-1} \text{ t}^{-1}$ and $54.7 \text{ g t}^{-1} \text{ t}^{-1}$, respectively in 2017. Mean yield over 13 years of tapping was also the highest for RR11 5 ($74.7 \text{ g t}^{-1} \text{ t}^{-1}$) followed by RR11 3 ($60.5 \text{ g t}^{-1} \text{ t}^{-1}$), while RR11 105 recorded a mean yield of $42.6 \text{ g t}^{-1} \text{ t}^{-1}$.

In trial 1991 C with 13 clones, PB 28/59 ($87.9 \text{ g t}^{-1} \text{ t}^{-1}$) had the highest yield in 2017, while GT 1 and RR11 600 recorded $74.6 \text{ g t}^{-1} \text{ t}^{-1}$ and $42.9 \text{ g t}^{-1} \text{ t}^{-1}$, respectively during the period. Clone PB 28/59 had the highest girth in the trial (103.9 cm) while RR11 600 had 68.4 cm girth at 26 years after planting.

3. Large scale trial (2000)

The large scale trial planted for the evaluation of RRII 400 series clones viz. RRII 403, RRII 407, RRII 414, RRII 422, RRII 429 and RRII 430 along with their parent clones viz. RRIC 100 and RRII 105 in the year 2000 is in progress. Although there was no significant difference between the tested clones

in 2017, RRII 430 recorded the highest mean yield ($121.1 \text{ g t}^{-1} \text{ t}^{-1}$) followed by RRII 414 ($87.1 \text{ g t}^{-1} \text{ t}^{-1}$) (Table Kad. 1). Mean yield over nine years was the highest in RRII 430 ($80.4 \text{ g t}^{-1} \text{ t}^{-1}$) and this was significantly superior to RRII 105 ($53.8 \text{ g t}^{-1} \text{ t}^{-1}$). Mean girth at 17 years after planting was significantly superior in clones RRII 414 (87.3 cm) and RRII 430 compared to RRII 105 (69.8 cm).

Table Kad.1. Yield and growth performance of clones in large scale trial (2000)

Clone	Mean yield in 2017 ($\text{g t}^{-1} \text{ t}^{-1}$)	Mean yield over 9 years ($\text{g t}^{-1} \text{ t}^{-1}$)	Mean girth at 17 years after planting (cm)
RRII 100	69.5	51.9 ^{cd}	77.3 ^{bc}
RRII 105	74.3	53.8 ^{bcd}	69.8 ^{cd}
RRII 403	81.3	56.2 ^{bcd}	65.5 ^d
RRII 407	51.9	7.7 ^d	72.8 ^{bcd}
RRII 414	87.1	72.4 ^{ab}	87.3 ^a
RRII 422	77.7	72.2 ^{ab}	67.1 ^d
RRII 429	73.9	68.0 ^{abc}	73.2 ^{bcd}
RRII 430	121.1	80.4 ^a	80.9 ^{ab}
CD(0.05)	NS	19.29	8.7

Values followed by the same letter are not statistically different at 5 per cent level of probability

4. Polycross garden (1995)

The polycross seed garden planted as per Simmonds (1986) design in 1995, consisting of nine pre-potent clones (RRII 105, PB 215, PB 217, PB 242, RRII 203, PB 5/51, PB 28/83, AVT

73 and Ch 26) is being maintained for collection of seeds for screening and selection.

The research farm also has a well-established Class B Agro-meteorological Observatory.

HEVEA BREEDING SUB STATION, THADIKKARANKONAM, TAMIL NADU

1. Genetic Improvement

1.1. Conventional breeding

Four projects are being pursued in the Station 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 various trials was studied. The block trials initiated in Palazhi and Bethany estates were under fourth year of tapping. Girth of the trial clones along with RR11 105 after 11 years of growth indicated that highest growth was recorded for clones RR11 429, RR11 414 and RR11 422.

The commercial yield data over four years indicated clones RR11 422 and RR11 430 to be the top yielders with 57.9 and 57.8 g t⁻¹ t⁻¹, respectively in the block trial at Bethany estate.

The yield data over four years of tapping recorded at Palazhi estate indicated that the clone RR11 430 was the highest yielder with 93.1 g t⁻¹ t⁻¹.

During the same period, the yield of RR11 105 was 51.3 g t⁻¹ t⁻¹ and 82.2 g t⁻¹ t⁻¹, respectively, at Bethany and Palazhi estates. Further, considerable damage was inflicted to the trees in both the trials as a consequence of the cyclone Ockhi which hit the region during November 2017.

A block trial was planned for evaluating the yield performance of the high yielding clones of the region viz. PB 255, PB 314, IRCA 109, IRCA 111, RR11 203, RR11 414, RR11 417, RR11 422, RR11 430 and RR11 105. Bud grafting of the

clones was undertaken in the central nursery of Arasu Rubber Corporation (ARC) Ltd., at Kuttiyar during the period under report. Planting of the trial is proposed to be taken at Chithar Division of ARC Ltd., during the ensuing season.

1.1.2. Hybridization and clonal selection

The two breeding orchards comprising of 51 parental clones which were properly maintained at the research farm of Thadikkarankonam. During the reporting year, flowering was very sparse and due to the high day temperature followed by a spell of summer shower, the entire flowers in the selected parents were lost. The hybrids obtained during 2017 were raised in seedling nursery for preliminary evaluation. Two hybrids from the previous seasons were cut back and maintained for further evaluation. The hybrids of 2016 were test tapped to evaluate their performance.

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 2016 were test tapped and the promising ones were pollarded for multiplication. Around 1500 healthy polyclonal seeds were collected and a seedling nursery was raised for evaluation.

1.1.4. Participatory clone evaluation

The annual growth of 11 pipeline clones and three check clones planted at Tharuvaiyur estate were recorded and the data tabulated. The clones P 10 (54.4 cm) and P 21 (54.4 cm) were more vigorous based on the data after nine years of growth. The average tappareability was only around 76 per cent. The trial is proposed to be opened for tapping in the ensuing season.

In the OFT at Bethany estate planted during 2010, of the 14 trial clones assessed for growth, clones P 44 (56.5 cm) and P 27 (55.4 cm) recorded relatively higher girth. The trial was opened for tapping during June 2017. However, severe damage was inflicted to the trial due to the cyclone Ockhi during November 2017 and tapping was stopped.

Assessment of the annual girth of the 11 trial clones in the OFT at Bethany estate planted in 2012 indicated that the trial clones P 102 (38.9 cm), P 104 (38.0 cm) and P 49 (40.0 cm) were better than the rest of the clones in relative growth.

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Library continued the information and literature support to its in-house and institutional users by providing reference services, current awareness services and reprographic services. Fourteen books and one standard were added to the stock. Received and registered 170 issues of journals as subscription/exchange.

Compiled information bulletins, viz. Documentation List (1-4) 2017, New Additions List 2017, Staff Publications list 2017-2018, Cumulative index to *Rubber Science* Vol. 1-30, and Library members Directory with details of 884 members. Databases were updated with the details of 19 books, 158 articles, 63 theses, 243 reprints and 7500 bound journals. Bound and accessioned 353 volumes of journals, circulated 726 books, filed 2467 press clippings of relevant articles and provided 6558 photocopies. Library membership issued to 44 users and extended library services to 449 outsiders.

As a part of sales promotion of RRII publications, library organized the sale and distribution of 442 copies of the journal *Rubber Science* and 246 other publications including RRII Annual Report.

SCIENTIFIC ADVISORY COMMITTEE RECOMMENDATIONS

During the reporting year, the following recommendations were approved by the Scientific Advisory Committee of RRII.

1. Paired row system of planting (3.2 x 5.0 m within the paired row and 9.0 m between the paired rows with 440 plants ha⁻¹), can be recommended as an alternate planting system for the grower who wants to remuneratively cultivate intercrops for the entire immaturity period in traditional (Kerala) area and North East India.

Upland rice, maize, cowpea and tuber crops like colocasia and elephant foot yam can be grown as suitable intercrops in immature rubber in North East India.

2. Recommendation of use of water-based fungicide thiophanate methyl (0.07%) for

effective control of *Corynespora* Leaf Disease (CLD) in immature rubber plantations. The cost of the new fungicide is on par with recommended fungicide, carbendazim. It also adopted recommendation of a combination fungicide, (SAAF) containing carbendazim (12%) and mancozeb (63%) at 0.2 per cent of the product for effective control of *Corynespora* Leaf Disease in immature rubber plantations.

3. A new region specific advisory of clones suited to each of the nine agro climatically distinct zones in the traditional and non-traditional regions of rubber cultivation India was formulated and approved in the reporting year for communicating to the rubber plantation sector

Region-Specific Clone Advisory:

Region	States/ Districts	Clones recommended
North East India	Tripura, Assam, Northern West Bengal, Meghalaya	RRII 208, RRII 429, RRII 600
East and West Central India	Maharashtra, Odisha	RRII 429, RRII 208, RRII 430, RRII 600
South-West Karnataka and Goa	Goa and Dakshina Kannada	RRII 430, RRII 414, GT 1, RRII 203
North Kerala	Kozhikode, Malappuram, Kannur, Kasargode	RRII 430, RRII 417, RRII 105
North Central Kerala	Trichur, Palakkad	RRII 417, RRII 429, RRII 430
Central Kerala	Ernakulam, Kottayam, Pathanamthitta, Alapuzha	RRII 430, RRII 414, RRII 417
South Kerala	Kollam, Trivandrum	RRII 422, RRII 430, RRII 417
Tropical high altitude regions	Wyanad, Idukki, Kulathupuzha, Vithura	RRII 422, RRII 429, RRII 417
South Tamil Nadu	Kanyakumari	RRII 430, RRII 105, RRII 429

In the OFT at Bethany estate planted during 2010, of the 14 trial clones assessed for growth, clones P 44 (56.5 cm) and P 27 (55.4 cm) recorded relatively higher girth. The trial was opened for tapping during June 2017. However, severe damage was inflicted to the trial due to the cyclone Ockhi during November 2017 and tapping was stopped.

Assessment of the annual girth of the 11 trial clones in the OFT at Bethany estate planted in 2012 indicated that the trial clones P 102 (38.9 cm), P 104 (38.0 cm) and P 49 (40.0 cm) were better than the rest of the clones in relative growth.

LIBRARY AND DOCUMENTATION CENTRE

The Library and Documentation Centre attached to Rubber Research Institute is well maintained with a collection of 22953 books, 23802 bound volumes of periodicals, 6003 standards, 1563 reprints, 189 Theses/ Dissertations and 1200 Microfiche/Microfilms. Subject bibliographies and computer based bibliographic databases of all books, research articles, standards, theses, reprints are also accessible to the users.

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Tropical high altitude regions	Wyanad, Idukki, Kulathupuzha, Vithura	RRII 422, RRII 429, RRII 417
South Tamil Nadu	Kanyakumari	RRII 430, RRII 105, RRII 429

4. Conservation pits @ 250 ha⁻¹ may be maintained in immature rubber to facilitate rain water harvesting and soil moisture conservation. The pits may be opened in between two plants, alternatively in the interspace and closer to the platform. For the initial three years of immature phase forking the plant basin during the month of January followed by heavy mulching. Minimum three rounds of mulching, *i.e.*, immediately after planting, after the end of rainy season and after forking the basin in January - for initial three years.
5. Recommended TAFE compact tractor MF 6028 manufactured by M/s. Tractors and Farm Equipment Limited (TAFE), Chennai, as suitable for low volume spraying in rubber plantations using Aspee-RRII tractor-mounted high tree mistblower. This compact tractor can be used for low volume spraying in mature rubber plantations for delivering spray fluid upto 80 feet height with a discharge of 1000ml per minute.
6. Recommendation of an additional rolling of wet ribbed sheet coagula (after turning the sheet upside down), through a roller set with upper plain and lower grooved rollers or a roller set having pair of grooved rollers that can reduce the sheet thickness and increase the surface area. This leads to saving of drying time by 25 per cent without affecting the sheet quality.

Annual Expenditure 2017-18

Expenditure at a glance

Head of Account	Expenditure (Rs. in lakhs)
Plan	
North East	513.17
Other than North East	2,402.69
Total	2,915.86
Non-Plan	1,291.74
Grand Total	4,207.60

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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 development of transgenic plants incorporating agronomically important genes for improvement of *Hevea* are the important areas in which the Biotechnology Division is engaged. The Advanced Centre for Molecular Biology and Biotechnology (ACMBB) is a functional grouping of scientists working in the areas of Molecular Biology, Biotechnology, Genome Analysis, Molecular Physiology and Molecular Pathology. The important fields of research of the Botany Division are breeding, evaluation and selection of new clones, propagation techniques, planting methods, anatomical studies and cytogenetic investigations. The Climate Change & Ecosystems Studies Division is pursuing studies on climate change process in traditional and non-traditional rubber growing regions of India and developing information system on rubber cultivation using remote sensing (RS) platform to identify area under rubber cultivation and

suitable area where rubber plantations can be extended. The Germplasm Division is concentrating on the introduction, conservation and evaluation of *Hevea* germplasm. The Plant Pathology Division is engaged in investigations on the diseases and pests of rubber and associated cover crops and their control. The Plant Physiology Division conducts studies on both fundamental and applied aspects of *Hevea* tree physiology. The Latex Harvest Technology Division is concentrating on all applied aspects of crop harvesting in rubber. The Rubber Technology Division concentrates on improvement in primary processing of rubber, its chemical modification, rubber product manufacture and quality control of processed rubber. The Technical Consultancy Division provides consultancy services for the promotion of the rubber industry. The Rubber Technology Division and Technical consultancy Division together forms the Advanced Centre for Rubber Technology (ACRT). The Economics Division undertakes studies on economic aspects related to rubber plantations.

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

Central Experiment Station

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

Regional Research Stations

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

Regional soil testing laboratories have been established at Kozhikode, Thrissur, Muvattupuzha, Pala, Kanjirappally, Adoor and Nedumangad. Mobile units for soil and leaf analysis are available at Kozhikode laboratory, apart from that at the headquarters.

National/International collaboration

RRII is a member of the International Rubber Research and Development Board (IRRDB), an association of national organizations devoted to research and development on natural rubber. Rubber Board is a member of the Association of Natural Rubber Producing Countries (ANRPC) and International Rubber Study Group (IRSG).

The RRII has research/academic linkages with the Banaras Hindu University (Varanasi), Kerala Agricultural University (Thrissur), Kerala University (Thiruvananthapuram), Mahatma Gandhi University (Kottayam), Cochin University of Science and Technology (Kochi), Indian Agricultural Research Institute (New Delhi), Indian Institute of Sciences (Bangalore), Indian Institute of Technology (Kharagpur), National Chemical Laboratory (Pune), Sree Chitra Tirunal Institute of Medical Sciences and Technology (Thiruvananthapuram), Tamil Nadu Agricultural University (Coimbatore), University of Agricultural Sciences (Bangalore) and University of Goa (Goa).

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