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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 to RRII.

Organization

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

Continued on inside back cover

ANNUAL REPORT 2009-2010



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The Indian Rubber Board was constituted under the Rubber (Production and Marketing) Act, 1947, which came into force on 19 April 1947. This Act was amended in 1954, 1960 and 1982. The Act was again amended by the Rubber (Amendment) Act, 1994 (Act 33 of 1994), which is now in force.

Organization

The Chairman is the principal executive officer and exercises control over all departments of the Rubber Board. The Rubber Research Institute of India (RRII) works under the administrative control of the Board, the Director being the head of the institution. Besides RRII, there are eight departments under the Board viz. Administration, Rubber Production, Processing & Product Development, Finance & Accounts, Training, Licensing & Excise Duty, Statistics & Planning and Marketing Promotion.

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DIRECTOR'S REVIEW

Development of high yielding clones and agro-management practices, upkeep of plant and soil health, elucidating the molecular basis of growth and yield, mechanism of tolerance to diseases and environmental stress, optimization of latex harvesting, development of healthy planting materials etc. were some of the priority areas of research during the reporting year.

Breeding and evaluation of clones continued to be one of the major activities of RRII. Several promising clones, both indigenous and exotic were evaluated in fields. Hybrids have been produced by crossing elite Wickham clones (female) with selected germplasm accessions (male) and a large number of these hybrids expressed heterosis in yield and timber production. Several half-sibs and polycross progenies were under field evaluation. Some promising ortets have been identified from the old plantations belonging to Rubber Board in South Andamans. These selections have been made from the PBIG seedling progenies.

Second phase of participatory clone evaluation was initiated in which on-farm evaluation of several pipeline clones will be done simultaneously at different locations. A programme for monitoring the growth and yield of newly released RRII 400 series clones in growers' fields was also initiated. In addition, studies on genotype x environment interactions, physical, mechanical and anatomical properties of wood, anatomy of TPD trees etc. were continued. Draft guidelines for DUS testing in rubber, as required under the Protection

of Plant Varieties and Farmers' Rights Act were also prepared. Characterization of the 1981 germplasm collection was continued for yield and secondary traits such as timber production, tolerance to drought and diseases etc. at the physiological, molecular and phenotype levels.

The root trainer technique, optimized for *Hevea* single handedly by Dr. T.A. Soman, offers an effective planting technique rapidly adopted by rubber growers. Plants developed in root trainers possess a compact and well developed root system, which ensures their successful establishment in the field. Plants raised in root trainers have uniform growth and have the potential to attain early tappareability. Root trainer plants are much lighter than polybag plants and easier for transportation, and hence, there is considerable reduction in labour costs in planting. Further research efforts are also on the way to refine the potting media etc. for root trainers.

In view of growing concern of deteriorating soil health (in terms of soil organic matter content and micro and macro nutrients), the various research programs of Agronomy/Soils team aimed towards development, as well as refinement of agro management practices to improve growth and yield of rubber while soil health



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was maintained sustainably. Optimizing ways to increase land productivity without increasing cost of cultivation through efficient agro-management practices was another major objective of the team. Developing a state-of-the-art rubber information system using recent advances in remote sensing and GIS has been attempted. Efforts were made to study the feasibility of managing rubber plant nutrition through revision of existing general fertilizer recommendation.

Experiments were continued on management of active and microbial carbon pools in rubber growing soils with the objective of improving original carbon status through addition of biofertilizers/green manures. It was found that potassium can be partly substituted by sodium. Experiments were carried out to find out the feasibility of growing perennial intercrops viz., coffee, vanilla, *Garcinia* and nutmeg. Another experiment was initiated to evaluate the performance of shade tolerant medicinal plants in mature rubber plantation. It was found that there was significantly more available K in banana intercropped area. Experiment on the effect of density of planting on growth and yield of rubber was continued. Development of agro management techniques for reducing the gestation period was another important area in which various experiments were carried out. Soil properties between different cycles of rubber cultivation and the adjacent forest were also compared. A new study was initiated to assess the soil quality in mature rubber plantation under two situations, with standard weed control practices and with restricted weed control.

Advanced Centre for Molecular Biology and Biotechnology under RRII continued its studies in the molecular aspects of economic traits and using advanced biotechnological tools for genetic improvement of *Hevea*

brasiliensis. The research programmes were aimed at development of *in vitro* propagation methods for elite *Hevea* clones, development of transgenic *Hevea* plants for better adaptation to environmental stress and tapping panel dryness, development of *in vitro* fertilization techniques and embryo rescue to complement conventional breeding programmes, understand molecular mechanism and characterization of genes controlling tolerance to diseases, abiotic stresses, latex biosynthesis and characterization of laticifer cell specific promoters. Using *in vitro* derived leaf explants, an efficient somatic embryogenesis pathway as well as subsequent acclimatization of the regenerated plants were standardized. Experiments to induce polyembryony were continued.

Development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping, development of genetic markers for biotic and abiotic stress tolerance and understanding their stress adaptation processes through transcriptome analysis and cloning and characterization of agronomically important genes (latex yield and tolerance to biotic and abiotic stresses) remained priorities of the Genome Analysis team. Thirty-seven polymorphic SSR markers were used to genotype the progeny population and segregation data was recorded for linkage map construction. Resistant Gene Analogue approach was adopted to identify disease resistant genes in *Hevea*. Transcript profiling in stress tolerant *Hevea* clones was continued for functional genomic studies. Studies were also continued on the development of expressed sequence tags for *Hevea*.

Focus of Pathology research was on refining existing disease/pest control measures for developing an integrated

disease and pest management through economic and ecofriendly measures. Evaluation of sprayers and spray oils, assessment of field loss due to diseases, evaluation of newly evolved clones for disease tolerance, studies on tapping panel dryness, bee keeping in rubber plantations, use of beneficial microorganisms for plant growth and treatment of sheet processing effluents were continued. Studies were carried out to find the factors influencing *Phytophthora* infection in rubber plantations in North-East region. A bio-degradable oil was found effective in spraying for controlling *Phytophthora* leaf disease.

Gene expression profiles of *Corynespora* challenged leaf samples were studied using DD-RT-PCR technique. The role of low molecular weight RNA in tapping panel dryness was also investigated. Bacterial isolates were screened for antagonistic activity against major pathogens of rubber. Experiments were carried out to assess the factors governing *Corynespora* leaf fall disease using agrometeorological parameters from diseased plantations. Experiments were also carried out on improvement of spraying technology for pest and disease management of natural rubber.

An experiment with an anaerobic hybrid reactor with a packing media inside for increasing the attached microbial population showed that the hybrid reactor was highly effective in reducing pollution load in RSS processing effluents. With an aim to develop an economical alternative to diffused aeration, a reed bed system has been developed. Development and demonstration of integrated vector control system for prevention of Chikungunya/ dengue in rubber plantation area was another research area.

Plant Physiology team continued its research in major areas of plant physiology

viz., environmental physiology, physiology of growth and yield, tapping panel dryness, gene expression, secondary metabolites and ecological impact of NR cultivation. Experiments were carried out on identification of physiological factors influencing drought and cold resistance in *Hevea* clones. Investigations on molecular basis of adaptation of clones to drought stress showed up-regulation of certain genes in drought tolerant clones. Results of another experiment revealed variations in CO₂ assimilation and stomatal conductance among the RRII 400 series clones.

Expression pattern of endogenous control genes during cold expression was studied in *Hevea* clones by qPCR analyses using real time PCR. Experiments were also carried out to find physiological basis for drought tolerance in wild germplasm. Studies were carried out to find the relationship of ATP status of latex, luteoid membrane composition and ATPase activity with rubber yield. Using radioactively labelled amino acids, clonal variations in latex regeneration mechanism in *Hevea brasiliensis* was studied. Experiments were continued on decipher effects of the ethylene compounds to latex production and stress responses and results indicated enhanced stress responses in the bark tissues of the stimulated tree compared to trees tapped without stimulation. Attempts were made for cloning and characterization of TPD responsive genes through subtractive hybridization.

Rubber Technology team of the Advanced Centre for Rubber Technology under RRII focused its work on evolving improved techniques in processing, refinement in test procedures, chemical modification of NR, development of blends of NR with other rubbers, polymeric fillers for reinforcement of NR, scorch control of

peroxide vulcanization and rubber nano composites. Experiments were continued to improve the quality of radiation vulcanized natural rubber latex. The effect of particle size of latex, maturity of latex and leaching conditions of films were evaluated. Two polymeric filler systems which can reinforce natural rubber were identified. Studies were continued on preparation and stabilization of nano zinc oxide using chemical route. The team also carried out experiments on development of epoxidised natural rubber (ENR), nano composites, footwear sole for physically-handicapped and Hurth coupling for rail locomotive.

Technical Consultancy team of Advanced Centre for Rubber Technology under RRII continued to cater to the needs of rubber based industries by assisting in product development, quality control, advisory services, training and schemes on diversified uses of NR. Latex based products like latex carpet backing, latex adhesives etc. were developed. The team also assisted in research, development and testing of natural rubber latex products as per national and international standards. The team also offered consultancy services for the preparation and stabilization of dispersions/emulsions which are essential ingredients of latex compounds.

Economic research team continued its studies in five major areas, *viz.* farm management, primary processing and marketing of NR, rubber products manufacturing industry and foreign trade, inter crops and by-products and inter-divisional collaborative projects. Analysis of relative contributions of price and productivity in sustaining the tempo of growth in farm income revealed that trends in real farm income had been influenced more by productivity than by price. The study on labour shortage under rubber Block

Planting Scheme in Tripura found growing shortage of family labour in the immature rubber plantations in the context of varied wage rates.

Latex Harvest Technology team continued its research on developing sustainable latex harvesting techniques. The team initiated a collaborative programme for developing a mechanized tapping tool. The team carried out evaluation of gaseous stimulant (ethylene) for further improvement in yield and further reduction in the length of tapping cut. Experiments on Low Frequency Tapping (LFT) with different levels of yield stimulation were continued. It was found that low frequency tapping (d4 and d7 tapping) with rain guarding was successful. Experiment was also carried out on Low Frequency Controlled Upward Tapping (LFCUT), as an alternate tapping method.

Besides meeting the research needs of RRII, Central Experiment Station, Chethackal realized 1266980 kg of total crop. The Regional Research Stations of RRII continued their research programme on developing clones specific to respective regions as well as assessing adaptation of newly released clones to the clonal agro-climatic conditions. The Library & Documentation Centre of RRII added 92 books, subscribed 51 international journals and 77 national journals, updated library databases and developed new databases for Thesis/Dissertations, Rubber Standards and Rubber Board Publications. Scientists of RRII published 43 research papers in journals and 20 chapters in various books. RRII also published a book and a monograph during the reporting year. Besides, RRII scientists presented 34 research papers/articles in international and national seminars. RRII also secured one patent during the reporting period.

AGRONOMY/SOILS DIVISION

The various research programs of the division are aimed at development and periodic refinement of agro management practices to improve growth and yield of rubber, sustain soil quality, increase land productivity and reduce cost of cultivation. Development of the rubber information system using remote sensing and GIS continued. The Division also functions as a centre for dissemination of knowledge on various soil and crop management techniques.

1. Nutrient management

Seedling nursery experiment was conducted to revise the current general fertilizer recommendation. Among the treatments, highest green buddability was noticed in the treatment N and P@ 150 kg/ha as ammophos (20-20) in combination with PGPR which was on par with standard practice and N and P @150kg/ha as ammophos, four months after planting (Table Ag.1).

In the seedling nursery, another experiment was conducted to develop an integrated nutrient management package incorporating bio-fertilizers (BF). Growth of the plants and budding success were comparable in standard practice, standard

practice + BF and 50 per cent N and P + full K + BF treatments and significantly superior to control and BF alone treatments. No significant difference was recorded in the buddability per cent. *Pseudomonas* population was significantly higher in BF alone, standard practice +BF and 50 per cent N and P +full K+ BF. VAM infection per cent in the root indicated 70 to 80 per cent infection in all the plant roots irrespective of treatments.

An experiment was initiated in the glass house with polybag plants of clone RR11 105 during 2009-10 to study the effect of potassium and silicon on moisture stress tolerance of young rubber plants. The experiment consisted of two main treatments, viz. with and without water stress and under each main treatment, there were four sub treatments, viz, control, Si, K and Si +K. Observations on chlorophyll content index and leaf water potential indicated positive effect of potassium and silicon in overcoming moisture stress effects in young plants. After imposing water stress for 30 days, there was 54 per cent reduction in water stressed plants without K or Si compared to plants without stress. The reduction in CCI was 25, 33 and 34 per cent for water stressed plants supplemented

Table Ag.1. Effect of fertilizer levels on diameter and buddability four months after planting

Treatment	Buddability (%)
T1- Control	63.62
T2- Standard practice (NPK @ 500-250-100-kg/ha)	71.83
T3- N&P@ 150 kg/ha (ammophos) + K @ 30 kg/ha	72.29
T4- N&P@ 250 kg/ha (ammophos) + K @ 50 kg/ha	67.03
T5- N&P@ 150 kg/ha (ammophos) + K @ 30 kg/ha +PGPR	73.33
SE	1.85
CD (P = 0.05)	5.65

with K. After imposing water stress for 35 days, all the plants were irrigated uniformly and it was observed that K supplemented plants recouped at a faster rate and showed better survival percentage.

Experiments initiated in 2008 for developing an integrated nutrient management package for young rubber is being continued. Girth of plants from the experiment in young rubber with cover crop system conducted in two locations indicated no significant difference between treatments. Population of the introduced microbes in the soil was recorded during May and September. Build up of Phosphobacteria was significant at both the locations in both recordings during 2009.

The experiment initiated at CES, Chethackal to develop an integrated nutrient management package for rubber + banana (Nendran) intercropping system was continued. The treatment which received 100 per cent chemical fertilizer and bio fertilizers recorded the highest girth and it was comparable with that of 25 per cent inorganic + bio fertilizer and 75 per cent inorganic + bio fertilizer. Soil nutrient status and pH were not significantly influenced by treatments.

Population of Phosphobacteria three months after biofertilizer application was higher in biofertilizer applied treatments. There was significant difference in the population of total bacteria also, however it did not indicate any definite trend.

The project on management of active and microbial carbon pools in rubber growing soils with the objective of improving carbon quality of rubber growing soils through the addition of bio fertilizers / green manures at Pottamkulam Estate, Yendayar continued. Soil samples were analyzed for OC, pH, Mineral-N and

available Ca, Mg, P and K. It was observed that C and N were significantly higher in treatments which received *Glyricidia* as green manure. Soil microbial population and girth of the rubber plants were not significantly different.

Continued the field trial to study the effect of coir pith organic manure (CPOM) as soil amendment in marginal soils, at Thanneermukkom near Cherthala. In terms of growth of rubber and microbial population in soil, both FYM and CPOM were comparable. Good establishment and satisfactory growth of rubber was observed in the sandy area. Dry matter accumulation of *Pueraria* in the second year was 10.5 t/ha.

The field experiment initiated in 2001 at CES, Chethackal to study of the effect of long term application of inorganic and organic manures on the growth and yield of rubber and on the physico-chemical properties of soil was continued (Table Ag.2). Application of 25 per cent chemical fertilizer and 75 per cent farm yard manure recorded significantly higher girth compared to all other treatments.

Table Ag.2. Effect of integrated nutrient management on growth of rubber

Treatment	Girth(cm)
T1- No fertilizer/no manure (Control)	49.54
T2- Farm yard manure (FYM) alone	52.95
T3-Fertilizers (Standard recommendation)	52.32
T4- 25%Fertilizers +75%FYM	56.21
T5- 50%Fertilizers+50%FYM	53.54
T6- 75% Fertilizers+25%FYM	51.33
SE	0.78
CD (P = 0.05)	2.35

The experiment at Malankara Estate, Thodupuzha to study the effect of zinc application on growth of rubber was continued. Application of Zn did not influence the girth of plants.

The experiment on sequential skipping of fertilizer application in mature rubber was in progress. Yield (2009-10) and girth increment (2002-10) of rubber were not significantly influenced by skipping of fertilizers. Significant difference was not noticed either in soil or leaf nutrient status among the treatments.

Study on partial substitution of K with sodium Na in mature rubber plantations at Malankara Estate, Thodupuzha was completed. Substitution of K with Na up to 50 per cent showed no difference in girth and girth increment. Substitution of various levels of K by Na did not affect the N, P, Ca and Mg content of leaf. All the treatments recorded higher K content compared to the control plots which received neither K nor Na. The different treatments did not influence EC, pH, organic carbon, available P, Ca, Mg and Na status of soil. Soil available K status was higher in all the treatments compared to control.

In the experiment on nutrient uptake by prominent *Hevea* clones, the nutrient uptake by different clones was compared. The nutrient uptake was comparatively higher for RR11 414, RR11 429 and RR11 417 compared to other clones. Carbon sequestration potential was also higher for these clones (Table Ag.3).

Table Ag. 3. Carbon sequestration potential (350 trees/ha)

Clone	C-stock (kg/tree)	C-seq. potential (t/ha)
RR11 414	324.95	113.73
RR11 430	301.51	105.53
RR11 429	292.34	102.28
RR11 417	171.74	60.11
RR11 422	154.36	54.03
RR11 105	163.18	57.11
CD (P = 0.05)	41.35	5.06

2. Soil and water conservation

A field experiment was initiated at Mundakayam Estate during 2009 to evaluate the effectiveness of biological hedges for soil conservation. Vetiver, guinea grass, pineapple and karimkurinji were established as biological hedges. The experiment was laid out in RBD with four replications. All the biological hedges established well in young rubber plantation.

3. Intercropping and cropping systems

Experiment to find out the feasibility of growing perennial intercrops in rubber was continued. Perennial intercrops viz., coffee, vanilla, *Garcinea* and nutmeg are being evaluated in the normal and paired row system of planting of rubber. In the paired row system of planting a strip of 1.5 m width was available at the centre of wide inter row area without shading for intercropping. There was no significant difference between treatments with respect to girth and girth increment of rubber in the paired row system of planting. However, in the normal system of planting, girth and girth increment of rubber was higher in the intercropped plots.

Yield of *Garcinea* has started to decline in the normal system of planting due to the intense shade and yield was highest in the paired row system of planting. Yield of vanilla and coffee was higher in the paired row system of planting.

Experiment on inter planting of rubber with timber trees viz., teak, wild jack and mahogany was continued. Girth of rubber was not significantly influenced by row spacing, type of timber intercrop and their interactions. However, growth of rubber was comparatively better in normal spacing compared to wide row spacing. Trees were

opened for tapping and yield recording was started.

Another experiment was initiated at Mundakayam Estate during 2009 to evaluate the performance of shade tolerant medicinal plants in mature rubber plantation. The medicinal plants which are being evaluated are *Adathoda beddomei*, *Alpinia calcarata*, *Andrographis paniculata*, *Asparagus racemosus*, *Desmodium gangeticum*, *Holostemma ada-kodien*, *Piper longum*, *Pseudarthria viscida*, *Rauvolfia serpentina* and *Strobilanthes cuspidate*. The medicinal plants were established in a 20 year old PB 28/59 plantation. The plot size was 911 m². All the medicinal plants established well in rubber plantation.

Experiment to find out the change in soil physical and chemical properties by planting different inter crops initiated during 2003 recorded significantly higher available K status in banana intercropped area. With respect to the other nutrients, there was no significant difference between treatments. Bulk density was also not affected by planting different intercrops.

4. Planting systems

The experiment on planting design/ geometry initiated during 2007 to study the effect of different planting geometries on

canopy development, growth and yield of rubber is in progress. Growth of rubber in the triangular system of planting and twin system of planting was superior to that of control (square system of planting). The canopies in altered planting systems started to exhibit asymmetrical pattern of growth.

Experiment on the effect of density of planting on growth and yield of rubber at CES, Chethackal is being continued. Plants in the lowest density of 420 trees/ha had significantly higher girth compared to all other treatments.

Plants in the lowest density of 420 trees/ha recorded significantly higher yield (g/t) compared to all other treatments. The annual yield per hectare was significantly higher in the highest density (749t/ha) which was on par with the yield in the density 549 trees/ha (Table Ag.4).

5. Development of agro management techniques for reducing the gestation period

The poly bag nursery experiments on comparison of growth performance of two types of planting materials *viz.*, direct seeded and budded stump rubber plants and the effectiveness of inorganic and organic sources of fertilizers on growth were

Table Ag. 4. Effect of different planting densities on yield

Treatments	M1	M2	Mean gm/tree/tap	M1	M2	Mean kg/ha/year
D ₁ - (420 trees/ha)	95	92	93.5	1976	2126	2051
D ₂ - (479 trees/ha)	70	71	70.5	2074	2355	2214
D ₃ - (549 trees/ ha)	80	67	73.6	3065	3156	3111
D ₄ - (638 trees/ha)	72	59	68.1	3021	2847	2934
D ₅ - (749 trees/ha)	64	61	59.9	3531	3217	3374
Mean	78	70				
Main plot	SE - 4.72			Main plot	SE - 173.55	
CD				-	10.27	CD - 378.17
Interaction				-	NS	Interaction - NS

Table Ag.5. Effect of inorganic and organic fertilizers on growth of planting materials four months after cut back

Treatment	Direct seeded				Budded stump			
	Diameter (mm)	Height (cm)	Fibrous root (g)	DMP (g)	Diameter (mm)	Height (cm)	Fibrous root (g)	DMP (g)
T1- NPKMg@ 6-6-2-4-1 g/plant	8.40	93.40	3.12	40.40	4.75	28.10	0.75	10.90
T2- T1 + Cowdung slurry	8.60	95.30	3.85	51.60	5.14	36.50	0.98	13.55
T3- T2 +Ground nut cake	8.40	91.10	3.96	62.10	5.06	34.30	1.50	16.40
T4- NPKMg@ 6-6-2-4-1 g/plant (N&P as ammophos)	9.10	96.70	3.26	56.60	5.31	32.60	1.80	22.70
T5- T4 + Cowdung slurry	8.50	96.50	8.02	62.00	5.45	34.30	2.20	25.70
T6- T5 + Ground nut cake	8.10	87.80	7.13	59.60	5.61	34.60	2.0	24.00
T7- T5+ Neem cake	8.20	92.30	7.42	64.90	5.34	33.00	2.90	22.80
T8 - 50% of T4+ PGPR	8.60	89.00	6.59	64.50	5.71	31.90	2.40	24.30
SE	0.11	1.50	0.29	2.90	0.07	1.26	0.14	1.30
CD (P = 0.05)	0.32	4.50	0.88	8.80	0.21	3.79	0.42	3.90

repeated. It was observed that irrespective of treatments, planting material produced through direct-seeding was significantly superior in diameter, height, number of whorls, fibrous root production and in dry matter production over the budded stump plants four months after cut back (Table Ag.5). Integration of various inorganic and organic fertilizers significantly influenced the growth of planting materials.

The field experiment initiated at Malankara Estate to develop an agronomic package to reduce the immaturity period of *Hevea* continued. Growth of rubber was positively influenced by the management

practices. The girth of the plants under integrated management was significantly superior to all other treatments (Table Ag. 6).

The experiment initiated with the same objective of developing an agronomic package to reduce the immaturity period of *Hevea* at CES, Chethackal is in progress. The girth of green - budded stumps raised in polybags and the girth and height of direct seeded green- budded plants with integrated management was found to be superior to those of respective type of planting material under standard practice.

The field experiment to study the effect of different types of planting material (polybag - one whorl, two whorl and three whorl and root trainer - one whorl, two whorl and three whorl) on growth of rubber was continued. There was significant difference in the girth of the plants between different stages of the same type of planting material (polybag - one whorl, two whorl and three whorl and root trainer - one whorl, two whorl and three whorl). Three whorl plants were significantly superior to two whorl which in turn were superior to one whorl plants irrespective of the type of planting material. However, there was no

Table Ag.6. Effect of agromanagement practices on growth of rubber

Treatment	Girth 2010 (cm)
Standard practice	32.93
Selective manuring	35.78
Enhanced nutrient application	34.75
Conservation oriented tillage	37.41
Irrigation	37.32
Irrigation+ Enhanced nutrient application	37.76
Integrated management	41.22
SE	0.37
CD(P = 0.05)	1.14

significant difference between the same stage of different types of planting material except for the three whorl stage where polybag three whorl plants were significantly superior to root trainer three whorl plants.

Field experiment was also initiated to compare the growth performance of age of the planting materials, viz., brown and green budded poly bag plants with difference in age of the root stock and direct seeding in poly bags and young budded poly bag plants. The girth increment of different types of planting materials was significantly influenced during the initial six months of growth. The girth increment was highest in brown budded polybag plants- (10 months old rootstock with polybag size 55 x 25 cm), which was on par with green budded polybag plants which were retained in polybag for 16-18 months

(polybag – 45x18 cm.), green budded polybag plants (5-6 month old rootstock with poly bag size 55 x 25 cm.) and young budded polybag plants (polybag – 45 x 18 cm.). During the later phase of growth (6-12 months) significant difference in girth increment was not noticed among different types of planting materials (Table Ag.7).

6. Rubber growing soils

Assessment of the status and distribution of Zn in the nine major soil series in the traditional belt of rubber cultivation indicated that the total Zn status of the soil was comparatively low. Major share of the Zn was in the residual fraction and is not available for plant growth. The water soluble plus exchangeable fraction of Zn was very low and the values reduced down the horizons. Even though the water

Table Ag. 7. Girth increment of plant during the 1st year

Treatments	Girth increment (mm)		
	0-6 th months	6-12 th months	0-12 th months
1. Brown budded – 18-20m rootstock (polybag-45 x 18 cm)	15.66	7.54	23.21
2. Green budded polybag plants – retained in polybag for 16-18 months (polybag – 45 x 18 cm.)	19.39	7.86	27.29
3. Direct seeded rootstock (polybag – 45 x 18 cm.)	15.41	7.32	22.77
4. Young budded rootstock (polybag – 45 x 18 cm.)	17.16	7.51	24.68
5. Brown polybag plants – 10 m. old rootstock (polybag – 45 x 18 cm.)	16.87	5.98	22.89
6. Greenpolybg plants – 5-6 m. old rootstock (polybag – 45 x 18 cm.)	16.40	7.48	23.88
7. Brown polybag plants – polybag 55 x 25 cm. (control)	20.06	5.95	22.86
8. Green polybag plants – polybag 55 x 25 cm. (control)	17.51	7.00	24.55
SE	0.968	0.557	1.388
CD (P = 0.05)	2.936	NS	NS

Table Ag.8. Change in fertility status of surface soil (0-30 cm)

Nutrient	Year		Significance
	1977	2009	
Organic carbon (%)	2.025	2.129	NS
Available P (mg/100g soil)	0.535	3.413	*
Available K (mg/100g soil)	2.759	5.546	**
Available Ca (mg/100g soil)	3.973	11.982	**
Available Mg (mg/100g soil)	3.996	2.517	NS
pH	4.606	4.398	**

Table Ag.9. Change in fertility status of sub surface soil (30-60 cm)

Nutrient	Year		Significance
	1977	2009	
Organic carbon (%)	1.324	1.291	NS
Available P (mg/100g soil)	0.245	0.296	NS
Available K (mg/100g soil)	2.184	4.376	**
Available Ca (mg/100g soil)	2.907	7.857	**
Available Mg (mg/100g soil)	4.067	1.503	**
pH	4.55	4.45	NS

soluble plus exchangeable fraction was high in the Ap horizon in some series, drastic reduction in the values were recorded in the subsurface horizons indicating that Zn

availability is restricted to the Ap horizon (0-15 cm) of the soil and down the layers the availability is low. Kanjirappally, Lahai, Thiruvanchoor and Kunnathur series were found to be extremely low in available fractions of the Zn.

In a separate study status of available Zn (DTPA-Zn) the replanting fields was assessed. The values ranged from 0.106 to 2.36 ppm. 61.0 per cent of the soil samples tested was found to be deficient in available Zn.

Effect of continuous cultivation of rubber on soil fertility by comparing the values generated during 1977 and 2009 was assessed and presented in Tables Ag. 8 and 9.

No decline in OC content was noted at both depths. A build up of available P was noted and the difference was significant at 0-30 cm depth. Similarly, significant improvement in available K and Ca status was noted in both depths. A reduction in

TableAg. 10. Comparison of soil fertility status of three cycles of rubber and adjacent forest - Pullengode Estate, Nilambur

Soil properties	Forest	1 st rubber cycle	2 nd rubber cycle	3 rd rubber cycle	CD
Bulk density (g/cc)	1.10	1.19	1.26	1.31	-
Soil pH	4.98	4.69	4.51	4.40	0.2308
OC (%)	2.32	2.23	1.85	1.56	0.3590
Av. P (mg/100g)	0.30	0.36	0.34	0.54	NS
Av. K (mg/100g)	24.57	13.90	10.21	8.54	6.1480
Av. Mg (mg/100g)	12.76	8.72	-	3.67	6.1247
Av. Ca (mg/100g)	64.77	39.31	-	18.38	29.2914

Table Ag.11. Comparison of soil fertility status of two cycles of rubber and forest estate - Shaliacary, Punalur

Soil properties	Forest	2 nd rubber cycle	3 rd rubber cycle	CD
Bulk density (g/cc)	1.05	1.10	1.16	0.0500
Soil pH	4.51	4.40	4.24	0.1379
OC (%)	1.95	1.82	1.34	0.3452
AvailableP (mg/100g)	0.34	0.30	0.60	NS
Available K (mg/100g)	6.98	5.67	5.37	NS
Available Mg (mg/100g)	2.82	0.92	0.89	0.9495
Available Ca (mg/100g)	8.83	4.00	3.34	3.0504

available Mg was noted and the difference was significant at lower depth. Decline in pH was noted and the difference was significant at 0-30 cm depth.

Soil properties between different cycles of rubber cultivation and the adjacent forest were compared at different locations. At Pullengode Estate (Nilambur) bulk density of surface soil showed increasing trend from forest to 3rd cycle rubber (Table Ag.10). Soil pH, OC and available K were significantly higher in forest compared to rubber plantation. Between cycles of rubber cultivation though a decreasing trend was recorded for pH, OC, available K, Ca and Mg, there was no significant difference.

At the second location (Shaliacary, near Punalur) also, the same trend was observed except for available K. There was no significant difference for available K status. However, bulk density was significantly higher in rubber plantation compared to forest (Table Ag.11).

In the third location (New Ambadi Estate-Kanyakumari) OC was significantly higher in the first cycle compared to 2nd cycle. With respect to other nutrients, there was no significant difference between the two cycles though a decreasing trend was observed in the second cycle.

The study on comparison of six soil ecosystems viz. mature rubber, rubber+ cover crop (*Mucuna*), rubber + pineapple (intercrop), cassava (monocrop), mature teak plantation and forest for soil quality and health continued. Nitrogen mineralization rate of these soils were estimated by laboratory incubation method. Soil samples were collected from these systems and incubated for different time intervals. The N mineralization rates (calculated based on the net N mineralized in 40 days) of soils in the different systems

varied and it decreased in the order forest > *Mucuna* > pineapple > teak > rubber > cassava. Estimations of organic carbon and total N as well as soil quality indices such as soluble carbons and particulate organic matter were initiated.

The sub project to study faunal and microbial population in these soil systems was completed. The data of three years were pooled and analyzed. Bacterial, fungal and nematode populations in different systems are presented. It is observed that significantly higher bacterial population was present in forest while significantly higher fungal population was in mature rubber. The nematode populations were not significantly different in the systems studied. Soil fauna were grouped into shredders, predators and herbivores and their population in different systems were examined. Ants, spiders and mites / ticks were included in predators group, earthworm, termites, chilopodes and fungi (above ground) were included in shredders group while in herbivores' group beetles / grubs, caterpillars, snails, silverfish, blattoidea/crickets / grasshoppers were included. The shredders group distinctly varied in different systems and in general, this fauna group had much higher representation in number in teak and forest soils. Earthworms were significantly higher in teak and forest soils while scanty in mature rubber and rubber-pineapple and almost absent in rubber-*Mucuna* and cassava soils. The herbivores were less in number in soils under cassava and rubber-pineapple. The predators group of fauna was generally less in cassava and rubber-pineapple soils and higher in teak and forest soils. In general soil faunal population had declined in cultivated systems compared to forest soil.

A new study was initiated to assess the soil quality in mature rubber plantation under two situations, with standard weed control practices and with restricted weed control to facilitate movement of tapper. Eight fields in each situation were identified. The clone was RR11 105 and all fields were planted in 1994-96 period. The girth of all rubber plants and other trees in all the sixteen fields were measured. The average girth of rubber plants were in general higher in fields where weed flora was not controlled.

An observational trial was initiated at Malankara estate, Thodupuzha during 2009 to explore the feasibility of establishing *Mucuna* in the later immaturity phase and mature phase of rubber plantations and fodder crops in rubber plantations under limited availability of light (after removing pineapple). It was observed that per cent establishment and growth of *Mucuna* under partial shade was higher than that under shade (mature rubber). Among the different fodder crops guinea grass, *stentrophrum*,

Stylosanthes scabra and *Stylosanthes ciat* 136 grows comparatively better under partial shade.

7. Development of rubber information system using remote sensing and GIS techniques

Project on developing rubber information system in the traditional region of rubber cultivation using remote sensing and GIS was continued. Completed registration of toposheet (Fig. Ag. 2), soil series association maps and IRS P6 satellite images (Fig. Ag. 1) covering Kerala and Kanyakumari district of Tamil Nadu. Completed vectorisation and data base creation of soil information for the traditional rubber growing areas. Preparation of rubber distribution map of four districts of Kerala is in progress. Did accuracy assessment of satellite base rubber area using ground GPS reading and it was 81 per cent for the Kanyakumari district.



Fig. Ag.1. IRS P6 satellite images covering traditional rubber growing areas



Fig. Ag.2. 1:250,000 toposheets covering the traditional rubber growing areas

BIOTECHNOLOGY DIVISION

Genetic improvement of *Hevea brasiliensis* using modern tools is the major goal of Biotechnology research at RRII. The major ongoing research programmes in Biotechnology Division are 1) development of *in vitro* propagation methods for elite *Hevea* clones 2) development of transgenic *Hevea* plants for better adaptation to environmental stress & tapping panel dryness, latex yield and disease tolerance 3) development of *in vitro* fertilization techniques & embryo rescue to complement conventional breeding programmes 4) study of molecular mechanism and characterization of genes controlling tolerance to diseases, abiotic stresses and latex biosynthesis and 5) study of laticifer cell specific gene expression and characterization of laticifer cell specific promoters.

1. Somatic embryogenesis

New leaf cultures were raised for callus induction from leaf explants collected from six month old bud grafted plants of clone RRII 105 maintained in the glass house. Calli formed were excised from the explant after 45-50 days in culture and sub-cultured for proliferation. With 2-3 serial subcultures in proliferation medium by gradually increasing the cytokinin/auxin ratio, the texture of the callus could be improved. Simultaneously a separate experiment was also carried out by including silver nitrate (0-30 mg/l) in the proliferation medium. Proliferated callus obtained from medium supplemented with 20 mg/l silver nitrate was light yellow in appearance with improvement in the callus texture. Embryogenic callus formation from the friable proliferating callus was obtained in

modified MS medium containing $\text{Ca}(\text{NO}_3)_2$, $4\text{H}_2\text{O}$ (360 mg/l), silver nitrate (10 mg/l) and sucrose (80 g/l). The medium also contained phytohormones, 0.2 % activated charcoal and 0.5% phytigel. Embryogenic callus clumps proliferated in the same medium with simultaneous embryo induction. Embryo induction was obtained in proliferated embryogenic callus in modified MS basal medium ($\text{Ca}(\text{NO}_3)_2$, $4\text{H}_2\text{O}$ - 360 mg/l and KH_2PO_4 - 170 mg/l) containing B5 vitamins, amino acids, organic supplements such as coconut water (5%), malt extract (50 mg l^{-1}), casein hydrolysate (300 mg l^{-1}), sucrose (60 g/l), BA (0.2 mg/l), kin (0.3 mg/l), GA_3 (0.8 mg/l) and NAA (0.1 mg/l). Callus clumps that produced embryogenic calli were maintained by subculture to fresh medium every month and fresh embryogenic calli could be obtained from them for 6-8 months. Maturation and apex induction could be achieved in WPM medium containing organic supplements, CW (10%), malt extract (200 mg/l), casein hydrolysate (400 mg/l), BA (0.5 mg/l), IBA (0.1 mg/l) and GA_3 (1.0 mg/l). Plant development occurred in media devoid of hormones and containing organic supplements such as coconut water, malt extract and sucrose (40 g/l). Plant development with 1-2 sets of leaves occurred within one month and transferred for hardening.

Using *in vitro* derived leaf explants, an efficient somatic embryogenesis pathway as well as subsequent acclimatization of the regenerated plants were standardized. Mature leaves of *in vitro* plants developed through somatic embryogenesis from immature inflorescence of clone RRII 105 were used as initial explant for inducing

Table Biotech. 1 Comparison of initial survival rate of somatic plants during hardening (%)

Pre-treatment	Potting medium				
	Soil	Soilrite	Soil & sand	Soilrite & sand	Soil, soilrite & sand
Without preconditioning	0	10	8	42	17
With preconditioning	0	29	33	70	45

somatic embryogenesis. Conditions for callus induction, embryo induction, maturation and germination of the embryos and acclimatization of the regenerated plants were standardised. Maximum callus induction frequency (90%) was obtained within one month in modified MS medium supplemented with 2,4-D (4.5 μ M), NAA (2.0 μ M) and Kin (3.0 μ M). Further callus proliferation was brought about by reducing the auxin levels to one fourth and the cytokinin level to half. Embryogenic callus was induced at a high frequency (71%) from the proliferated callus within three months of transfer to the embryo induction medium consisting of WPM basal fortified with GA₃ (3.6 μ M) and BA (4.4 μ M) in the presence of low levels of NAA (1.0 μ M). Embryo maturation was favored by a combination of 0.2 μ M ABA and 0.5% phytagel. Thirty per cent of the mature embryos germinated in the presence of BA (8.8 μ M) and GA₃ (2.9 μ M) and advanced into well developed plants.

Several experiments on standardization of the planting substrates as well as preconditioning treatments of the somatic plants prior to transfer for hardening, were carried out. As a result of these experiments, survival rate of the somatic plants could be enhanced considerably when plants were preconditioned in a medium with reduced level of basal salts and sucrose and were subsequently planted in earthenware pots filled with soilrite - sand mixture and kept in an environmentally controlled growth chamber (Table Biotech.1). Later, these

hardened plants were transferred to polybags and maintained in the shade house. A final survival rate of 50% could be obtained.

2. Embryo rescue and induction of polyembryony in *Hevea brasiliensis*

Experiments were conducted for the refinement of the media already developed for the rescue of immature embryos. Open pollinated fruits were collected at different maturity (1 to 5 weeks) and inoculated in different combinations of nutrient medium for the recovery of plantlets. Fruits were dipped in 80% alcohol for 10 minutes and were allowed to dry on a filter paper. Using Nistch basal medium, effect of BA & Kin (1-5 mg/l) on embryo development, in presence of GA₃ (1mg/l) was evaluated. The results revealed that Kin had a positive influence on embryo development whereas BA had no positive influence in embryo development. Gradual drying of the ovules was observed in the medium supplemented with BA. It was observed that when Kin was given in the same concentration as GA₃ (1 mg/l), a slight reduction in the percentage of embryo recovery was observed compared to the medium supplemented with GA₃ alone (Table Biotech.2). The frequency of embryo recovery increased gradually with increase in Kin concentration. The frequency of embryo recovery could be increased to 32.4% when 5.0 mg/l Kin along with 1.0 mg l⁻¹GA₃ was used (Table Biotech. 2). Further experiments are required to understand the effect of Kin at higher levels (>5.0 mg/l).

Table Biotech. 2. Effect of Kinetin on embryo development in presence of GA₃ (1 mg/l)

Kinetin (mg/l)	Embryo recovery (%)
1	18 (4.24) ^a
2	23(4.79) ^b
3	24(4.90) ^b
4	26.4(5.14) ^c
5	32.4(5.69) ^d
CD (5%)	0.18
CV	2.81

Experiments to induce polyembryony and production of multiple seedlings from a single ovule were continued. Immature fruits of 8-10 weeks were collected from the field and inoculated in Nitsch medium with three growth regulators GA₃, Kin and Zeatin. Weekly observations were taken. Polyembryony could be repeatedly induced in a few combinations. The number of embryos varied from 2-50. Plants were developed, hardened and field planted.

3. Genetic transformation

Genetic transformation experiments were continued to develop transgenic plants with increased tolerance to abiotic stresses, tapping panel dryness and higher latex yield. The genes coding for superoxide dismutase under the control of *CaMV* 35S and *FMV* 34S promoters separately, sorbitol-6-phosphate dehydrogenase, isopentenyl transferase, *hmgR1* and osmotin protein were used in these studies.

Agrobacterium infections were carried out with *MnSOD* gene constructs containing *CaMV*35S and *FMV* 34S promoter separately using the callus derived from immature anther. Many putatively transformed cell lines were obtained. Transformation event was confirmed by GUS staining and PCR with

gene specific primers. The influence of salinity stress, ABA and osmotica (PEG and sucrose) on induction of embryogenic callus from the transgenic cell lines of *Hevea brasiliensis* incorporated with *MnSOD* gene under *CaMV* 35S promoter was evaluated. When the transgenic callus was subjected to osmotic stress using PEG, there was significant improvement in the induction of friable calli. The frequency of friable callus formation was 13 % when the medium osmoticum was altered using 6% PEG whereas in presence of 8 % PEG, the frequency was raised to 20% (Table Biotech. 3).

Table Biotech. 3. Influence of PEG on friable callus initiation (%)

PEG (%)	Transgenic callus (%) ^a	Control callus (%) ^a
0	2.0 (1.62)	1.0 (1.31)
4	5.0 (2.43)	9.0 (3.15)
6	13 (3.73)	6.0 (2.62)
8	20 (4.57)	5.0 (2.43)
10	7.0 (2.81)	— (1.00)
12	3.0 (1.93)	— (1.00)
CD (P = 0.05)	0.71	0.55

^aAnalysis was carried out using square root transformation. The values given in parenthesis are the transformed values.

In order to improve the rate of formation of embryogenic callus, different levels of ABA (0.1-8.0 mg/l) were incorporated the embryo induction medium in combination with 6 and 8% PEG. Later the transgenic callus was subjected to salt stress (0 - 300 mM) and the emergence of embryogenic calli was assessed after 30 days of transfer to medium devoid of NaCl. Among the different osmotica tested, friable embryogenic calli was obtained when 8% PEG was used along with 1.0 mg/l⁻¹ ABA (68 %). Salt stress (300 mM) also had a beneficial role in promoting embryogenic

Table Biotech. 4. Frequency of embryogenic callus formation in NaCl treated callus

Treatment	NaCl (mM)*				
	100	150	200	250	300
Control	34.8(5.89)	37.8(6.14)	53.2(7.29)	68.8(8.29)	76.2(8.73)
CD (P = 0.05)	—	—	—	—	—

* Analysis was carried out using the transformed values. The values given in parenthesis are the transformed values.

calli formation from the transgenic cell lines (76 %) upon transfer to medium devoid of NaCl (Table Biotech. 4).

The friable embryogenic calli from these treatments were further subcultured to the embryo induction medium. The callus obtained with 8% PEG and 1.0 mg/l ABA produced numerous transgenic embryos.

Experiments were also carried out to explore the possibility of using leaf derived callus as the initial explant for *Hevea* genetic transformation. Callus induced in leaf sections and maintained in an actively proliferating stage was used for *Agrobacterium* infection. Modifications were made in the infection, co-cultivation and selection media so as to control bacterial overgrowth and improve transformation efficiency. The effect of silver nitrate in controlling bacterial over growth and improving the texture of callus in newly emerged lines was studied. When silver nitrate (0-10 mg/l) was added in the infection and co-cultivation medium and (0-30 mg/l) in the selection media, it was found that overgrowth of bacteria could be controlled without reducing the transformation frequency. The optimum concentration of silver nitrate used in different steps for transformation was identified as 5.0 mg/l in infection and co-cultivation medium and 10 mg/l in selection medium. When the selection medium was supplemented with silver nitrate (10 mg/l),

overgrowth of bacteria could be controlled by 80%. Infected tissues maintained in this medium could be recovered without overgrowth after one month culture.

The effect of thiol compound, L-cysteine (0-300 mg/l) in the infection, co-cultivation and selection medium and addition of dithiothreitol (5.0 mg/l) in the infection medium in improving transformation frequency and transgenic tissue regeneration was studied. It was observed that inclusion of dithiothreitol (5 mg/l) in the infection and co-cultivation medium and L-cysteine (100 mg/l) in the infection, cocultivation and selection medium helped in improving the transformation efficiency. Presence of thiol compounds along with silver nitrate helped in reducing overgrowth and improving callus texture in newly

Table Biotech. 5. Effect of L-cysteine and 5 mg/l DTT in presence of AgNO₃ on transformation efficiency

L-cysteine (mg/l)	Bacterial Strain	
	EHA 101	LBA 4404
	Transformation efficiency (Mean %)*	
0	9.1 (1.64)	15.8 (2.17)
50	13.3 (1.99)	20.8 (2.49)
100	14.6 (2.23)	24.6 (2.82)
200	14.6 (2.23)	22.5 (2.59)
250	13.0 (2.12)	18.3 (2.30)
300	13.5 (2.06)	21.6 (2.55)
CD (P = 0.05)	0.29	NS

* Data from four replicate experiments using 30 callus clumps per treatment. Values in parenthesis are transformed values

formed transgenic lines. Addition of the surfactant pluronic F68 (300 mg/l) to the modified infection medium also helped in increasing the transformation efficiency. Improvement in the transformation frequency by 10% was obtained by the inclusion of the thiol compound dithiothreitol (5 mg/l) in the infection and co-cultivation medium, L-cysteine (100 mg/l) in the infection, cocultivation and selection medium and addition of the surfactant pluronic F68 (300 mg/l) in the infection medium. It was also observed that the frequency of transformation was found to vary with the binary vector, *Agrobacterium* strain and the texture of target tissue. After about 5 subcultures, new lines started emerging from the infected tissues and proliferated in the same medium. Twelve SOD transgenic lines, 19 TB antigen gene incorporated transgenic lines and 38 IPT gene incorporated transgenic lines could be obtained. Proliferating new lines were subcultured further in selection medium containing kanamycin (200 mg/l). Eight SOD transgenic lines, 15 TB antigen transgenic lines and 23 IPT transgenic lines turned friable, yellow and proliferating and they were transferred to embryo induction medium fortified with charcoal. Four transgenic lines from SOD, five from IPT and seven from TB antigen produced embryogenic callus after five months culture. Embryo induction was initiated from the proliferated embryogenic callus.

The MnSOD transgenic plants developed earlier were multiplied by bud grafting. Water stress was induced in control and transgenic plants by withholding water for two weeks. Leaf samples were collected and total RNA was isolated for northern hybridization analysis. Northern hybridization was

carried out with the RNA isolated from all the samples using MnSOD probe according to the standard protocol. Higher MnSOD transcript level was observed in the stress induced transgenic leaves indicating over expression of the integrated gene under environmental stress conditions.

Attempts were continued to develop transgenic plants integrated with the gene coding for osmotin protein, for imparting biotic and abiotic stress tolerance. Seven different explants such as embryogenic calli of anther, ovule, leaf and 2-month-old calli derived from leaf and anther and leaf discs were tried. 15 transgenic lines showing resistance to kanamycin were obtained from embryogenic calli of anther. Ten transgenic lines were obtained from 2-month-old calli derived from anther. No transgenic calli were obtained from leaf discs. Infected cultures could not be from embryogenic calli of leaf due to *Agrobacterium* overgrowth. Four transgenic cell lines were obtained from ovule derived embryogenic calli.

Among different growth regulators tried for proliferation, a combination of NAA with 2, 4-D favored callus growth and the proliferation rate could be improved considerably (Table Biotech. 6). A steady increase in the callus proliferation rate with increase in 2, 4-D was observed. It was observed that up to 1.5 mg/l 2, 4-D, the calli were yellow and friable whereas, with higher concentrations the calli appeared white and watery. The calli was proliferated in modified MS medium supplemented with 2, 4-D (1.5 mg/l) and NAA (1 mg/l). Proliferated calli were transferred to embryo induction medium.

For embryo induction, modified MS medium with different levels (0.1-5 mg/l) of growth regulators, viz. NAA, ABA, Kin, BA and 2, 4-D were tried. Embryo induction

was observed in modified MS medium supplemented with NAA and mannitol after 2 months in culture. Maximum embryo induction frequency (36%) could be obtained in a combination of 10 g/l¹ mannitol and 2 mg/l NAA. It was also noticed that when NAA and mannitol were supplied individually, no embryos could be obtained. Maximum embryo induction was observed at a higher concentration of NAA (2 mg/l) in the presence of mannitol. However, higher concentrations beyond 3.0 mg/l resulted in reduction in the embryo induction frequency. Embryos were matured (15%) and a few germinated (2%).

A study was also carried out to develop transgenic plants integrated with *HMGR-1* gene for increased rubber biosynthesis. The embryogenic callus developed from immature anther was used as the explant for *Agrobacterium* infection. The effect of different factors such as antinecrotic agents, media components, co-cultivation temperature, desiccation of the culture after infection, different *Agrobacterium* strains etc. contributing to increased transformation frequency was assessed. In order to improve the explant viability, the embryogenic callus was treated with antinecrotic mixture

containing 15 mg/l ascorbic acid, 40 mg/l cysteine and 2.0 mg/l silver nitrate for a period of 5-10 h. The co-cultivation medium (MS) containing acetosyringone (20 mg/l) and glycine betaine HCl (154 mg/l) was additionally supplemented with varying concentrations of L-cysteine (0, 100, 200, 300 mg/l) and AgNO₃ (0, 20, 40, 60 µM). In the experiments on antinecrotic treatment, co-cultivation media components and desiccation after infection were not having any positive influence. In order to identify the ideal co-cultivation temperature, the *Agrobacterium* infected tissues were subjected to different temperature regimes such as 4, 20 and 28°C for three days. It was observed that when the plates were co-cultured at 20°C, the callus remained yellow after two sub cultures and emergence of transgenic cell lines was observed.

Agrobacterium strain is considered as one of the key parameters which plays an important role in transformation efficiency. When different *Agrobacterium* strains were used for infection, there was significant variation in the transformation frequencies. Tissues infected with *Agrobacterium* strains EHA 105 and pGV 1303 produced transgenic cell lines with varying frequencies, but no transgenic line was produced from the callus infected with LBA 4404. Efficiency of transgenic cell line formation was observed to be higher with the strain EHA 105 compared to pGV 1303. Using fresh anther callus as the explant, the frequency of transformation was 8% with EHA 105 strain and 4% with the strain pGV 1303 (Table Biotech. 7). Therefore the strain EHA 105 was better than pGV 1303 while LBA 4404 was the least responsive strain. From the results obtained, it can be concluded that the efficiency of *Hevea* transformation with *HMGR-1* gene callus can be significantly

Table Biotech. 6. Effect of 2,4-D on callus proliferation in presence of NAA (1 mg/l)

2,4-D (mg/l)	Callus proliferation (%) ^a	Appearance of calli
0.5	19.53 (11.2)	Yellow friable calli
1	34.57 (32.2)	Yellow embryogenic calli
1.5	60.00 (75)	Yellow embryogenic calli
2	73.38 (91.8)	Loose watery calli
2.5	93.2 (74.94)	Loose white calli

CD (P = 0.5) 1.39

^a The analysis was performed using Arc sine transformation and transformed values are given in parenthesis

enhanced by using the strain EHA 105 co cultured at 20°C.

Table Biotech. 7. Influence of bacterial strain on transformation efficiency

Strain	No. of callus clumps cultured	No. of lines emerged	Transformation frequency (%)
EHA105	50	4	8
pGV 1303	50	2	4
LBA 4404	50	—	—

4. Molecular studies

4.1. Molecular mechanism of disease tolerance

In our earlier attempts to amplify the promoter of the gene coding β -1,3-glucanase enzyme involved in abnormal leaf fall disease tolerance, four different promoter fragments (910, 550, 579 and 198 bp) were obtained when inverse PCR was carried out with β -1,3-glucanase gene specific primers. Attempts were continued to amplify these promoter forms along with coding regions of the genomic DNA using promoter-specific forward and gene-specific reverse primers. The reverse primers were designed from the conserved regions upstream to the stop codon. During these experiments, two more isoforms were obtained. The 847 bp promoter showed more coding sequence similarity with an earlier reported sequence AY 325498 (Thanseem *et al.* 2003) and exhibited only four nucleotide differences. Another form is from *Hevea* clone FX-516 which is more tolerant to abnormal leaf fall disease. These results clearly indicated the existence of at least five forms of β -1,3-glucanase gene controlled by separate promoters in a single *Hevea* clone RRII 105. Out of the newly isolated six forms of β -1,3-glucanase including one isolated from the

clone FX-516, the one which had a 579 bp promoter sequence (*Gln 4*) showed more dissimilarity with the other forms. This particular form had a different stop codon, 'TAA' (amber codon) rather than the 'TGA' (ochre codon) in other forms. *Gln 4* showed one amino acid deletion (21st amino acid methionine) with respect to other forms thus having a total of 373 amino acids while 374 amino acids with the other forms. These changes in the open reading frame could lead to structural and functional changes of the different forms. This can be proved through specific expression of the proteins through qualitative as well as quantitative analysis.

The 3' untranslated regions (3' UTR) of genes control the stability and translocation of mRNAs. In order to understand the stability of mRNA and translocation of the protein of different forms of glucanases, trials were carried out to amplify the different isoforms along with their 3' UTR with different sets of primers. The isoform-specific forward primers were designed from the promoter region showing clear nucleotide sequence variations in their promoter sequence and the reverse primers from different regions of the 3' UTR of the cDNA reported earlier by Chye *et al.* (1996). To study the function of the fully characterized isoforms of glucanase gene, attempts were made to obtain the translated protein in its original form. For this purpose yeast expression vectors were constructed with β -1,3-glucanase gene isoforms for over expression of the protein in the yeast, *Pichia pastoris*. Two types of expression vectors were constructed for each of these different forms of glucanases in secretory (pPICZ 8) as well as in non secretory (pPICZ) vectors. The restriction digestion of the vectors as well as the inserts (amplified from the cloned gene insert) were done successfully with *EcoRI* and *KpnI* for the secretory system and *XbaI*

and *KpnI* for the non- secretory systems. Twelve vectors were constructed with all the inserts in appropriate reading frame in the vector. The vectors were amplified in DH5 α cells using Zeocin antibiotic as the selection marker. The clones harbouring the recombinant plasmids were subjected to plasmid isolation. Later the linearized plasmids were introduced in the competent *Pichia pastoris* and selection was made in YPD5 plates with 100 μ g/ml Zeocin. The *Pichia* strain X-33 was used as the host for the expression of the recombinant proteins (X-33 is a wild type strain with *Mut^r* phenotype). The screening of the host strains was done in MM and MGY plates for non- secretory and BMM/ BMGY plates for the secretory type.

4.2 Tissue specific gene expression and characterization of promoters

An attempt was made to clone and characterize the promoter of *cis-prenyltransferase* gene from RR11 105. The upstream promoter region was amplified through inverse PCR using genomic DNA. PCR was performed with oligonucleotide primers designed based on sequences registered earlier in GenBank database (Accession nos. AY712939, AY299405 EF587242 and EU675683) and the restriction enzyme sites in the known sequence. Two amplicons of size 700 bp and 400 bp were obtained with *DnaI* digest. DNA bands were eluted from the gel, purified, cloned into plasmid vector (Strataclone cloning kit for sequencing) and nucleotide sequenced. The larger DNA fragment contained 610 base pairs which included the forward and reverse primers, gene portion and 5' UTR. By excluding the nucleotides belonging to the known gene portion and 5' UTR, a 504 bp promoter region was obtained. The known portion of the amplified fragment

showed similarity to the HbCPT mRNA reported by Sando *et al* (2008) isolated from the *Hevea* clone RRIM 600 (NCBI Acc No: AB294715). This is an indication of the occurrence of similar forms/isoforms of *cis-prenyltransferases* across various *Hevea* clones. The smaller fragment contained 361 bp including forward and reverse primers and 5' UTR. A 241 bp region, upstream to the early reported portion was obtained. The known portion showed significant levels of identity to the *cis-prenyltransferase-2* reported by Saleena *et al* (2008) from *Hevea* clone RR11 105 (NCBI Acc No: EU675683). In the 610 bp fragment, the TATA box was present at the 257th position.

The relative expression of two isoforms of *cis-prenyltransferase*, *cis-1* and *cis-2* reported earlier were studied through $\Delta\Delta Ct$ Method. Latex cDNA prepared from two clones, representing a high latex yielding (RR11 105) and a low latex yielding clone (RR11 33) were used for Real Time PCR analysis. The relative expression was calculated by setting the value of *cis-1* as reference ("1") and the value of *cis-2* was compared. The relative expression of *cis-2* was lower in both the clones. In the clone RR11 105, the *cis-2* gene expression was only about 75% and in RR11 33 it was about 60 % in comparison with *cis-1*. Analysis of *cis-1* and *cis-2* gene expression was carried out by using *cis-1* of RR11 105 alone as the reference. In RR11 33, the expression of *cis-1* was higher compared to RR11 105. However, in clone RR11 105, the expression of *cis-2* was more compared with the clone RR11 33. The expression rate of *cis-1* & 2 genes in the high and low yielding plants of the same clone was also compared. The expression of *cis-1* and *cis-2* were higher in the high yielding plants of RR11 105 and RR11 33. More studies are needed to understand the factors controlling the

differential expression of *cis-1* and *cis-2* and their role in rubber yield and chain elongation.

An attempt was also made to understand the relative expression of *cis-1* and *cis-2* genes with respect to enhanced rubber production when stimulated with ethephon. Real Time PCR analysis shows a higher accumulation of the *cis-prenyltransferase* transcripts in the latex of ethephon treated trees compared to that of control. The relative expression was

calculated by using the value of *cis-1* and *cis-2* of 0 hr as reference ('1'). In RRII 105, *cis-1* showed a two fold increase and *cis-2* showed a four fold increase after 48 hr of stimulation. In RRII 33, *cis-1* showed a 1.75 fold and *cis-2* a 3 fold increase. A significant increase in mRNA accumulation was detected in stimulated trees after 24 and 48 h. The stimulatory effect was found to be high at 48 h. of treatment where the maximum *cis-1* and *cis-2* mRNA levels could be detected as compared to 0 h treatment.

BOTANY DIVISION

Evolving new and improved rubber clones, evaluation of clones for latex and timber yield, drought and disease tolerance, need based investigations on propagation aspects and studies on the anatomy of bark and wood were the thrust areas of work in the Botany Division during the reporting year. The second phase of the participatory clone evaluation project was initiated for multi-locational on-farm and large scale evaluations of the third batch of promising clones in the pipeline. Post-release follow-up observations on the yield of the RRII 400 series clones planted in small holdings were initiated during the year.

1. Evolving high yielding clones for the traditional area

1.1 Hybridization and clonal selection

The yield of 42 promising selections from among 200 hybrid clones resultant of the 1986 hybridization programme, which were in the 12th year of tapping (Table Bot. 1) in four small scale trials laid out at CES, Chethackal in 1989, was assessed.

Twenty nine hybrid clones maintained higher annual mean yield than RRII 105 during the period, and 27 clones maintained a higher summer yield than RRII 105.

Out of the 37 promising selections among 196 hybrid clones under observation in eight small scale trials planted in 1990, 22 clones maintained higher yield than RRII 105 in the 11th year of tapping. Among 21 hybrid clones under evaluation in two small scale trials planted in 1992, five clones recorded superiority in yield over the check, RRII 105 in the tenth year of tapping with yield ranging from 55.73 to 65.72 g/t/t.

Thirty four hybrid clones are under evaluation in the SST planted in 1995. Of these, 25 clones recorded promising yield compared to RRII 105 in the 7th year of tapping (Table Bot. 2).

Among the 26 W x A hybrid clones under evaluation in an SST planted in 1995, 15 clones were promising compared to the check clone RRII 105 in the 7th year of tapping (Table Bot. 3).

Table Bot.1. Yield (g/t/t) of selections in the small scale trials in the 12th year of tapping

Trial A		Trial B		Trial C		Trial D	
Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield
86/244	120.62	86/32	90.98	86/428	166.13	86/188	106.29
86/110	59.43	86/44	82.68	86/424	87.22	86/674	89.46
86/60	97.77	86/64	80.55	86/650	104.08	86/599	31.65
86/120	87.70	86/79	84.02	86/778	64.09	86/400	79.26
86/34	99.67	86/111	112.20	86/908	53.54	86/191	35.65
86/59	84.73	86/117	68.97	86/968	61.35	86/597	160.45
86/23	78.30	86/122	140.90	86/966	75.98	86/99	103.07
86/5	40.15	86/174	97.38	RRII 105	57.68	86/98	92.98
86/157	100.19	86/306	50.95			86/70	41.79
86/304	90.83	86/651	48.79			86/68	48.96
86/902	72.21	RRII 105	75.71			RRII 105	55.04
86/957	71.52						
86/607	66.63						
86/602	91.61						
86/613	88.17						
86/660	60.10						
RRII 105	74.74						

In another small scale trial of 17 hybrids obtained by crossing RRII 105 with six wild germplasm accessions, one hybrid clone, 90/109, from the cross RRII 105 x RO 26 was better in yield than RRII 105 and four hybrid clones were comparable with the check in the 7th year of tapping.

In a clonal nursery of 36 hybrid clones (Wickham x Amazonian germplasm), comprised of the progenies of 1997 HP, test tapping was conducted for three consecutive years. Mean test tap yield over three years revealed that five clones were superior over the check (RRII 105). Clone 97/300 was the highest yielder (29.32 g/t/10 tap) followed by 97/213 (28.83 g/t/10 tap). The check clone showed 16.08 g/t/10 tap. Highest girth (25.25 cm) was observed in clone 97/300 followed by clone 97/213 (21.17 cm). Estimates of standard heterosis for yield and girth were worked out (Table Bot. 4).

Out of the 26 hybrid clones and their parents under evaluation in the two trials

planted in 1998, one clone was superior and 13 clones were on par with RRII 105 in terms of yield in the fifth year of tapping. Among 40 hybrid clones under evaluation in two small scale trials planted in 1999, four clones were superior and 15 clones were comparable in yield to RRII 105 which registered 49.9 and 68.5 g/t/t in trials 1 and 2 respectively in the fourth year of tapping. Among 24 hybrid clones under evaluation in another small scale trial, in the second year of tapping, nine clones with yield ranging from 37.0 to 69.1 g/t/t were promising compared to the check RRII 105 which registered 34.5 g/t/t. Among 54 clones under evaluation in three small scale trials planted in 2001, in the second year of tapping, 22 hybrid clones registered better yield compared to the high yielding check, RRII 105, while 39 clones were superior in girth compared to the check.

Among the 22 clones of full-sib and half-sib origin generated during the 2002

Table Bot. 2. Performance of hybrids from the 1989 HP programme

Sl. No.	Clones	Parentage	Mean girth in the 7 th year of tapping (cm)	Mean yield in the 7 th year (g/l/l)
1	89/7	PB 260 x RRIM 600	76.13	89.18
2	89/21	PB 260 x RRIM 600	61.83	51.49
3	89/27	PB 260 x RRIM 600	83.25	96.14
4	89/30	PB 260 x RRIM 600	61.34	51.06
5	89/49	RRIM 600 x PB 242	59.22	52.93
6	89/63	RRIM 600 x PB 242	72.93	72.62
7	89/64	RRIM 600 x PB 242	78.04	60.05
8	89/79	PB 311 x RRII 105	68.22	71.69
9	89/88	PB 311 x RRII 105	67.54	59.12
10	89/95	PB 311 x RRII 105	71.90	91.46
11	89/102	PB 311 x RRII 105	66.89	65.82
12	89/103	PB 311 x RRII 105	68.15	47.28
13	89/124	PB 311 x RRII 105	69.33	61.66
14	89/128	PB 311 x RRII 105	67.46	48.41
15	89/145	RRII 105 x RRII 118	58.41	44.20
16	89/230	RRII 105 x RRII 118	66.17	43.79
17	89/240	RRII 105 x RRII 118	60.46	54.54
18	89/243	RRII 105 x RRII 118	60.06	65.73
19	89/262	IAN 873 x RRII 105	64.42	49.00
20	89/308	GT 1 x PB 311	67.37	62.80
21	89/309	GT 1 x RRIM 600	68.71	79.90
22	89/318	GT 1 x RRIM 600	73.79	62.07
23	89/344	GT 1 x RRII 105	59.71	51.06
24	89/349	GT 1 x RRII 49	71.25	72.08
25	89/356	IAN 873 x RRII 118	73.71	71.69
26	RRII 105	Tjir 1 x GI 1	56.00	42.24
CD (0.05)			10.57	17.26

hybridization and polycross breeding programmes, which are under evaluation in a clonal nursery planted in 2007 along with the check clones RRII 105, RRII 414 and RRII 429, progeny of the cross combination PB 330 x RRII 414 showed highest growth (13.8 cm) followed by progeny of PB 330 x RRII 414 with 12.8 cm girth, in the second year after planting. These two clones also exhibited good branching habit.

Six full-sib families and three half-sib families consisting of 465 seedlings resultant of the 2005 hybridization and polycross breeding programmes were subjected to

detailed evaluation. Seedling progenies exhibited wide variability in terms of growth, test tap yield and morphological characters. Thirty five promising progenies were selected based on growth and vigour in the early stage for further evaluation. Selection of medium to high growth seedlings could maximize genetic gain. The present study could also identify potential parents for repeated recombination to generate more productive progenies. Recovery of high frequency of elite progenies from the half-sib progeny evaluation underlined the importance of this approach

Table Bot. 3. Mean girth and yield of promising clones

Sl. No.	Clone	Parentage	Mean girth in the 7 th year	Mean yield in the 7 th year
			of tapping (cm)	(g/tl)
1.	90/3	RRII 105 x RO 142	74.65	47.58
2.	90/10	RRII 105 x RO 142	81.66	120.93
3.	90/21	RRII 105 x RO 142	80.83	56.38
4.	90/25	RRII 105 x RO 142	63.42	57.47
5.	90/29	RRII 105 x RO 142	88.33	56.05
6.	90/34	RRII 105 x RO 142	74.91	74.43
7.	90/241	RRII 105 x RO 142	74.95	68.32
8.	90/322	RRIM 600 x RO 142	74.00	49.37
9.	90/340	RRIM 600 x RO 142	62.21	63.17
10.	90/170	RRIM 600 x RO 87	71.87	65.58
11.	90/171	RRIM 600 x RO 87	83.88	53.57
12.	90/174	RRIM 600 x RO 87	81.46	51.41
13.	90/193	RRIM 600 x RO 87	73.93	81.07
14.	90/352	RRIM 600 x RO 87	69.58	53.83
15.	90/271	RRIM 600 x RO 87	78.92	73.46
16.	RRII 105	Tjir 1 x GI 1	55.69	58.45
CD (0.05)			10.15	12.23

Table Bot. 4. Standard heterosis for girth and yield of W x A hybrid clones

Sl. No.	Clone	Parentage	Standard heterosis	Standard heterosis
			for girth (%)	for yield (%)
1.	97/4	RRII 105 x MT 1014	73.89	14.00
2.	97/10	RRII 105 x MT 1027	68.82	-
3.	97/213	RRII 105 x MT 1005	75.97	79.29
4.	97/219	RRII 105 x MT 1005	73.15	-
5.	97/224	RRII 105 x MT 1005	24.68	-
6.	97/225	RRII 105 x MT 1005	38.57	-
7.	97/238	RRII 105 x MT 1021	32.33	-
8.	97/13	RRIM 600 x MT 999	50.29	-
9.	97/47	RRIM 600 x RO 380	67.33	21.64
10.	97/62	RRIM 600 x MT 1021	60.68	-
11.	97/63	RRIM 600 x MT 1021	55.19	-
12.	97/72	RRIM 600 x MT 1021	40.81	-
13.	97/72	RRIM 600 x MT 1005	39.23	-
14.	97/98	RRIM 600 x MT 1005	67.66	-
15.	97/245	RRIM 600 X MT 1005	7.14	-
16.	97/255	RRIM 600 X MT 1014	46.38	-
17.	97/125	RRIM 600 x MT 1014	82.21	-
18.	97/181	RRIM 600 x MT 1014	68.99	17.03
19.	97/182	RRIM 600 x MT 1014	110.00	82.34
20.	97/300	RRIM 600 x AC 495	15.87	-
21.	97/166	RRIM 600 x AC 495	82.87	-
22.	97/170	RRIM 600 x AC 495	75.22	-
23.	97/173	RRIM 600 x AC 495	70.40	-
24.	97/279	RRIM 600 x AC 495	60.26	-
25.	97/289	RRIM 600 x MT 1027	30.92	-
26.	97/189	RRIM 600 x MT 1027	54.44	-
27.	97/196	RRIM 600 x MT 1027	42.89	-
27.	97/199	RRIM 600 x MT 1027	-	-

Table Bot. 5 a. Narrow sense heritability of yield and growth traits

Parent progeny relationship	Heritability equations	AMY (g/t/t)	SY (g/t/t)	G (cm)	BH (m)
Narrow sense heritability estimates (h^2)					
Offspring - female parent	$h^2 = 2b$	-0.18	-0.22	-0.14	-0.28
Offspring - male parent	$h^2 = 2b$	0.56	0.52	0.36	0.18
Offspring: midparent mean	$h^2 = b$	0.34	0.36	0.17	-0.11

AMY- Annual mean yield; SY- Summer yield; G- Girth; BH- Branching height

b = parent-offspring regression.

Table Bot. 5 b. Genetic correlation (r_g) of yield and growth traits based on offspring-parent 'cross-variance' estimates

	Summer yield	Girth	Branching height
Annual mean yield	0.8557	0.8432	-0.1709
Summer yield		1.3448	-1.3714
Girth			0.1464

which can be repeated over the years to consolidate productive seedlings.

Based on data from the full-sibs and their parent clones, narrow sense heritability of yield and growth traits and their genetic correlation were estimated based on parent-offspring regression. The heritability for annual mean rubber yield based on parent-offspring regression was moderate ($h^2=34\%$ in offspring-midparent grouping) to high ($h^2=56\%$ in offspring-male parent grouping). In respect of summer yield also, the offspring-male parent grouping showed comparatively high heritability ($h^2=52\%$) than the offspring-midparent grouping ($h^2=36\%$), based on regression analysis. Heritability for girth was low ($h^2=17\%$ in offspring-mid parent analysis) to moderate ($h^2=36\%$ in offspring-male parent grouping) using regression. Regarding branching height, offspring-male parent grouping showed a low heritability ($h^2=18\%$) in regression analysis. Based on parent-offspring cross-variances, annual mean rubber yield showed high genetic correlation with summer yield and girth.

Summer yield showed very high correlation with girth (Tables Bot. 5 a and b).

1.2. Ortet selection

A total of 115 ortets selected from various small holdings and large plantations are under evaluation in six small scale trials and two large scale trials at CES, Chethackal. In the two small scale trials of ortets selected from PCK Ltd. Kodumon estate (Table Bot.6), OKn 36, a potential latex timber clone in one SST continued to perform better (71.09 g/t/t) followed by OKn 49 (68.13 g/t/t) and OKn 73 (61.30 g/t/t) during the 7th year of tapping, whereas RRII 105 recorded an average yield of 66.59 g/t/t during the period. Rising yield trend of these clones was observed during 7th year of tapping. In the other SST where clones are in the 8th year of tapping, OKn 28, OKn 75 and OKn 39 recorded comparable yield with RRII 105 which recorded 62.75 g/t/t. OKn 39, OKn 4 and OKn 73 were superior in girth compared to RRII 105.

In the small scale trial of ortets selected from various small holdings, yield in the 5th year showed that O 73 (selection from a small holding at Kanjirappally) and O 72 (selection from the selfed progeny of RRII 105), recorded superior yield with 87.31 g/t/t and 82.68 g/t/t respectively and girth compared to check clone RRII 105 (55.03 g/t/t) (Table Bot.7). Growth of clone O 73 was very high recording 82.79 cm during 5th year of

Table Bot. 6. Yield and girth of ortet selections (Kodumon estate)

Trial 1 (1993 SST)			Trial 2 (1994 SST)		
Clone	Yield (g/t/t) 8 th year of tapping	Girth 8 th year of tapping	Clone	Yield (g/t/t) 7 th year of tapping	Girth (cm) 7 th year of tapping
OKn 28	56.91	87.91	OKn 50	35.86	79.55
OKn 11	41.08	81.17	OKn 4	27.52	91.34
OKn 59	26.20	75.64	OKn 73	61.30	73.96
OKn 54	37.43	85.70	OKn 51	48.65	68.29
OKn 63	32.11	67.02	OKn 38	39.65	68.88
OKn 75	58.70	83.80	OKn 10	18.88	67.75
OKn 22	48.68	71.81	OKn 29	33.45	61.65
OKn 25	25.91	69.22	OKn 32	31.53	68.37
OKn 01	21.97	62.65	OKn 17	49.17	85.36
OKn 12	39.87	79.43	OKn 68	30.60	63.27
OKn 39	61.59	85.45	OKn 61	24.49	48.52
OKn 74	37.04	74.62	O Kn 26	46.66	80.24
OKn 27	28.61	70.07	O Kn 15	20.86	59.88
OKn 19	23.21	74.42	O Kn 37	46.59	75.71
RRIM 600	51.37	69.76	OKn 2	33.90	70.32
RRII 105	62.75	66.02	OKn 36	71.09	77.00
			OKn 49	68.13	77.14
			OKn 44	39.19	81.90
			OKn 48	38.11	62.94
			OKn 46	31.50	65.15
			OKn 41	58.77	71.82
			OKn 24	32.80	67.07
			OKn 55	40.30	64.95
			OKn 23	44.81	80.27
			OKn 35	39.22	64.07
			RRII 105	66.59	62.20
CD (P = 0.05)	23.69	16.14	CD (P = 0.05)	21.88	

tapping indicating the clone as a very promising latex timber clone.

In the small scale trial of 12 ortets selected from HML Kaliyar, Thodupuzha, laid out at CES in 1998, OKr 75, OKr 48 and OKr 14 recorded comparable yield with the check RRII 105 in the fourth year of tapping. In another small scale trial laid out in 2000, one ortet selection from Kasargode, OKgd 3 (46.74 g/t/t) recorded yield superior to RRII 105 (35.38 g/t/t) in the second year of tapping.

In a small scale trial planted in 1997 four clones showed higher yield than the check clone in the 5th year of tapping. Highest yield was recorded by the ortet clone 12 (85.46 g/t/t) followed by clone 35 (70.18 g/t/t) and 33/8 (69.70 g/t/t), while the check, RRII 105 recorded 60.47 g/t/t.

In the large scale trials of ortets selected from large estates, which are in the 4th year of growth, clone MO 28 in one trial recorded the highest girth (32.58 cm) followed by RRII 430 (32.17 cm) whereas the girth of RRII

Table Bot. 7. Yield and girth of ortet selections from small holdings in the 5th year of tapping

Clone	Yield	Girth
O 49 (PCK Kodumon)	36.33	79.94
O 77 (Erumely - EB1)	29.61	63.92
O 74 (Kanjirappally)	42.28	79.25
O 73 (Kanjirappally)	87.31	82.79
O 75 (Kanjirappally)	20.26	73.34
O 76 (Kanjirappally)	18.27	64.82
O 21 (Kattappana)	38.06	81.13
O 72 (Progeny of RRII 105)	82.68	77.47
O 36 (Kothamangalam)	31.83	78.36
O 81 (CES, Chethackal)	39.78	89.62
O 79 (CES, Chethackal)	18.21	68.50
O 80 (CES, Chethackal)	28.04	61.62
O 78 (CES, Chethackal)	30.64	69.98
O 66 (PCK Kodumon)	30.14	64.28
RRII 105	55.03	67.37
RRIM 600	31.23	60.93
CD (P = 0.0 5)	20.2	17.81

105 was 22.95 cm. In another large scale trial, the highest girth was recorded by clone OChy 35 (33.44 cm) followed by OChy 48 (33.31 cm) whereas the girth of RRII 105 was 27.69 cm.

Twenty one ortets selected from Guwahati, Padiyoor and Ambalavayal are under small scale evaluation in a clonal nursery laid out in 2007 at RRII along with check clones viz., RRII 105, RRII 414 and RRII 430. Three ortets from Paralair, viz. Par O 10, Par O 18 and Par O 11 were vigorous in growth in the second year after planting compared to the fast growing check clone RRII 414.

An ortet selection programme was undertaken at Andamans in an area comprising 206.36 ha. planted with GGI, GGII, and GGIII series of PBIG seedlings. Initially, 75 trees were selected and yield and secondary characters recorded for one year. Of these, 23 trees were finally selected and the clones were multiplied for establishment of source bush nursery.

2. Evaluation of clones

2.1. Large scale evaluation

In the multidisciplinary evaluation of 26 clones, which is in the 12th year of tapping in two large scale trials planted in 1989 at RRII Farm, RRII 5 and RRII 118 continued to be the top yielders in Trial 1, showing better performance over RRII 105. In Trial 2, PB 255 followed by PB 314 were the top yielders (Table Bot. 8). Clones PB 280, PB 311 and PB 235 also recorded significantly high yields.

Table Bot. 8. Yield of introduced and indigenous clones in the 12th year of tapping

Trial 1		Trial 2	
Clone	Yield (g/t/t)	Clone	Yield (g/t/t)
RRII 5	93.51	PB 217	49.33
RRII 118	84.77	PB 235	70.14
RRII 208	30.84	PB 255	93.07
RRII 300	37.92	PB 260	65.70
RRII 308	58.02	PB 280	83.63
RRIM 600	43.26	PB 310	53.47
RRIIM 703	37.95	PB 311	75.39
PCK 1	56.25	PB 312	52.83
PCK 2	32.61	PB 314	52.43
SCATC 88/13	38.74	KRS 25	44.57
SCATC 93/114	21.15	KRS 128	46.71
HN 1	19.50	KRS 163	66.94
RRII 105	54.62	RRII 105	34.64
GM*	46.86	GM	60.68

*G.M. - General mean

Clones PB 280, PB 314 and PB 217 recorded significantly higher yield than RRII 105 in the 13th year of tapping in another large scale evaluation of introduced and indigenous clones at CES. Yield in the summer months was high in clones PB 311, PB 280, PB 314 and PB 217 when compared to RRII 105.

In the large scale trials planted at CES in 1993, Clones RRII 414 and RRII 417 in

trial 1 and clones RR11 422 and RR11 430 in trial 2 were superior to RR11 105 in terms of mean yield over nine years of tapping. In general the clones showed a rise in yield in the third year of tapping in panel BO 2, compared to that of the previous year. Clones RR11 417 and RR11 430 were significantly superior to the rest in the ninth year of tapping. The other clones yielding better than RR11 105 were RR11 414 and RR11 55 in trial 1 and PB 330 in trial 2. Based on long term yield, an attempt was made to determine the period of stabilisation of rubber yield by employing the Spearman's rank correlations. In general the clones attained stable yield levels after the third year of tapping as evidenced by significant positive and high correlations in yield rankings among the third and subsequent years of tapping.

In terms of structural features of the virgin and renewed bark of the RR11 400 series clones, there was no clonal variation for bark thickness, but the number of laticifers varied significantly. In the virgin bark, the number of laticifers was highest (>23 nos.) in clones RR11 402 and RR11 429 in trial 1 and RR11 52 and RR11 422 in trial 2. These clones were superior to RR11 105. Clones RR11 402 (>24 nos.) and RR11 422 (>23 nos.) maintained superiority with the highest number of latex vessel rows in the renewed bark also.

In a collaborative study on the RR11 400 series clones, parameters related to rubber biosynthesis such as sucrose content, ATP and C-serum invertase, and antioxidant enzymes (SOD and peroxidase), and other biochemical components such as thiols and proteins were measured during peak yielding season (Sep-Nov) and low yielding summer season (Feb-April) for two consecutive years (2008 and 2009). The

results indicated that RR11 422, followed by RR11 430 and RR11 414, had potential for better tolerance to stress situation than the other clones.

Yield in the seventh year in the large scale evaluation of 12 clones of exotic and indigenous origin at CES, Chethakkal, showed that clones, viz. 86/120 (91.73 g/t/t), 86/44 (77.80 g/t/t) and RR11 712 (84.60 g/t/t) were showing yield comparable with RR11 105 which recorded 95.87 g/t/t. The clone 86/44 performed better than RR11 105 in the large scale trial at RRS, Padiyoor, showing 8.3% yield improvement with 49.40 g/t/t whereas RR11 105 recorded 45.60 g/t/t during the second year of tapping. In another large scale trial at RRS, Padiyoor, clone 86/468 (55.31 g/t/t) recorded 21.34% yield improvement over RR11 105 which recorded 45.58 g/t/t followed by clone 86/613 (51.25 g/t/t) with yield improvement of 12.43%.

2.2. On farm evaluation

Two indigenous and seven introduced clones are under evaluation in the on-farm trial at Sasthamkotta. Mean yield in the sixth year (Table Bot.9) revealed that clone RR11 105 was the highest yielder (2584 kg/ha/yr) followed by PR 255 (2219 kg/ha/yr). Clone PB 255 showed the highest girth (66.00 cm) followed by RR11 203 (62.00 cm).

Table Bot.9. Performance of clones at Sasthamkotta in the 6th year of tapping

Clones	Girth (cm)	Yield (kg/ha/yr)
RR11 105	57.60	2584
GT 1	56.65	1707
RR11 203	62.00	1850
PB 217	60.74	1721
PB 255	66.00	2198
PB 260	56.71	1764
PB 311	57.85	2007
PB 314	58.15	2137
PR 255	61.54	2219

In an on-farm evaluation in the 10th year of tapping at Shaliackary Estate, Punalur, PB 280 (2473 kg/ha), RRII 5 (2345 kg/ha) and PB 260 (2336 kg/ha) recorded better yield than RRII 105 (1979 kg/ha.). In another on-farm evaluation at the same estate, among five clones, RRII 176 a hybrid clone evolved in India with 4035 kg/ha was superior in yield compared to RRII 105 (3913 kg/ha) in the 12th year of tapping. Clone PB 280 recorded higher yield and girth followed by PR 261, compared to RRII 105 in the 2nd year of tapping in an on-farm evaluation at Ayiranallur, Punalur.

The RRII 400 series clones, under tapping in various large estates, continued to maintain better yield and growth compared to RRII 105. At Cheruvally Estate, among the five recommended RRII 400 series clones, RRII 430 was consistently the top yielder over six years of tapping. In the OFT at Shaliackary Estate at Punalur, RRII 429 followed by RRII 414 and RRII 417, were superior in girth and yield compared to RRII 105.

As part of post-release follow-up on the RRII 400 series clones, more detailed studies on the growth and yield of these clones were initiated in small holdings scattered across Kerala. Data on girth, yield and disease incidence were collected from five small growers fields in Central Kerala, two small holdings in South Kerala and three small holdings in North Kerala. At Malayattoor, clones RRII 417, RRII 422 and RRII 429 recorded higher yield than RRII 105. At Oonnukal, RRII 429 and RRII 430 performed better than RRII 105. At Ponkunnam, tapping was initiated in 100% trees in the 5th year after planting and yield was promising. In the adjacent plot tapping was not yet initiated in RRII 105 due to inadequate girth. Growth data of RRII 400

series clones in the fifth year from the OFT at a small holding in Kanjirapally showed that RRII 429 recorded highest girth (45.5 cm) followed by RRII 414 (44.4 cm) compared to RRII 105 (38.8 cm). In another small holding in Ayur, Kollam district, RRII 422 followed by RRII 414, RRII 417 and RRII 429 were superior in yield compared to RRII 105. Among the locations in North Kerala, at Ottappalam, clone RRII 417 performed better than the check. At Malappuram, RRII 422 exhibited better performance compared to the other RRII 400 series clones and RRII 105. At Kunnamkulam, RRII 414 exhibited better yield and girth. The incidence of pink disease at Ottappalam was much lower in clone RRII 417 (14.4%) compared to RRII 105 (22.5%).

2.3. Investigations on genotype x environment interactions

Data on mean yield for the 4th to 6th year of tapping (yield stabilization period) from 3 locations, viz. Kanyakumari, Agartala and Nagrakata, which were opened for tapping simultaneously, were subjected to AMMI (Additive Main effects and Multiplicative Interaction) analysis to estimate G x E interaction. Attempt was also made to study the influence of meteorological variables on rubber yield. At IMMT, Bhubaneswar, yield recording was initiated in 2009, and the yield data for the first year was summarized.

The ANOVA for AMMI model showed that genotypic variation contributed to only 27 % of the total variation whereas environmental variation and GEI together contributed to more than 70 % of the total variation.

Genotype evaluation based on AMMI-1 biplot is given in Fig. Bot.1. Clones RRII 429, RRII 422, RRII 417, RRII 430 and RRII 105 were the high yielding clones across environment. RRIM 600, which showed the least interaction, was found to be the most

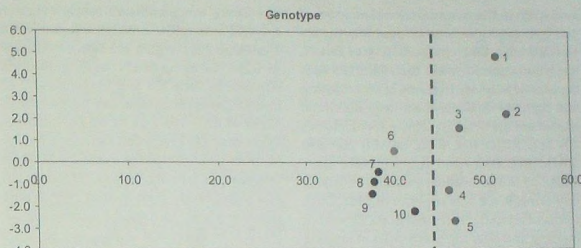


Fig. Bot.1. Genotype evaluation based on biplot AMMI-1
Clones: 1. RRII 429, 2. RRII 417, 3. RRII 422, 4. RRII 430, 5. RRII 105, 6. RRII 600,
7. RRII 414, 8. PB 217, 9. RRII 176, 10. RRII 203

stable clone. RRII 429 was the least stable. RRII 430 combined high yield and stability.

3. Participatory evaluation of rubber clones

On-farm trials of twenty pipeline clones and three high yielding check clones, *viz.* RRII 105, RRII 414 and RRII 430, were established across 13 locations in Kerala, Karnataka and Tamil Nadu. Casualty replacements were carried out in 12 on-farm trials and two central large scale trials. Diseases were recorded and soil samples were analysed. Phase II of the project was initiated using 17 clones (14 pipeline clones and three check clones) in 10 OFT locations and at CES where the central LST will be established.

4. Breeding for other specific objectives

4.1. Compact canopy

Three progenies of crosses between the genetic variant with compact canopy and

popular clones, *viz.* 'Genetic variant' x RRII 118 and RRII 600 x 'Genetic variant' exhibited normal growth with compact canopy in the 9th year. The recombinants registered less yield compared to the check clone RRII 105. However, two hybrid clones of parentage 'Genetic variant' x RRII 118 and RRII 105 x 'Genetic variant' were comparable with check RRII 105 in terms of yield.

Of the half-sib progenies of the genetic variant under evaluation, intermediate (74.6 cm) and normal type (71.4 cm) registered higher girth than RRII 105, whereas the mean yield of the above morphotypes was 28.8 and 33.9 g/t respectively compared to RRII 105 (40.1 g/t) in the 5th year of tapping.

4.2. Drought tolerance

The yield of the 24 hybrid clones and seven parents in two small scale trials planted in 1998 was monitored at fortnightly intervals in the fifth year of tapping. There was significant clonal variation with respect to annual mean yield

and girth in the two trials. Yield of seven of the hybrid clones was comparable to that of RRII 105. One clone, 93/214 in Trial 1, recorded superior yield than RRII 105 over 5 years of tapping. In terms of yield during the summer months there was no clonal variation in Trial 1. The parent clones PB 217, RRIC 104, RRIC 52, PB 260 and RRII 600 recorded higher yield than RRII 105 in the summer months. In Trial 2, one clone, 93/58 was superior to RRII 105 in terms of annual mean yield in the 5th year of tapping and over the first 5 years of tapping and yield of clone 93/92 was comparable. In terms of summer yield, clone 93/92 was superior, while three clones were comparable to the check. Broad sense heritability estimates for the two trials were higher for annual mean yield (0.48 and 0.62) than that for summer yield (0.15 and 0.37).

Among the 14 hybrid clones from the 1994 HP progeny under evaluation in small scale trial 1 planted in 1999 at CES, yield ranged from 23.32 to 71.25 g/t/t in the fourth year of tapping and four clones exhibited promising yield compared to RRII 105. Two clones, 94/41 and 94/50 were superior to RRII 105 in mean yield over the first four years of tapping.

Data from Trial 2 (progenies of the 1995 hybridization programme) revealed significant clonal variation. Forty six clones showed higher yield than the check clone RRII 105. While 13 clones were significantly superior in terms of annual mean yield, 11 clones were better in terms of summer yield in the fourth year of tapping. Annual mean yield of the 56 clones ranged from 22.08 to 87.12 g/t/t with a general mean of 43.28 g/t/t. Significant clonal variation was evident for yield over four years of tapping and 11 clones were superior in yield to clone RRII 105.

There was significant clonal variation in yield among the drought tolerant ortets from Dapchari, which are being evaluated at CES. The clone D 111 was significantly superior to RRII 105 and two clones, D 37 and D 236 were comparable to the check. Yield of RRII 105 was 81.82 g/t/t. Clones D 111 and D 37 recorded significantly high summer yield compared to the check. Over five years of tapping, clone D 111 emerged as a significantly high yielder with a high summer yield, followed by clones D 37, D 173, D 95, D 236 and D 35.

Significant clonal variation for yield and girth in the second year of tapping was observed in the two small scale trials planted in 2001. Twenty nine clones were superior in growth and seven clones, with yield ranging from 42.1 to 66.1 g/t/t, were superior in yield compared to RRII 105. Clone RRII 105 registered 34.2 and 35.9 g/t/t in Trials 1 and 2, respectively.

4.3. Polycross progeny evaluation

In the two evaluation trials of polycross progenies comprised of 150 clones significant variation existed in dry rubber yield and girth among and within progenies in the ninth year of tapping. The mean yield of the progenies in Trial 1 ranged from 38.94 to 54.76 g/t/t. Progenies of PB 215 gave the highest mean yield, followed by those of PB 217 and RRII 105. In Trial 1, 34 clones with mean yield ranging from 54.32 to 104.34 g/t/t performed better than RRII 105. The mean yield of the progenies in Trial 2 ranged from 32.62 to 52.18 g/t/t in the ninth year of tapping. Ten clones with a mean yield ranging from 62.47 to 137.72 g/t/t performed better than RRII 105. The highest mean yield and recovery of high yielding clones was recorded by the progeny of PB 252, followed by that of Ch 26 and RRII 105.

In the clonal nursery evaluations of polycross progenies planted in 2005, the growth and test tap yield of the 3-year-old clones were better at CES, Chethackal, compared to that at the drought prone location of RRS, Padiyoor. Another batch of clones was also test tapped in both the locations. The clones showed test tap yield ranging from 17.85 to 127.25 g/10 tap with a mean of 59.57 g/10 tap at RRS Padiyoor and yield ranging from 23.33 to 141.67 g/10 tap with a mean of 78.60 g/10 tap at CES. The clones 263, 329 and 98 were high yielders in both the locations.

Twenty four clones selected from half-sib progenies are in the third year of evaluation in a clonal nursery planted in RRII in 2007. Progenies of LCB 1320 (25.2 cm) and BD 5 (24.8 cm) showed vigorous growth in the third year when compared to check clones RRII 105, RRII 414, RRII 430 and PB 235.

5. Anatomical investigations

As part of investigations related to tapping panel dryness, the distribution,

structure, senescence and biochemical characteristics of protein storing cells (PSC) in the bark of healthy, TPD affected and stimulated trees of rubber were studied. In healthy trees, the PSC was found in one or two layers surrounding the phloic rays and also adjacent to the sieve tubes, where as its density was more in TPD affected trees (4-5 layers) as well as in stimulated trees. The protein analysis also showed higher amount of protein in the bark of TPD trees than healthy trees. As the senescence starts the protenaceous materials completely disappeared and lignification was initiated.

Rapidly moving objects (similar to brownian movement) could be observed in the tissue of *Hevea* bark preserved in Formalin - Acetic acid - Alcohol mixture for one year. These moving objects may be starch granules, cellular inclusions or endophytic bacteria. Since the above movement was observed only in healthy trees and not in fully TPD affected trees, it assumes importance in TPD related studies.

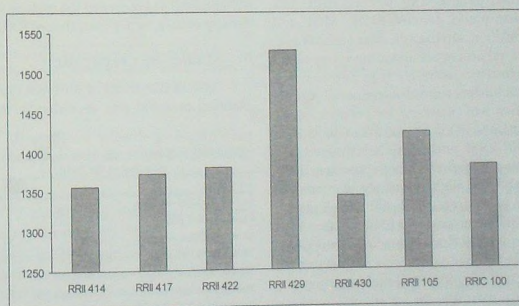


Fig. Bot. 2a. Variation in fibre length (µm) among different clones

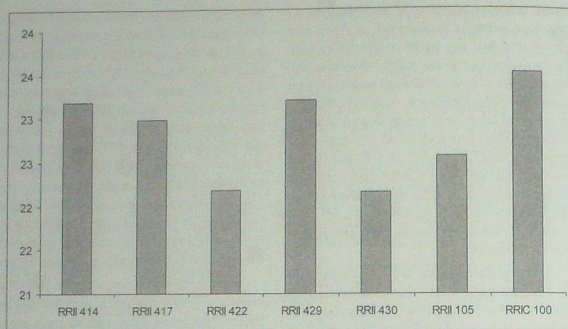


Fig Bot. 2 b. Variation in fibre width (µm) among different clones

Studies were carried out on physical, mechanical and anatomical properties of wood of 23-year-old trees of RRII 414, RRII 417, RRII 420, RRII 422, RRII 429, RRII 430 and their parents RRII 105 and RRIC 100. Data on wood density, MOR, MOE and volumetric shrinkage were analysed. Structural properties including xylem vessel characteristics in relation to porosity and fibre characters were also compared. Clonal variation was observed for porosity, based on number and total vessel area. Clones RRII 414, RRII 429 and RRIC 100 showed low frequency of pores but high pore area. RRII 430 exhibited high frequency of pores but lower pore area. RRII 400 series clones recorded lower incidence of tylosis than RRII 105. In all the clones, tension wood fibres recorded higher wall thickness than normal fibres with significant clonal variation.

The RRII 400 series clones recorded shorter fibre length and higher fibre width

compared to RRII 105, indicating their faster rate of growth (Figs Bot. 2 a & b). Among these RRII 429 recorded maximum fibre length and width, RRII 430 followed by RRII 417 and RRII 414 recorded the highest wood density values, better than that of RRII 105.

5. Studies on propagation

Girth of the plants of different types of planting material was recorded in the 8th

Table Bot. 11. Girth of the trees eight years after planting in the field

Treatments	Girth (cm)
Brown budded stumps	54.82
Field budded plants	49.83
Green budded plants	57.90
Brown budded plants	55.83
Young budded plants – stock 42 days old	55.13
Young budded plants – stock 49 days old	53.51
Young budded plants – stock 56 days old	55.43
CD (P = 0.05)	4.03

Table Bot. 12. Growth and tappareability of young-budded and brown-budded plants

Region/Place	Type of planting material	Girth (cm)			Tappareability (%)
		1 st year (2002)	3 rd year (2004)	7 th year (2009) [#]	7 th year (2009)
Kozhikode	Y	4.23*	14.55*	49.55* (9.72)	86.00
	B	3.66	12.27	45.50 (11.41)	60.00
Mannarkkad	Y	5.31	20.92	52.90 (7.68)	92.00
	B	5.28	20.33	52.50 (8.25)	92.00
Shoranur	Y	4.47	15.00	47.21 (11.43)	59.00
	B	4.65	15.10	45.80 (12.08)	57.00
Kothamangalam	Y	3.61*	18.35	48.96 (13.31)	80.76
	G	2.31	17.82	47.81 (11.97)	77.00
Muvattupuzha I	Y	3.26*	15.48*	44.97* (15.08)	57.00
	B	2.71	13.72	39.66 (16.77)	33.00
Muvattupuzha II	Y	3.90	16.76	48.75 (11.43)	82.00
	B	3.80	16.78	49.20 (11.90)	88.00
Thodupuzha	Y	3.19*	13.56*	45.18* (10.38)	70.00
	B	4.20	14.76	52.30 (8.14)	95.00
Kottayam	Y	4.44*	16.50*	47.40 (10.57)	87.00
	G	3.84	13.21	48.50 (6.94)	89.00
Kanjirappally	Y	4.31*	18.32*	44.21 (13.57)	46.00
	B	3.52	15.00	43.40 (17.46)	43.00
Pathanamthitta	Y	4.57	17.54	50.38 (8.53)	92.00
	B	4.05	16.95	48.65 (6.30)	90.00
Punalur	Y	3.20	16.00	50.95 (9.13)	89.00
	G	3.31	17.58	52.05 (12.12)	90.00
Mean girth	Y	4.04	16.63	48.22	76.43
	B/G	3.75	15.77	47.76	74.00

* Significantly different from respective control plants;

Y Young budded plants; B: Brown budded plants; G: Green budded plants;

Values given in parentheses are coefficients of variation.

year after field planting. Significant difference for girth between field budded plants and other planting materials was noted. Green budded plants showed better girth (Table Bot. 11). Field budded plants recorded lowest girth.

In a study on young budded plants under commercial evaluation, during the 7th year, young budded plants were superior (Table Bot. 12) in Kozhikode and Muvattupuzha whereas in Thodupuzha

brown budded plants were superior. In other locations growth of both type of planting material was comparable. The results revealed that under similar management conditions there was no significant difference in growth of planting material generated by different budding techniques.

In a study on the influence of age of bud wood stock and fresh seed stock on field performance after three years of growth in

the field, growth of plants from fresh seed stock was found to be uniform and better compared to plants raised from the stored seeds stock. No significant girth difference was noted in the field and polybag nursery between the plants raised from budwood collected from four year and 20 year budwood stock. Plants raised from buds collected directly from the trees were inferior in growth with higher co-efficient of variation compared to others.

In another study, there was no significant difference in girth of the plants raised from brown coloured leaf axil buds, scale buds or light green buds, three years after planting in the field. Trees raised from light green buds showed higher variability and lowest girth compared to trees raised from other type of buds.

A study was conducted on the effect of polyhouse ecosystem on growth of polybag plants. Green budded stumps and brown budded stumps were planted in polybags

and separately kept in polyhouse and outdoor. Height of scion, stem diameter, number of whorls, biomass content and number of lateral roots, were recorded. Plants raised in polyhouse were better in terms of mean height of scion, mean diameter, number of whorls, total biomass content and number of lateral roots/plant. Polybag plants of both types recorded early emergence of buds and better and uniform growth inside polyhouse when compared to plants maintained outdoor. The study indicated that good quality planting materials could be raised in polyhouse in large scale.

7. Morphological characterization of popular clones

Draft guidelines for DUS testing in rubber, as required under the Protection of Plant Varieties and Farmers' Rights Act (PPV and FRA) was prepared.

GERMPLASM DIVISION

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

1. Introduction, conservation and documentation

1.1 Domesticated gene pool from secondary centers (Wickham collection)

183 Wickham clones are being conserved in field gene banks (one clone museum at RRII Farm, Kottayam, and two germplasm gardens established at CES, Chethackal).

Among the five IRCA clones in Germplasm Garden IV, IRCA 130 and IRCA 111 continued to show superiority over RRII 105 (Table Ger.1). Significant clonal differences were recorded for yield girth,

Table Ger. 1. Performance of IRCA clones at the age of 18 years

Clones	Dry rubber yield (7 th year of tapping) (g/t/t)	Girth in the 18 th year of planting (cm)	Bole height (m)	Bole volume (m ³)
IRCA 130	110.21	75.13	6.77	0.23
IRCA 109	44.77	65.64	5.87	0.16
IRCA 111	67.58	75.24	4.77	0.17
IRCA 18	51.47	69.07	4.64	0.14
IRCA 230	57.85	66.00	4.64	0.13
RRII 105	73.70	69.38	3.58	0.11
CD (P = 0.05)	21.31	6.33	1.19	0.03

bole height and bole volume. IRCA 130 continued to be far superior to all the other clones in terms of yield, girth and bole volume. IRCA 111 was on par with RRII 105 for yield, and superior for girth and bole volume.

The clones IRCA 130, IRCA 111 and IRCA 109 were multiplied for On-Farm Evaluation in five different locations.

Among the 20 clones in Germplasm garden V (Table Ger. 2), RRIC 100, RRII 23 and RRIM 609 continued to be the best clones in terms of yield (90.7-82.8 g/t/t), while the control clone RRII 105 had an yield of 62.1 g/t/t. Plugging index recorded in the peak season (December) was also relatively low for these clones, ranging from 3.1 (RRIM 609) to 3.9 (RRIC 100), while RRII 105 had a value of 4.1. These three clones also had high girth ranging from 88.9 cm (RRIC 100) to 82.6 cm (RRIM 609), while the control clone RRII 105 had a girth of 68.3 cm.

1.1.1. Formulation of Distinctiveness, Uniformity and Stability (DUS) testing norms

Steps were initiated to formulate DUS norms for *Hevea* clones for plant variety protection. Available descriptors from different sources were compiled into the format required by PPVFR&A. However, comparison of the descriptors prepared by

various authors showed lack of uniformity not only with regard to the characters proposed but also for the classes (states) of a character, although there were also many similarities. An attempt was made to use this format to collect data on juvenile growth traits and mature fruit characteristics from available clones. A high degree of intraclonal variation for many traits was observed, which could make these characters unsuitable for use in DUS testing. Hence, additional traits will have to be identified, and all descriptors will have to be harmonised for DUS testing. A multilocation project has been proposed for this.

1.2. 1981 IRRDB wild gene pool

Field conservation of a total of 3576 wild accessions was continued in the different conservation-cum-source bush nurseries (SBNs). All the wild accessions were maintained with proper identity.

Re-establishment of the conservation nurseries has been carried out. The first re-established nursery, SBN 2003, was cut back. Out of 975 wild accessions in SBN 2004, based on two rounds of test tapping, 13 accessions having 80% or more yield of RRII 105 were identified and selected for Further Evaluation Trial and 15 accessions having 50-80% yield of RRII 105 were identified and selected for Clonal Nursery Evaluation.

Table Ger. 2. Performance of 20 Wickham clones in the sixth year of tapping

Clone	Yield (g/t/t)	Girth (cm)	PI	Clone	Yield (g/t/t)	Girth (cm)	PI
RRIC 100	90.7	88.9	3.9	PCK 1	38.9	57.0	7.4
RRII 23	84.6	84.6	3.7	RRII 22	35.2	57.0	3.9
RRIM 609	82.8	82.6	3.1	RRII 27	32.8	53.9	5.2
RRII 105	62.1	68.3	4.1	RRII 15	29.6	72.6	3.0
SCATC 93/114	61.2	65.5	4.6	RRII 20	29.2	68.8	7.0
RRII 178	58.7	84.4	4.4	PB 255	22.8	87.3	6.1
RRIC 148	57.5	88.6	3.3	SCATC 88/13	18.6	70.2	5.5
RRIC 102	53.9	70.2	4.2	RRIM 618	13.6	55.1	14.1
Haiken 1	48.3	73.6	4.3	RRII 12	11.9	73.0	8.1
RRIC 36	42.1	68.5	4.8				
RRII 108	40.9	72.9	3.3	CD (P = 0.05)	23.66	12.55	2.95

These selected accessions were multiplied and raised in a polybag nursery for field planting during next year. Accessions with good girth and girth increment were also identified in this SBN. The 35 selections identified from the first round of test tapping in the 701 accessions in SBN 2005, were subjected to a second round, along with check clones. While the check clones yielded 5.7 g/t/t (RRII 105) to 7.2 g/t/t (PB 235), MT 201 gave the highest yield of 9.3 g/t/t, followed by ten other accessions, whose yield ranged from 9.04 to 5.97 g/t/t. Four other accessions had at least 80% of the test tap yield of RRII 105, while 13 accessions had between 50- 80% of that of RRII 105. Bark samples were collected for profiling the anatomical traits contributing to yield. The fourth set of 806 accessions planted in conservation nursery 2006 in an augmented RBD with four check clones is maintained properly. Test tapping of 806 accessions was conducted and 63 accessions identified with more than 50% test tap yield of that of RRII 105 for second round of test tapping. The conservation nursery 2007 comprising of 500 accessions were monitored for early growth. The 201 wild accessions in SBN 2008 were characterized morphologically at

the age of one year using 22 qualitative traits and four quantitative traits.

1.3. Other *Hevea* species

The arboretum comprising all the species of *Hevea* and their hybrids available at RRII (*H. benthamiana*, *H. spruceana*, *H. nitida*, *H. camargoana* and two accessions of *H. pauciflora*, two *H. brasiliensis* clones, five natural putative interspecific hybrids and FX 516 (an interspecific cross between *H. brasiliensis* and *H. benthamiana*) planted at CES in 2006, was maintained.

2. Characterization and preliminary evaluation

In Preliminary Evaluation Trial (PET) Ortets- 99 at CES, Chethackal, girth of two wild accessions OR 1182 (61.8 cm) and OM 1107 (59.6 cm) were on par with that of the check clones RRIM 600, PB 260 and RRII 105, which ranged from 58.7 cm – 65.7 cm. Three wild accessions OR 1147, OR 1184 and OR 1185 also showed yield ranging from 33.6 - 28.9 g/t/t, on par with RRII 105 (34.5 g/t/t) in the first year of regular tapping.

Six wild accessions out of 47 in PET 2000 at RRII had girth ranging from 49 cm - 61.6 cm, while that of the check clones RRII

105 and RRIM 600 were 47.2 cm and 35 cm respectively. Three accessions 11/76, 3/82 and 16/39 showed promising yield levels, while six accessions showed relatively high number of latex vessels (NLV) which will be useful in crop improvement (Table Ger.3).

Among the evaluation trials at RRS, Padiyoor, annual girth recorded in PET 2000A was the maximum in AC 2537 (49.40 cm) followed by AC 3609 (49.00 cm) and AC 3075 (47.50 cm), while the check clones RR11 105, RR11 208 and RRIM 600 recorded 31.41 cm, 36.11 cm and 31.67 cm respectively. In PET 2000 B with 166 wild accessions, annual girth and girth at the end of summer period was recorded for identifying accessions with better summer period growth. Trees were opened for regular tapping during the peak season and yield collection started in the form of cup lumps. In PET 2002, AC 567 recorded the highest girth followed by AC 1964 and MT 1717.

3. Further evaluation and selection

The annual girth, dry rubber yield and volumetric timber (bole) yield were recorded and analysed at the age of 15 years (6th year after tapping) in the Further Evaluation Trial (FET)-95. The annual girth was maximum for the accession MT 1032 (79.63 cm) and minimum for MT 188 (36.5 cm) after 6th year of tapping. Five accessions (MT 1032, MT 941,

MT 1674, MT 1630 and MT 915) showed annual girth significantly higher than that of the check clone RR11 105 (61.01 cm) and 67 accessions had the girth statistically on par with the check clone.

The accession AC 166 had the highest dry rubber yield (58.78 g/t/t) which is statistically on par with the yield of RR11 105 (64.74 g/t/t) after 6th year of tapping. This accession showed 91% of the yield of the check clone RR11 105, with high ATP content during the peak and stress seasons. 12 accessions showed 50 to 69% yield of the check (Table Ger. 4). The biochemical analysis indicated that two accessions viz. AC 166 (265.9 i mol) and AC 2004 (275 i mol.) showed high ATP content than that of RR11 105 (250.2 i mol). Considering the consistent yield performance for the last three years, AC 166 has been multiplied for On-Farm evaluation trials in five locations under different environments.

The volumetric timber in terms of bole volume was maximum for MT 941 (0.13 m³) and minimum for MT 188 (0.03 m³). 13 accessions (Acre - 3, Rondonia - 3, Mato Grosso - 7) had the bole volume significantly higher than that of RR11 105 and in 64 accessions it was on par.

In the FET 2003, observations were recorded on early growth performance. Accession RO 2629 (47.75 cm) recorded

Table Ger. 3. Performance of top accessions in PET 2000

Accession No.	Girth (cm)	Accession No.	NLV	Accession No.	Yield (g/t/t)
8/1	61.6	3/82	16.0	11/76	46.0
25/24	51.0	1/76	15.3	3/82	21.6
3/65	49.9	8/1	11.8	16/39	16.8
4/4	49.5	21/29	11.3	RR11 105	22.7
16/39	49.3	25/113	9.0	RRIM 600	16.1
3/54	49.0	12/39	8.5		
RR11 105	47.2	RR11 105	12.5		
RRIM 600	35.0	RRIM 600	9.0		

highest girth followed by AC 4149 (43.25 cm) and AC 626 (42.79 cm), while the check clones RRIM 600, RRII 105 and RRII 208 recorded 34.25 cm, 36.19 cm and 32.69 cm respectively.

Significant clonal differences were seen for annual girth in FET 2005 comprising 22 wild accessions and three checks. MT 2217 and AC 2004 continued to have the highest girth in the fifth year of growth (33.7 and 31.2 cm respectively), on par with the checks PB 235 (28.7 cm) and RRII 105 (28.4 cm), while MT 4788, RO 2731, MT 185 and RO 3012 (28.2 cm – 25.7 cm) were also on par with the best accessions. Bark samples were collected for anatomical profiling.

In the 26 wild accessions in FET 2008, along with the two checks, growth in terms of girth, height and number of whorls was recorded, and data are being analysed.

13 wild accessions selected from SBN 2004 having more than 80% test tap yield of RRII 105 were multiplied and raised in polybag nursery at CES, Chethackal for field planting of FET 2010 A. 23 wild accessions

selected from SBNs 2003 and 2005, along with 3 check clones were multiplied and raised in the polybag nursery for planting in ensuing season at CES, Chethackal (FET 2010B) and 11 selected wild accessions along with 2 check clones were multiplied and raised in the polybag nursery for planting in ensuing season at RRS, Dapchhari (FET 2010C).

4. Screening for stress tolerance

4.1. Biotic stress resistance

Screening of the wild accessions for resistance to major diseases, in collaboration with Plant Pathology Division could not be carried out.

4.2. Abiotic stress resistance

4.2.1. Drought tolerance

In the field screening trial planted in 2003 at RRS, Dapchhari with 130 wild accessions, twig samples were collected for anatomical studies (for studying drought related intraxylary phloem characters) and processing is going on. Seven potential accessions out of 130 could be identified based on drought related growth parameters and juvenile rubber yield. These accessions were multiplied and raised in polybag nursery at RRS, Dapchhari for further evaluation.

In the further field evaluation of selected *Hevea* clones at RRS, Dapchhari in collaboration with Botany Division, assessed the growth during the summer and peak period of growth at 6 months interval in the 34 selected *Hevea* clones comprising 23 wild accessions, 5 HP clones and 6 check clones, viz. RRII 430, RRII 414, RRII 105, RRIM 600, RRII 208 and Tjir 1. Among the 23 wild accessions, accession MT 4788 recorded the highest girth and it showed consistent performance throughout the period. The

Table Ger.4. Accessions showing better performance for dry rubber yield (g/t)

Sl.No.	Accessions	Dry rubber yield (g/t)	% of the yield of RRII 105
1	AC 166	58.78	90.77
2	RO 2908	44.73	69.07
3	MT 922	42.20	65.16
4	MT 54	41.97	64.80
5	AC 692	40.36	62.30
6	RO 2385	39.58	61.11
7	RO 2976	39.47	60.95
8	AC 655	38.09	58.82
9	MT 1020	37.28	57.56
10	AC 635	36.28	56.02
11	AC 638	35.84	55.34
12	AC 2004	34.81	53.75
13	AC 650	32.36	50.00
	RRII 105	64.76	
	CD (P = 0.05)	16.01	

foliage production at the age of six months was also highest in this accession. After experiencing two summer periods of 2008 and 2009, 4 wild accessions out of 23 and one among the hybrid clones recorded higher girth than the proven drought tolerant clones RRIM 600 and RRII 208. Among the five hybrid clones, 93/53, 93/270 and 93/105 were superior to check clones for annual girth increment of which one clone 93/270 was superior for girth in the third year after planting. Summer period girth increment was high in accessions MT 67, MT 1619, RO 1769 and MT 1627. Visual scoring on leaf yellowing and drying at weekly intervals was done during the summer period of 2009. There was differential response among the clones for these two characters which ranged from 0-10 % for leaf yellowing and 3-23 % for leaf drying in the year 2009.

With regard to the performance of the clones RRII 430 and RRII 414 at Dapchari, the annual girth increment in these two clones was higher than the rest of the four check clones, viz. RRII 105, RRII 208, RRIM 600 and Tjir 1. RRII 430 was superior in terms of absolute girth in the third year after planting. Clone RRII 430 also showed higher annual girth increment whereas the clone RRII 414 showed a higher summer period girth increment than RRII 430. Although high summer girth increment was noted for RRII 414 compared to RRII 430, but while assessing the overall growth response, clone RRII 430 appears more suitable than RRII 414 in the drought prone area. Compared to RRII 414, leaf yellowing was less in the clone RRII 430 indicating the better suitability of RRII 430 for establishment in a drought prone region.

Another experiment conducted was the evaluation of drought tolerance potential of half-sib progenies raised in a seedling

nursery at RRII, using the seeds from nine prepotent *Hevea* clones of polyclonal seed garden at HBSS, Nettana (collaborative study with Botany Division). Conducted test tapping during both peak and summer seasons and assessed the family wise performance of clones. Family wise performance with respect to test tap yield and percentage recovery of seedlings above the overall mean of summer yield is shown in Table Ger.5. The number of seedling progenies evaluated in the nine clones ranged from 19-67. The mean peak season yield (2008) of the nine progenies in general was 6.54 g/10 tappings whereas it was 7.61 g/10 tappings during the summer season (2009). Summer season test tap yield was highest in the family of the clone PB 28/83 (10.2 g/10 tappings) followed by that of PB 215 (9.5 g) whereas in the family of clone RRII 105, it was 7.3 g.

The highest percentage recovery of superior seedlings based on population mean of summer period test tap yield was from the family of clone PB 215 (44.8%) followed by PB 5/51 (42.1%), PB 28/83 (42%) and PB 242 (41.7%). Altogether, 115 potential seedlings out of 359 (32.03 %) could be identified as having high summer test tap yield, showing potential for cloning and nursery evaluation at drought prone regions as an attempt to evolve drought tolerant and high yielding clones.

Vigour in terms of seedling girth is an important juvenile trait as far as reduction in immaturity period is concerned. The mean girth was highest in the family of clone PB 5/51 (17.7 cm) followed by PB 215 (17.63 cm) and PB 217 (17.35 cm) whereas it was 15.19 cm in the family of RRII 105 indicating the general vigour of PB clones (Table Ger. 6).

Out of 115 potential half sibs identified as having high summer test tap yield, 50

Table Ger. 5. Family wise performance on test tap yield and percentage of seedlings above the overall mean of summery yield.

Progeny	No. of seedlings evaluated	Mean yield (g/10 tap.) (Peak season)	Range in peak season yield	CV % (Peak season)	Mean yield (g/10 tap.) (summer season)	Range in summer yield	CV % Summer season	% of superior seedlings (summer)
PB 242	48	7.0	2.83-14.73	49.3	8.3	0.06-26.27	86.8	41.7
RRII 203	67	5.4	2.01-16.84	62.9	5.0	0-44.9	162.3	20.9
PB 217	22	6.9	2.92-17.54	56.52	6.0	0-22.12	102.9	26.09
Ch 26	45	6.2	2.91-23.54	68.5	6.9	0-34.28	105.7	33.3
RRII 105	28	6.1	3.23-17.57	53.6	7.3	0.21-26.24	94.8	32.1
AVT 73	50	5.2	2.49-22.86	66.8	6.1	0-34.88	146.6	18.0
PB 5/51	19	7.1	2.98-14.69	59.4	9.2	0-35.65	107.9	42.1
PB 215	29	7.8	0-28.17	74.8	9.5	0-37.67	92.5	44.8
PB 28/83	50	7.2	0-23.83	63.6	10.2	0-35.51	96.14	42.0
Mean		6.54			7.61			

and 34 were multiplied and raised in polybag nurseries at RRS, Dapchari and RRS, Padiyoor for clonal nursery evaluations.

Another experiment was a pot study in a set of 16 wild *Hevea* germplasm to identify the drought tolerance potential, based on growth and drought related morphological, physiological and anatomical parameters. The anatomical parameters studied were number of primary xylem points and intra xylary phloem using one year old twig. The linear relationship observed for IXP tissues and growth and the possible activation of

this tissue under conditions of stress assumes significance. Potential accessions for structural parameters are shown in Table Ger.7.

Accession MT 1623 showed maximum number of IXP and its drought tolerance potential in relation to this character has to be investigated.

In collaboration with Crop Physiology Division, attempts were made to identify accessions that have intrinsic drought tolerance traits. Symptoms of drought on the leaves were visually scored in one year old plants in the field. The percentage of leaf

Table Ger. 6: Mean girth and test tap yield / unit length of cut of nine clones

Progeny	Mean girth (cm)	Range in girth (cm)	CV %	Yield/cm girth (g)	Range in yield/cm. (g)	CV %
PB 242	15.44	8.0-34.0	35.89	0.449	0-1.56	74.30
RRII 203	14.13	8.0-27.0	34.37	0.329	0-4.49	191.29
PB 217	17.35	9.0-22.0	45.41	0.346	0-1.55	104.55
Ch 26	15.09	8.0-28.0	34.70	0.529	0-1.43	91.23
RRII 105	15.18	9.0-24.0	31.25	0.393	0-1.13	77.25
AVT 73	14.96	8.0-35.0	38.95	0.257	0-2.57	133.09
PB 5/51	17.70	10.0-27.0	34.28	0.401	0-1.32	89.69
PB 215	17.63	8.0-30.0	30.84	0.457	0-1.51	80.29
PB 28/83	16.04	9.0-27.0	31.02	0.545	0-1.68	72.44
Mean	15.95			0.412		

yellowing and leaf senescence was recorded in these plants by empirical scoring. Accessions were then sorted numerically and ranked for leaf senescence and leaf yellowing. About 3772 germplasm accessions were screened for visual drought tolerance traits and analyzed. Accessions that were exhibiting less senescence and yellowing in field were ranked in top as the most drought tolerant ones. Similarly accessions exhibiting high rate of senescence and severe leaf yellowing were ranked as the most drought susceptible ones. A total of 168 wild accessions were selected as drought tolerant and 125 wild accessions as drought susceptible ones. The short listed accessions were subjected to *in-vitro* water deficit stress with PEG and changes in the effective quantum yield of PS II were estimated. A significant relationship was obtained between the visual drought tolerance scoring and PS II activity. The selected accessions with intrinsic drought tolerance qualities would be included in future breeding programmes for evolving clones that can better tolerate adverse environmental conditions.

4.2.2. Cold tolerance

A total of 64 wild accessions were screened for cold resistance in two trials at

Regional Experiment Station, Nagrakata, West Bengal. Girth of the ten-year-old accessions recorded during pre- and post-winter period, showed significant variation. Monthly yield was also recorded. Higher annual girth was observed in RO 2902 (71.68 cm), MT 5105 (71.01 cm) and RO 2387 (68.96 cm) as compared to clones SCATC 93/114 (60.79 cm) and RRIM 600 (58.69 cm) in trial 1. In trial 2, clone RO 2727 recorded the highest girth of 73.51 cm followed by MT 915 (73.37 cm) and MT 900 (69.01 cm), while the checks Haiken 1 and RRIM 600 recorded 68.52 cm and 58.27 cm respectively.

5. Screening for timber characteristics

5.1. Field screening

Annual girth and bole height were recorded and estimated the bole volume at the age of 9 years. Among the wild accessions, the girth ranged from 34.26 cm (AC 651) to 53.21 cm (MT 919). The maximum girth was recorded by MT 919 (53.21 cm) and minimum for AC 651 (34.26 cm). 11 accessions had the girth statistically on par with PB 260 and PB 235; 14 accessions were on par with RR118; 15 accessions were on par with RR113; 18 accessions were on par with RR115 and all the 19 accessions were on par with RRIM 600

Table Ger. 7. Potential accessions for structural traits along with twig diameter

Accession/ Clone	No. of primary xylem points (PXP)	No. of intraxylary phloem points (IXP)	Diameter of twig (mm)	IXP/mm diameter of twig
MT 1623	104.50	77.00	15.60	4.94
MT 200	81.80	75.38	20.30	1.82
MT 3714	74.25	59.25	17.90	3.31
MT 48	54.67	38.79	12.80	3.03
Tjir 1	60.83	30.67	12.50	2.45
RR11 105	48.60	38.20	9.00	4.24
RR11 208	56.00	39.88	11.90	3.35
RRIM 600	57.48	49.15	7.70	6.38
CD ($P = 0.05$)	30.15	30.40	3.88	
CV (%)	26.53	42.04	15.08	

for girth (Table Ger. 8). Among the six Wickham clones, PB 260 showed the highest girth (58 cm) followed by PB 235 (58.0 cm), RRII 118 (54.41 cm), RRII 33 (51.54 cm), RRII 105 (49.34 cm) and the lowest girth was recorded in RRIM 600 (45.9 cm).

One wild accession AC 650 had the bole volume equal to that of the check clone PB 235 and RRII 118 and four accessions were on par. Seven accessions had the bole volume equal to that of PB 260 and RRII 33 and all the wild accessions showed the bole volume statistically on par with RRII 105 and RRIM 600.

6. Utilisation of *Hevea* germplasm

6.1. W X A Open pollination Garden 2005

The open-pollination garden at RRS, Padiyoor planted in 2005, comprising 24 selected Amazonian and 11 Wickham clones, was maintained.

6.2. Breeding Garden at RRII

A breeding garden established in 2007 at RRII head quarter's campus comprising 46 potential domesticated (Wickham) clones and 6 selected wild accessions is maintained properly.

6.3. Hand pollination programmes

A hand pollination programme conducted during 2009 at CES, Chethackal involving 3 wild accessions showing potential for yield, and 6 cultivated Wickham clones resulted in 10 cross combinations. The hybrid seeds were harvested from the trees and 75 successful hybrid seedlings were established and early growth was monitored. Another HP programme involving two popular clones and seven wild accessions was also carried out at RRS, Padiyoor, with the objective of combining yield with drought tolerance. Of a total of 506 crosses, 5.1% success was

Table Ger. 8. Annual girth and bole volume of wild and Wickham clones

Sl.No.	Accessions	Girth (cm)	Bole volume (m ³)
1	MT 919	53.21	0.04
2	MT 941	52.08	0.04
3	AC 685	52.33	0.02
4	MT 999	49.18	0.04
5	RO 255	49.41	0.03
6	MT 915	48.33	0.04
7	MT 935	47.64	0.03
8	MT 1032	47.92	0.03
9	RO 322	47.23	0.04
10	MT 922	45.87	0.04
11	AC 707	46.99	0.02
12	AC 635	42.44	0.02
13	AC 650	42.33	0.05
14	AC 655	42.23	0.02
15	AC 1021	39.64	0.03
16	MT 1020	36.80	0.03
17	RO 879	35.79	0.02
18	AC 637	35.41	0.02
19	AC 651	34.26	0.02
Wickham Clones			
1	PB 260	58.77	0.04
2	PB 235	58.00	0.05
3	RRII 118	54.41	0.05
4	RRII 33	51.54	0.04
5	RRII 105	49.34	0.03
6	RRIM 600	45.95	0.03
CD (P = 0.05)		14.56	0.02

obtained. Recorded growth parameters (plant height, girth and number of leaves) of 29 progenies derived from 2 crosses and 25 OP seedlings (Table Ger. 9).

6.4 Generation of mapping population

The interspecific cross between the popular *H. brasiliensis* clone RRII 105 and the disease tolerant *H. benthamiana* clone F 4542 to raise a mapping population, resulted in 112 seeds, which were planted in a tray for germination. 104 sprouted, including a few reciprocals. The putative hybrids were

Table Ger. 9. Growth performance of hybrid and open pollinated seedlings

Parentage	Height (cm)	Girth (cm)	No. of leaves	Recovery of superior seedlings
RRII 105 x AC 675	203.7	7.97	32.6	40%
RRII 105 x RO 368	220.5	7.07	45.1	37%
OP seedlings of RRII 105	230.5	8.04	42.7	36%

planted in a seedling nursery at 1 m x 1 m spacing, along with the budgrafted parents (replicated) to be used as checks. The hybridity will be confirmed using molecular tools before taking up further studies in this direction.

7. Other studies

7.1. Feasibility of ratooning in *Hevea*

Ratoons continued to be superior to their corresponding polybag grown counterparts in the tenth year of growth in terms of girth (Fig. Ger.1). 94.2% of the ratoon plants have attained tappable girth so far, while only 22.4% of the polybag raised plants have done so. Additional trees were brought under tapping this year: 128 ratoons could be tapped, while only 17 polybag plants of the same age could be opened for tapping (Table Ger.10). Among

the ratoon and polybag raised plants, 12 and 26 respectively were lost in very strong winds in April this year.

7.2. Effect of stimulation in the laticiferous tissues of *H. brasiliensis*

Bark samples were collected from BO-1 and B1-2 panels before and after stimulation (Clone-RRII 105) and recorded the bark thickness, number of functional and disorganized laticifers. The bark thickness increased significantly during stimulation in both panels. The number of both functional and disorganized laticifers was increased significantly in both panels. The total number of laticifers significantly increased in the case of BO-1 panel whereas in B1-1 panel it was not significant (Fig. Ger. 2-4).

7.3. Studies on the inclination of laticifers and clonal variability in *H. brasiliensis*

The conventional (left top to right bottom slope) and modified tapping (right top to left bottom slope) was carried out in the clone PB 86 which showed leftward inclination of laticifers at HBSS, Nettana. Compared the volume of latex, DRC and Plugging Index for one year at monthly intervals between the two tapping systems. The results indicated that the mean volume of latex was significantly higher during the modified tapping (34.76 ml) than that of conventional tapping (28.97 ml). DRC and PI was not significant (Table Ger.11).

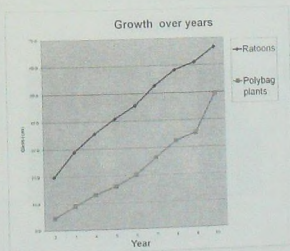


Fig. Ger.1. Growth over years of ratoons and polybag plants

Table Ger. 10. Growth and yield of ratoon plants

	Ratoons	Polybag plants
Number of plants	139	174
Girth range (cm)	40 - 100	20 - 77
Mean girth (cm)	66.6	44.2
>50 cm	131 (94.2%)	39 (22.4%)
Number of trees tapped last year	128	17
Yield (g/t/t)	32.6	21.8
Range (g/t/t)	141.7-3.3	48.6-3.4

7.4. Evolving location specific *Hevea* clones through Ortet selection programme from Rampachodavaram, Andhra Pradesh (Collaboration with RP Department)

The objective of the study was screening and identification of potential ortet clones from the 15 - 18 year old block plantings of Rubber Board, comprising poly clonal seedling trees. Initiated preliminary

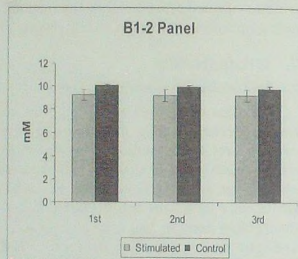
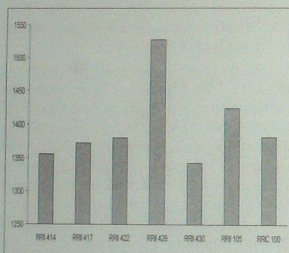


Fig. Ger.2. Bark thickness of stimulated and unstimulated bark in BO-1 and B1-2 panels of RRII 105

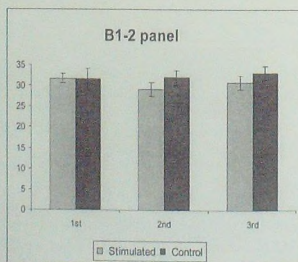
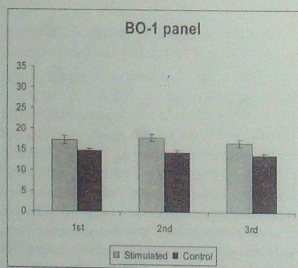


Fig. Ger.3. Number of functional latex vessels in stimulated and unstimulated bark in BO-1 and B1-2 panels of RRII 105.

discussion with the concerned officials and farmers.

7.5. Assessment of the performance of new rubber plantations of ITDA, Andhra Pradesh (Collaboration with ITDA, RP Department & Agronomy/Soils Division of RRII)

The study was taken up with the objective of monitoring growth of new large

scale plantings in AP by the ITDA in 2008. Initiated preliminary discussion with the concerned officials and visited eight farmers' fields. It was found that poor growth in some fields was due to poor after care.

8. Participatory clone evaluation:

8.1. On farm evaluation of pipeline clones- Phase I

Gap filling and plot demarcation were

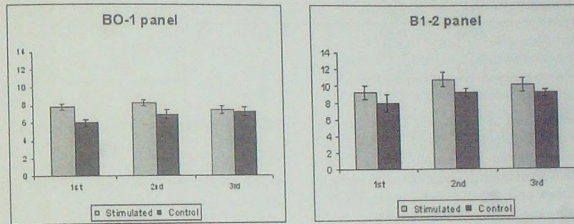


Fig. Ger.4. Number of disorganised latex vessels in stimulated and unstimulated bark in BO-1 and B1-2 panels of RRII 105

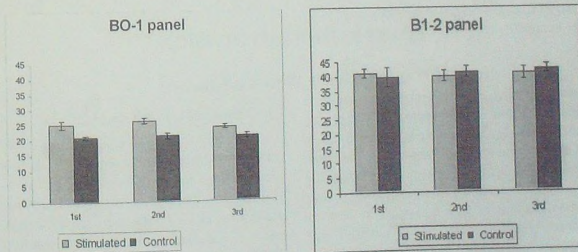


Fig. Ger.5. Total number of latex vessels in stimulated and unstimulated bark in BO-1 and B1-2 panels of RRII 105.

Table Ger.11. Variation in latex volume, DRC and Plugging index in different tapping systems

Tree nos.	LVR inclination towards the left (degrees)	Volume of latex (ml)		Dry Rubber Content (g)		Plugging Index	
		Normal tapping	Modified tapping	Normal tapping	Modified tapping	Normal tapping	Modified tapping
1	6.8°	38.5	42.4	25.7	25.4	3.8	5.5
2	13.4°	16.8	23.6	36.3	35.3	5.8	5.6
3	4.2°	21.7	37.5	36.1	35.8	2.8	3.6
4	4.9°	37.7	46.6	29.1	32.5	5.2	3.8
5	5.4°	20.9	25.9	26.8	29.5	4.7	4.8
6	3.4°	38.2	35.1	31.9	35.9	3.1	4.1
7	7.8°	18.5	23.4	31.0	32.0	4.8	4.9
8	2.6°	43.9	55.1	23.7	23.0	3.8	3.1
9	3.0°	24.5	23.1	25.3	36.4	4.7	5.5
Mean		28.97	34.76	29.54	31.76	4.30	4.55
SD		10.42	11.66	4.62	4.88	1.02	0.92
CV (%)		35.98	33.55	15.65	15.36	23.59	20.19

carried out at Shaliacary Estate, Punalur, Mooply Estate, Trissur, TR&T Estate, Mundakayam and Calicut Estate, Calicut. Disease incidence was evaluated in the test clones and three checks, with RRII 105 being used for covariance analysis in each plot.

8.2. On farm evaluation of pipeline clones- Phase II

Polybag planting was carried out in the test clones and three controls, with RRII 105 being used for covariance analysis in each plot at B C Cheruvally Estate, Erumeli and Malankara Estate, Thodupuzha.

PLANT PATHOLOGY DIVISION

The division is primarily concentrating on studies on the economic and ecofriendly management of diseases and pests. Evaluation sprayers and spray oils assessment of field loss due to diseases, evaluation of newly evolved clones for disease resistance, studies on tapping panel dryness, bee keeping in rubber plantations, use of beneficial microorganisms for plant growth, treatment of sheet processing effluents were the other areas of research.

1. Leaf diseases

1.1. Abnormal leaf fall disease

A study was taken up to detect likely presence of *Phytophthora* inoculum in rubber growing regions of Tripura (NE India), to examine why *Phytophthora* disease in rubber plantation is less prevalent in NE India, relate ALF disease incidence with weather parameters at Tripura and Kottayam and finally presume possibility of ALF disease outbreak in NE Indian rubber plantations.

Phytophthora isolated from soil and litter collected from rubber plantations in Agartala, Tripura revealed that inoculum density was low in Tripura and were less virulent. As weather plays an important role in development and spread of *Phytophthora* disease, a comparison of weather parameters between Tripura and Kottayam during May to August 1988 to 2009 was made. Agartala weather was observed to be hotter during the rainy season, which may be one of the factors inimical to the development of *Phytophthora*. During the same period, RH was found to be higher in Kottayam (85%) compared with that of Agartala (82.6%), yet another factor possibly associated with inoculum establishment. Thus, hot weather with low RH coupled with less number of rain spells may be some of the important factors preventing inoculum build up for initiating *Phytophthora* infection in rubber plantations of Tripura. The trial on evaluation of biodegradable oil for ALF disease control at the Puthukkad estate, Thrissur and Lahai estate, Punalur on the clone RRIM 600 was continued. The bio-degradable oil was found good for oil based COC micron spraying with a leaf retention of 68.6% over conventional spray oil (50.6%) and control (8.0%).

To select potential biocontrol agent against *P. meadii*, 252 bacterial endophytes were collected by disinfection and trituration method from root, leaf and petiole tissues of rubber clones RR11 105 and RRIM 600 from five locations.

Root tissues showed higher bacterial population compared to petiole and leaf tissues. Forty two isolates showed growth inhibition of *Phytophthora meadii* in dual culture plate.

Eleven selected endophytes showing more antagonism were identified through

16S rDNA sequencing, eight showed similarity to *Pseudomonas aeruginosa* and others were *Bacillus subtilis*, *Klebsiella oxytoca* and *Alcaligenes faecalis*. These isolates were under evaluation for their biocontrol efficiency.

1.2. Powdery mildew disease

A crop loss trial was initiated with clone RR11 105 at New Ambadi Estate in Kanyakumari district of Tamil Nadu. Two blocks were demarcated and pre-treatment application of sulphur was undertaken at recommended dose using micron duster. In the subsequent years, one plot was maintained as dusted and other as undusted. The disease intensity was assessed on a 0-5 scale (very light, light, moderate, severe and very severe). Moderate disease intensity (3.0%) was observed during pre-treatment year. Subsequent year (2009) the intensity was very low (0.3%) and no crop loss was recorded.

1.3. *Corynespora* leaf disease

Gene expression profiles of *Corynespora* challenged leaf samples along with the uninfected leaves of RR11 105 as control were studied using DD-RT-PCR technique. Differentially expressed up-regulated bands were cloned and sequenced. Many of the

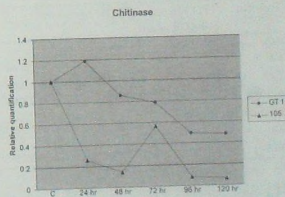


Fig.Path. 1. Chitinase activity in tolerant (GT1) and susceptible (RR11 105)

cDNA clones showed consistent over-expression in all the treated samples when compared with the control. Among the up-regulated clones, two of them showed significant homology with Anthocyanidin 3-O-glucosyltransferase and GRAS transcription factor.

Tolerant (GT 1) and susceptible (RRII 105) *Hevea* plants were challenged with *C. cassiicola* to quantify the chitinase activity through real time PCR. The induced chitinase activity was observed at 24th hour in GT 1 (The tolerant clone) and eventually it decreased compared to un-inoculated control. Whereas in RRII 105, chitinase activity level had been lesser in treated plants than their control (Fig.Path.1). The results indicated that level of chitinase activity increased only in early stages infection in tolerant clone.

2. Tapping panel dryness (TPD)

To characterise the LMW RNA, RT-PCR from total RNA was carried out with viroid specific primers which yielded product in the range of viroid only in TPD affected plants (Table.Path 1 & Fig.Path.2) and the

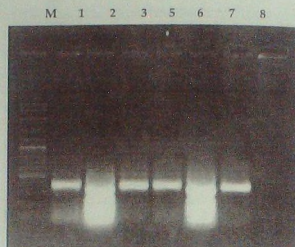


Fig. Path. 2 Agarose gel electrophoresis of PCR products (M- marker, lane 1-7 TPD, lane 8 Apparently healthy)

same was cloned. The sequencing of the cloned product was performed which showed homology to Potato Spindle Tuber Viroid (PSTVd) on BLAST analysis showing that the LMW RNA could be a viroid. The viroid sequence obtained from rubber was deposited in GenBank (HM107843.1).

Under the study on the transmission of LMW-RNA on rubber seedlings and indicator hosts it was found that viroid is transmissible. Seedlings that were LMW RNA +ve or -ve were bud grafted with scion collected from LMW RNA +ve TPD affected trees and LMW RNA -ve healthy trees. The results (evaluated by R-PAGE) showed that all the plants tested from the group in which both stock and scion were viroid +ve, showed viroid bands in R-PAGE. Plants budded with scion taken from TPD affected trees as well as healthy trees were test tapped to study the appearance of TPD symptoms. The results after six months tapping showed that TPD was observed in both group of plants, namely scion taken from TPD as well as healthy trees.

Table. Path 1. Presence of viroid in healthy (R-PAGE -ve) and TPD affected (R-PAGE +ve) trees

Tree No	TPD Status/ R-PAGE result	PCR	Sequencing
4/4	+ve	+ve	Viroid
62	+ve	+ve	Not sequenced
120	+ve	+ve	Not sequenced
6/15	+ve	+ve	Viroid
95	+ve	+ve	Viroid
2	+ve	+ve	Viroid
116	+ve	+ve	Not sequenced
24	-ve	-ve	
98	-ve	-ve	
103	-ve	-ve	
36	-ve	-ve	

3. Pests of rubber

To compare the efficacy of insecticides and bio control agents against white grub *Holotrichia serrata* infesting rubber seedlings field experiment was repeated at Nagapuzha Estate. The treatments included entomopathogenic nematode (EPN) 272 lakhs in 5 splits, Beaumet 65 x 106 (a combination product of *Beauveria bassiana* and *Metarrhizium*) in 4 splits, *Beauveria brongniatii* 72 x 106 in 4 splits and insecticides viz., Imidacloprid 0.005% 4 splits, Imidacloprid 0.005% + Carbaryl 0.2% 4 splits and phorate 10% 25g/plant in 4 splits. The results indicated that all the treatments were significantly superior to control and at par in effectiveness giving 71 to 77 percent control after two years.

Studies were carried out to find the effect of different treatments such as entomopathogenic nematodes, entomopathogenic fungus, Azadirachtin (0.03%) and fenvalerate against mooply beetle, *Luprops curticolis* in rubber plantations. Fenvalerate (0.02%) gave 98% mortality followed by Azadirachtin (0.03%) (75%). EPN (6 lakh/m²) gave 52.20% larval mortality on the 10th day of application.

A field trial was laid out at PCK, Kodumon to control *Aetherastis circulata*. Among the treatments, the combination of fenvalerate and carbaryl showed 88% mortality of *A. circulata* followed by neem oil (75.82%) and neemark (52%) (Table Path. 2).

Mealy bug, *Paracoccus marginatus* was found infesting on matured tree branches, stem, foliage, inflorescence and seeds in Palakkad and Ernakulam districts. Imidacloprid (0.05%) was the effective insecticide giving 90% control after two days. Bioformulations viz. verticillium and metarrhizium combinations were not effective. However, Neem oil (0.1%) reduced the numbers (12%) after two weeks.

4. Improvement of spraying technology for pest and disease management of natural rubber

A study was carried in collaboration with M/s. Aspee Co. Ltd. in Pudukkad and Echipara Estates, Trissur for the evaluation of the mini-tractor with additional attachment for the micron sprayer. The clone RRIM 600 was selected for the study. Prophylactic spraying was carried out as per the standard practice. For comparison micron spraying was used. Leaf retention after the treatments was assessed (Table Path. 3).

Table Path. 3. Leaf retention in clone RRIM 600 after micron spraying

Type of spraying	Leaf retention (%)	
	Pudukad	Echipara
Mini-tractor with micron	28.0	42.5
Manual spraying with micron	30.5	38.0
	NS	NS

Table Path. 2. Comparative evaluation of ecofriendly formulations and insecticides against bark feeding caterpillar, *Aetherastis circulata*

Treatments	Initial population	Mean percentage population reduction			
		after 7 days	% of mortality	after 45 days	% of mortality
Neemark 0.03%	151	135	- 10.59	72	-52.00
Neem oil 0.1%	91	79	- 13.18	22	-75.82
Myco jaal 10%	26	21	- 19.23	32	23.07
Fenval+carbaryl	70	6	- 91.42	8	-88.57
Control	27	33	22	51	88.00

5. Bee keeping in rubber plantations

Observation was made on symptoms of TSBV disease in honey bee colonies. Due to sudden out break of the disease the colonies were lost and hence the experiment was discontinued. Subsequently, healthy colonies were established. About 20kg honey could be extracted from the colonies.

6. Microorganisms for improving growth of rubber and cover crops

Competitive ability of three bacterial isolates, that nodulate *M. bracteata*, viz. NE 2, (from Agarthala), NE4 (from Tura), and RR11-M (Kerala), to occupy *Mucuna* nodules, when inoculated together was studied in polybags. Ten plants were uprooted after 4 months growth and the occupancy of the isolates in the nodules was estimated using the antibiotics at the IAR levels. The results are given in Table Path. 4.

Out of the 10 inoculated plants, 3 plants were infected with isolate RR11-M, 4 plants each with isolate NE-2 and NE-4. Only one plant showed mixed infection.

Characterisation of twelve isolates of growth promoting rhizobacteria selected from previous studies was carried out.

Colony characters of the isolates were studied after 5 days growth in agar media and biochemical characterisation by their reaction to 18 biochemical tests. Functional characters of the isolates were recorded by their growth promoting activities under *in vitro* conditions (Table Path. 5).

Eighty bacterial isolates were screened for antagonistic activity against 5 major pathogens of rubber viz., *Phytophthora meadii*, *Corynespora cassicola*, *Colletotrichum acutatum*, *Corticium salmonicolor* and *Phellinus noxius* by dual culturing. Five isolates were selected based on zone of inhibition and their compatibility with the fungicides viz. tridemorph, hexaconazole, propiconazole, carbendazim, mancozeb and wettable sulphur was studied by incorporating in growth media at 25% to 200% recommended level. Carbendazim and wettable sulphur were safe to the antagonists tested while mancozeb was more harmful.

The colony characters of three selected *Azospirillum* isolates, viz. isolate Azo-5, Azo-12 and Azo-13 from rubber plantations were studied in malic acid agar plates. They were small, circular, translucent, flat and with entire margin. The biochemical

Table Path.4. Nodule occupancy of the isolates in *Mucuna* plants (cfu/plant)

No. of plants	RR11-M (str _{sp} rif ₁₀₀)	NE-2 (gen _{sp} cyc ₁₀₀)	NE-4 (tet ₁₀₀ rif ₁₀₀)	Total bacteria without antibiotics
1	—	66.5 × 10 ² (80%)	—	82.5 × 10 ²
2	93.5 × 10 ² (74%)	—	32.5 × 10 ² (26%)	125 × 10 ²
3	—	—	109 × 10 ² (76%)	143.5 × 10 ²
4	—	74.0 × 10 ² (87%)	—	85 × 10 ²
5	—	75.0 × 10 ² (58%)	—	130 × 10 ²
6	—	69.0 × 10 ² (56%)	—	123 × 10 ²
7	—	—	83 × 10 ² (90%)	92 × 10 ²
8	98 × 10 ² (79%)	—	—	124 × 10 ²
9	76 × 10 ² (90%)	—	—	84 × 10 ²
10	—	—	74 × 10 ² (90%)	82 × 10 ²

Table Path.5. Growth promoting activities of selected rhizobacterial isolates

Isolate	IAA production ($\mu\text{g/ml}$)	Phosphate solubilisation (P released in $\mu\text{g/ml}$)					Ammonia production	Phosphatase activity ($\mu\text{gPNP/ml}$)	Sidero- phore production	N ₂ fixation
		SE %	Ca ₃ (PO ₄) ₂	FePO ₄	AlPO ₄	Raiphos				
Ri 25	23.1	220.00	509.0	131.6	252.3	95.5	Medium	0.78	High	-
K 43	25.06	127.27	380.0	84.45	117.0	-	Medium	0.08	-	-
K 52	17.4	112.50	407.5	117.1	219.85	-	High	0.08	-	-
RB 88	6.92	208.33	337.5	71.0	108.45	20.5	Low	1.16	High	-
K 24	13.4	106.67	418.5	-	147.55	-	-	0.0	Low	-
A 1	9.04	162.50	420.0	-	107.9	-	High	0.16	Low	-
RH104	5.36	200.00	479.0	36.6	101.7	47.5	High	3.1	Medium	-
RH 34	10.80	184.62	445.0	13.2	229.5	-	High	1.71	-	-
3 pt	4.68	121.43	246.5	15.15	125.3	-	High	0.78	Low	-
Ps 20	20.0	128.57	402.0	80.5	234.3	-	High	0.08	Medium	-
Ri 10	25.46	110.00	349.0	49.40	106.75	-	Medium	0.23	Low	-
F 1	15.90	118.19	420.0	73.7	104.85	-	Medium	0.08	Low	+

characterisation of the isolates was also studied by different biochemical tests.

Growth characters of nine phosphofungi isolates collected from rubber plantations were recorded. They varied in their growth rate and spore colour. Based on spore formation, 5 isolates were identified as *Penicillium* and *Aspergillus*.

In the experiment on biofarming of rubber using beneficial microorganisms green budded stumps of RR11 430 were planted in polybags for field planting. The treatments imposed to the plants in the polybags were, microbial cultures + cow dung slurry, biological management + chemical management of nutrients and diseases at 50% of recommendation level chemical management of nutrients and diseases as per recommendations. Nitrogen fixing *Azotobacter* isolate G, PGPR isolates RH 104, RH 34 and K 24 were used in polybags studies. The plants were transplanted to the field keeping the same treatments. Gross 200 plants were planted for each treatment in one acre area. An antagonistic bacteria (Ps 24) of the root pathogen, *Phellinus* was also

added to the soil at the time of planting. The initial girth, height and whorls of all the plants in each treatment were also recorded (Table Path. 6).

Table Path. 6. Growth measurements of plants in the bio farming trial at the time of planting (average of 200 plants)

Treatment	Height (cm)	Girth (cm diameter)	Whorls(no)
Biological	28.63	0.696	1.79
Integrated	32.18	0.789	1.99
Chemical	25.69	0.668	1.82

In order to protect the tender leaves from powdery mildew attack, prophylactic application of the antagonistic bacteria-RH 104 in talc was applied to the plants in the biological treatments and Sulfex to the plants in the chemical treatments at 14 days intervals. For the plants in the integrated treatments, alternate application of chemical and bio agent was given. Disease incidence was very less in all the treatments.

Sixty five bacterial isolates were collected for studying their ACC deaminase activity from soil, rhizosphere and roots of

Table Path 6. Efficiency of Integrated waste water treatment system (Coir filter + Hybrid reactor+ diffused aeration + sedimentation + sand filtration)

Parameter	Raw effluent	Filtered Effluent	Discharge from hybrid reactor	Efficiency of hybrid reactor (% Reduction)	After diffused aeration and sedimentation	Combined efficiency of aeration and sedimentation (% Reduction)	Final (After filtration)	Overall efficiency of ETP (% Reduction)
pH	4.7-6.0	4.8-6.3	6.8-7.2	—	6.5-7.7	—	6.6-8.0	—
COD	10964	9037	441	95.12	183	58.50	150	98.63
BOD	5349	4859	264	94.57	30	88.64	28	99.48
TS	13119	11362	2856	74.87	2562	10.29	2432	81.46
DS	12003	10388	2437	76.50	2371	2.71	2219	81.51

Average values are taken (values are in mg/l, except pH)

Table Path 7. Performance of the reed bed system.

Parameter	Inflow (discharge from hybrid reactor)	Out flow (Discharge from reed bed 1)	% Reduction	Outflow (Discharge from reed bed 2)	% Reduction (After biomethanation-reed bed 2)
pH	7.0-7.2	6.8-7.5	—	7.3-7.5	—
COD	494	293	40.70	89	82
BOD	285	57.5	80.00	14	95.08
TS	2849	2413	15.30	2738	—
DS	2426	2232	8.00	2638	—

Average values are taken (values are in mg/l, except pH)

rubber from RRS, Padiyoor, North Kerala, one of the drought prone area. A standard ACC deaminase bacteria, *Ochrobacterium* sp. from IARI, will be used for comparison.

7. Waste management in rubber processing

An experimental anaerobic hybrid reactor with a packing media inside for increasing the attached microbial population was designed and built at Elavampadam Model Rubber Producers' Society in Palakkad Dist. The evaluation showed that the reactor is highly effective in reducing pollution load. It could reduce

BOD by 94.57 %, COD by 95.12% total solids by 74.87%, and dissolved solids by 76.50%. The pH also increased from the range of 4.8-6.3 to 6.8-7.2. The average biogas production from the anaerobic hybrid reactor was 4 m³/day (Table Path. 6)

With an aim to develop an economical alternative to diffused aeration, the reed bed system has been designed and developed. The preliminary analysis of the system showed promising results. The system is found to have an efficiency which is in par with that of the diffused aeration system. The parameters of the treated water were found within safe limits (Table Path. 7).

PLANT PHYSIOLOGY DIVISION

Crop Physiology Division is involved in a wide range of research activities such as environmental physiology, physiology of growth and yield, tapping panel dryness, gene expression studies, secondary metabolites and ecological impact of NR cultivation.

1. Environmental physiology

1.1. Physiological adaptation to high light and drought stress

Effective quantum yield of PSII (Φ PSII) was studied in RR11 400 series clones and three check clones under soil moisture deficit conditions. The clones RR11 430 and RR11 600 recorded a small inhibition in PSII as compared to other clones under drought conditions. The clones RR11 414, RR11 417 and RR11 422 were shown to be drought susceptible. Leaf discs from RR11 600 was incubated in 40% PEG and high light condition ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 12 hrs under growth chamber conditions. The level of expression of the 23 k Da chloroplast sHSP was prominent in PEG incubated leaves compared to water control and it is proved that the stress protein can be induced in leaf discs also.

1.2. Identification of molecular basis for drought tolerance

Polybag plants of clones RR11 105, RR11 600 and RR11 208 were subjected to drought for 15 days in natural conditions at RRS, Dapchhari during the summer period (March-April) of 2009. Leaf samples were collected after assessing the drought status of the plants by physiological measurements. Gene expression analyses were performed using four drought responsive genes viz., HbDRT 5b (NAC transcription factor), HbNRG 18 (rps 14 protein), HbNRG 21 (unknown) and HbDRT 50 (unknown).

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as endogenous control for normalization. The quantitative expression of each gene had been analyzed with reference to irrigated plants of RR11 105.

The quantitative expression analysis indicated up-regulation of HbNRG 18 and HbDRT 5b in both irrigated and drought imposed plants of RR11 600 and RR11 208. This confirms the association of HbDRT 5b and HbNRG 18 with drought tolerance as both RR11 600 and RR11 208 are known drought tolerant clones.

Another set of polybag plants of four *Hevea* clones (RR11 105, RR11 600, RR11 414 and RR11 430) were imposed with drought for 18 days. Gene expression analyses were performed using seven stress responsive genes viz., HbNRG 18 (rps 14 protein), HbDRT 50 (unknown), peroxidase, ABC transporter, LEA 5 protein, CRT/DRE binding factor and glutathione peroxidase (GPX). Two housekeeping genes, ADP ribosylation factor and polyubiquitin were used as endogenous control for normalization and quantitative expression of each gene was analyzed with reference to irrigated plants of RR11 105.

When RR11 105 control plants were taken as the calibrator (Table Ph. 1), expression of C-repeat element/dehydration-responsive element (CRT/DRE) binding factor was found more in drought tolerant clones (RR11 600 and RR11 430) and it was remarkably high in clone RR11 600. CRT/DRE binding factors are known to be upstream transcription factors that bind to promoter *cis*-element CRT/DRE and play vital regulatory roles in abiotic stress responses in plants. The up-regulation of this factor may be vital for drought tolerance in RR11 600 and RR11 430. ABC transporter

Table Phy.1. Relative quantification of seven stress responsive genes in *Hevea* under drought stress (irrigated plants of RRII 105 as calibrator)

Gene	RRII 105		RRIM 600		RRII 414		RRII 430	
	C	D	C	D	C	D	C	D
HbNRG18	1	1.57	1.61	1.84	1.97	1.89	2.71	7.10
HbDRT 50	1	0.65	1.18	1.52	1.68	1.09	2.55	0.98
Peroxidase	1	0.28	2.64	3.98	1.45	0.56	2.57	0.44
ABC transporter	1	1.25	0.59	0.18	1.06	1.52	0.66	0.42
LEA 5	1	4.95	0.80	1.03	1.12	20.38	0.77	1.47
CRT/ DRE bf	1	0.36	2.93	7.51	0.07	0.93	0.90	1.45
GPX	1	1.12	1.03	0.85	1.10	1.18	1.05	0.97

protein had been found down regulated in RRIM 600 and RRII 430 (drought tolerant clones) where as it did not vary much in RRII 105 and RRII 414 (drought susceptible clones). ATP-binding cassette (ABC) transporters are involved in the membrane transport of wide range of structurally and functionally unrelated compounds such as ions, inorganic acids, peptides, secondary metabolites, toxins and drugs. Down regulation of ABC transporter (ABCT) in the drought tolerant clones (RRIM 600 and RRII 430) implies that this might be contributing for drought stress tolerance.

HbNRG 18 was remarkably higher in the drought tolerant clone, RRII 430 indicating its association with drought tolerance. Expression of the gene LEA 5 was more in the drought susceptible clones (RRII 105 and RRII 414) and was not much altered in the tolerant clones (RRIM 600 and RRII 430) after imposition of drought stress. Expression of peroxidase was relatively lesser under drought condition in all clones except RRIM 600 in which it was higher. Expression of glutathione peroxidase and HbDRT 50 did not show any significant trend with drought susceptibility/tolerance of different *Hevea* clones.

1.3. Molecular studies on cold stress

Polybag plants of clone RRII 105 and RRIM 600 were cold acclimatized in growth

chamber at 16°C for two days and were treated with low temperature at a minimum of 8°C for three days. Cold induced photoinhibition was confirmed with gas exchange parameters before collecting the leaf samples for gene expression studies. mRNA isolation and cDNA synthesis were carried out from the leaf samples. Expression pattern of four endogenous control genes was studied by qPCR analyses using real time PCR. qPCR was performed with selected stress associated genes such as WRKY transcription factor (WRKY tf), ABC transporter protein (ABCT), transcription factor MBF (TfMBF), LEA 5 protein, CRT/DRE binding factor (CRT/DRE bf), glutathione peroxidase (GPX), a hypothetical protein (33HP), Dna J protein and peroxidase. Suitable endogenous control genes were selected (ADP ribosylation factor and polyubiquitin gene) from four primers after performing qPCR and calculating their gene stability (M value) using GeNorm software.

One set of quantitative expression analysis was performed with control plants of RRII 105 as calibrator and the other set with control plants of each clone as calibrator. Out of the nine genes analyzed, only two genes showed remarkable fold change (over control) under cold conditions in both the clones. When relative gene expression analysis was performed using

control plants of each clone as calibrator (Table Phy. 2), both LEA5 protein as well as peroxidase were found to be up-regulated by 8.14 and 5.8 fold in the low temperature treated plants of RR11 105 and RRIM 600, respectively. Though the expression of TfMBF and 33HP were 2.66 and 3.26 fold higher in low temperature treated plants of RR11 105, their expression was lesser than control plants of RRIM 600. In contrast, ABCT protein was 3.67 fold higher in cold treated plants of RRIM 600 and was lesser than control in the case of RR11 105. These results indicate that both peroxidase and LEA5 protein were up-regulated in both the clones. However, expression of ABCT protein was found higher only in RRIM 600 indicating its association with cold tolerance.

Table Phy. 2. Relative quantification of nine genes in low temperature stressed plants of RR11 105 and RRIM 600

Gene	RR11 105 (C)*	RR11 105 (LT)	RRIM 600 (C)	RRIM 600 (LT)*
WRKY II	1	1.26	0.99	0.70
ABCT	1	0.37	1.09	3.98
Tf MBF	1	2.65	0.50	0.46
LEA 5	1	3.85	2.15	12.57
CRT/DRE b1	1	0.15	0.13	0.02
GPX	1	1.23	0.88	0.74
33 HP	1	3.25	0.87	0.33
DnaJ protein	1	0.84	0.75	0.39
Peroxidase	1	8.14	6.29	36.45

* C = Control, LT = Low temperature

1.4. Xylem sap-flow measurements in mature rubber plants

Sap-flow measurements were carried out in 20-year-old plants of two clones, viz. RR11 5 and PR 255. The seasonal variation in xylem sap flow-rate was determined. There was summer decline in transpiration rate in clone RR11 5. Correlations were established between sap flow rate and

meteorological parameters viz. maximum temperature (Tmax), sun shine hours and evapotranspiration (ET). The xylem sap flow rate was small during intensive rainy days (June-July) and it is attributed to low transpiration pull due to stomatal closure. The sap-flow rate was directly influenced by the sunlight intensity and sunshine hours during summer season.

1.5. Measurement of flux in CO₂ and water vapour in rubber canopy

The ecosystem level carbon dioxide and water flux and canopy level photosynthesis in a rubber plantation were continuously measured throughout the reporting period (April 2009 to September 2010) by using an eddy covariance system at Central Experimental Station, Chethackal, installed inside a four-five year old plantation in a field standing tower. The daily NEE (net ecosystem exchange) by the rubber plantation was continuously recorded and ranged from 1-25g/m²/day during the study period (Table Phy. 3). Most of the days, recorded CO₂ influx in to the

Table Phy. 3. Mean daily net carbon dioxide (g/m²/day) and water vapour (mm/day) flux in a four-five year old immature rubber plantation

Monthly mean	Carbon dioxide flux (Net carbon assimilation) NEE (g/m ² /day)	Water vapour flux-Evaporation (mm/day)
April 2009	12.3±0.6	3.1±0.15
May	12.1±0.8	3.3±0.20
June	9.3±0.6	3.3±0.25
July	12±1.1	3.8±0.24
August	15.2±0.9	3.4±0.20
September	3±0.4	2.9±0.15
October	14±0.6	3.2±0.10
November	11.4±0.9	2.1±0.15
December	9.4±0.8	2.73±0.20
January 2010	13.1±1.0	3±0.10
February	10.5±0.9	2.4±0.10
March	12±1.2	2.7±0.14

plantation; however, a few days recorded net carbon efflux from the plantation to the atmosphere. On those days, around 1-7g CO₂/m²/day was released to the atmosphere and there was rain and relatively less sunshine hours. Heavy rainy days witnessed a net efflux of CO₂ to the atmosphere, most probably, due to a low rate of canopy photosynthesis and possible sudden spurt in release of locked up CO₂ from the soil. The mean NEE was 11g CO₂/m²/day which is equivalent to 33.5 tons CO₂/ha/year. The evopotranspiration (ET) rate calculated from the latent heat of vapourization (LE) indicated a 3-4 mm net evaporative water loss from the rubber plantation per day.

The amount of carbon sequestered by the rubber trees was estimated during the same period by estimating the annual shoot biomass increment during this period. According to this method, the CO₂ sequestration realized as shoot biomass increment was 13.5 ton CO₂/ha/year (which does not include root biomass, soil respiration, litter decomposition and sequestration by weeds and cover crops).

1.6. Evaluation of *Hevea* clones for drought tolerance

1.6.1. Physiological evaluation of 400 series clones for drought tolerance- Field trial at CES Chethackal

Polybag plants of seven clones were raised and planted at CES, Chethackal during the planting season of 2009. The impact of drought on six months old plants in the field was assessed. Summer casualty (Dec. 2009-March 2010) was lesser in clones RR11 429, RR11 430 and RR11 600 when compared to RR11 414, RR11 417, RR11 422 and RR11 105. The percent of plants which shed two whorls of leaves during summer was the lowest in clone RR11 430 followed by RR11 600.

1.6.2. Physiological evaluation of RR11 400 series clones for drought tolerance- Studies in polybag plants

Polybag plants of five modern clones viz., RR11 414, RR11 417, RR11 422, RR11 429 and RR11 430; ten pipeline clones viz. P 10, P 15, P 21, P 60, P 61, P 67, P 68, P 76, P 84 and P 88 and three check clones (RR11 105, RR11 600 and Tjir 1) were studied for their drought tolerance potentials by withholding irrigation continuously for 15 days while irrigated plants of the respective clones served as control. Plants were grown both in open ambient and glass house conditions.

Results showed that among the 400 series clones, RR11 430 had the highest CO₂ assimilation (A) and stomatal conductance (g_s) whereas, clone RR11 414 was severely affected by drought. RR11 600 a proven drought tolerant clone showed least decline in 'A' and g_s among all the clones studied. Similar results were obtained in glass house experiment also. Effective quantum yield (Φ PSII) was higher in RR11 600 and RR11 430 under drought while all other clones recorded more than 50% reduction in Φ PSII activity compared to their irrigated control.

The leaf water potential was more negative in drought imposed plants than the irrigated control in all clones. A significant relationship was established between assimilation rate (A) and leaf water potential (Ψ_L). Similar trend of relationship existed between leaf water potential and stomatal conductance (g_s) also. The intrinsic water use efficiency was found to be better in RR11 430.

1.7.3. Evaluation of pipeline clones for drought tolerance

Among the pipeline clones tested P 10, P 21, P 60, P 67 and P 68 showed higher 'A' under drought condition while clones P10, P 67, P 68, P 76 and P 88 showed better PSII

activity. CO_2 assimilation under glass house condition was high for clones P10, P 67, P 68 and P 84 after 10 days of drought imposition.

1.7. Rapid screening of *Hevea* germplasm lines for intrinsic drought tolerance traits

1.7.1. Field scoring of wild germplasm accessions for drought tolerance

Empirical scoring of leaf yellowing and leaf senescence in wild germplasm collection was carried out during summer in SBN 2008 at CES, Chethackal. A total of 202 wild accessions were visually scored in the month of March, 2010 for leaf traits, sorted numerically and ranked for percent leaf senescence and yellowing. Genotypes showing less senescence and yellowing in field were ranked top as tolerant ones and *vice versa*. Accordingly, they were classified as 7 accessions in top ranking, 10 in medium ranking and 2 in bottom ranking. Wild accessions RO 3160, RO 2806, RO 1370, AC 3325, AC 1846, AC 1969 and RO 3223 were identified as the most intrinsic drought tolerant ones.

PS II photochemical efficiency was assessed in germplasm lines selected by field scoring for drought tolerance traits from germplasm source bush nursery. Visual scoring of leaf yellowing and leaf senescence was carried out in summer and accessions were ranked as top, middle and bottom ranking ones based on the extent of leaf injury caused by drought and high light stress. The selected accessions were validated by *in vitro* drought assay using polyethylene glycol (PEG 6000) incubation followed by measurement of PS II photochemical activity of leaf. The percentage reduction in PS II quantum yield (Φ PSII) of treated samples was estimated. All data were pooled together and regression lines were drawn to validate the

field scoring. Significant relationship was observed between percent leaf yellowing in field and reduction in PS II activity ($R^2=0.29$). The results of the lab experiment confirmed the field scoring as a valuable tool for the evaluation of intrinsic drought tolerance in *Hevea* plants.

1.7.2. Nursery evaluation of selected germplasm accessions

Two wild germplasm lines MT 5078 and MT 5100 selected by rapid screening using a laboratory technique were further evaluated in polybags at nursery stage by imposing soil moisture deficit for 18 days. Net photosynthetic rate (Δ) and wax content on leaf surfaces were estimated in these selected accessions. Compared to RR11 105, a known drought susceptible clone, the two germplasm lines exhibited higher photosynthetic activity under soil moisture deficit condition. More than 70% reduction in 'A' was observed in RR11 105 and it ranged between 55-60% in germplasm lines. Epicuticular wax content was found to be high in leaves of RR11 105 than the two wild accessions.

1.7.3. Field scoring of wild germplasm accessions for drought tolerance traits at RRS, Dapchari

Intrinsic drought tolerance capacity of few *Hevea* germplasm accessions was evaluated at RRS, Dapchari, a non-traditional drought prone locality. The clones included were 24 wild accessions, 5 HP lines and 4 known drought tolerant and drought susceptible check clones. Leaf traits such as yellowing and senescence were empirically scored during summer and classified into top and bottom ranks. Seven wild accessions and three HP clones were identified as most intrinsic drought tolerant at Dapchari conditions (Table Phy. 4).

Table Phy. 4. The wild germplasm accessions selected from field planting for drought tolerance traits

Top ranking	Top ranking	Bottom ranking
MT 1627	RRIM 600	RRII 105
MT 1623	MT 1681	MT 41
MT 43	RO 2387	MT 4856
HP 225	RO 2153	RRII 414
MT 1668	HP 92	RO 85
HP 105		

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

Two field experiments were initiated at Haileyburia Tea Estate Ltd., Elappara, a high altitude location in the traditional belt, to evaluate the growth of *Hevea* plants in low temperature and high wind prevailing area. Annual girth of plants was recorded at 150 cm height from the bud union. Clones PB 260 and GT 1 showed the highest girth and PR 261 recorded the lowest girth in trial 2006. In 2007 experiments RRIM 600 and RRII 208 experiment recorded maximum girth (10 cm) whereas clone RRII 105 recorded the lowest girth (5.5 cm). In general, serious symptoms were not observed either due to drought or low temperature stress.

2. Physiology of growth and yield

2.1. Yield and yield components

Twelve clones planted at CES, Chethackal were tapped for 16 years under normal S/2 d2 6d/7 system and yield potential of these clones was evaluated. The yield dropped from 11th-12th year of tapping in most of the clones. Many clones exhibited a good yield trend for ten years between 4th - 13th year of tapping. To overcome the declining trend of yield the trees were subjected to controlled upward tapping (CUT) in all clones (S/3 d2 6d/7 ET 5%). Trees

were stimulated on alternate months and cup lump yield was recorded. In most of the clones considerable increase in yield was observed in the first year of tapping and yield declined subsequently. Significant reduction was noticed in clones PB 235 and RRIM 501 whereas, clone RRIM 612 continued to record higher yield. At the end of the third year of tapping under CUT around 36% decrease in yield from the first year CUT yield was noticed (Table Phy.5).

Table Phy.5. Comparative yield performance of clones in the third year of tapping under CUT

Clone	Mean yield (g/t/t)	
	2007-2008 (S/3 d2)	2009-10 (S/3 d2)
RRII 300	93.6±8.10	43.42±2.83
PB 235	187.2±17.90	56.53±2.95
RRII 105	97.5±10.26	60.45±4.46
RRIM 600	90.8±7.34	74.9±5.55
GT 1	132.3±10.73	84.9±5.61
PR 107	62.5±4.21	51.9±3.49
GI 1	71.0±3.55	56.6±1.97
RRIM 501	67.0±6.27	37.8±3.66
RRII 118	136.6±8.20	95.0±5.91
RRIM 703	67.5±5.95	48.5±3.82
Tjir 1	74.0±8.71	53.7±4.98
RRIM 612	56.3±6.11	62.5±4.45

2.2. Tapping induced loss of biomass

The clone RRII 105 had recorded consistently less annual biomass increment upon tapping by d2 system. Around 39% of shoot biomass was lost in d2 system of tapping compared to the untapped trees. The annual biomass increment in d3 tapping system was higher than d2 system in clones RRII 105, RRII 300 and PB 311. Clone PB 235 followed by PB 260 and PB 311 recorded continuously higher biomass in the untapped trees. The loss of biomass is smaller in PB clones than RRII clones. The

clone RR11 105 is continuously proved to be a tapping sensitive clone.

The yield potential of five different clones in d2 and d3 system of tapping showed that PB clones have greater yield potential than RR11 clones in South Kanara District of Karnataka (Table Phy. 6). The

Table Phy. 6. The yield potential of five different clones in d2 and d3 system of tapping at HBSS, Nettana (2009-10)

Clones	Annual dry rubber yield (kg/ha)	
	d2	d3
RR11 105	1687 ^a ± 76	1769 ^a ± 226
RR11 300	755 ^a ± 109	1085 ^a ± 202
PB 235	2137 ^b ± 254	2185 ^b ± 140
PB 260	2130 ^a ± 201	1791 ^a ± 220
PB 311	1602 ^a ± 80	1396 ^a ± 147

annual rubber yield was significantly different among the clones. PB 235 recorded the highest yield followed by PB 260 and RR11 300 recorded the lowest yield. The clones PB 235 and PB 260 consistently recorded better biomass increment as well as high yield, indicating their suitability for the South Kanara district of Karnataka.

2.3. On-farm trial for the selection of latex-timber clones

With an aim of selecting latex-timber clones, four clones tapping on S/2 d3 system were selected during 2006-07 at Malankara Estate, Thodupuzha to find out the relationship between yield and annual girth increment. The annual girth increment and annual block yield during 2009-2010 were recorded. Among the four clones, RR11 105 was shown to be the highest yielding followed by PB 260. The annual shoot biomass increment was the highest in PB 235. The study is being continued.

2.4. Relationship of ATP status of latex, luitoid membrane composition and ATPase activity with rubber yield.

Biochemical parameters in latex (ATP, sucrose and thiols) were estimated in eight high yielding and five low yielding germplasm accessions along with the check clone RR11 105 (RRS, Padiyoor). Accessions included were AC 166, MT 1020, MT 179, RO 2629, AC 675, RO 2385, AC 2004, AC 655 (high yielding) and AC 655, AC 707, AC 637,

Table Phy. 7. Latex biochemical parameters in different germplasm accessions

Category	Accessions	Yield (g/l/t)	ATP (μM)	Sucrose (mM)	Thiol (mM)
High Yielding	AC 166	53.4 ± 9.1	265.9 ± 7.1	12.6 ± 2.6	0.126 ± 0.003
	MT 1020	36.3 ± 4.8	175.9 ± 4.2	7.9 ± 0.86	0.12 ± 0.009
	MT 179	31.6 ± 8.8	183.1 ± 12.3	13.9 ± 2.2	0.146 ± 0.021
	RO 2629	44.9 ± 1.3	230.8 ± 16.8	9.4 ± 0.45	0.129 ± 0.014
	AC 675	32.5 ± 2.5	191.5 ± 5.02	12.3 ± 2.3	0.138 ± 0.015
	RO 2385	26.1 ± 5.6	241.6 ± 7.5	13.9 ± 2.5	0.197 ± 0.045
	AC 2004	35.7 ± 4.6	270.8 ± 3.9	8.8 ± 0.6	0.160 ± 0.024
	AC 655	37.6 ± 8.7	197.8 ± 6.9	11.2 ± 1.1	0.135 ± 0.021
Low Yielding	AC 707	4.8 ± 1.0	128.8 ± 3.8	11.3 ± 1.8	0.131 ± 0.012
	AC 637	8.5 ± 1.2	110.0 ± 3.7	24.2 ± 4.4	0.159 ± 0.032
	AC 158	5.6 ± 1.41	150.8 ± 7.2	16.0 ± 0.7	0.116 ± 0.001
	AC 661	7.2 ± 2.6	137.9 ± 3.5	6.1 ± 0.22	0.103 ± 0.011
	AC 162	4.7 ± 0.73	147.9 ± 7.5	19.1 ± 2.8	0.374 ± 0.051
	Check clone RR11 105	64.8 ± 3.8	263.2 ± 5.6	7.7 ± 1.1	0.171 ± 0.024

AC 158, AC 661 AC 162 (low yielding). Among the accessions, AC 166 and AC 2004 showed high ATP content in latex similar to RRII 105 and the correlation between yield and ATP was positive. Thiol content in latex was also high in AC 2004. Accession AC 162 also showed higher content of thiol and sucrose but very low ATP content compared to the high yielding accessions (Table Phy. 7).

2.5. Clonal variations in latex regeneration mechanism in *Hevea brasiliensis*

Incorporation of radioactively labelled amino acids (Leucine, Arginine, Lysine and Phenylalanine - labelled with ^{14}C) into protein in C-serum of five RRII 400 series clones along with check clone RRII 105 were measured *in vitro*. Amino acid incorporation in cytosol proteins showed differences between clones. Higher rate of incorporation was noticed in clones RRII 414 and RRII 429 than in clone RRII 105.

2.6. Studies on rubber biosynthesis in *Hevea*

Prenyltransferase (RuT) enzyme activity in the whole latex was measured using ^{14}C -labelled IPP as the substrate in 5 year old plants of ten *Hevea* clones of varying yield potential. The clones selected were five high yielding clones (RRII 105, RRII 600, PB 217, PB 235, PB 260), three medium yielding clones (GT1, Tjir 1, PB 5/51) and two low yielding clones (RRII 33, RRII 38). Along with this five 400 series clones (RRII 414, RRII 417, RRII 422, RRII 429, RRII 430) were also studied.

2.7. Cloning and production of β -hydroxy β -methylglutaryl CoA-(HMGR) protein of *Hevea* for immunoassay analysis

Studies on HMGR enzyme protein in the bark tissues of different *Hevea* clones in relation to varying yield potential were

initiated. Primers have been designed and synthesized for HMGR protein and PCR amplification and cloning are in progress.

2.8. Seasonal variations in yield and associated biochemical changes in RRII 400 series clones

The study was carried out in five 400 series clones *viz.*, RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430 along with the popular check clone RRII 105, planted in 1993 at CES, Chethackal. Nine trees each of uniform girth and yield were selected for biochemical studies. The trees were under S/2 d3 6d/7 system of tapping.

Parameters related to rubber biosynthesis such as sucrose content, ATP, C-serum invertase, antioxidant enzymes such as SOD and peroxidase and biochemical components such as thiols and proteins were determined during low yielding summer season. The data was compared with the peak yielding season. Mean peak season yield (Sep-Nov), summer yield (Feb-April) and yield depression during stress periods were also calculated for all the clones.

The data was statistically analyzed using split-plot design taking clones as main plot and season as sub plot to observe the

Table Phy. 8. Mean yield (g/t/t) in different clones during peak yielding (Sep-Nov) and stress season (Feb-April)

Clone	Yield (g/t/t) Peak season	Stress season	Yield depression (%)
RRII 414	90.0	49.9	44.5
RRII 417	105.5	39.0	63.0
RRII 422	86.5	43.5	49.6
RRII 429	82.5	34.5	59.1
RRII 430	102.0	39.2	58.7
RRII 105	87.0	34.2	61.0
CD (clone)	12.92	CD (season)	15.75

Table Phy. 9. Biochemical parameters associated with rubber synthesis in different clones during peak yielding (Sep-Nov) and stress season (Feb-April)

Clone	Sucrose (mM)		ATP (μ M)	
	Peak season	Stress season	Peak season	Stress season
RRII 414	5.69	3.07	289.18	270.14
RRII 417	7.88	8.17	270.16	240.19
RRII 422	7.98	3.46	278.90	259.28
RRII 429	7.76	10.00	290.90	237.70
RRII 430	6.15	3.92	277.20	261.07
RRII 105	5.27	9.18	257.20	224.70
CD (clone)	2.52		CD (clone)	12.71
CD (season)	4.78		CD (season)	27.90

seasonal variations in biochemical parameters and their interaction with different seasons. The analysis showed that mean yield depression during the stress period compared to the peak season over two year period (Table Phy. 8) was 45.29% (RRII 414), 63.35% (RRII 417), 50.03% (RRII 422), 57.0% (RRII 429), 58.7% (RRII 430) and 64.5% (RRII 105). Among the biochemical parameters studied, latex sucrose content increased in clones RRII 417, RRII 429 and RRII 105 during stress season and maintained the ATP level (Table Phy. 9) compared to peak yielding season and conversely invertase activity was low in these clones. All the clones showed an increased peroxidase activity in C-serum during the stress period compared to peak yielding season. A general decrease in protein content in latex was also observed in all the clones during summer compared to peak season.

Significant clonal variations were observed in thiols, SOD and peroxidase in C-serum during the stress season and clone \times season interaction was also observed. RRII 422, RRII 430 and RRII 414 showed higher thiols, invertase and SOD in C-serum during stress season. Clones RRII 429, RRII 417 and RRII 105 showed very low SOD and invertase during this period.

3. Tapping panel dryness (TPD)

3.1. Effect of ethylene compounds on yield and stress responses

Trees under regular tapping (S2 d3; B1-2 panel) with uniform yield and ethylene production capacity were identified from a mature population of RRII 105 clone. Similarly, trees newly opened for tapping (BO-1 panel) of the same clone and age were used for this stimulation experiment. One set of both the regular tapping trees (BO-1 and B1-2 panels) were stimulated with 5% ethephon (bark application) as per the stimulation schedule May, November and April during 2009-2010. Bark samples were collected one month after the successive stimulation period for biochemical analysis. The tissues were analysed for various abiotic stress components such as hydrogen peroxide (H_2O_2), malondialdehyde (MDA) cyanide (CN) and the scavenging enzymes like peroxidase (PX) and β -cyanoalanine synthase (β -CAS). The β -hydroxy β -methyl glutaryl CoA reductase (HMGR) protein content was also analysed using ELISA technique. Latex yield and TPD incidence were recorded at monthly intervals in both experimental and control trees.

CN, H_2O_2 and MDA in the bark tissue were increased significantly due to

stimulation both in BO-1 and B1-2 panels compared to their corresponding unstimulated controls. PX activity decreased in the stimulated bark tissues. However, PX activity showed seasonal variations both in BO-1 and B1-2 panels. On the contrary to the increased tissue CN, the cyanide scavenging enzyme, β -CAS activity was decreased in stimulated bark tissue. β -CAS activity was significantly high in the unstimulated control trees. It indicates that stimulation with ethephon reduces the levels of cyanide detoxifying enzyme and therefore accumulating cyanide in the soft bark tissue.

Stimulated virgin panel (BO-1) always showed higher latex yield compared to the renewed panel (B1-2) which showed an initial marginal increase in latex volume that declined subsequently. Compared to virgin panel, stimulation could not induce a sustainable yield response in the renewed tapping panel. Bark thickness of the tapping panel increased significantly during stimulation in both BO-1 and B1-2 panels. Both the functional and disorganized latex vessel rows (LVRs) were also increased due to stimulation effect. However, total LVRs increased in BO-1 panel but remained unchanged in B1-2 panel and hence the high latex yield in BO-1 panel.

Studies indicated enhanced stress responses in the bark tissues of the stimulated trees compared to trees without stimulation. Apart from wounding stress, stimulated trees are also exposed to ethylene mediated stress responses that may cause the accumulation of stress components such as H_2O_2 , CN, MDA, etc. in the bark tissues of the tapping panel indicating oxidative stress.

3.2. Molecular basis of TPD

3.2.1. Cloning and characterization of TPD responsive genes by subtractive hybridization

The EST sequences obtained through subtractive hybridization experiment were prepared in the required format and about 805 ESTs were submitted in the dbEST database of GenBank. The accession numbers for the same have been obtained (GR305128 – GR305928). qPCR primers were designed and synthesized for endogenous control genes such as cyclophilin, ADP ribosylation factor, GAPDH, elongation factor, polyubiquitin, H1/H5, H3, ACBP and ferredoxin. Based on the primer efficiency (slope value) and Ct value, primers for cyclophilin, ADP ribosylation factor, GAPDH and polyubiquitin were selected for further analysis. The primers were tested for their efficiency and a set of 15 primers were short-listed for expression analysis.

About 100 trees were selected and TPD scoring was done. Bark samples were collected from TPD (late dripping trees, 10%, 25%, 50%, 75% TPD affected) and normal trees. mRNA was isolated from bark tissue samples and cDNA was synthesized. Primers for cyclophilin and GAPDH genes were as endogenous control. A set of nine genes viz. WRKY tf, CRT/DREbf, HP 33, LEA5, peroxidase, glutathione peroxidase, ABCT protein, tf MBF and DnaJ protein were selected for this study and their expression under different levels of TPD was quantified by quantitative PCR (qPCR). cDNA synthesized using mRNA of bark samples (from trees with varying levels of TPD) were used as template for the qPCR analysis. Among the nine genes tested by qPCR (Table Phy.10), expression of tf MBF remained up-regulated in all stages of TPD with a maximum of 3.5 fold in 80% TPD than the

Table Phy. 10: Relative quantification of nine transcripts under TPD and healthy conditions (with Cyclophilin and GAPDH as multiple reference genes)

Gene	Normal	LD	10%	20-30%	40-60%	80%	100%
ABCT protein	1.00	1.99	3.14	0.71	1.80	0.91	0.61
DnaJ	1.00	1.21	1.45	0.89	0.89	1.23	1.38
Tf MBF	1.00	0.59	1.22	3.06	1.80	3.64	2.87
Peroxidase	1.00	6.25	3.96	5.37	1.22	2.53	3.78
GPX	1.00	0.84	0.67	0.35	0.47	0.96	0.55
33 HP	1.00	0.12	0.31	0.71	0.95	1.08	1.95
WRKY tf	1.00	4.67	5.24	1.56	0.09	1.58	1.07
LEA 5 protein	1.00	0.53	1.03	0.76	0.74	0.76	0.56
CRT/DRE bf	1.00	0.42	1.76	0.07	0.01	0.10	0.03

Values given are as fold change over normal trees

normal tree. In the case of peroxidase, expression was found more in all the TPD groups with a maximum in 20-30% TPD trees (5.4 fold). Expression of DnaJ protein remained only marginally higher in 10%, 80% and 100% TPD trees and it was lesser than normal in 20-30% and 40-60 % trees. Expression of ABCT protein was 3 fold higher in 10% and 1.5 fold in 40-60% TPD trees, but remained lesser than normal trees in rest of the TPD groups. Similarly, level of WRKY tf also increased five fold in 10% TPD group and it remained lesser or more or less equal to the normal trees in the rest of the TPD groups.

In the case of CRT/DRE bf, expression was much lesser across all TPD groups except in 10% TPD group (1.8 times) than normal trees. Similarly, LEA5 protein levels had been more or less equal to control in the initial stage of TPD (10%) but was lesser among other group of TPD trees. Expression of HP33, had been very low in the initial stages of TPD which reached almost the level of normal trees in 80% and doubled in 100% TPD trees. GPX in general showed a declining trend except in 80% TPD trees. On the whole, the data indicated the significant up-regulation of two genes (peroxidase, tf MBF) and down regulation of one gene (CRT/

DRE bf) in TPD trees. Higher levels of peroxidase in TPD trees reveals the possibility of increased production of reactive oxygen species (ROS) since it was substrate inducible. Higher levels of MBF1 proteins in TPD trees indicate that this might be involved in triggering the transcription of stress alleviating genes that are related to ethylene stress as found in TPD trees. Senescence associated protein, zink finger protein and SUMO associated protein were highly up-regulated under TPD conditions when compared to normal bark tissues. Similarly levels of ETR1 and ETR2 were also greater under TPD conditions.

3.3. Molecular and biochemical basis of ethylene induced latex production in *Hevea brasiliensis* (Ethylene receptors and signal transduction mechanism)

mRNA was isolated from control and stimulated trees of clone RR11 105. cDNA was synthesized and RT-PCR was carried out using primers of known and newly identified ETR. Expression of ETR was confirmed in both the samples. Nucleic acid sequence data of ETR2, ERS1 and EIN4 were taken from the database and aligned. Degenerate primers were designed to identify new ethylene receptors in *Hevea*.

4. Gene expression studies

4.1. Construction of expression vector for *Bacillus subtilis* and over-expression of chitinase in endophytes

An expression vector pHT43 was used to clone coding region of chitinase gene from *Hevea*. The construct was used for transforming *E. coli* cells. In another experiment, chitinase cloned in pGEM-T vector was sequenced and clone with right sequence was identified.

5. Secondary metabolites

5.1. Quantification and identification of inositols

The experiment was conducted for the identification of other inositol forms from the latex. For this latex samples from RRII 105 were collected and processed as per the protocol developed by the Division. The separation of individual components was achieved and the experiment is in progress to identify individual peaks. In connection with the first examination report of the patent application (A process for obtaining substantially pure L-quebrachitol from natural rubber latex), clarifications were made and submitted for further processing of the patent. The patent application was granted (IP 238511, dated 9-2-2010).

5.2. Water relations of latex with reference to the contents of inositols and sugars in the latex during drought condition

Latex and leaf osmolyte composition was analyzed to see their role in latex water relations. C-serum was separated from the latex samples and assessed the osmotic potential and invertase activity. The osmotic concentration of C-serum was calculated from the osmotic potential values. The data obtained were statistically analysed. There was co-elution of osmolytes during HPLC analyses. So we couldn't get the composition of latex and leaf osmolytes. The clonal difference in osmoregulation was confirmed. Osmotic concentration was found high during the stress season than during the peak yielding season in all the clones. The invertase activity was found high in high yielding clones.

6. Ecological impact of rubber cultivation

6.1. Impact of climate change on Indian plantation sectors

6.1.1. *Historic climate change that has occurred in the rubber growing regions of India*

Monthly datasets for maximum and minimum temperatures were plotted for the

Table Phy. 11. Trend in long term temperature change in rubber growing regions in India

Station	Period	Temperature	Mean	Rate/year
Tura	1995-2008	Tmax	29.3	0.12
		Tmin	16.9	0.05
Agarthala	1984-2007	Tmax	30.6	0.02
		Tmin	19.9	0.06
Padiyoor	1998-2009	Tmax	32.8	0.01
		Tmin	21.8	0.11
Dapchari	1987-2009	Tmax	33.2	0.08
		Tmin	20.6	0.03
Kottayam	1957-2009	Tmax	31.2	0.05
		Tmin	22.7	0.03

first five year mean and the last five year mean for Kottayam, Chethackal, Parliar, Padiyoor, Dapchari, Agarthala and Tura. The data indicated that the days were warmed up by 2.6°C and the nights were warmed up by 1.6°C in Kottayam for the last 53 years. In Chethackal, the days were warmed up by 0.5°C and the nights were cooled down by 0.7°C for the last 23 years. In Parliar, the days were warmed up by 1.0°C and the nights were cooled down by 0.3°C for the last 15 years. In Padiyoor the days were warmed up by 0.6°C and the

nights were warmed up by 1.3°C for the last 12 years. In Dapchari the days and nights were warmed up by 1.8°C and 0.7°C respectively, for the last 23 years. In Agarthala the days and nights were warmed up by 0.5°C and 1.4°C respectively, for the last 24 years. In Tura the days were warmed up by 1.5°C and the nights were warmed up by 0.7°C for the last 14 years. The trend analysis was done for all stations to understand the rate of change in the T_{max} and T_{min} of the concerned regional stations (Table Phy. 11).

RUBBER TECHNOLOGY DIVISION

The activities of the Division were focused mainly on evolving improved techniques in processing, refinement in test procedures, chemical modification of NR, development of blends of NR with other rubbers, polymeric fillers for reinforcement of NR, scorch control of peroxide vulcanisation and rubber nano composites.

1. Primary processing

1.1 Preservation of latex

1.1.1. Low temperature preservation

Low temperature preserved latex was used to prepare ISNR 3L. Though the quality of the rubber obtained meets the specifications of ISNR 3L, there is batch to batch variation in colour and change in color during storage. Hence the experiments are being continued to improve the colour.

1.1.2. Chemical preservation

One chemical which was found to be effective as secondary preservative for latex was further evaluated in field latex. It was observed that this new chemical could

effectively replace TMTD used in low ammonia preservation systems. However, the toxicity of the new chemical needs to be assessed.

Cationic fatty amide derivatives were synthesised by varying the cationicity using solid phase synthesis. Antimicrobial activity of the synthesised molecule was evaluated against different bacterial strains and it was found that the antimicrobial activity increased with cationic charge.

1.2. Fabrication of sheeting machine

Initiated further modification of a new rubber sheet making machine, which requires 50 % less space for installation and cheaper than the conventional dual rollers, with the help of a private engineering machine manufacturer. An Indian patent was filed for the invention.

1.3. Skim latex processing

The latex concentrate manufacturing industry produces around 1.5-2 lakhs tones of skim latex per year as by-product. The

conventional method of recovering rubber from skim latex is time consuming, environmentally polluting, labour intensive and can cause health hazard to workers. A new method has been developed which involves deproteinisation of the latex using a proteolytic enzyme followed by creaming and coagulation using sulphuric acid. The rubber recovered by this process has lower nitrogen content and high PRI compared to conventional skim rubber (Table Chem.1). The HAF filled skim rubber obtained by the new method has also showed better vulcanisate properties (Table Chem. 2)

Table Chem.1. Comparison of raw rubber properties

Skim rubber	Nitrogen content (%w/w)	Initial plasticity, Po	PRI	Ash content (% w/w)
Conventional	1.18	38	56	0.24
New method	0.58	39	60	0.21

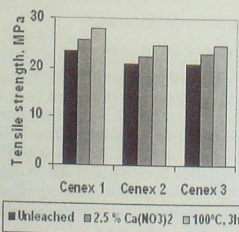
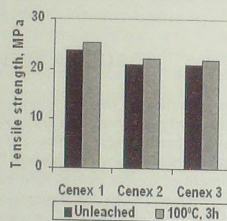
Table Chem. 2 Comparison of vulcanisate properties

Parameter	Skim rubber (40phr HAF filled)	
	Conventional	New method
Scorch time (t_{90}) at 150°C (min.)	3.2	4.5
Optimum cure time (t_{90}) at 150°C (min.)	12.4	13.8
300 % Modulus (MPa)	13.8	12.0
Tensile strength (MPa)	20.1	25.0
Elongation at break (%)	425	520
Tear strength (N/mm)	74.5	78.3
Heat build-up (DT°C)	29.0	25.0
Resilience (%)	61.0	65.0
Compression set (%)	38.9	34.0
Retention after ageing at 70° C for 7 days		
Hardness (Shore A)	64.0	60.0
300 % Modulus (%)	95.0	104.0
Tensile strength (%)	76.0	86.0
Elongation at break (%)	84.0	90.0

2. Latex technology

2.1. Radiation vulcanised latex(RVNRL)

To improve the quality of radiation vulcanized natural rubber Latex (RVNRL) the effect of particle size of latex, maturity of latex and leaching conditions of films were evaluated. It is possible to obtain RVNRL of good tensile strength at a comparatively low dose rate of 1.26 kGy/h by selecting concentrated latex of higher green strength. The tensile strength was also improved by leaching in 2.5% calcium nitrate solution and by heating the RVNRL films at higher



(The average particle size of latex concentrate was in the order Cenex1 > Cenex 2 > Cenex 3)

Fig.Chem.1. Effect of post treatment on tensile strength of RVNRL film

temperature for a short duration. The ageing resistance of the RVNRL films in terms of tensile strength was comparable with that of sulphur pre-vulcanised NR films.

3. Rubber Technology

3.1. Reinforcement

3.1.1. NR/ Polymeric filler system

Two polymeric filler systems were identified which can reinforce natural rubber and are designated as PF1 and PF2. Among the two polymeric fillers identified to reinforce NR, PF1 showed better efficiency in reinforcing compared to PF2. In order to improve the vulcanisate properties of NR/ PF2 system different proportions of HAF was added and compared the properties with that of 50 phr HAF filled NR vulcanisate. NR/PF2 system with 20 phr HAF showed comparable results with 50 phr HAF filled NR (Table Chem.3). Laboratory evaluation of NR/PF1 system was carried out using typical formulations of products like tyre side wall, conveyor belt, and microcellular soles etc. The results showed comparable properties with the control formulations.

3.1.2 Latex - filler master batch

Latex carbon black master batch was prepared by in situ soap sensitised

coagulation method and its mechanical properties were found to be better compared to dry mixing (Table Chem. 4). The loss of carbon black during the coagulation of latex and filler dispersion was negligible.

3.2. Blends

NR/NBR blends prepared by latex stage blending using skim latex and deproteinised skim latex had comparatively good mechanical properties though dynamic properties were inferior. They also had better air and solvent ageing characteristics as compared to dry rubber mixing. The fuel ageing resistance of latex stage blended NR/ NBR (30/70) blends using pure and deproteinised latex in comparison with NR/ NBR dry blends is given in Figure 1. Blend based on both pure/deproteinised skim latex have better fuel resistance than dry rubber blends.

3.3. Nano composites

Polymer coated nano silver was prepared using gamma radiation. The stabilised nanosilver dispersion was assayed. For its antibacterial activity against different bacterial strains. The nano silver with and without polymer coating gave comparable antibacterial property.

Table Chem. 3. Effect of Carbon black addition on properties of NR/PF2 system

Properties	NR/PF2	NR/PF2/ HAF 10	NR/PF2/ HAF 20	NR/PF2/ HAF 30	NR HAF 50
Tensile strength (MPa)	29.3	29.1	28.7	29.0	25.7
Modulus 100% (MPa)	2.4	2.7	3.7	4.3	2.8
Modulus 300% (MPa)	7.3	9.9	13.2	15.1	12.6
Elongation at break (%)	602	559	545	531	550
Tear strength (Nmm ³)	66.3	72.5	79.6	92.5	72.3
Hardness (Shore A)	54.0	58.0	62.0	64.0	60.0
DIN Abrasion loss (mm ³)	81.0	80.0	78.0	74.0	80.0
Heat build up (DT° C)	8.0	10.0	12.0	14.0	18.0

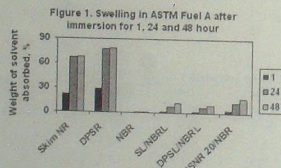
Table Chem. 4. Mechanical properties of carbon black master batches in comparison with conventional mixes

Parameters	NR/ carbon black masterbatch		NR/ carbon black dry mixed	
	30	40	30	40
Modulus 100% (Mpa)	1.91	2.15	1.50	2.06
Modulus 300% (MPa)	7.80	9.76	5.73	8.77
Elongation at break (%)	549.00	526.00	627.00	606.00
Tensile Strength (MPa)	26.50	28.40	24.00	26.70
Hardness (Shore A)	56.00	60.00	50.00	56.00
Compression set (%)	35.00	37.00	35.00	38.00
Heat buildup (DT° C)	23.00	24.00	24.00	25.00
Abrasion loss (mm ³)	105.00	103.00	120.00	114.00
Resilience (%)	75.00	72.00	76.00	74.00
Tear strength (N/mm)	57.00	65.00	43.00	63.00

Continued the work on preparation of nano zinc oxide using chemical route and its stabilization was attempted using potassium oleate and SDS

3.4. Peroxide vulcanisation

Sulphur/accelerator like scorch control could be achieved in peroxide vulcanisation using a stable free radical (TEMPO). The loss of crosslink density due to scavenging of polymeric radical by TEMPO could be well compensated using a coagent without affecting the scorch time (Fig.Chem.3).



(DPSR-deproteinised skim rubber, DPSL-deproteinised skim latex)

Fig.Chem.2. Swelling behaviour of skim rubber and its blends

3.5. Epoxidised natural rubber (ENR)

Evaluation of the raw rubber properties of ENR stored for one year at ambient conditions showed that the viscosity of the rubber increased only in range similar to the hardening of NR stored under similar conditions.

ENR silica composites with varying mole per cent of epoxide groups were prepared and the vulcanisates prepared were analysed using AFM for silica dispersion and SEM EDAX for the dispersion of silica, ZnO and sulphur. ENR of medium epoxy content showed good silica dispersion. ZnO showed non uniform distribution.

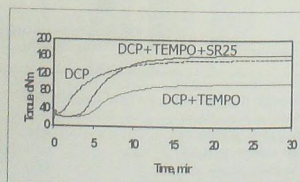


Figure Chem.3. Scorch control using TEMPO

4. Collaborative project

4.1. Development of footwear sole for physically handicapped

Six hard sole compounds were prepared in the laboratory and were evaluated at Scheffli Leprosy Research and Training Centre (SLR&TC), Karigiri. The feed back is being awaited.

4.2. Development of Hurth coupling for rail locomotive

In connection with the development of Hurth coupling membrane for Chithranjan Locomotive Works (CLW), West Bengal, 6 nos. of Hurth coupling membranes were sent to CLW for application trials. Work is being continued to use commercially treated fabric for preparation of Hurth coupling membrane.

TECHNICAL CONSULTANCY DIVISION

The Technical Consultancy Division of the Rubber Research Institute of India provides technical assistance to promote rubber goods manufacturing industries in the country. The Division acts as a link between technology, knowledge, innovations, applied research and development to the entrepreneurs and industrialists in the rubber industrial sector. The wide range of services offered are advices in project proposals and investment decisions, quality control by testing of rubber/rubber chemicals/rubber compound/rubber products etc as per national and international standards, technical problem solving, training etc. to the rubber industry. The Division also had undertaken product development, and training programs as per the need and requirement from the clients. Besides these, academic activities and R&D activities are also undertaken.

Project profiles of various rubber goods manufacturing units (dry rubber and latex based) have been prepared by the Division. Project Profiles provide the basic details of typical rubber products manufacturing units in the SSI sector like capital investment, infrastructure facilities

required, profitability and general overview of the manufacture of the product. Technical Bulletins which cover the technical details of various products are also available in the Division.

Detailed Project Reports for ascertaining the techno-economic feasibility of an industrial unit can be prepared by the Division on specific request from entrepreneurs. This gives all the general information for the establishment of an industry.

With the aim of increasing the internal consumption of natural rubber, the Division is engaged in the activities of Rubber Industrial Parks in Kerala, Tamil Nadu, and Tripura

The above activities were continued this year also and some of the major items for the period under report are summarized below:

1. Product Development

The Division assists entrepreneurs to develop new products as well as substitutes for imported ones. Also helps the manufacturers to produce and market products successfully meeting specifications prescribed by the BIS, Railways, Defence

Department and various other Government/ Public Institutions.

The following products were developed based on the enquiries from existing industries/entrepreneurs

a) Latex Based Products

1. Latex carpet backing
2. Prevulcanised latex for rubber moulds used in statue making
3. Latex adhesive
4. Special purpose latex adhesive
5. Latex vulcanised sheet
6. Fluorescent rubber band
7. Carpet backing using waste rubber crumb

b) Dry Rubber Based Products

1. NR/EPDM Valves
2. Autorickshaw engine mounting
3. Rubber sleeve
4. Pressure cooker gasket
5. EPDM coated NR tile mould
6. Sponge rubber mat
7. Rubber sheets similar to linatex sheets
8. Low hardness sheet (black)
9. Low hardness sheet (coloured)
10. Insulating mat
11. Sponge pad
12. Special rubber component for floor cleaning device
13. Submarine rubber component

2. Quality Control

The Division is equipped with a quality control laboratory. Almost all the quality tests essentially required for the rubber industrial sector can be carried out in this laboratory on nominal charges. This helps manufacturers to maintain the quality of

their products and suitable modifications can be done for improving the quality, if required.

The TC Division has established a well equipped latex technology laboratory for research, development and testing of natural rubber latex products as per national and international standards. Latex products include:

- Gloves - all types
- Uridrain condoms
- Medical tubings
- Condoms
- Latex foam products
- Rubberised coir foam
- Latex backed carpets
- Balloons
- Rubber bands
- Latex adhesives
- Elastic thread
- Other dipped goods

During the reporting period the Division has tested 6550 parameters on consultancy basis. Evaluation of chemicals viz. accelerators, stabilizers, reclaimed rubber, plasticizers etc. was also done.

3. Advisory Services

Advisory and consultancy services are also offered on technical matters and other problems during trial run/commercial operation of the units. Based on specific requests from product manufacturers, factory visits are conducted by experts to rectify the defects, streamline the operations and also to solve production problems.

The Division also offers consultancy services for the preparation and stabilization of dispersions/emulsions which are essential ingredients of latex compounds.

Technical advice was given for the queries received from 580 firms.

4. Training Activities

TC Division also provides assistance to the Training Department in conducting training courses for the benefit of latex and dry rubber goods manufacturers. Also specialised training on certain rubber products is conducted by the Division on specific request from the clients.

During the period entrepreneurs from three firms were imparted training on rubber adhesives. Training was also given for carpet backing using waste rubber crumb on consultancy basis.

Besides technology transfer, our technical staffs serve as faculty members of various industrial and academic institutions.

ECONOMICS DIVISION

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

1. Trends in farm income and wages in the era of market uncertainty: an exploratory analysis of natural rubber sector in Kerala

The major objectives of the study were (i) to understand the trends in productivity, prices, farm income and wages during the pre and post-reforms phases, (ii) to analyse the comparative stability of productivity, prices, farm income and wages, (iii) to examine the relative contributions of productivity and price in the observed trends in farm income and (iv) to highlight the policy implications.

Documented field level time series data on wages from primary sources and published official data on productivity and price during the 28 year period from 1980-81 to 2007-08 were used for the analysis. The trends in statutory basic daily wages plus dearness allowance in the estate sector and the rate of tapping wages in the smallholdings sector were considered for the analysis of wages. Since time series data on net farm income based on reliable cost of production are not readily available, the trends in total annual farm income per hectare was estimated by multiplying the reported annual average productivity in Kerala with the annual average price of RSS 4.

The study revealed that during the 28 year period, real farm income grew at an annual average rate of 3.13 per cent despite a negative annual average growth rate (-0.55%) in real price (Table Eco. 1). The observed positive growth rate in farm income had been contributed by a higher growth rate in productivity (3.71%). Real wages in both the smallholdings and estate sector had shown positive growth rates of 1.88 and 1.20 per cent respectively. To a

large extent, the potential erosion in net farm income due to the cumulative effect of rise in real wages and a negative growth in real prices was prevented by a higher growth rate in productivity. The analysis during the pre and post- reforms phases (1980-81 to 1990-91 and 1991-92 to 2007-08, respectively) showed that even with a negative growth in price (- 2.04%) farm income grew at the rate 1.07% during the pre-reforms phase due to higher growth rate in productivity (3.18%). During the post-reforms phase, a higher growth rate exhibited by farm income (4.15%) despite a lower growth rate in productivity (2.91%) was due to a positive growth rate in price (1.21%). The growth rates in wages in both estates and smallholdings were higher during the post-reforms phase (2.26 and 4.31%) compared to pre-reforms phase (0.52 and 0.56%). Compared to pre-reforms phase, price, productivity and farm income were unstable during post-reforms phase. The high instability in farm income during post-reforms phase (30.52 compared to 6.71 during pre-reforms phase) was mainly due to the higher instability in price (27.84) during the phase. Wage rates of both smallholdings and estates were comparatively more stable with higher growth rates during the post-reforms phase. An important feature of price during post-reforms phase was higher rate of

growth (1.21%) with higher instability (27.84).

The analysis of relative contributions of price and productivity in sustaining the tempo of growth in farm income revealed that during the 28 year period, trends in real farm income had been influenced more by productivity (55%) compared to that of real price (45%). Disaggregate level analysis of the trends in the relative contributions of price and productivity (during the pre and post-reforms phase) revealed that during pre-reforms phase the movements in farm income was influenced more by productivity (54%) but during the post-reforms phase uncertain prices attained prominence with a comparative influence of 65 per cent.

2. Family labour shortage under rubber Block Planting Scheme in Tripura

The specific objectives of the study were (i) to summarise the region-specific factors instrumental in the unique developmental experience of NR sector in Tripura, (ii) to focus on the recent developments posing serious constraints on the supply of family labour to the immature plantations in the context of the growing popularity of employment opportunities under the National Rural Employment

Table Eco. 1. Comparative growth rates and instability indices

Variables	Growth rate (%)			Instability index		
	Pre-reforms phase	Post-reforms phase	Total period	Pre-reforms phase	Post-reforms phase	Total period
Price	(-) 2.04	1.21	(-) 0.55	5.97	27.84	21.95
Productivity	3.18	2.91	3.71	1.99	4.44	5.08
Farm income	1.07	4.15	3.13	6.71	30.52	17.94
Wages (S.H.)	0.56	4.31	1.88	7.97	6.77	14.15
Wages (Estates)	0.52	2.26	1.20	4.38	3.93	6.44

Table Eco. 2. Labour availability to BPS during the pre and post-NREGA phases

Year of Planting	Estimated annual labour requirement (Mandays/ha)	Excess/deficit of labour availability during	
		Pre-NREGA phase (%)	Post-NREGA phase (%)
1	557	196.23	(-) 41.83
2	137	78.83	84.67
3	102	26.47	27.45
4	77	46.75	14.29
5	80	45.00	7.50
6	70	(-) 25.71	(-) 40.00
Total	1023	125.32	(-) 9.78

Guarantee Act, 2005 (NREGA) and (iii) to suggest policy options to overcome the observed problems from a long-term perspective.

The study used secondary data on field level availability of labour during the pre and post phases of NREGA collected from the weekly abstracts of Block Planting Units (BPU) from Nucleus Rubber Estate and Training Centre (NRETC), Agartala. The data collected were compared with the estimated annual requirement of labour for raising the plantation. For comparison, time series data on wage rates of BPUs and NREGA were collected respectively from NRETC and notifications of minimum agricultural wage rate of Government of Tripura.

The study found growing shortage of family labour in the immature rubber plantations in the context of higher wage rates offered under the NREGA Scheme in Tripura. Since the scheme primarily depended on family labour from the beneficiary households, shortage of labour

lead to the postponement of operations and resultant prolongation of the immature phase. It also affected the uniform and the healthy growth of plants. Labour availability to Block Planting Scheme (BPS) during the pre and post- NREGA phases is given in Table Eco. 2.

Higher wage rates offered under NREGA schemes was found to be the main contributory factor for the shortage of the family labour. It was suggested to include the development work during the immature phase of rubber plantations under the NREGA schemes so as to ensure adequate availability of labour, timely completion of the prescribed cultural practices and to avoid potential conflict between the wage rates. The saved funds of the participating agencies of the BPS can be effectively utilized for building up region-specific infrastructural facilities for group processing, marketing and community based development projects for empowerment of the Economically and Socially Marginalised Groups (ESMGs) and women.

LATEX HARVEST TECHNOLOGY DIVISION

The International Rubber Research Development Board (IRRDB) during its annual meeting 2008 at Kuala Lumpur resolved to change the terminology of Exploitation Studies into Latex Harvest Technology. In accordance with this decision and as approved in the 160th meeting of the Rubber Board, the Exploitation Studies Division was renamed as Latex Harvest Technology (LHT) division. In addition to the ongoing activities on various latex harvesting techniques, the division initiated a few ambitious programmes. Availability of skilled rubber tappers is becoming a serious issue year after year. In view of the very small size of the holdings in India, one approach to solve this is to tap the rubber trees by the growers themselves. Since tapping is a highly skilled operation, it is not a simple task. Hence, the division in collaboration with Amal Jyothi Engineering College, Kanjirappally, has entered into a collaborative programme for developing a battery operated tapping tool by which skill in tapping can be eliminated. We have made good progress by developing a prototype

and further modifications are going on. Scientists of the division were instrumental in the latest revision (2009) of International tapping notations.

The standard practice in Controlled Upward Tapping (CUT) is to tap a quarter spiral cut with ethephon stimulation. The division carried out evaluation of gaseous stimulant (ethylene) for further improvement in yield and or further reduction in the length of tapping cut to S/8. The preliminary results were promising. However, due to certain technical hurdles like gas leakage, after one year the programme is kept in abeyance. The collaborative programme on extension of low frequency d3 tapping with ethephon application in select small holdings along with the Rubber Production Department is progressing well.

1. Low frequency tapping systems

Experiment on Low Frequency Tapping (LFT) with different levels of yield stimulation in clone RR11 105 was continued

Table LHT.1. Yield performance of Low Frequency Tapping systems with stimulation in clone RR11 105

Tapping system	Yield** (Kg/ha)	No. of taps	Panel
S/2 d2 6d/7 (control)	2949 a	144	BI-2(1)
S/2 d3 6d/7. ET2.5% Pa1(1.5) 3/y*	2454 bcd	100	BI-1(3)
S/2 d3 6d/7. ET2.5% Pa1(1.5) 4/y*	2290 cd	99	BI-1(3)
S/2 d3 6d/7. ET2.5% Pa1(1.5) 5/y*	2624 abc	99	BI-1(3)
S/2 d4 6d/7. ET2.5% Pa1(1.5) 5/y*	2507 abc	74	BI-1(1)
S/2 d4 6d/7. ET2.5% Pa1(1.5) 7/y*	2570 abc	73	BI-1(1)
S/2 d4 6d/7. ET2.5% Pa1(1.5) 9/y*	2836 ab	73	BI-1(1)
S/2 d6 6d/7. ET2.5% Pa1(1.5) 10/y*	2180 cd	51	BO-2(6)
S/2 d6 6d/7. ET2.5% Pa1(1.5) 12/y	2009 d	51	BO-2(6)
S/2 d6 6d/7. ET2.5% Pa1(1.5) 16/y*	1993 d	51	BO-2(6)
CD (P = 0.05) - 493			

** Values followed by same letter/s are not critically different from each other

at Experimental Farm Unit (EFU), RIT, Pampady. Considerably higher and comparable yield under d2 and d4 frequencies of tapping was due to panel change (BI-2 first year during 2009-10) effect (Table LHT.1). Under d6 tapping yield was not comparable to other frequencies of tapping, in the report period since the tapping cut is closer to bud union.

Two LFT trials with rainguarding were laid out in Kulasekharam region, Tamil Nadu in the year 2002. At Kanthimathy Estate, there were 7 tapping blocks under weekly system of tapping and monthly yield stimulation throughout the trial period. Whereas at Hariharaputhra Estate, there

were 4 tapping blocks under d4 frequency of tapping with rainguarding. Other tapping blocks in the estate were not rain guarded in the initial years. Yield stimulation as per the schedule was carried out throughout the trial period. Six years data on yield and related parameter were collected, consolidated and compiled.

In the weekly tapping trial at Kanthimathy Estate, mean yield of 2334 kg/400 trees could be obtained during the six years trial period (Table LHT.2). Benefit–Cost analysis of the data indicated a BCR of 11.33. Whereas, at Hariharaputhra Estate the yield was 1933 kg/400 trees (11 months tapping / year) could be obtained under d4 system (Table LHT.3).

Table LHT.2. Dry rubber yield under weekly tapping with rainguarding at Kanthimathy Estate, Kulasekharam (Tamil Nadu)

Particulars	03-04	04-05	05-06	06-07	07-08	08-09	Mean
Kg/400 trees	2334.0	2503.0	2596.0	2203.0	2625.0	1743.0*	2334.0
Kg/tap	50.7	49.0	52.0	44.0	57.1	36.6	51.0
DRC (%)	38.6	37.1	37.5	38.7	40.1	38.5	38.4
Scrap (%)	23.2	23.8	23.6	22.8	21.2	16.5	21.9
Kg/tree	5.8	6.3	6.5	5.5	6.5	4.4	5.8
g/tree/tap	126.1	122.7	130.0	110.0	143.5	95.7	121.3
Tapping days	46.0	51.0	50.0	50.0	46.0	46.0	48.0
Rainfall (cm)	0.1	109.8	125.2	06.6	105.4	71.6	94.8
Rainy days			71.0	62.0	75.0	54.0	66.0

* ET10/Y

PD – 7 % (March 09)

Table LHT. 3. Dry rubber yield under d4 frequency of tapping with rainguarding at Hariharaputhra estate, Kulasekharam (Tamil Nadu)

Particulars	03-04	04-05	05-06	06-07	07-08	08-09	Mean
Kg/400 trees	1961	1956	2337	1978	1788	1576	1933
Kg/tap	33.8	31.1	33.9	26.7	34.5	30.9	33.5
DRC (%)	37.9	34.3	34.8	35.5	38.4	37.7	36.4
Scrap (%)	25.9	32.6	27.9	26.3	25.5	15.9	25.7
Kg/tree	4.9	4.9	5.8	4.5	4.5	3.9	4.8
g/tree/tap	84.5	77.8	84.0	80.4	83.3	75.0	80.8
Tapping days	58	63	69	56	54	52	59
Rainfall (cm)	-	153.9	156.2	128.8	161.4	90.1	138.1
Rainy Days	-	79	80	79	84	63	77
TPD - 9 %							

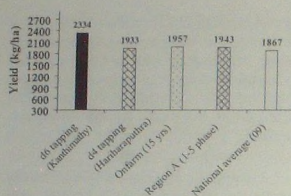


Fig. LHT.1. Yield comparison of Low Frequency Tapping with rainguarding in clone RRII 105 (Kulasekharam, Tamil Nadu)

Global mean yield of clone RRII 105 (1-10 year phase) in Kulasekharam region is 1904 kg/ha. Onfarm mean yield of clone RRII 105 (1-15 years) is 1957 kg/ha. National average yield is less than 1900 kg/ha. The yield under both d4 and d7 frequencies in the region was equal or better than these means (Fig. LHT.1).

Results of the study indicated feasibility of successful adoption of low frequency tapping (d4 and d7 tapping) with

rainguarding in Kulasekharam region of Tamil Nadu. Based on the success, these estates have shifted their entire area into LFT. Rainguarding is essential even in low rainfall regions like Kulasekharam for the success of LFT. The technology can address current shortage of skilled tappers to a great extent.

The demonstration plot at CES under weekly tapping with monthly stimulation continued to give promising yield during 2009-10 (Table LHT. 4). It was 8.2 kg/tree/year and 62.7 kg (mean per tap). Current year yield is 75% of 2008-09 (1st year yield on BO-2 panel). Incidence of tapping panel dryness is very low (1.6 %).

Exploratory trial on LFT (d10) in clone RRII 105

Initial two years, the trees were tapped under d6 frequency in BO-2 panel. From third year onwards frequency was modified to d10. Promising yield of more than 2 tonnes/400 trees was obtained in the 8th year of BO-2 panel (Table LHT. 5). TPD percentage is very low (1.4 %)

Table LHT. 4. Monthly yield response of Low Frequency Tapping (S/2 d6 d7) in clone RRII 105

Months	Kg/400 trees	Kg/tap	No. taps	DRC (%)
Apr' 09	200.5	40.1	5	38.3
May	145.5	36.4	4	40.2
June	232.4	58.1	4	38.3
July	480.0	96.0	5	33.2
Aug	336.0	84.0	4	35.5
Sep	285.6	71.4	4	36.1
Oct	349.2	69.8	5	38.9
Nov	282.1	70.5	4	38.3
Dec	402.7	80.5	5	40.0
Jan' 10	287.1	71.8	4	39.2
Feb	130.9	32.7	4	36.2
Mar	163.1	40.8	4	37.1
Total	3295	62.7	52	37.6
Kg/tree	8.2	(mean)		(mean)

Table LHT. 5. Monthly yield response of Low Frequency Tapping (S/2 d10) in clone RRH 105 at CES, Chethackal

Months	Yield (kg/400 trees)	Yield (Kg/tap)
Apr '09	160.3	53.4
May	160.8	53.6
June	215.6	71.9
July	243.2	81.1
Aug	239.2	79.7
Sep	221.5	73.8
Oct	271.7	90.6
Nov	200.2	66.7
Dec	207.0	69.0
Jan'10	178.8	59.6
Feb	129.5	43.2
Mar	167.1	55.7
Total	2394	66.5
Kg/tree	6.0	(mean)

Demonstration plots on d3 frequency tapping in Small holdings: Collaborative programme of RRH and Regional offices of Rubber Board

The programme schedule was to cover all Regional offices in Kerala, Tamil Nadu and Karnataka in two phases. Accordingly,

in the 1st phase (2009), grower meetings were held in 10 Regional Offices to give awareness about LFT and yield stimulation. After the meeting, growers were given the option to join for the 3 year collaborative programme. Subsequently plot selection was carried out and base information about the plots were also collected. Growers were given practical training in their own field on dilution of ethephon and method of application. They were requested to record yield of all tapping days. The programme is continuing successfully. In spite of the delayed starting in 2009 (June-July), an average yield of over 4 kg/tree was realized from 75 tapping days in all the plots. There was no report on increase in TPD from any of the growers.

2. Low Frequency Controlled Upward Tapping (LFCUT)

An experiment on LFCUT was carried out in clone RRH 105 at EFU, RTI, Pampady. Continuous CUT under d4 (T3) and d6 (T7 and T11) frequencies of tapping were comparable to conventional CUT (periodic

Table LHT. 6. Performances of Low Frequency Tapping with different harvesting practices in clone RRH 105

Tapping system	Yield* (Kg/400 trees)	No. of Taps	No. of stim/yr
S/2 d3 6d/7. ET2.5% 2/6m ; S/4U d3 6d/7. ET5.0% 6/6m	3215 ab	100	5
S/2 d4 6d/7. ET2.5% 4/6m ; S/4U d4 6d/7. ET5.0% 9/6m	2544 bcd	72	8
S/4U d4 6d/7. ET5.0% 18/y	2991 abc	72	15
S/4U d4 6d/7. ET5.0% 18/y (APC)	2421 cd	72	15
S/4U d4 6d/7. ET5.0% 18/y (BPC)	2911 abc	72	15
S/2 d6 6d/7. ET2.5% 6/6m ; S/4U d6 6d/7. ET5.0% 12/6m	2427 cd	52	15
S/4U d6 6d/7. ET5.0% 24/y	3403 a	51	23
S/4U d6 6d/7. ET5.0% 24/y (APC)	2664 abcd	51	23
S/4U d6 6d/7. ET5.0% 24/y (BPC)	2591 bcd	51	23
S/2 d6 6d/7. ET2.5% 6/6m ; S/3U d/6 6d/7. ET5.0% 9/6m	2398 cd	52	13
S/3U d6 6d/7. ET5.0% 18/y	2821 abcd	52	16
S/3U d6 6d/7. ET5.0% 18/y (APC)	2117 d	52	16
S/3U d6 6d/7. ET5.0% 18/y (BPC)	2104 d	52	16

CD (P = 0.05) - 781 *Values followed by same letter/s are not critically different from each other

Table LHT.7. Yield response of Low Frequency Controlled Upward Tapping with rainguard in clone RRII 105 at CES, Chethackal

Tapping system	Yield* (Kg/400 trees)	No. of Taps
S/2 d3 6d/7. ET2.5% Pa1(1.5) 3/y*	3164 d	100
S/4U d3 6d/7. ET5% La1 18/y(3w)	7989 a	100
S/2 d4 6d/7. ET2.5% Pa1(1.5) 6/y*	5153 cd	75
S/2 d4 6d/7. ET2.5% Pa1(1.5) 6/y*	3967 cd	75
S/4U d/4 6d/7. ET5% La1 24/y(2w)	7368 ab	75
S/2 d/6 6d/7. ET2.5% Pa1(1.5) 12/y(m)	4913 cd	50
S/2 d/6 6d/7. ET5% Ga1 18/y(3w)	5589 bc	50
S/2 d/6 6d/7. ET2.5% Pa1(1.5) 12/y(m)	3383 cd	50

* Values followed by same letter/s are not critically different from each other.

panel change) d3 frequency of tapping (Table LHT.6). Good yield was obtained during the fifth year of tapping also. For continuous CUT, rainguarding using wider polythene (90 cm) is required. Mean yield of five years under S/4 d3 frequency with periodic panel change is comparable with S/4 d4 (periodic panel change), S/4 d4 (annual and biennial panel change), continuous CUT of S/4 d4 and S/4 d6 and S/4 d6 (APC) with fortnightly stimulation.

In another experiment at CES, Chethackal during sixth year of tapping, T2, T5 and T7 are shifted to CUT and others are shifted to basal panel. Yield under S/4 d3 (once in 3 weeks stim) frequency of tapping was comparable with S/4 d4 with stimulation after every third tapping (Table LHT. 7). Yield of 19.9 kg per tree was obtained in S/4 d3 with once in three week's stimulation and 18 kg/tree in S/4 d4 with

stimulation after every 3rd tapping. Similarly trees tapped under different frequencies were comparable in the basal panel tapping.

In the LFCUT experiment in clone RRII 118 at CES, Chethackal yield under S/4 d3 and S/3 d4 with once in six weeks stimulation and S/3 d6 with once in three weeks and monthly stimulations were comparable (Table LHT. 8). Highest yield of 7.8 Kg/tree was obtained under S/4 d4 with once in three weeks stimulation.

3. Other Experiments

Long term evaluation of rainguard:
Problem of production losses due to rain and recovery through stimulation

During the year it was observed that yield loss under d2 or d3 frequency can be compensated by stimulation in the absence

Table LHT. 8. Yield response of Low Frequency Controlled Upward Tapping with rainguard in clone RRII 118

Tapping system	Yield* (kg/400 trees)	Kg/tree	No. of Taps
S/4U d3 6d/7. ET5.0% La1 9/y(6w)	2308 b	5.8	99
S/4U d4 6d/7. ET3.0% La1 18/y(3w)	3113 a	7.8	76
S/3U d4 6d/7. ET5.0% La1 9/y(6w)	2633 ab	6.6	76
S/3U d6 6d/7. ET5.0% Ga1 18/y(3w)	2579 ab	6.4	52
S/3U d6 6d/7. ET3.0% Ga1 12/y(m)	2411 b	6.0	52

*Values followed by same letter/s are not critically different from each other

Table LHT. 9. Effect of stimulation under d₁ and d₂ frequencies of tapping in clone RR11 105 without rainguarding on recovery of yield loss at EFU, RIT, Pampady

Tapping system	Kg/ha*	Kg/ha/tap*	g/t/t	Kg/tree*
S/2 (RG) d2 6d/7	2296 b	16.6 c	42.8 b	5.9 bc
S/2 d2 6d/7	1753 b	18.6 c	70.3 b	6.7 abc
S/2 d2 6d/7. ET2.5% Pa. 3/y	2488 b	25.8 c	68.8 b	6.6 abc
S/2 d2 6d/7. ET2.5% Pa. 5/y	2637 ab	27.5 bc	72.4 b	6.9 abc
S/2 (RG) d3 6d/7. ET2.5% Pa. 3/y	2274 b	24.2 c	66.4 b	6.2 bc
S/2 d3 6d/7. ET2.5% Pa. 3/y	1706 b	28.1 bc	75.1 b	4.6 c
S/2 d3 6d/7. ET2.5% Pa. 5/y	2576 b	40.3 b	122.0 a	7.8 ab
S/2 d3 6d/7. ET2.5% Pa. 7/y	3700 a	57.8 a	148.7 a	9.5 a
LSD (0.05)	1068.0	14.42	34.76	3.006

* Values followed by same letter/s are not critically different from each other

of rainguard. Higher per tap yield was observed under d3 system of tapping with out rainguarding at higher levels of stimulation. (Table LHT. 9).

Evaluation of "Mortex" as an yield stimulant in rubber

In the preliminary evaluation trial of mortex, an yield stimulant formulation, comparable yield to that under S/2 d3 6d/7. ET2.5% Pa. 3/y was obtained under three or six rounds of mortex per year. However, the cost of Mortex is very high (approximately 7 times higher) compared to ethephon.

Evaluation of bio-degradable polythene for rainguarding rubber trees

Possibility of using bio-degradable polythene for rainguarding of rubber trees

were initiated. Heat aging studies of the samples were conducted at 70°C for 5 and 10 days as well as UV exposure for 5 days at room temperature. In heat aging only 50% deterioration was noticed by RRII where as RAPRA test result indicated that samples were too weak for testing (Table LHT.13).

UV aging values at RRII (at room temperature) cannot be compared to RAPRA as the later did it at 50°C. Since the polythene is ultimately to be used in field, we have used it for rainguarding trees. Along with it, conventional LDPE (300 gauge) was used as control, for comparison. Within 3 months, the first sample of biodegradable polythene started deteriorating faster than required period of 8-9 months. Modified polythene with longer life of around 9 months is being developed.

Table LHT. 13. Accelerated aging of bio degradable polythene

Test	Tensile strength, MPa		Elongation at break, %		Modulus at 50%, MPa	
	RRII	RAPRA	RRII	RAPRA	RRII	RAPRA
Unaged	31.6	28.5	752.2	96.8	16.7	22.3
5 days heat aged -70° C	29.9	Too weak to test	735	Too weak to test	17.3	Too weak to test
10 days heat aged -70° C	17	Too weak to test	31.4	Too weak to test	2.6	Too weak to test
5 days UV exposure						
50° C	31.5	11.5	791.4	5.3	16.9	—

GENOME ANALYSIS LABORATORY

Genome Analysis laboratory is primarily working on the following areas: (I) the development, optimization and validation of molecular tools for the assessment of genetic diversity in rubber, clonal identification and genome mapping, (II) development of genetic markers for biotic and abiotic stress tolerance and understanding the stress adaptation processes through transcriptome analysis and (III) cloning and characterization of agronomically important genes for improvement of *Hevea* clones in terms of latex yield and tolerance to biotic and abiotic stresses.

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

It is essential to generate a large number of polymorphic DNA markers for their use in genetic mapping and marker-assisted selection (MAS) in rubber. Moreover, different markers might reveal different classes of variation in the genome. Therefore, different marker technologies have been adopted and successfully established to characterize rubber genome. The details of the progress achieved so far are described.

1.1. Microsatellites and its application in the characterization of *Hevea* germplasm

Development of microsatellite markers in *Hevea* was continued with the isolation and characterization of *Hevea* genomic clones containing microsatellite/ simple sequence repeats (SSR). We used eight polymorphic microsatellites for diversity analysis in

cultivated rubber clones including RRII 400 series clones. Five microsatellites were used for characterization of 60 wild *Hevea* germplasm accessions.

Enriched genomic libraries for trinucleotide repeats were made in lambda as well as in plasmid vector. One hundred and four positive clones were sequenced, of which 38 contained the following trinucleotide motifs: AAG/CTT, GGT/ACC and GTG/CAC. Primers were synthesized, based on the flanking sequences of the repeats, to develop markers.

1.2. Single Nucleotide Polymorphisms (SNPs) in *Hevea*

Direct analysis of genetic variation at the sequence level (SNPs) offers several advantages over other types of DNA marker system. SNPs are rapidly becoming the marker of choice for many applications in genome analysis due to their abundance in the genome. Four representatives of wild *Hevea* accessions from each of the three different provinces: Acre, Mato Grosso and Rondonia were used in SNP detection. Twelve genes were amplified at their 3' untranslated region (3'UTR) and sequenced. We identified 18 SNPs and 1 indel in *glutathione peroxidase*, 7 SNPs in *mevalonate kinase*, 3 in *HMG Co-A synthase* and 2 SNPs in *latex abundant protein* genes at their 3'UTR and its associated coding sequences. In our earlier studies with cultivated clones we could not detect any SNP in *glutathione peroxidase* and *latex abundant protein* genes. SNPs detected in the Mato Grosso genotypes showed similarity with popular clones. While analyzing SNPs in *glutathione peroxidase* gene, we could observe clear discrimination of the accessions province-wise indicating geographic distinctness of

the wild accessions. This observation supports our earlier findings on genetic relationship using RAPDs and microsatellites. Effective haplotypes were predicted which could be used as geographical markers for wild accessions.

Characterization of SNPs in latex biosynthesis genes in RR11 400 series clones. Four major genes: *geranyl geranyl diphosphate synthase*, *farnesyl diphosphate synthase*, *mevalonate kinase* and *HMG Co-A synthase* involved in rubber biosynthetic pathway were assessed for SNPs in high yielding RR11 400 series rubber clones developed through hybridization between RR11 105 and RR11 100. Mainly untranslated regions were preferentially amplified for SNP detection. Specific amplification of the desired gene fragments was obtained. All the four genes except *geranyl geranyl diphosphate synthase* possess introns resulting in large amplicon size than the expected ones. PCR products were sequenced directly and the chromatograms were analyzed. Only the PCR products derived from parental clones (RR11 105 and RR11 100) were cloned and sequenced for haplotype detection. A total of 20 SNPs were identified in the four genes. Four SNPs were detected in *geranyl geranyl diphosphate synthase* coding region, six intronic SNPs were detected both in *farnesyl diphosphate synthase* and *HMG Co-A synthase* genes and four SNPs (one coding along with three intronic SNPs) in *mevalonate kinase* were detected. Frequency of SNPs in each gene was calculated using the SNP data.

Allele-specific PCR amplification was optimized for validation of SNPs in *Hevea*. Touchdown PCR amplification procedure along with the use of proof reading polymerase enzyme, was adopted. Allele-specific amplification of SNP was found consistent either with C or G alleles.

1.3. Genetic linkage map in rubber

Construction of a genetic linkage map is important, which presents the linear order of markers in their respective linkage groups and the distance between adjacent markers as a function of recombination distance. The linkage map allows revelation of more and more restricted segments of the genome and undoubtedly enhances our understanding in many areas of plant systematics. Informative markers developed in rubber were used to generate banding profiles in a progeny population including parents.

Total 52 RAPDs (20 RAPDs - RR11 105 specific, 19 RAPDs - RR11 118 specific and 13 RAPDs - present in both the parents) were analyzed for segregation in progeny population. Genomic microsatellite markers (di- and tri-nucleotide repeats) developed in our laboratory and EST derived (gene-derived) microsatellite markers, generated through mining of the GenBank sequences, were tested for polymorphisms using two parental clones (RR11 105 and RR11 118) of a progeny population being used for linkage mapping. Thirty-seven polymorphic SSR markers (13 genomic and 24 EST-derived SSRs) were used to genotype the progeny population and segregation data was recorded for linkage map construction.

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

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

2.1.1. Resistance gene analogue (RGA) in rubber

RGA approach was adopted to identify disease resistant genes in *Hevea*. We

reported isolation and characterization of genomic RGAs in rubber from RR11 105. For comparative analysis, RGAs were also amplified from RRIM 600 (RGA-M) and *Hevea benthamiana* (RGA-B). Sequences were analyzed to generate phylogram. Recently we made an attempt to identify the functionally active RGAs in rubber along with the isolation of genomic RGAs. Reverse transcription (RT) polymerase chain reaction technique was adopted to amplify cDNA from RNA isolated from the *Corynespora* challenged leaf samples of RR11 105. A degenerated primer-pair was used for amplification of functional RGAs (cDNA). Approximately 0.6 kb band was gel-purified and cloned. Thirty-five cDNA (RT-RGA) clones were sequenced to get an idea about the functional disease resistance gene in rubber. Sequences of 28 clones were analyzed as they coded for open reading frame (ORF). Conceptual translation of the 28 clones revealed characteristic motifs of "R" genes. Substantial sequence variability was detected among these RT-RGAs. Alignment of amino acid sequences of RT-RGAs also revealed variation at translation level and ultimately clustered into 9 major groups. Reverse Northern analysis of the representative RT-RGA clone of each group was carried out both with control and treated (*Corynespora* infected) cDNA probes (radio-labelled). Both constitutive (expressed both in control as well as in infected plants) and induced (only in infected plants) expression of RT-RGAs was identified which had significant levels of sequence variability.

2.1.2. Genes involved in host tolerance to *Corynespora* leaf disease

Understanding of molecular mechanisms underlying host-pathogen interactions is of primary importance in

devising strategies to control disease. Therefore, an effort was made to isolate genes induced during the interaction of *Corynespora cassiicola* with RR11 105 during disease development, using differential display of mRNA (DDRT-PCR) by challenge inoculation of rubber plants with spore suspension of *C. cassiicola* in glass house condition. Fifty-seven differentially expressed (over-expressed) transcripts/bands from *C. cassiicola* challenged leaf samples were cloned and sequenced. Full-length cloning was initiated for two genes anthocyanidin 3-O-glucosyltransferase and transcriptional regulators of the GRAS family. All the cDNA clones were subjected to reverse northern analysis using infected and non-infected total RNA from RR11 105 and RRIM 600 as probe to identify the cDNA clones showing consistent expression profiles.

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

Plants have the ability to respond to environmental changes by altering the expression of complex gene networks through sensing environmental cues. These transcriptional changes can result in successful adaptations leading to tolerance in tolerant genotypes. To understand stress adaptation processes, transcript profiling of both tolerant and sensitive responses under different abiotic stresses have been initiated. This will provide a comprehensive understanding of stress adaptation and clues for identification of genes, which are useful for improvement of abiotic stress tolerance.

2.2.1. Cold tolerance in rubber

Transcript profiling in two stress tolerant *Hevea* clones PR 261 and RR11 208 in

relation to cold stress was continued for functional genomic studies. Out of 180 differentially expressed major bands/cDNA (down or up-regulated), 144 bands could successfully be cloned and sequenced. Finally 131 cDNA sequences (59 down-regulated and 72 up-regulated cDNAs) were analyzed, which revealed 110 unique sequences comprised of 13 clusters/contigs and 97 singletons. Most of them had no match with the GenBank sequences. However, several differentially expressed genes i.e., *catalase*, *phosphatidylinositol/phosphatidylcholine transfer protein*, *NADH dehydrogenase*, *myb transcription factor*, *downward leaf curling protein*, *epimerase/dehydratase*, *Na⁺/H⁺ antiporter*, *chloroplast Ycf2* and *chloroplast FtsH protease* involved in cold adaptation process in rubber were also identified along with the unique transcripts. Cold-induced accumulation of FtsH-like protease has already been detected in *Medicago sativa*, and induction of *FtsH* transcripts is specifically regulated by low temperature and light independently and is not part of a general response to stressful conditions.

2.2.2. Rubber EST Project

A directional cDNA library was constructed from cold stressed leaf samples of rubber in Lambda 'ZAP Express' vector for the expressed sequence tag (EST) sequencing project. The objective of our EST sequencing project is to establish and provide a well-characterized, non-redundant EST resource for genetic enhancement of this important crop. To identify cold responsive genes, around 873 clones were isolated from the library and checked for their insert size. *In-vitro* excision of the recombinant lambda clones for sequencing is in progress.

To generate more number of cold responsive ESTs, we constructed a 2nd

subtracted cDNA library containing more than 600 subtracted cDNA clones.

2.3. Methylation dynamics of *Hevea brasiliensis* genome

DNA methylation is one of the most abundant epigenetic modifications in higher plants and animals, which play an important role in regulating development and developmental processes. Plants respond and adapt to biotic and abiotic stress conditions by the controlled regulation of expression of several genes and methylation plays a major role in this process. Studying the methylation pattern of *Hevea* clones will help to better understand the molecular process involved in the differential expression of characters by the same clone under varied stressed conditions and the stress adaptation mechanism of plants by regulating the rate of expression of various genes.

A preliminary study was conducted in *Hevea* to establish the presence of methylation in its genome and to understand the influence of stress on this phenomenon. Experiments were designed to analyze the methylation pattern variations within the selected promoter regions of few genes involved in rubber biosynthesis and disease resistance in three popular *Hevea brasiliensis* clones (RRII 105, RRIM 600 and PB-260) in response to cold stress. Existence of methylation in *Hevea* genome was proved through restriction digestion analysis using methylation sensitive/in-sensitive isoschizomers and bisulfite sequencing. Bisulfite sequencing data was collected from *HMG Co-A reductase-1* and *coronatine-insensitive-1* gene promoter regions for two consecutive years (2007-08) and from *HMG Co-A synthase*, *farnesyl diphosphate synthase* and *rubber elongation factor* gene promoters for a

single year (2008). The bisulfite sequencing data was analyzed using CyMATE software dedicated for methylation analysis. Significant difference in the methylation pattern was observed in the selected promoter regions of all the five genes when samples from two different locations were compared. In *HMG Co-A reductase-1* gene promoter, 5.67% of the probable sites appeared to be methylated. RRII 105 plants were found to be more prone to methylation changes in the above promoter region. Significant changes in methylation pattern were observed at important motifs related to stress response, defense and promoter enhancement of this gene. In *HMG Co-A synthase*, on average 6.34% sites were found methylated. It also showed the same trend as that of *HMG Co-A reductase-1* in terms of methylation at the *CAAT-box*, an important promoter enhancer regulatory motif. In *coronatine insensitive-1* gene promoter region, 8.96% of the probable sites were found methylated. Characteristic methylation patterns were observed in the *ACE* and *CAAT* motifs of RRII 105 plants from Elappara. In *farnesyl diphosphate synthase* gene promoter region, 3.57% sites were methylated, which comprised of CGN and CHH types of methylation whereas all three types of methylation, CGN, CHG and CHH in varying degree were noticed in *rubber elongation factor* gene promoter at a frequency of 5.59%. The results from this study indicate that RRII 105 is more sensitive to methylation variations than RRIM 600 and PB 260. Moreover the study throws light on the epigenetic mode of gene regulation mechanism in *Hevea* as part of their stress adaptation process in response to changing environmental conditions.

3. Cloning and characterization of agronomically important genes

3.1. Cloning and characterization of lignin biosynthesis gene(s) in *Hevea* for their over-expression in timber clones

As wood quality relies on secondary xylem formation, and more particularly on lignin deposition in secondary cell walls, a substantial effort has been put on the functional characterization of genes involved in lignin production. Last year we reported successful cloning and characterization of cDNA encoding cinnamyl alcohol dehydrogenase (CAD), which catalyzes the final step in lignin precursor synthesis reducing the cinnamyl aldehydes (*para*-coumaryl, coniferyl and sinapyl aldehydes) to the corresponding alcohols in the presence of NADPH. Corresponding full-length genomic clone was also generated from RRII 105. Nucleotide sequence information showed that the protein-coding region of the gene is ~2091 bp in size and the coding sequence was interrupted by the presence of 4 introns.

To identify promoter sequences in the upstream to the 5' end of the CAD gene, we generated two clones through genome walking. Among these two, only the '*CAD_Stu*' fragment (713 bp) showed homology in the coding region of the CAD gene at the down stream (from 547 bp) of the sequence. Therefore the upstream sequence of 546 bp of this clone is a putative promoter sequence characterized by the presence of a *TATA box* ("TATATATAA") at position 88 bp from ATG start codon of the gene. Further characterization of the promoter sequence and also the expression study with the CAD gene are in progress.

A bacterial expression cassette for the CAD gene controlling lignin biosynthesis in

rubber was constructed. About 49 kD protein (fusion protein) band was noticed on SDS-PAGE within an hour of induction of the transformed *E. coli* cells with 1 mM IPTG.

Sequence information of the full-length cDNA encoding cinnamoyl-CoA reductase (CCR), involved in lignification, was deduced through the sequencing of both 5' and 3' RACE (rapid amplification of cDNA ends) products of the gene. Full-length cDNA sequence of the CCR gene was found to be 1397 bp in length including 1017 bp of coding and 380 bp of non-coding sequences (114 bp of 5'-UTR and 266 bp of 3'-UTR), which was cloned successfully.

3.2. Cloning of metallothionein gene (s) in rubber

Identification of metallothionein gene (MT) in subtracted cDNA library derived from cold stressed leaf samples of rubber prompted us to clone and characterize MT gene, as the same codes for a low molecular weight protein that acts as a reactive oxygen species (ROS) scavenger in the cells to protect plants from oxidative stress. An effort was made to identify the functional isoforms of the gene in stressed leaf samples. Reverse transcriptase reaction was carried out with gene specific primers to amplify the respective cDNA. The amplified product was cloned successfully, sequenced and confirmed as metallothionein gene.

CENTRAL EXPERIMENT STATION, CHETHACKAL

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

The Station meets the needs of the scientists of various disciplines of Crop Improvement, Crop Management, Crop Protection, Crop Physiology and Latex Harvest Technology. The station works under A and B Divisions of almost equal area. Apart from clone trials and bud wood nursery of pipeline clones, trials on low frequency tapping, CUT, Germplasm accessions, disease management and

fertilizer dosages make up bulk of the experimental areas. Specialised trials like gas based tapping (G-Flex), intercropping and immaturity reduction etc. also make part of the experimental area. A three part tree crown budded area with canopy form FX 516 is laid to study disease resistance mechanisms. An Eddy-covariance tower gives micro-environmental data.

During the reporting period, the total crop realized was 1266980 kg. A total of 299 tapping days was possible in the year and 42 tappers (per day) were engaged for tapping. The CES Dispensary attends to the medical needs of the workers and the total number of visits of patients during the period under report was 4640.

REGIONAL RESEARCH STATION, GUWAHATI, ASSAM

The priority areas of research included crop improvement, management, protection and latex harvesting technology.

1. Crop Improvement

1.1. Large scale trial for selection of location specific clones

Fifteen promising clones of *Hevea* viz. RRII 414, RRII 417, RRII 422, RRII 429, RRII430, RRIM 600, RRII 203, RRII 208, SCATC 88/13, IRCA 109, IRCA 111, IRCA 130, PB 280, PB 312 and PB 314, were multiplied and planted in polybag for field planting at RRTC, Hahara.

1.2. Large scale trial for evaluation of potential primary clones

Ten promising polyclonal selections viz. Gh 1, Gh 2, Gh 3, Gh 4, Gh 5, Gh 6, Gh 7, Gh 8, Gh 9 and Gh 10) of Sarutari farm under RRS, Guwahati along with two control clones RRIM 600 and RRII 429, were multiplied and planted in polybag for field planting at RRTC, Hahara.

1.3. On-Farm evaluation of selected clones of *Hevea* in Assam

Four promising clones of *Hevea*, viz. RRII 417, RRII 422, RRII 429 and RRII 43, along with two control clones, viz. RRIM 600 and SCATC 88/13, were multiplied at Sarutari farm under RRS, Guwahati and planted in polybag at four different farmers field (Umsiang, Byrnihat, Krishnanagar and Bhakuagoo) for field planting.

2. Crop management

2.1. Development of an Integrated Nutrient Management system for young rubber

An experiment for development of an Integrated Nutrient Management system for young rubber with cover crop was initiated

at RRTC, Hahara, RRS, Guwahati during 2008. The clone RRIM 600, with seven treatments, was panted in RBD. The treatments involved combination of different doses of inorganic fertilizers with and without biofertilisers and biofertiliser separately. The biofertilisers applied are *Azotobacter*, *Pseudomonas*, *Phosphobacteri* and *AMF*. There was no significant difference in girth of plants under various treatments (Table Ghy. 1)

Table Ghy. 1. Girth (cm) of plants at 150 cm height

Treatment	Girth (cm)	
	Dec 2009	June 2010
Control	7.27	7.53
Standard practice	7.69	7.99
25% N & P + BF *	7.44	7.75
50% N&P + BF *	8.12	8.82
75% N&P +BF *	7.73	7.95
Standard practice +BF	7.66	8.18
BF alone	8.00	8.51
SE	0.33	0.43
CD (P = 0.05)	NS	NS

* Full dose of K, BF - Biofertilizers

2.2. Evaluation of different biological bunds in soil and water conservation in rubber

The suitable location for starting the experiment was identified at RRTC, Hahara farm, RRS, Guwahati, Assam.

2.3. Effect of crop intensification with intercrops on establishment and growth of rubber in rubber plantation

The suitable location for starting the on farm intercropping experiment was identified at Morugdala, Byrnihat, Guwahati.

3. Crop Protection

3.1. Survey of diseases and pests

Survey on pests and diseases of rubber was carried out in 33 sites covering

17 different locations in Assam, Meghalaya and northern part of West Bengal. Severity of powdery mildew disease was high (above 60%) in private rubber plantation at Umling, Umsiang, Byrnihat, Biharuhat, Jiti and Rango and the infection caused die-back in lower branches of the affected trees (Table Ghy.2). High incidence of *Periconia* leaf blight disease (85%) was noticed in polybag nursery at Umling, Soil Conservation Department, Govt. of Meghalaya where 29.7% polybag plants dried due to die-back and the disease was controlled by spraying Bavistin. Minor incidence of brown root

disease (below 2%) was noticed in private rubber plantation at Agia and also in block plantation at RRTC, Hahara in Assam and RES, Nagrakata in northern part of West Bengal. Incidence of purple root disease (below 5%) was noticed in immature rubber plants (5-years-old) in block plantation of RP department at RRTC, Hahara for the first time in Assam and the disease was controlled by application of Calixin. Fructification of the pathogen causing purple root disease was also observed in *Pueraria* cover crop and the pathogen might have spread to rubber from cover crop. Minor

Table Ghy. 2. Incidence and severity of various pests and diseases of rubber during 2009-10

Location	Area (ha)	Pests and diseases of rubber (%)								
		PM	PLB	BR	Pink	TB	PR	SLF	Pod rot	Scale insect
Sarutari farm*	8.0	Below 20.0	-	-	-	-	-	Below 40.0	Below 10.0	Below 10.0
RRTC, Hahara*	5.0	20 to 60.0	-	0.5 to 1.9	-	-	Below 5.0	Below 40.0	Below 10.0	Below 10.0
Rangdoloit*	1.0	75.0	-	-	-	-	-	-	-	Below 10.0
Agia	4.5	Below 30.0	-	0.5	-	-	-	-	-	Below 10.0
Lemacona	2.0	40.0	-	-	-	-	-	-	10.0	Below 10.0
Mitani*	1.0	40.0	-	-	-	-	-	10.0	Below 10.0	Below 10.0
Umsiang*	2.0	70.0	-	-	-	-	-	20.0	10.0	-
Umling*	2.0	60.0	85.0	-	-	30.0	-	20.0	10.0	-
Byrnihat*	1.0	60.0	-	-	-	-	-	20.0	10.0	-
Killing*	1.0	-	-	-	-	-	-	-	-	-
Mendipathar	1.0	40.0	-	-	-	-	-	-	-	-
Nagrakata*	5.0	Below 10.0	-	Below 0.5	-	-	-	-	10.0	-
Rango*	2.0	65.0	-	-	-	-	-	10.0	-	-
Jiti*	2.0	65.0	-	-	-	-	-	-	-	Below 10.0
Lakhipara*	1.0	65.0	-	-	-	-	-	-	-	10.0
Biharuhat*	1.0	65.0	-	-	-	-	-	-	-	5.0
Neli	1.0	10.0	5.0	-	-	-	-	-	-	-

N. B. All locations marked with asterisk are susceptible pocket to powdery mildew disease. The incidence of powdery mildew disease at Sarutari farm, RES, Nagrakata and RRTC, Hahara (Res) is below 20% though these pockets are susceptible to powdery mildew disease which is due to dusting of sulphur as prophylactic measure against the disease. PM - Powdery mildew, PLB - *Periconia* leaf blight, BR - Brown root, TB - Thread blight, PR - Purple root, SLF: Secondary leaf fall.

infestation of scale insect (below 10%) was observed in nursery and also on RRII 422 in on-farm trial at Umsiang. Infestation of termite (8.4%) was observed in private rubber plantation at Killing in Meghalaya and it was controlled by spraying of Chlopyriphos. Advisory service was given to 14 small holdings for the management of pests and diseases of rubber.

3.2. Isolation and identification of fungal pathogens of rubber

Fungal pathogens isolated from diseased samples of pods and leaves of rubber collected during survey identified as *Colletotrichum gloeosporioides*, *Periconia heveae*, *Phellinus noxius* and *Helicobasidium compactum*. Soil samples from 8 different rubber plantations (Umling, Umsiang, Byrnihat, Sarutari, Hahara, Mitani, Lemacona and RES, Nagrakata) were tested for *Phytophthora* sp. using bright green rubber leaf and green mature rubber pod as baits. However, *Phytophthora* sp. could not be isolated from soil as well as affected rubber pods. From the same samples *Colletotrichum gloeosporioides*, *Fusarium* and *Penicillium* spp. were isolated. Out of seven isolates, four isolates of *Periconia heveae* caused die-back in seedling nursery.

3.3. Evaluation of wild germplasm against tolerance to powdery mildew disease

Severity of powdery mildew disease was assessed in short-listed accessions of wild germplasm (16 nos.). Nine accessions showed tolerance to powdery mildew disease. Three accessions, viz. RO 1737, AC 587 and AC 5302 appeared to be more tolerant to powdery mildew disease (Table Ghy. 3).

3.4. Relevance of inocula of AM fungi and growth responses of rubber in P deficient soils

Studies were continued on the growth of rubber seedlings inoculated with AM

Table Ghy. 3. Severity of powdery mildew disease on short-listed wild accessions of *Hevea* germplasm at Sarutari farm during 2009-10

Wild accessions	PDI
MT 5150	36.0
MT 4706	40.0
MT 5101	50.0
MT 4854	40.0
MT 4768	50.0
MT 4895	40.0
MT 4729	34.0
RO 1737 *	20.0
RO 4607	34.0
RO 5250	36.0
RO 6157	30.0
RO 4627	28.0
AC 587 *	20.0
AC 5302 *	28.0
AC 4603	36.0
AC 6147	32.0

fungi and other beneficial microorganisms. The results indicated that the treated plants were performing better in terms of girth and height (Table Ghy. 4a & b). The combined inoculation of beneficial organisms together with *G. fasciculatam* and *G. mosseae*, supported better growth performance (girth, 7.15 cm; height, 172.50 cm) as compared to single inoculum. Plants inoculated with *G. fasciculatam* showed maximum growth rate followed by *G. mosseae* and *G. margarita*. Similarly, nursery seedlings showed superior growth when treated with *G. fasciculatam* and PSM (girth, 7.69 cm; height, 244.94 cm). The seedlings treated with AM fungi attained maximum growth followed by those inoculated separately with PSM and azotobactor (Tables Ghy. 3a & b).

3.5. Microbial activity, disintegration of litter and nutrient release under a mature rubber plantation and a natural forest cover

'Litter bag method' was followed to assess the rate of disintegration of leaf litter

Table Ghy. 4a. Growth performance of rubber plants in polybag treated with AMF

Treatment	Girth (cm)	Height (cm)	Leaf No.	Whorl no.
<i>G.fasciculatum</i>	6.09	147.0	29	3
<i>G.mosseae</i>	5.79	137.25	29	4
<i>G.fasciculatum</i> + <i>G.mosseae</i>	7.15	172.58	28	4
Control	4.19	86.25	25	4

Table Ghy. 4b. Growth performance of rubber plants in nursery treated with AM and other beneficial organisms

Treatment	Girth (cm)	Height (cm)	Leaf No.	Whorl No.
AMF	6.23	122.49	53	5
PSM	5.41	114.36	48	5
Azotobacter	4.91	99.75	49	5
AMF+ Azotobacter	6.82	218.25	55	6
AMF +PSM	7.69	244.94	57	6

of sal, teak, bamboo and rubber, under two different agro ecological conditions. The results indicated that the rate of weight loss in leaf litter maintained under the forest cover was faster compared to those maintained under a mature rubber plantation. Leaf litter of rubber and bamboo were decomposing at a faster rate as compared to those of sal and teak (Table Ghy. 5). The quantity of microbial population isolated from the decomposed litters under the forest cover were found to be higher as compared to rubber system (Table Ghy. 6). Saprophytic soil fungi, viz. *Penicillium*, *Aspergillus* sp., *Trichoderma* sp., *Fusarium* sp. and filamentous yeasts were found to be dominant. The decomposed leaf litters were dried and processed for further analysis of litter components.

Table Ghy.5. Percentage weight loss in leaf litter exposed to rubber plantation and forest system

Litter type	Percentage weight loss	
	Forest system	Rubber system
Sal	27.75	15.50
Teak	29.60	16.75
Rubber	35.00	22.75
Bamboo	36.00	24.00

3.6. Development of integrated nutrient management system from non-traditional region – assessment of microbial populations

A set of beneficial microorganisms referred as biofertilizers namely azotobacter, pseudomonas and phosphate solubilizing microorganisms including inorganic fertilizers were applied in different doses to the rubber plantations to assess the growth of rubber and monitor the change of microbial populations. Soil samples collected from rhizosphere region after 3 months of treatment and observed an increase in the populations of microorganisms in the rhizosphere zone of rubber.

Table Ghy.6. Microbial populations associated with decomposed leaf litters

Litter type	Location	Fungi (x10 ³)	Bacteria (x10 ⁴)	Actinomycetes (x10 ⁵)
Sal	Rubber	17.52	26.78	16.59
	Forest	23.17	33.66	20.79
Teak	Rubber	19.14	27.57	15.79
	Forest	22.46	35.63	23.58
Rubber	Rubber	11.77	16.01	11.99
	Forest	14.49	21.14	14.84
Bamboo	Rubber	11.28	12.69	11.58
	Forest	12.48	20.64	13.84

Table Gh. 7. Distribution of micro flora and other beneficial organisms in the rhizosphere soil (after 3 months)

Treatment*	Depth (cm)	Fungi (10x ³)	Bacteria (10x ³)	Actinomy-cetes	Pseudo-monas	Azoto-bacter (10x ³)	PSM (10x ³)
T1-Control	0-10	30.48	4.06	2.79	2.40	2.82	3.29
	10-20	25.82	3.61	2.47	2.02	2.26	2.46
T2-IF	0-10	35.96	4.14	2.63	1.89	2.43	3.63
	10-20	26.78	3.57	2.46	1.69	2.05	2.76
T3-25%IF +BF	0-10	38.82	3.92	2.51	2.45	2.80	3.97
	10-20	19.18	3.29	2.40	1.96	2.16	2.70
T4 -50% IF+ BF	0-10	26.73	4.06	2.84	2.38	2.64	3.51
	10-20	19.88	3.41	2.46	1.96	1.81	3.01
T5 -75% IF + BF	0-10	30.68	4.50	2.84	2.48	3.12	3.77
	10-20	24.13	3.52	2.68	2.37	2.41	3.17
T6 - 100% IF +BF	0-10	31.74	4.40	2.62	2.59	3.06	3.69
	10-20	19.51	3.86	2.47	2.21	2.50	2.94
T7- BF	0-10	22.52	4.69	3.04	1.83	2.20	3.38
	10-20	20.57	3.83	2.26	1.63	1.88	2.45

*T - Treatment, IF - Inorganic fertilizer, BF- Biofertilizer

Dilution plate technique was employed for the estimation of microbial population using specific medium for the assessment of different microorganisms. Quantitatively, the bacterial population was high in all the treatments and not much variation was observed in the distribution of PSM, pseudomonas, and azotobacter populations. A decreasing trend was noted in their distribution along side of soil depth (Table Gh. 7).

4. Crop Physiology

4.1. Shallow tapping – an option to stress alleviation in *Hevea* plantation during winter season in NE regions

This experiment was initiated at Sarutari Research Farm in Assam in January 2010 with an objective to evaluate the impact of shallow tapping during winter

(January to March) and normal tapping during April to December on yield, DRC and TPD in RRIM 600 and RRII 105. Another experiment on normal tapping from January to December without rest and from January to March with rest was also conducted in RRIM 600 and RRII 105, for comparison. Yield (g/t/t), DRC (%) and volume of latex (ml/t/t) were recorded from 3 different blocks of each clone (50 trees in each block). Observations on TPD and soil moisture were also carried out.

5. Latex harvest technology

5.1. Controlled upward tapping(CUT)

A new experiment has been initiated on CUT at Sarutari farm. RRIM 600 clone has been selected with 4 treatments. Initial results indicated that T1 showed maximum yield followed by T2, T3 and lowest was in T4.

REGIONAL RESEARCH STATION, AGARTALA, TRIPURA

The station continued its research on cropping system models, nutritional requirement, breeding and clone evaluation, plant pathological problems, latex technology, property study and socio economic survey. The station also provided advisory services to rubber growers.

1. Crop improvement

1.1. Clone evaluation

In clone evaluation trial (1995) involving 10 clones, RRIM 600 (49 g/t/t), PB 260 (48 g/t/t) and PB 311 (47 g/t/t) gave better yield. In the G x E trial, RR12 422 (64 g/t/t) exhibited maximum yield followed by RR12 105 (54 g/t/t) and RR12 417 (53 g/t/t).

Clone RRIM 600 (913 kg/ha) exhibited maximum yield in one on-farm trial. In the on-farm trial of RR12 400 series clones, RR12 429 recorded maximum girth (22.2 cm) followed by RR12 417 (22 cm) and RR12 430 (21.9) in the fourth year.

In ortet evaluation trial, selection 114 (48 g/t/t) gave highest yield followed by 98 (47 g/t/t) and 315 (46 g/t/t), compared to check clone RRIM 600 (36 g/t/t). Half-sib progenies were planted in the field during the year. Four clonal nurseries were established with popular clones, pipeline clones, ortets and hybrid selections. Progenies of HP programmes (2007) were test tapped.

2. Crop management

An experiment was initiated to develop an integrated nutrient management package for young rubber in Tripura. Application of bio-fertilizer along with 50 % inorganic fertilizer showed encouraging result. In the study on response to double dose of fertilizer, RR12 429 recorded maximum girth (46 cm) in the sixth year compared to RR12 417,

RR12 430 and RRIM 600. Application of 20 kg FYM with 50% recommended dose of fertilizer supported better growth of RRIM 600 (13.8 cm) during immature period. In the root trainer potting media experiment, soil with cow dung was found suitable. In tea intercropping trial, average yield of rubber was 1621 kg per ha, in the fifth year of tapping. Green tea leaf yield was 544 kg per ha. during the year exhibiting declining trend due to increase of shade. In the cropping system models, two tuber crops, viz. Colocasia (*Colocasia esculenta*) and elephant-foot yam (*Amorphophallus paucifolius*) were planted as intercrop between rubber and pineapple during second year of the experiment. Fodder signal grass (*Brachiaria decumbens stapf*) was sown in the boundary.

3. Latex harvest technology

Clone PB 235 continued to exhibit highest yield under S/2 d3 system. In another experiment "S/3 double cut alternate panel tapping system (DCA)" with six stimulation, resulted in higher yield compared in RRIM 600, PB 235 and GT 1. Changing panel every year resulted in higher yield. An experiment on controlled up ward tapping (CUT) was carried out in clone RRIM 600. Trees were tapped in the virgin bark of the high panel (HO-1) in combination with short cut (S/4 U d2 6d7) and stimulation ET 5% La 12Y. Yield in CUT system was higher than S/2 d2 system downward tapping in BI panel. Downward tapping on first renewed bark was less productive than upward tapping on virgin bark.

4. Crop protection

Survey of diseases and pests of rubber was carried out in 11 locations covering 16

sites of west and south Tripura districts. Four leaf diseases, viz. Powdery mildew, *Colletotrichum* and *Corynespora* leaf disease and *Periconia* leaf blight, one stem disease (pink) and one root disease (brown root) were observed in varying intensities. Incidence of powdery mildew was low (15-20%). Germplasm accessions, viz., RO 3794, RO 5055, RO 5365 and MT 4859 were moderately tolerant to powdery mildew. Incidence of *Colletotrichum* and *Corynespora* leaf diseases was moderate (40 to 46 per cent) in young plants. Incidence of *Periconia* leaf blight was low. Pink disease was observed at Sabrum, (South Tripura) in very low intensity (0.8%). Low incidence (<5%) of brown root disease was observed in few locations. Minor incidences of scales and mealy bug infestation was observed. Severe bark damage (22%) due to animal feeding was recorded at Pathalia.

Three rounds of sulphur dusting (12 kg/ha/round) at 10 days interval reduced powdery mildew disease by 50% in RRII 105 and 20% in RRIM 600 in a 22-year-old plantation. RRIM 600 was moderately tolerant to powdery mildew disease, while RRII 105 was susceptible. In another experiment, two rounds of foliar spray with 1.0% monopotassium phosphate reduced powdery mildew disease in polybag plants of RRIM 600 and RRII 105 clone. Three antagonistic phylloplane bacteria, causing 64 to 72 per cent *in vitro* growth inhibition in *Colletotrichum gleosporioides*, were tested for their efficacy in controlling diseases in young poly bag plants of RRIM 600 in the field. There was 16-20 per cent reduction in disease incidences in treated plants compared to control.

5. Latex technology

The study on the seasonal variations of properties of rubber was concluded.

However, the particle study was continued and the winter samples from all seven clones were analyzed for the particle size. The study revealed that particle size was decreased during winter season.

In the study on transfer of technology to group processing (GP) centers in Tripura, it was observed that most of the group processing centers process latex into sheets and some sold ammoniated latex to other entrepreneurs. Even though trained personnel and good infrastructure were available in all centers, the recommended practices were not followed. Full capacity utilization was observed only during winter season. In centers where latex was sold, the infrastructure made for making sheets was unutilized. Many GP centers were not grading the sheets before sale and most of the centers were selling the sheets locally. The above study showed the need for refresher trainings and awareness programmes to emphasize importance of processing techniques.

6. Socio-economic studies

In continuation of the study on the impact of the block planting scheme on the socio economic status of the beneficiaries, it was observed that the average household income of mature BPUs was Rs.99168 compared to Rs.46824 of immature BPUs. An annual income difference of Rs.52344 (112%) was observed between households with and without income from rubber (mature and immature BPUs). The average household income of GPUs was Rs.158196. The study showed that rubber is the major source of income for GPUs (75%) and mature BPUs (66%). For immature BPUs, employment is the major source of income (77%). The average annual income of more than 68 per cent households under mature BPUs and 98 per cent households under

GPUs was more than Rs.60,000. Conversely, more than 46 per cent households under immature BPUs earned an average annual income of less than Rs.36,000. The composition of consumption expenditure is dominated by food, clothing, healthcare and education. The annual average value of household savings and assets was Rs. 72546, Rs.41966 and Rs. 183278 respectively for mature BPUs, immature BPUs and GPUs. The difference in annual savings and assets was 72.87 per cent between households with and without rubber income.

The BPS has also led to an explicit shift from the conventional shifting cultivation to a settled mode of NR cultivation. In the process of change, the BPS has ensured an equitable distribution and control over land

with an assured income to the beneficiary households. The community approach contained in the planting operations and group marketing had been helpful in retaining the traditional cooperative spirit among the beneficiary households.

7. Advisory work

Discriminatory fertilizer recommendation based on soil and leaf analysis were offered to 376 rubber growers. A total of 752 soil samples and 693 latex samples were analysed and 1235 m of bud wood of high yielding clones were supplied for distribution to growers. Plant disease control were also suggested based on request from growers.

REGIONAL RESEARCH STATION, TURA, MEGHALAYA

The station continued its research activities on evaluation of clones and polyclonal populations and latex harvest technology.

1. Crop Improvement

In the 1985 and 1986 clonal trials, highest yield was recorded in clones RRIM 600 (46.7 g/t/t), PB 311 (43.7 g/t/t), RRH 105 (42.3 g/t/t), RRH 203 (g/t/t) and PB 235 (39.2 g/t/t). Highest monthly yield was recorded in November. In the study on polycross progenies, more than 50% progenies survived and the progenies are maintained for further evaluation. Bud woods and budded stumps of selected clones were collected and a polybag nursery was raised for raising a large scale trial for generating sufficient planting materials of the selected clones.

For on-farm evaluation of clones in farmers fields RRH 417, RRH 422, RRH 429, RRIM 600, PB 235 and RRH 203 were selected and polybag nursery was raised. To evaluate the performance of half-sib progenies, collection of seeds were initiated in the 1985 and 1986 clone trials. Half-sib progenies of clones, viz. RRIM 600, RRH 203, PB 5/51, RRH 105 and PB 86 were collected and seedlings raised at RRS Tura, Ganogre Farm.

2. Crop Physiology & Latex Harvesting Technology

Low temperature ($< 10^{\circ}\text{C}$) during winter is one of the main factor for reduction of yield and percent dry rubber content under the agro-climatic condition of Garo Hill. Results indicated that maximum volume of latex, dry rubber yield and DRC (%) was recorded during September -

Table Nem.1. Mean of monthly yield (g/t/t) under different treatments of Ethephon application under controlled upward tapping system

Treatments	Yield (g/t/t)		
	February 2010	March 2010	Mean
T1- S/4U d2 (5% ET) 21 days interval- S/2 d/2 (2.5 % ET) twice in the tapping period.	67.30	61.14	64.22
T2- S/4U d2 (5% ET) Monthly interval- S/2 d/2 (2.5 % ET) twice in the tapping period.	58.60	61.14	59.87
T3- S/3U d2 (5% ET) Monthly interval- S/2 d/2 (2.5 % ET) twice in the tapping period.	73.10	70.97	72.03
T4- S/3U d2 (5% ET) 45 days interval- S/2 d/2 (2.5 % ET) twice in the tapping period.	51.20	60.83	56.01
SEm +	6.431	3.996	4.827
CD (P= 0.05)	NS	NS	NS

Table Nem.2 Soil analytical report for seedling nurseries at Jengchekgre (Meghalaya) and Darrengiri (Assam)

Table Nem 2. Soil analytical report for seedling nurseries at Jengchekgre (Meghalaya) and Darrengiri (Assam)					
Depth of profile (cm)	Soil	Soil pH	O.C. (%)	Available nutrient (mg/100g)	
	moisture (%)			Phosphorus	Potassium
DDC, Jengchekgre, seedling nursery, Meghalaya					
0-15	10.24	4.84	1.12	0.93	9.0
15-30	12.34	4.95	0.98	0.58	9.4
30-60	14.96	4.98	0.96	0.33	9.7
60-90	15.48	5.06	0.94	0.22	9.8
90-120	16.19	5.11	0.90	0.28	10.1
120-150	17.10	5.14	0.75	0.14	10.5
150-180	18.64	5.15	0.67	0.06	10.8
0-20	11.45	4.93	0.98	0.96	9.7
20-90	15.11	5.05	0.90	0.32	9.9
90-180	18.10	5.19	0.67	0.13	10.6
0-30	-	5.22	0.89	0.79	9.9
30-60	-	5.20	0.80	0.51	10.1
DDC, Darrengiri, seedling nursery, Assam					
0-15	15.50	4.58	0.82	3.88	10.4
15-30	20.60	4.61	0.79	3.85	10.5
30-60	23.40	4.63	0.68	2.49	10.7
60-90	25.40	4.64	0.65	1.20	11.4
90-120	25.60	4.69	0.55	0.73	11.7
120-150	25.60	4.72	0.55	0.41	11.9
150-180	25.80	4.74	0.52	0.47	12.3
0-20	21.32	4.59	0.85	3.80	10.4
20-90	24.80	4.67	0.76	2.19	11.2
90-180	26.10	4.73	0.66	0.37	12.4
0-30	*	4.57	1.22	0.31	10.8
30-60	*	4.67	0.96	0.14	11.0

*Mature area

November. Lowest DRC (26.82 %) was recorded in February which is the complete defoliation period. During defoliation and refoliation period DRC ranged from 26 – 28.5 %. Defoliation started during from last week of December and completed in the last week of January while refoliation occurred in the 3rd week of February. Lowest soil moisture was recorded in January – February.

A new project was initiated on CUT at RRS Ganolgre using RRIM 600. Initial results indicated that T1 showed maximum yield followed by T2 and T3 and lowest in T4 (Table Nem. 1).

In the experiment on the comparison of yield and yield parameters between normal and shallow tapping during tap rest period, initial results indicated that maximum volume of latex was recorded in normal tapping system (238.7 ml/t/t) than shallow tapping system (87.87 ml/t/t). Low DRC was recorded in normal tapping system (26.20%) than the shallow tapping system (29.11%).

3. Crop Management

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

Maximum girth (84.68 cm), girth increment (3.25 cm), yield (73.85 g/t/t), DRC (35.32%) and latex volume (220.83 ml/t/t) were recorded in $N_{60}P_{30}K_{45}$ kg/ha treatment and minimum in $N_0P_0K_0$. Application of NPK fertilizers significantly increased the O.C. content (0.75 – 1.36 %), available P (0.65-1.18 mg/100g) and K (7.8-10.4 mg/100 g) in the combination of $N_{60}P_{30}K_{45}$ kg /ha.

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

Soil samples were collected at the depth of 0-15 cm, 15-30 cm and 30-60 cm from the 1985 and 1986 field trials for soil moisture study. Soil moisture content showed increasing trend with increasing soil depth. Maximum soil moisture content was recorded during July and minimum in December and January.

3.3. Analytical/ Advisory work for fertilizer recommendation

Soil samples were collected from the rubber plantations and seedling nurseries of Meghalaya. Soil samples were analyzed for available nutrients and fertilizer recommendation was given to the growers. Majority of the soil samples showed medium O.C. content and available potassium. Available P was also low. Soil pH ranged from 4.7 to 5.2. Soil profile samples were collected from Jengichekgre (Meghalaya) and Darrengiri (Assam) for analysis of soil moisture content, soil pH, O.C content and available P and K. Details are shown in Table Nem. 2. Results showed that organic carbon content was low to medium (0.55 to 1.12 %), available P was low at Jengichekgre (0.14-0.93 mg/100 g) and medium to high at Darrengiri (1.2 to 3.88 mg/100 g) and available K was medium (9.0 to 11.9 mg/100 g) and soil pH was 4.58 to 5.22. Bulk density ranged from 1.19 to 1.38 g/cm³ at Jengichekgre and 1.06 to 1.16 g/cm³ at Darrengiri at the depth of 0-15 cm and 30-45 cm, respectively.

REGIONAL EXPERIMENT STATION NAGRAKATA, WEST BENGAL

1. Crop improvement

1.1. Evaluation of clone

Four clone evaluation trials were initiated to screen clones for performance in terms of growth and yield in the agro-climate conditions of North Bengal. In trial I, RR11 118, followed by SCATC 93/114, RR1M 703 and Haiken 1 exhibited higher girth (Table Nag. 1). Similarly, in trial II, RR1M 612 followed by RR1M 605, PB 86, RR1I 208 and PR 107 exhibited higher girth. In trial III, PB 310 showed higher girth followed by SCATC 93/114. In trial IV, SCATC 93/114 scored higher girth followed by PB 280.

In terms of yield (Table Nag. 2), RR1M 703 showed maximum yield followed by SCATC 88/13 and PB 235 in trial I. In trial II, RR1I 208, RR1M 605, PB 86 and PR 107 showed higher yield than the check clone RR1I 105. Compared to the check clone RR1M 600, three clones *viz.* PB 235, RR1I 208

and PB 310 showed high yield in trial III. In trial IV, RR1I 208 and Haiken 1 showed higher yield than the check clone RR1M 600.

1.2. Evaluation of germplasm

Performance of 21 genotypes including standard check clones was evaluated. Maximum girth was recorded in RO 2890 followed by RO 3172 within the wild accessions (Table Nag.3). Among the Wickham clones, PB 260 showed highest girth. In terms of yield among the germplasm accessions, RO 5363 followed by AC 1950 showed highest yield. RR1I 105 showed highest yield among the four Wickham collections.

1.3. Performance of polyclonal seedlings

Half-sib Polycross progenies raised from seeds collected from Kanyakumari (Tamil Nadu) were grown under the climatic condition of North Bengal. There were 240 trees in CRD (single tree single plot) planted at normal spacing (5 x 5 m). Mean

Table Nag. 1. Growth performance of clones in different trials

Trial I		Trial II		Trial III		Trial IV	
Clone	Girth (cm)	Clone	Girth (cm)	Clone	Girth (cm)	Clone	Girth (cm)
PB 5/51	63.42	GL 1	63.64	PB 260	61.84	RR1C 104	58.16
GT 1	64.93	PR 107	66.55	RR1I 208	62.79	RR1I 308	58.65
RR1I 203	65.43	RR1I 208	69.29	PR 107	64.40	RR1I 105	61.29
PB 311	66.92	PB 86	69.96	PB 86	66.69	RR1I 300	62.05
RR1I 300	68.51	RR1M 605	73.45	HK 1	68.34	PR 261	62.78
SCATC 88/13	69.59	RR1M 612	82.90	PB 235	68.40	RR1I 208	62.98
PB 235	70.82	RR1I 105	62.77	RR1C 102	69.49	PB 235	63.64
HK 1	74.23			RR1M 612	70.12	HK 1	65.45
RR1M 703	74.66			SCATC 93/114	73.47	PB 280	66.82
SCATC 93/114	74.69			PB 310	89.64	SCATC 93/114	67.42
RR1I 118	75.83			RR1M 600	68.53	RR1M 600	63.86
CD (P = 0.05)	2.52	CD (P = 0.05)	2.66	CD (P = 0.05)	3.72	CD (P = 0.05)	2.02

Table Nag. 2. Yield of clones (g/tree/leaf) in different trials

Trial I		Trial II		Trial III		Trial IV	
Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)	Clone	Yield (g/t/t)
PB 5/51	35.06	GL 1	31.25	RRIC 102	36.41	SCATC 93/114	38.19
PB 311	39.31	RRIM 612	46.31	PR 107	37.37	RRIC 104	40.24
RRII 300	39.42	PR 107	50.11	RRIM 612	37.57	PR 261	47.02
HK 1	40.29	PB 86	53.16	SCATC 93/114	41.03	RRII 300	48.53
RRII 203	45.69	RRIM 605	59.69	PB 86	41.63	PB 280	50.31
SCATC 93/114	46.26	RRII 208	62.20	PB 260	47.80	RRII 308	51.27
RRII 118	47.97	RRII 105	42.76	Haiken1	52.94	PB 235	51.80
GT 1	48.84			PB 310	59.07	RRII 105	56.87
PB 235	48.92			RRII 208	62.02	Haiken 1	60.03
SCAT C88/13	55.06			PB 235	62.46	RRII 208	63.29
RRIM 703	56.75			RRIM 600	59.22	RRIM 600	55.73
CD (P = 0.05)	3.42	CD (P = 0.05)	4.70	CD (P = 0.05)	5.03	CD (P = 0.05)	3.80

Table Nag. 3. Growth and yield in different accessions of Hevea

Accessions	Girth (cm)	Yield (g/t/t)	Accessions	Girth (cm)	Yield (g/t/t)
AC 1950	59.06	24.92	RO 2629	65.24	5.51
AC 607	35.64	5.72	RO 2635	66.72	4.75
AC 619	58.54	3.03	RO 2890	71.87	5.11
AC 623	45.04	2.45	RO 3172	70.94	4.51
AC 68	60.21	3.77	RO 5329	55.73	10.15
AC 763	53.74	15.72	RO 5348	67.66	5.73
GL 1	55.31	22.30	RO 5363	61.09	31.82
GT 1	61.05	24.29	RO 5408	55.00	5.02
MT 196	60.64	15.87	RO 5430	64.13	11.12
MT 2229	64.73	10.89	RO 5557	67.70	5.27
MT 2594	51.19	5.26	RO 6139	51.78	4.38
MT 44	65.45	6.34	RRII 105	58.26	41.60
PB 260	61.89	17.24	CD (P = 0.05)	3.14	3.59

girth of the trees was 65.6 cm, with average block yield of 44.9 g/t/t. Among the existing one seventy eight plants, 37.5% showed above average yield.

2. Crop Management

2.1. Nutritional trials

In the experiment on different doses of fertilizers, highest girth (126.5 cm) was

recorded in $N_{45}P_{40}K_0$ and highest yield (67.63 g/t/t) in $N_{45}P_0K_0$.

2.2. Cropping system

In the study on feasibility of intercropping rubber with tea in Dooars area of West Bengal, it was found that green tea leaf yield was significantly higher in pure tea plot. Low green tea leaf yield in rubber-tea combination plot was mainly

Table Nag. 4. Growth and yield of tea and rubber in intercrop trial

Treatments	Spacing	Tea yield (Kg)	Rubber girth (cm)	Rubber yield (Kg/tree/yr)
T1 (Pure Rubber)	Rubber - 5 x 5 m		55.6	5.6
T2 (Rubber + Tea)	Rubber - 10 x 2.5 m	1673	57.8	5.8
	Tea - 10x(1.0 x 0.6) m			
T3 (Rubber + Tea)	Rubber - 12 x 2.5m	1974	58.0	5.9
	Tea - 12x(1.0 x 0.6)m			
T4 (Rubber + Tea (paired row))	Rubber - 18x(3x3)m, 2rows	3870	60.1	6.1
	Tea - 18x(1.0 x 0.6)m			
T5 (Rubber + Tea)	Rubber - 10x 5.0m	2339	63.4	6.4
	Tea - 10x(1.0 x 0.6)m			
T6 (Pure Tea)	Tea - 1.0 x 0.6m	9349		
CD (P = 0.05)		2408	2.5	1.2

Table Nag. 5. Growth characteristics of *Hevea* seedlings raised from seeds developed in different climatic condition

Seed source	Growth data (January, 2010)		
	Plant height (cm)	Girth (cm)	Number of whorls
Nagrakata (Clone trial)	185	19.8	4.7
HBSS, Kanyakumari, Kerala	276	28.3	5.2
Guwahati (random sampling)	145	15.6	4.6
Tura (random sampling)	193	20.8	3.6

due to more shade imposed by mature rubber tree canopy that attracted pests. Details are given in Table Nag.4.

3. Crop Physiology

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

In the experiment the performance of the polycross progenies, premature leaf fall due to severe cold during January was observed. However, new flushes emerged in March. Details of growth performance are given in Table Nag. 5.

3.2. Performance of rubber clones in abandoned Tea growing areas of Dooars belt of North Bengal

An experiment was initiated to assess the performance of rubber under alkaline pH in the abandoned tea growing areas of Sub-Himalayan West Bengal. Four clones *viz.* RRIM 600, RRII 208, RRII 105 and RRII 429 were planted in blocks in soils having high pH (8.3) along with normal pH (5.8). The growth of rubber plants was healthy even at pH 8.3.

REGIONAL RESEARCH STATION DAPCHARI, MAHARASHTRA

Evaluation and screening of clones/wild *Hevea* accessions for desired characters like tolerance to water and high temperature stress, development of location specific agro technology and latex harvest technology, stress management technology continued to be the thrust areas of research.

1. Environmental Physiology

Three irrigation-based experiments were conducted to study the effect of irrigation and irrigation system on yield of rubber. The irrigation experiment started in 1987 with ETc based Basin (1.00 ETc, 0.75 ETc and 0.50 ETc) and drip (0.75 ETc, 0.50 ETc, 0.25 ETc) irrigation treatments in clone RR11105 was continued. The objective was to standardize and evaluate the advantages of drip irrigation system over basin irrigation in terms of water saving and total economy in the quantity of water and methods of irrigation. From February 2000 onwards, treatments viz. 0.75 ETc basin and 0.50 ETc drip were modified to 0.25 ETc (basin and drip) to test whether irrigation requirement can be further reduced. Observations on the fortnightly cup lump weight, monthly girth measurements and seasonal DRC, PI, TP were recorded. The trees under basin irrigation (1.0, 0.25, 0.50 ETc) showed higher girth (74.26, 69.88, 70.59 cm) as compared to drip irrigation system (69.94, 69.11, 66.79 cm). All other treatments were comparable. Trees under rainfed condition recorded lower girth (64.10 cm) than the other irrigation treatments. The basin irrigated trees recorded higher yield in comparison to drip and control (Table Dap. 1). Trees irrigated through basin method at 1.00 ETc recorded maximum yield (52.13 g/t/t). Under drip irrigation method, 0.75 ETc irrigation

(along with 0.25 ETc basin irrigation) recorded more yield. Reducing the irrigation to lower level (0.25 ETc) did not affect the growth and yield of rubber.

A trial was started in 1983 to study the effect of different levels of irrigation (1.00, 0.75, 0.05 and 0.25 ETc) on yield and yield

Table Dap. 1. Effect of irrigation on growth and yield

Treatments	Mean girth (cm) (March 2010)	Mean dry rubber yield (g/t/t)
Control		
(No irrigation)	64.10	40.56
1.00 ETc basin	74.26	52.13
0.25 ETc basin		
*(Earlier 0.75 ETc)	69.88	45.86
0.50 ETc basin	70.59	40.04
0.75 ETc drip	69.94	46.56
0.25 ETc drip*		
(Earlier 0.50 ETc)	69.11	41.17
0.25 ETc drip	66.79	36.31
SE	1.78	1.87
CD (P = 0.05)	3.89	4.07

* Changed from 0.75 ETc to 0.25 ETc from Feb-2000 onward

components in two clones, viz. RR11105 and RR11118. Fortnightly yield, monthly girth, seasonal DRC%, PI, BI and TP were monitored. Results indicated that RR11118 performed better in terms of growth while clone RR11105 recorded better yield in response to different levels of irrigation treatments.

In the cost evaluation trial, the expenses incurred towards various inputs, farm practices and irrigation were monitored trees since 1987 in irrigated and unirrigated trees of RRIM 600. Irrigated trees were assessed based on different soil depth, viz. 1/5th ETc (deep soil) and 1.0 ETc (shallow soil) levels of

irrigation. The irrigation level was reduced from 1/4 to 1/5 ETc to find out the optimum water requirement for mature trees in good soil depth area. Meteorological and physiological parameters (i.e. block yield, total latex volume, DRC %, PI, BI, girth) were also recorded.

The trees growing at the group B site (high soil depth, partially irrigated 1/5th ETc) recorded significantly higher yield than rainfed and low soil depth with fully irrigated trees (group C). The result shows that the soil moisture content also higher in higher soil depth area than the other two sites.

Latex Harvest Technology

In order to study the optimum stimulation schedule for maximum latex harvest from low yielding regenerated bark, one demonstration trial based on controlled upward tapping (CUT) is being carried out. The study was initiated during 2009 in BI-1 panel (regenerated bark) of RRII 105 planted

in 1983 with randomized block design. The objective was to identify CUT practice suitable to this region along with the enhancement of yield during low yielding phase (regenerated bark) of clone RRII 105 and to optimize the stimulation schedule (Table Dap. 3). Yield was recorded from all the tappings as cup lumps.

Results shows that treatment T2 under CUT recorded significantly higher yield of 111.66 g/t than the other treatments. Result revealed that under CUT, yield increased upto 138% (T2) than the previous year of basal panel tapping (Table Dap.3).

3. Crop Improvement

In the clone evaluation trial (1985) of fifteen modern clones (RRII 5, RRII 6, RRII 208, RRII 308, RRII 605, PB 260, PB 310, PB 311, RRIC 100, RRIC 102, RRIC 105, PR 255, PR 261 and RRII 105, clone RRII 208 performed better in terms of growth and yield compared to RRIC 52 (20.49 g/t). RRIC 52 recorded maximum girth (69.2 cm) (Table Dap. 4).

A trial was initiated during 2008 to evaluate the growth and yield performance of 14 ortets selected from polycross seedlings. There was no significant difference in plant height among the ortets and control clones (Table Dap. 5). Among the ortets, highest plant height was recorded in OS 34 (378.2 cm) and OS 173. Maximum number of

Table Dap. 2. Effect of irrigation and soil depth on summery yield

Irrigation type	Projected yield (kg/ha) (Jan-May 2009)	Yield (g/t) 2009
Unirrigated (A)	333.17	19.7
Partially irrigated (B) (deep soil)	1159.42	69.2
1.0 ETc (C) (shallow soil)	856.98	52.0

Table Dap. 3. Yield response of Low Frequency Controlled Upward Tapping Vs basal tapping

Treatments	No. of taps	CUT (November 2009 to March 2010)			Basal panel (S/2 d3 d4/7) (November 2008 to March 2009)		
		g/t/t	Kg/ha	Kg/tree	g/t/t	Kg/ha	Kg/ha
T1 - S/4U d3 d4/7 ET 5% La (3w)	61	92.08	2246	5.6	44.56	2.71	1087
T2 - S/3U d3 d4/7 ET 5% La (3 W)	61	111.6	2724	6.8	46.89	2.86	1144
T3 - S/4 U d3 d4/7 ET 5% La(m)	61	82.70	2018	5.0	37.96	2.31	926
T4 - S/3 U d3+ d3 6 d/7 ET 5% La (m)	61	88.54	2160	5.4	41.71	2.54	1017
SE		7.60			4.73		
CD (P = 0.05)		16.19			10.08		

Table Dap. 4. Growth and yield of *Hevea* clones

Treatments	Dry rubber yield (g/t/t)	Mean girth (March 2010) (cm)	PY* (kg/ha)
RRII 5	36.36	63.17	1088
RRII 6	41.80	66.99	1251
RRII 105	35.05	59.84	1049
RRII 208	46.50	68.42	1392
RRII 308	20.46	60.50	612
RRIM 605	26.08	62.71	780
PB 260	27.74	64.31	830
PB 310	30.52	64.70	913
PB 311	31.95	62.63	956
RRIC 52	20.49	69.19	613
RRIC 100	33.98	62.37	1017
RRIC 102	32.85	63.49	983
RRIC 105	20.87	60.53	625
PR 255	32.17	62.76	963
PR 261	24.83	60.70	743
CD (P = 0.05)	7.95	4.03	

* PY = g/t/t x No. of tapping days x No. of trees/0.9/1000

whorls was recorded in OS 42 (3.5) and OS 111 (3.5).

Screening of wild *Hevea* accessions for drought was initiated in 2003. Mato Grosso accessions showed better growth performance than Rondonia and Acre accessions. Twenty five potential drought tolerant accessions were identified for further detailed studies.

Evaluation of clones for drought tolerance (2007) using 25 drought tolerant wild Amazonian accessions revealed wide variability for all characters. MT 4788

Table Dap. 5. Performance of ortets at Dapchari

Ortets	Girth (cm)	Height (cm)	No. of whorls
OS 1	9.5	333.8	2.7
OS 8	8.1	306.3	2.7
OS 34	11.1	378.2	2.9
OS 35	10.4	369.2	3.0
OS 36	9.2	304.1	3.2
OS 37	9.5	364.4	2.9
OS 42	9.1	304.5	3.5
OS 111	10.3	367.0	3.5
OS 135	8.6	306.8	3.0
OS 136	7.1	300.4	2.2
OS 173	11.6	356.7	3.0
OS 216	10.3	358.5	3.1
OS 236	9.9	338.3	3.1
OS 317	10.6	367.9	2.8
RRII 105	8.2	279.4	2.3
RRII 208	9.5	329.0	3.4
RRII 430	8.5	279.6	2.6
RRIM 600	10.1	411.5	3.3
SE +/-	1.07	42.33	0.33
CD (P = 0.05)	2.18	NS	0.67

recorded maximum girth and showed consistent performance.

2. Crop Management

In the study on the effect of moisture conservation practices on growth of rubber initiated during 2009, there was no significant difference in growth performance (Table Dap. 6).

Table Dap. 6. Influence of soil moisture conservation practices on performance of RRII 105

Treatment	Plant height (cm)	Girth (cm)	Relative water content (%)	Soil moisture content (%) at 30 cm
T1 (Control)	235.6	6.24	91.24	32.05
T2 (Soil moisture conservation)	225.5	6.27	94.06	32.42
T test	0.96	0.75	0.23	0.87

REGIONAL RESEARCH STATION DHENKANAL, ORISSA

The station continued its research on identification of clones suitable for dry subhumid climate.

1. Crop Improvement

1.1. Clone Evaluation

In the clone trial (1987), GT 1 (75.1 cm) recorded highest girth closely followed by RRIM 600 (72.3 cm). Clones GT 1 and RRIM 600 recorded significantly higher mean girth over RRII 105 (67.9 cm). RRIM 600 recorded highest mean yield of 34.8 g/t/t, followed by RRII 105 (32.7 g/t/t). The clone RRIM 600 was found to superior in terms of both growth and yield (Table Or. 1)

Table Or. 1. Mean girth and yield of elite clones

Clone	Girth (cm) March 2009	Yield (g/t/t)
RRII 105	67.9	32.7
RRIM 600	72.3	34.8
GT 1	75.1	31.8
Mean	71.7	33.1
SE	1.10	1.31

In the second clone trial (1990), no significant different was recorded in girth among the clones. The clone SCATC 93/114 recorded the highest mean yield (44.5 g/t/t) followed by SCATC 88-13 (40.5 g/t/t). Even though the clone SCATC 93/114 recorded the highest girth, yield was lowest (22.0 g/t/t) among the clones. Clone RRII 208 showed superior growth, yield and adaptability compared to other clones (Table Or. 2).

In the third clone trial (1991), the performance of *Hevea* clones was compared with polyclonal seedlings. Polyclonal trees (96.5 cm) recorded highest girth and adaptability, closely followed by GT 1

Table Or. 2. Growth and yield performance of *Hevea* clones

Clone	Girth (cm) March 2009	Mean yield (g/t/t)
RRII 5	78.4	38.4
RRII 208	78.2	44.5
RRII 300	76.1	27.0
RRIM 600	78.5	38.6
RRIM 701	77.5	28.4
PB 310	76.7	33.8
PR 255	76.5	26.5
SCATC 88-13	78.5	40.5
SCATC 93-14	83.6	22.0
Haiken 1	74.1	34.4
Mean	77.8	33.4
SE	1.12	3.15

(86.9 cm) and RRII 208 (81.4 cm). RRII 208 recorded highest mean yield (62.8g/t/t) followed by RRII 600 (41.1 g/t/t). In this trial also, RRII 208 showed better growth, yield and adaptability. The lowest mean yield was recorded in polyclonal seedlings (28.0 g/t/t) (Table Or. 3).

In another trial on evaluation of modern clones (1999-2000), the growth of clones

Table Or. 3. Performance of various clones

Clone	Girth (cm) March 2010	Mean yield (g/t/t)
RRII 5	76.7	39.6
RRII 105	75.5	35.3
RRII 208	81.4	62.8
RRIM 300	79.1	30.9
RRIC 102	79.2	38.9
RRIM 600	73.0	41.1
GT 1	86.9	47.9
PR 255	79.6	32.3
PR 261	75.4	30.9
Polyclonal	96.5	28.0
Mean	80.3	38.7
SE	2.37	3.80

differed significantly. Among the clones, highest mean girth was recorded in IRCA 111 (48.4 cm) and RR11 208 (47.7 cm) closely followed by RR11 105. The lowest girth was observed in RR11 51 (37.5 cm) (Table Or. 4).

Clone	Girth (cm) March 2010
RR11 51	37.5
RR11 105	45.4
RR11 208	47.7
RR11 300	50.2
RR11 351	43.0
RR11 352	45.2
RR11 357	43.3
PB 28/59	46.6
RR11 600	46.3
IRCA 109	37.0
IRCA 111	48.4
Mean	49.0
SE	3.07

1.2. Polyclonal trial

To evaluate the growth, yield performance and adaptability of polyclonal

seedlings under Orissa conditions, a trial was laid out in 1989. Highest mean girth was recorded in tree No. 319 (127.6 cm) followed by tree No.93 (127.3 cm). Highest annual mean yield was recorded in tree No. 383 (106 g/t/t) followed by tree No. 638 (103.8 g/t/t). Ten elite polyclonal selections were identified and field planted for further evaluation.

1.3. Ortet Evaluation

Ten ortets were planted along with few modern clones for further evaluation during 2008. Ortets OR 7 and OR 8 and the clone SCATC 93/114 showed better growth in terms of girth and height.

2. Latex Harvest Technology

2.1. Controlled upward tapping trial

Initial observation showed that clone RR11 600 under treatment T3 (S/3 d2 ET 5% - monthly; S/2 d2 ET 2.5% twice tapping) showed maximum yield of 28.6 g/t/t, followed by T2 (S/4 d2 ET 5% monthly :S/2 d2 ET 2.5% twice tapping).

REGIONAL RESEARCH STATION, PADIYOOR, KERALA

The station continued research on identification of clones suitable to the region and evaluation of tolerance of clones to drought and disease. Studies on agro-management practices for reduction of the maturity period for tapping are also in progress.

1. Crop Management

1.1. Physico-chemical characterization of soil

Spatial variability in terrain characteristics was studied. Digital

elevation model acquired by the Shuttle Radar Topography Mission (SRTM) at 90 m resolution was used. Interpolated maps of coarse fragments, actual soil volume, slope and depth characteristics were plotted. Spatial variability mapping enabled better understanding of the study site for adoption of different agro-management practices.

1.2. Water requirement studies

The experiment initiated in immature rubber with irrigation levels at IW/CPE

ratio of 0.3, 0.6, 0.9, 1.2 and an unirrigated control was continued to the mature phase. The response of irrigation on stand establishment and per cent tappable trees was significant. Irrigation in the immature phase reduced tree losses leading to more uniformity in tree growth. Girth differences between the treatments and control trees narrowed down. The trees have been opened for tapping (Table Pad.1).

Table Pad.1. Effect of different irrigation levels on growth of Hevea

Treatment (IW/CPE)	Girth (9 th Year)	Girth increment	
		Dry months (December-May)	Wet months (June-November)
1.2	54.5	1.76	3.31
0.9	53.2	2.20	2.91
0.6	55.9	2.76	3.60
0.3	51.8	2.44	3.68
Control	48.9	2.46	4.05
CD (P = 0.05)	NS	NS	NS

1.3. Response of fertilizer application

The experiment with three clones (RRII 105, RRII 414 and RRII 429) under four fertilizer levels (30:30:20, 60:30:20, 90:60:40 and 120:60:40 kg/ha of N, P₂O₅ and K₂O) indicated no significant response in growth of the different clones with varying levels of fertilizer (Table Pad. 2).

Table Pad. 2. Effect of fertilizer on growth of clones

Treatment	Girth (cm)		
	RRII 105	RRII 429	RRII 414
30:30:20	39.0	33.0	38.1
60:30:20	39.1	37.6	40.2
90:60:40	35.2	35.3	38.6
120:60:40	39.2	34.0	37.3
CD (P = 0.05)	NS		

2. Crop Improvement

2.1. Large scale evaluation of clones

In the 1996 clone trial with 11 clones, significant growth difference was observed.

PB 330 was significantly superior to RRII 105 with respect to girth. IRCA 130 showed better annual and summer yield than RRII 105.

Table Pad. 3. Growth performance of modern Hevea clones

Clone	Girth (cm)	Yield	Summer yield
		(g/t/t)	(g/t/t)
RRII 105	56.81	48.39	28.56
PB 314	59.33	44.61	25.48
IRCA 130	61.19	65.58	43.28
PB 28/59	56.24	47.73	25.24
IRCA 109	56.05	32.81	19.18
PB 330	62.06	41.34	15.71
IRCA 18	61.43	39.73	13.02
RRIM 703	50.65	37.78	16.29
IRCA 111	56.36	35.23	20.12
PB 255	56.38	51.93	31.99
IRCA 230	57.88	39.38	15.69
CD (P = 0.05)	4.47	14.72	13.98

Table Pad. 4. Performance of clones and selection at high altitude area

Clone	Girth	Bark thickness	Latex vessel rows
RRII 105	42.4	4.5	8.5
RRII 203	60.5	6.09	5.89
RRIC 100	64.3	5.1	6.3
RRIC 102	53.3	4.68	7.28
PB 86	63.1	5.11	7.39
P 296	58.4	4.62	9.11
P 90	54.9	4.8	5.56
P 270	69.9	5.2	8.78
P 280	54.9	4.27	8.06
P2	54.7	4.6	4.67
P 121	54.4	4.09	4.5
P 213	66.8	4.3	5.75
P 1	63.3	4.9	5.83
P 155	45.3	3.72	4.5
Irrity 1	57.9	4.51	7.4
CD (P = 0.05)	12.7	NS	*

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

The trial field planted in 1996 at high altitude (974 m msL) with 10 selections and

5 clones indicated significant superiority of the clones PB 86, RRIC 100, RR12 203 and the selections P 270, P 213, P 296, P1, Iritty 1 over that of RR12 105 with respect to girth. Iritty 1 showed high degree of disease tolerance to powdery mildew while P 90

and P 270 and clones RR12 105 and RR12 203 were highly susceptible. Significant differences in latex vessel rows were observed among the clones and ortet selections while the difference in bark thickness was not significant (Table Pad. 4).

HEVEA BREEDING SUB STATION NETTANA, KARNATAKA

The major thrust areas of research are to evaluate clones under different biotic and abiotic stress conditions and to identify clones suitable for commercial cultivation.

1. Crop Improvement

1.1. Small scale trials

In the trial 1988 A, 15 ortet clones and three control clones are under evaluation.

Growth and yield performance of clones during 2009 and over eight years of tapping (2002-2009) are presented in Table Kar.1. Mean yield over eight years of tapping indicated T2 to be the highest yielder with 73.7 g/t/t closely followed by the ortets O 17 (69.1 g/t/t), O15 (66.1 g/t/t) and GT 1 (65.9 g/t/t). RR12 600 and RR12 105 maintained the same

Table Kar.1. Growth and yield performance of clones in the small scale ortet trial (1988A)

Clone	Girth(cm) at age 22 years (Dec. 2009)	Mean yield during 2009 (g/t/t)	Mean yield over eight years of tapping (g/t/t)
T 2	108.4	78.6	73.7
O 17	104.6	80.2	69.1
O 15	93.1	69.8	66.1
GT 1	80.8	67.5	65.9
C 42	98.6	52.9	59.1
C 1/2	87.8	45.2	58.5
O 41	86.4	58.7	57.7
C 70	89.7	62.5	56.4
O 47	121.3	64.7	54.4
O 34	84.6	43.9	51.6
RR12 105	75.9	44.3	49.5
RR12 600	72.1	25.7	47.9
O 44	76.9	27.6	37.9
C 7/2	74.8	30.3	35.8
O 50	67.1	27.2	31.8
O 46	85.2	20.2	31.2
O 45	81.0	10.7	26.5
O 19	66.4	16.1	25.1
CV (%)	12.6	36.9	16.9
SE (±)	6.3	9.7	4.9
CD (P<0.05)	18.1	27.9	14.0

Table Kar. 2. Growth and yield performance of clones in the small scale ortet trial (1988B)

Clone	Girth(cm) at age 22 years (Dec. 2009)	Mean yield during 2009 (g/t/t)	Mean yield over eight years of tapping (g/t/t)
T 1	109.1	74.2	70.0
GT 1	90.7	74.9	70.0
O 40	85.6	37.4	51.7
O 53	89.1	34.3	50.2
RR12 105	71.5	37.5	48.9
RR12 600	64.3	32.2	43.6
O 54	82.7	29.9	43.2
C 150	78.9	36.6	41.3
O 38	84.8	32.3	41.1
O 16	75.2	36.8	38.3
PO	78.4	38.3	34.3
O 46	81.9	25.0	33.8
O 14	72.7	31.7	31.2
C 151	69.8	26.3	28.7
O 37	73.6	21.2	26.9
O 23	62.8	22.5	26.4
O 22	68.1	11.2	24.2
O 57	60.9	12.1	21.1
C 9	64.9	8.9	16.9
CV (%)	13.4	43.9	25.7
SE (±)	6.0	8.3	5.8
CD (P<0.05)	17.2	23.8	16.6

positions as that of the previous years with yields of 49.5 and 47.9 g/t/t respectively. Ortet 47 recorded highest girth (121.3 cm) followed by T 2 (108 cm). Though O 47 had highest girth, its yield was only 54.4 g/t/t. Sixteen ortets and three control clones are under evaluation in the second trial (1988B). Eight years of yield recording indicated clones T 1, GT 1, O 40, O 53 and RRII 105 were the highest yielding clones (Table Kar. 2). In the third trial (1988C) leading clones were GT 1, C 140 and RRII 105 with a yield of 85.1 g/t/t, 77.1 g/t/t and 68.6 g/t/t respectively (Table Kar. 3).

Table Kar. 3. Growth and yield performance of clones in the small scale ortet trial (1988 C)

CClone	Girth(cm) at age 22 years (Dec. 2009)	Mean yield during 2009 (g/t/t)	Mean yield over eight years of tapping (g/t/t)
GT 1	92.2	90.2	85.1
C 140	97.2	74.0	77.1
RRII 105	75.6	47.2	68.6
O 49	77.4	74.2	67.7
O 26	93.9	52.8	67.0
O 55	118.9	69.3	65.2
O 11	99.1	44.5	60.5
O 56	92.3	64.4	56.3
C 6	58.4	16.1	50.6
O 30	87.8	49.8	49.7
C 10/9	89.0	25.2	49.7
O 64	75.4	47.5	49.0
O 39 A	88.1	30.8	44.0
O 51	83.8	33.7	39.9
C 32	79.6	16.5	38.5
RRII 600	72.9	26.8	31.6
CV (%)	15.1	53.2	24.8
SE (±)	7.5	14.7	8.1
CD (P=0.05)	21.8	42.3	23.3

A total of 54 trial clones and three control clones are under evaluation in three trials. In 1991 A after six years of tapping maximum yield was noted in clones PB 235 (80.7 g/t/t), PB 314 (79.0 g/t/t), PB 280 (77.0 g/t/t),

Table Kar. 4. Growth and yield performance of clones in the small scale clone trial (1991A)

Clone	Girth (cm) at age 19 years (Dec., 2009)	Mean yield during 2009 (g/t/t)	Mean yield over six years of tapping (g/t/t)
PB 235	87.0	57.5	80.7
PB 314	71.5	52.3	79.0
PB 280	74.8	73.7	77.0
PB 312	68.7	63.3	73.3
PB 311	72.3	48.5	67.8
RRII 300	84.5	58.8	60.1
RRII 105	64.1	31.6	59.6
RRII 203	86.0	57.4	59.4
PB 217	80.7	54.0	59.2
RRII 100	75.6	45.6	56.3
RRII 6	73.9	52.8	55.5
PB 260	72.8	46.9	53.0
PB 310	78.0	41.3	48.3
KRS 163	64.1	40.1	48.2
PB 255	55.8	30.1	47.5
GT 1	74.1	38.6	45.5
RRII 104	65.7	32.3	41.8
KRS 25	63.5	21.1	39.0
RRII 36	55.9	15.2	38.9
KRS 128	60.2	32.5	38.6
SCATC 88-13	54.2	29.0	37.5
CH 26	67.1	26.1	35.7
RRII 703	53.7	14.2	33.2
LCB 1320	72.5	29.8	32.1
PB 5/60	56.0	19.3	31.4
RRII 605	65.6	18.6	31.0
RRII 600	47.5	13.9	30.2
O 63	64.4	16.8	26.4
RRII 501	52.3	16.7	25.0
RRII 701	50.3	16.3	25.6
WARRING 4	49.6	15.3	25.0
HAIKEN 1	46.8	19.7	22.7
P 46	61.8	17.7	21.2
SCATC 93-11457.0	51.1	10.0	16.6
AVROS 352	51.1	10.0	16.6
CH 4	53.3	11.5	14.1
CV (%)	7.6	25.1	17.8
SE(±)	3.5	5.8	5.4
CD (P=0.05)	10.0	16.7	15.6

Table Kar. 5. Growth and yield performance of clones in the small scale clone trial (1991B)

Clone	Girth (cm) at age 19 years during (Dec., 2009)	Mean yield during 2009 (g/t/t)	Mean yield over six years tapping (g/t/t)
RRII 5	78.1	51.6	66.2
RRII 3	71.5	52.4	59.2
Nab 17	79.9	34.0	46.9
RRII 105	60.9	23.9	41.1
RRII 308	70.4	27.9	38.1
RRII 118	76.1	33.9	35.2
RRII 208	66.2	35.9	33.6
GT 1	69.5	29.7	32.0
AVROS 255	62.9	22.3	30.4
RRIC 102	56.6	21.0	26.0
Ch 2	65.3	14.8	22.6
PB 5/139	58.2	15.0	22.6
Ch 3	70.1	15.2	19.3
Ch 3	60.7	9.8	16.5
CV (%)	13.1	54.3	44.4
SE (\pm)	3.7	6.2	5.2
CD (P=0.05)	10.8	18.0	15.0

Table Kar. 6. Growth and yield performance of clones in the small scale clone trial (1991C)

Clone	Girth (cm) at age 19 years during (Dec., 2009)	Mean yield during 2009 (g/t/t)	Mean yield six years of tapping (g/t/t)
HP 83/224	83.3	58.0	61.2
PB 28/59	85.3	55.7	54.3
HP 83/225	68.5	41.5	45.1
GT 1	77.6	40.9	44.8
PR 261	73.2	29.9	43.0
HP 83/236	75.5	36.3	39.0
RRIC 105	56.7	23.1	32.9
PR 255	60.6	18.7	32.0
RRIM 600	59.5	22.4	28.6
PII B/84	58.4	18.9	26.2
PB 5/51	74.3	16.8	25.4
Ch 2	66.4	13.1	24.3
AVROS 49	56.8	14.4	16.8
CV (%)	9.1	25.2	21.6
SE (\pm)	4.5	5.3	5.6
CD (P=0.05)	13.7	16.5	17.2

Table Kar. 7. Growth and yield performance of clones in the large scale clone trial (1989)

Clone	Girth (cm) at age 21 years during (Dec. 2009)	Mean yield during 2009 (g/t/t)	Mean yield over eight years tapping (g/t/t)
RRII 203	94.5	64.8	67.5
KRS 25	82.1	43.8	57.6
PB 255	76.0	53.4	52.5
KRS 163	74.5	41.2	50.8
KRS 128	80.6	44.5	50.5
RRII 105	73.0	39.8	47.4
RRII 308	82.8	46.4	46.2
SCATC 88/13	64.8	33.5	40.6
PR 255	62.9	39.1	38.3
HAIKEN 1	57.1	22.9	34.8
RRIM 600	70.9	45.8	33.8
PR 261	69.6	27.8	32.2
RRII 300	74.8	33.3	30.5
SCATC 93/114	66.4	20.8	13.4
CV (%)	5.5	28.1	21.3
SE (\pm)	2.4	6.5	5.2
CD (P=0.05)	6.8	18.8	15.2

Table Kar. 8. Growth and yield performance of clones in the large scale clone trial (1990A)

Clone	Girth (cm) at age 20 years during (Dec. 2009)	Mean yield during 2009 (g/t/t)	Mean yield over seven years tapping (g/t/t)
PB 260	83.9	67.1	62.0
PB 235	88.4	60.7	59.9
HP 372	91.2	80.2	57.1
PB 217	78.0	54.7	55.3
PB 311	76.2	56.3	55.2
RRII 105	72.0	52.3	51.8
HP 223	83.0	65.1	51.2
GT 1	77.3	45.3	47.9
HP 185	74.8	43.0	30.7
Mil 3/2	80.1	39.3	30.1
GI 1	67.3	19.6	28.8
HP 187	72.9	24.1	27.7
Hil 28	72.4	26.1	25.9
HP 204	67.3	19.7	21.8
Tjir 1	62.1	17.0	19.4
CV (%)	4.3	15.7	9.8
SE (\pm)	1.9	4.1	2.4
CD (P=0.05)	5.5	11.7	6.8

Table Kar. 9. Growth and yield performance of clones in the large scale clone trial (2000)

Clone	Girth (cm) at age 10 years (Dec 2009)	Mean yield over fourteen months of tapping (g/t/t)
RRII 414	63.4	62.8
RRII 430	62.2	52.0
RRII 422	42.3	51.0
RRII 429	55.6	50.3
RRII 403	46.5	42.7
RRII 407	52.0	33.1
RRII 105	50.7	32.9
RRIC 100	57.8	31.4
CV (%)	6.2	13.9
SE(±)	1.9	3.6
CD (P=0.05)	5.9	10.8

PB 312 (73.3 g/t/t) and PB 311 (67.8 g/t/t) (Table Kar. 4). In 1991 B clones RRII 5 and RRII 3 are performing well (Table Kar. 5). In 1991 C, highest yielding clone was HP 83/224 (61.2 g/t/t) after six years of tapping (Table Kar. 6).

1.2. Large scale trial

In 1989 trial, 14 clones are under evaluation. Growth and yield performance of clones during 2009 and over eight years of tapping (2002-2009) are given in Table Kar. 7. After eight years of tapping, maximum yield

was recorded in clone RRII 203 (67.5 g/t/t) followed by KRS 25 (57.6 g/t/t) and PB 255 (52.5 g/t/t). RRII 600, PR 261 and SCATC 93-114 were the lowest yielding clones with a yield of 33.8, 30.5 and 13.4 g/t/t respectively. In 1990 trial, 15 clones are under evaluation. Growth and yield performance of clones during 2009 and over seven years of tapping (2003-2009) are given in Table Kar. 8. Clone PB 260 recorded maximum yield (62.0 g/t/t) followed by PB 235 (59.9 g/t/t) and HP 372 (57.1 g/t/t). Tjir 1 was the lowest yielder (19.4 g/t/t). Eight modern clones including RRII 400 series clones are under evaluation in 2000 trial. Initial yield recording (Table Kar. 9) indicate good yield in RRII 414 (62.8 g/t/t).

2. Estimation of genetic parameters

This trial was planted in 1990 to evaluate parents and their half-sib progenies for estimating genetic parameters that are essential for planning plant breeding activities. Twelve clones and their progenies are under evaluation. After seven years of tapping, parent clones PB 235, RRII 203 and RRII 105 were the leading in yield with a yield of 77.1, 54.9 and 50.8 g/t/t respectively. Among the progenies, half-sibs of RRII 203,

Table Kar. 10. Details of clones/genotypes having tolerance to ALF caused by *Phytophthora*

Trial	No of clones/ genotypes tolerant to ALF	Clones/genotypes
1987 Trial	2	PB 260, RRII 105
1988 Ortet trials	13	O 34, O 15, O 41, O 17, O 47, O 40, O 46, O 53, C 140, O 30, O 51, O 55, O 26
1989 LST	4	RRII 203, RRII 105, KRS 25, KRS 163
1990 LST	5	HP 223, HP 372, PB 217, GT 1, RRII 105
1991 SST	9	RRII 203, RRII 300, PB 260, RRII 5, RRII 3, NAB 17, HP 83/224, GT1, PB 217
Genotypes (Seedling trees)	75	G 1 to G 75
Total	108	

PB 235 and GT 1 recorded a yield of 45.1, 41.7 and 41.1 g/t/t respectively.

3. Reaction of *Hevea* clones/genotypes to *Phytophthora* leaf disease

Response of various clones and genotypes to *Phytophthora* leaf disease was

studied under non-sprayed conditions. Based on three years observation (2007-2009), it was been found that 108 clones/genotypes showed tolerance to *Phytophthora* leaf disease. Details are provided in Table Kar. 10.

HEVEA BREEDING SUBSTATION PARALIAR, TAMIL NADU

Hybridization and evaluation of clones, standardisation of root trainer technique and influence of solar radiation on TPD are the thrust areas of research.

1. Crop Improvement

1.1. Clone evaluation

In the large-scale clone trial initiated at Keeriparai (1994) in panel B01 in the panel B01 PB 314, IRCA 109, IRCA 111 and PB 235, gave better yield than RRII 105 (Table Par. 1). Towards the end of the eighth year of

tapping, PB 255 (84.26 g/t/t) performed better than RRII 105 (65.94 g/t/t). Two clones from Ivory Coast, viz. IRCA 109 (77.65 g/t/t) and IRCA 111 (77.97 g/t/t) also showed promising yield. Clone PB 314 (77.67 g/t/t) showed maximum incidence of TPD (51.96%).

In the block evaluation experiment (1994), RRII 105 (61.91 g/t/t) continued its status as the best yielder, closely followed by PB 28/59 (59.76 g/t/t) (Table Par. 2). Although PB 311 (58.9 g/t/t) exhibited

Table Par. 1. Mean girth, yield, DRC and TPD incidences in large scale clone trial (1994)

Clone	Mean girth (cm)	Mean yield (g/t/t)			Mean DRC over 8 years	Percentages of TPD over (8 years)
		B0-1 (6 years)	B0-2 (2 years)	Pooled 8 years		
RRII 105	78.64	57.52	89.08	65.94	39.76	13.33
PB 314	83.01	75.79*	82.82	77.67	33.27	51.96
IRCA 130	77.01	66.66	89.03	72.71	35.78	39.27
PB 28/59	74.92	58.71	76.15	63.36	40.66	15.76
IRCA 109	80.78	75.39*	83.86	77.65	39.66	15.38
PB 330	81.04	53.38	73.92	58.86	38.33	30.17
IRCA 18	80.07	57.31	80.07	63.38	37.82	20.20
RRII 703	83.32	62.22	84.86	69.72	36.17	34.47
IRCA 111	86.93	73.97*	88.96	77.97	35.77	19.47
PB 255	86.54	74.17*	112.01*	84.26*	38.72	20.55
IRCA 230	84.64	57.62	85.99	65.19	37.53	7.43
Mean	81.54	64.79	86.10	70.61	37.58	33.81
CD (P = 0.05)	12.79	15.28	22.11	16.99	3.82	30.17

Table Par. 2. Mean girth and yield in the block trial (1994)

Clone	Girth (cm)	Yield (g/t/t)	
		2009-10	8 years
RRII 5	71.00	45.74	44.53
RRII 50	73.26	53.68	45.43
RRII 51	73.96	46.15	38.68
RRII 105	68.41	73.82	61.91
RRII 176	82.57	52.27	43.54
RRIC 102	78.23	70.42	52.70
PB 217	80.65	47.85	48.64
PB 235	81.12	49.37	55.44
PB 260	79.55	55.05	50.96
PB 311	73.77	54.31	58.90
PB 28/59	73.48	72.44	59.76
PR 255	72.18	49.56	50.01
PR 261	71.99	53.13	47.87
Mean	75.39	55.67	50.64
SE	2.11	3.14	2.63

promising yield, the clone was highly susceptible to wind damage.

In the multi-location clone trial (1996), RRII 203 (58.3 g/t/t) exhibited better yield than RRII 105 (57.21 g/t/t) (Table Par 3). Among RRII 400 series clones, RRII 430 (52.96

g/t/t) was the best yielder, followed by RRII 422 (50.44 g/t/t) and RRII 417 (50.09 g/t/t). In the observational trial at Vaikundam Estate (2000), all hybrid clones, except RRII 427, exhibited better yield (49.39 to 54.84 g/t/t) than RRII 105 (45.78 g/t/t).

Since RRII 400 series clones exhibited variations in yield in two adjacent estates in the Kanyakumari region, five on-farm trials were initiated in five locations. Observations were made on juvenile growth characteristics of the clone.

1.2. Hybridization and clonal selection

Two breeding orchards, consisting 51 modern clones as parents, were maintained. Hand pollinations were attempted using various parental combinations and the hybrids were evaluated in nurseries. Ten selections were included in a large-scale clone evaluation experiment at RRII, Kottayam. Potential high yielders were multiplied for conducting small-scale clone evaluation experiments. A new experiment was initiated during 2010 and hand pollinations were carried out.

1.3. New generation polyclonal seed garden

Polycross seeds collected from the garden were raised at HBSS, Paraliar and Nagamalai Estate, Punalur. The progenies at Paraliar were test tapped during 2010 and 22 potential high yielders were selected for further evaluation in a small-scale trial. The polycross progenies raised at Nagamalai Estate were test tapped and field planted.

2. Root trainer planting technique

In the field trial at Churulacode (2002), root trainer plants continued to exhibit better growth and yield (46.28 g/t/t) than polybag plants (41.98 g/t/t). Root trainer plants showed more uniformity in growth compared to polybag plants.

Table Par. 3. Mean girth, yield and TPD in the GxE trial (1996)

Clone	Girth (cm)	Yield (g/t/t)		Incidence of TPD
		2009-10	7 years	
RRII 414	74.21	45.31	46.14	1
RRII 417	75.30	70.17	50.09	1
RRII 422	70.01	49.85	50.44	1
RRII 429	78.94	45.18	40.95	3
RRII 430	70.21	53.78	52.96	—
RRII 51	71.71	39.32	37.14	1
RRII 176	77.84	68.60	42.77	—
RRII 203	72.64	75.53	58.30	—
RRIC 100	73.05	55.82	44.38	—
PB 217	72.37	49.60	37.33	1
RRIM 600	74.61	45.94	42.96	2
RRII 105	67.64	75.45	57.21	3
Mean	73.21	56.21	46.72	
CD	3.03	3.58	2.66	

In the experiment entitled *in situ* young budding on stocks raised in root trainers, lateral root formation was better in *in situ* budding than in stump planting in root trainers or polybags. *In situ* budding had several advantages, viz. better budding success and initial establishment, sturdy growth, less expensive etc. Training on root trainer planting technique was imparted to five batches of extension officers, one batch of Farm Officers and Farm Managers and another batch of nursery owners.

3. Participatory clone evaluation (PCE)

In PCE trial (2008) at Tharuvaiyar, plots were demarcated and fertilizer was applied based on soil analysis. Observations were

recorded on growth characteristics like branching height etc.

Advanced planting materials were raised in polybags to initiate two more on-farm experiments under the project at Bethany Estate during the year 2010. Plots were demarcated and pre-planting operations were completed by March 2010.

4. Solar radiations and TPD

In clone trial (Ponmanai, 2008) and block trial (Bethany Estate, 2008), the bark protection device was replaced with areca nut spatula to protect the tapping panel from the harmful effects of solar radiations. Observations were recorded on initial establishment and juvenile growth.

LIBRARY AND DOCUMENTATION CENTRE

During the year, 92 books were added to the stock of the library. The library subscribed 51 international journals and 77 national journals. About 32 other journals were also received as gift/exchange. Literature searches from AGRIS and RAPRA CDs were carried out.

Four issues of *Documentation List*, four issues of *Rubber Alerts*, 27 issues of *Current Content Bulletins* and one issue of *New Additions List* 2008 were compiled and distributed. Databases were updated by adding 131 books and 383 journal articles. Three databases were developed for Thesis/

Dissertations, Rubber Standards and Rubber Board Publications. Distribution of 724 press clippings and 199 SDI bulletins was also carried out.

Natural Rubber Research, the Institute's journal (Vol. 21; 2008) was published and 277 copies of the journal including the back volumes were distributed. Organized the distribution of 111 numbers of *Annual Report* (2006-07). The sale of 71 numbers of *RRIL* publications were arranged. Photocopies of about 60,919 numbers of information materials were provided during this period.

AGROMETEOROLOGY

1. Climate resource characteristics of rubber growing tracts

Data on Rainfall, Maximum temperature, Minimum temperature, Mean temperature, Rainfall, Rainy days and Sunshine hours have been analyzed for long term trends in respect of five Regional Research Stations, viz. Kottayam (09° 32'N 76° 36'E 73m above MSL) in Central Kerala, Padiyoor (11° 58'N 75°N 36'E 20m above MSL) in North Kerala, Parliar in South Tamil Nadu (8° 26'N 77° 19'E 33m above MSL), Nettana in South Karnataka (12° 43'N 75° 42'E 110m above MSL) and Dapchari (20° 04'N 72° 04' E 58m above MSL). The analysis for weekly, monthly, seasonal and annual trends were carried out with daily datasets ranging from 10 to 53 years. All the variables analyzed for trends were further tested by the help of the standard Mann-Kendall statistics for significance. Extreme climatic events were also considered in the study to detect any significant change in the trends.

1.1. Seasonal and annual trends

Maximum temperature was positively significant for all seasons in Kottayam). The highest increase in maximum temperature (0.04°C/year) was seen during all seasons in Kottayam.

1.2. Extreme climatic events

Stations were compared for extreme climatic events based on the total annual number of days above respective threshold limits for temperature, sunshine and rainfall. Increase in the hot days was observed only for Kottayam while decrease in warm nights was seen for Parliar and Nettana.

2. Forewarning of pests and diseases

Incidence of Abnormal Leaf Fall (ALF),

Powdery Mildew and *Corynespora* Leaf Fall (CLF) were related with weather and data on the disease onset was analysed for the second year in the traditional rubber growing regions.

2.1 *Corynespora* Leaf Fall (CLF)

Spore traps with slides coated with petroleum jelly fixed to a rope at four levels of 1m height from the top canopy, were installed in Pinavoorudi, Ernakulam. The amount of daily leaf fall due to disease was studied with the daily weather parameters.

The parameter known as Humidity Thermal Index (HTI), which is known to influence plant diseases was worked out by taking the ratio of humidity and temperature of the corresponding timings and included in the time series analysis. The leaf collection was also compared with that of the Percent Disease Intensity (PDI), which was recorded periodically in different plots in the field. The HTI ratio between morning relative humidity and minimum temperature were highly related to daily leaf fall. The HTI was also compared with that of the Percent Disease Intensity (PDI), which was recorded periodically.

It was found that during the periods of 2009 and 2010, HTI could be related with the triggering of CLF incidence after three to four days of HTI = 4.0 and leaf fall after seven to eight consecutive days of HTI = 5.5. Leaf fall intensity (at seven days lag) increased with the duration of days = 5.5 HTI. This was mainly observed in Jadkal during 2009 when the disease severity (PDI) was 100 per cent. Increase in the intensity of leaf fall showed a high positive serial correlation of 0.40 with the increase in HTI values above 5.5 after seven days. Similar results were also observed during the same refoliation period in 2010 (Fig.

Agromet. 1). However, 100 per cent disease was observed only in trees which had leaves at the most vulnerable stage (light-green). In Pinavoorkudi (Fig Agromet. 2), the maximum spore count was observed after a period of 6 to 8 days with HTI = 5.5 for seven consecutive days. Spot appearance accompanied by

maximum spore count was seen after 3 to 4 days with HTI = 5.5 mark. This could also be related to the development and severity of the disease as was observed in Guthigar and Jadkal. The serial correlation worked out between the 7-day lag period HTI and maximum spore count was 0.85.

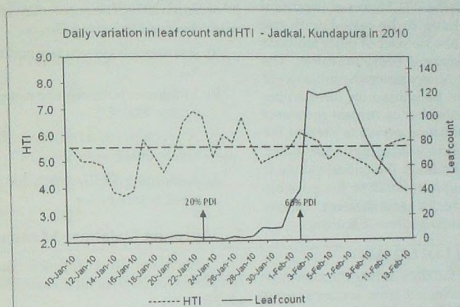


Fig. Agromet. 1. Variation of daily HTI with leaf fall during the refoliation period of RR11 105 at Jadkal, Kundapura in 2010

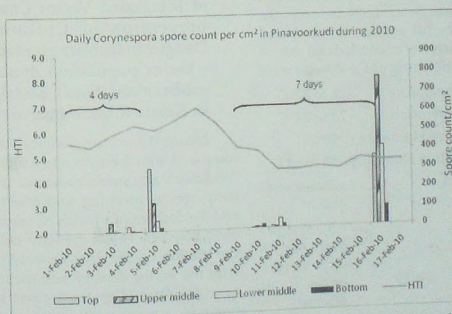


Fig. Agromet. 2. Daily HTI variation with Corynespora spore count/cm during the refoliation period of RR11 105 at Pinavoorkudi in 2010.

The study indicated that HTI = 4.0 for consecutive three to four days triggered the disease and a minimum duration of seven days HTI = 5.5 can favour the incidence and severity of the disease resulting in leaf fall. It also revealed that incidence and intensity of the disease is greatly influenced by the microclimate over a particular location, especially with the HTI. However, the critical values will have to be tested with the microclimate of a number of other locations where the disease is being prevalent. The critical values observed for the initiation of the disease can be utilised for forewarning and timely adoption of control measures during the refoilation period to prevent the development and spread of the disease. Based on the above findings, the critical values, it was also possible to analyse the possible occurrence of the disease in different locations within the changing climatic scenario.

2.2. Abnormal Leaf fall (ALF)

Daily leaf fall data during the monsoon season was collected from an unsprayed area in RRS Padiyoor for the period from June to September 2009. The data was then compared with the antecedent and ambient atmospheric parameters collected from the agrometeorological station. It was found that the atmospheric conditions which favoured the triggering of the disease were

Maximum temperature	- 26 to 32°C
Minimum temperature	- 23 to 24°C
Morning Relative Humidity	- >90%
Afternoon Relative Humidity	- 55 to 93%
Sunshine hours	- > 5 days with >1.0 hr
Rainfall	- > 50.0 mm / day for 3 days before onset of disease

ALF incidences were also observed in Chemoni Estate in Thrissur. No ALF

incidence was recorded in Vaikundam Estate in Nagercoil, Kanyakumari district.

2.3. Oidium Leaf fall

During the second year of the experimental trial on Oidium leaf fall, incidences were identified in RRS Padiyoor with leaf fall from the 20th of February to the 3rd of March 2010. The antecedent conditions were observed to be as follows.

- Average Maximum temperature of 35 °C.
- Minimum temperature ranging from 21.8 to 24.4 °C.
- A week with afternoon RH >50% before onset.
- A morning RH of >90% for almost a week before onset.

3. Agromet Database Mangaeement

Archival in computer has been completed for 2009 for all the stations of RRJI till 2009.

4. Development and demonstration of Integrated Vector Control for prevention of Chikungunya/ Dengue in Rubber plantation area - Vector population dynamics in rubber plantation/ Coastal regions in Kerala (Collaborative project)

Mosquito vector density have been compared with the antecedent atmospheric conditions *viz.* maximum and minimum temperature, morning and afternoon RH and the daily rainfall with number of rainy days between vector density collections. Correlation coefficients were worked out with the vector density and mean as well as individual climatic factors with lag periods up to 10 days from the corresponding dates of collected vector population density.

ANNUAL EXPENDITURE

Expenditure at a glance (2009-10)

Head of Account	Expenditure (Rs. In lakhs)
Non-Plan	
Non-plan (Research)	528.76
Projects (CES)	330.68
Total	859.44
Plan	
Plan (Research)	1548.45
NERDS Research Component	320.66
Total	1869.11
Grand Total	2728.55

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Deputy Director

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Scientist C

Scientist B

Scientist B

Scientist S3

Scientist S3

Scientist B

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