

IRC 2012

INTERNATIONAL RUBBER CONFERENCE

Kovalam, Kerala, India

28-31, October 2012

ABSTRACTS



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MESSAGE

I am pleased to note that the abstracts of papers selected for presenting at the International Rubber Conference hosted by Rubber Board under the auspices of the International Rubber Research and Development Board from 28-3 October, 2012 at Kovalam, India are compiled and brought out as a book. It is important to document scientific findings for benefit of the wider community. These abstracts reflect the present state of R & D in natural rubber cultivation and allied industries in the different countries where this crop is cultivated. I hope and expect that the research findings appearing in these abstracts will result in practical applications for benefit of the natural rubber sector and society at large.

There are delegates from nearly 20 nations attending this Conference and it is my pleasure to welcome all of them to Kovalam. I wish them a fruitful Conference and pleasant stay in India.

I congratulate all those who have worked behind organizing this Conference and wish the event every success.

Kottayam
27 October, 2012

Sheela Thomas, IAS
Chairman Rubber Board India
& Patron, IRC 2012



MESSAGE

I am glad that the organizers of the International Rubber Conference (IRC) have been able to put together the abstracts of the IRC presentations into a structured and readable form at very short notice. There are a large number of papers on wide ranging subjects from Rubber Technology and Genomics to Extension and Social Science, making this book of abstracts a rich source of valuable scientific information.

The International Rubber Research and Development Board (IRRDB) is highly grateful to Government of India for graciously agreeing to host the IRRDB Annual Meetings for 2012 in India and the IRC preceding the same. For the past ten years, it has been customary for the country that hosts the IRRDB Annual Meetings to organize an IRC which is attended by delegates from all member countries/ institutes/organizations of IRRDB.

I would also like to place on record the sincere appreciation of IRRDB for Madam Sheela Thomas, Chairman, Rubber Board India for taking every effort for the success of the IRC and the IRRDB Annual Meetings.

Kuala Lumpur
26 October, 2012

Datuk Dr. Abdul Aziz
Secretary General, IRRDB

PREAMBLE

Welcome to IRC 2012!

There has been overwhelming response to call for papers for presentation at the 10th International Rubber Conference organized in connection with the Annual Meetings of the International Rubber Research and Development Board (IRRDB) by Rubber Board India at Hotel Samudra, Kovalam, Kerala, India from 28 to 31 October, 2012. More than 180 abstracts spanning all aspects of natural rubber cultivation, processing and products making have been received from delegates from Cambodia, China, Cote d' Ivore, France, Ghana, Guatemala, India, Indonesia, Malaysia, Myanmar, Nigeria, The Philippines, Sri Lanka, Thailand, U.S., U.K. and Vietnam. These abstracts fairly represent the present status of rubber research that is going on in these countries.

Concerns about supply, quality and price of natural rubber and the raw materials are reflected in the papers presented by the rubber- goods manufacturing industry. Recent developments in rubber technology for making products with better performance and environment standards are covered in several papers scheduled for the first day of the Conference. An exclusive session on Growers' Innovations is a unique feature of this Conference. Several new innovations and findings in natural rubber cultivation covering diverse areas such as Genomics and molecular marker assisted breeding for development of elite clones, zero tillage, increased use of farm machinery for agronomic and plant protection purposes, declining soil fertility, root trainer planting techniques, utilization of wild *Hevea* accessions, application of geo-informatics and remote sensing, ecosystem processes, modern extension techniques, climate change, climate resilient clones and farming practices, reducing gestation period, intercropping, labour shortage, low frequency tapping, nursery management, high density

planting, micro nutrient deficiency, genetic transformation *etc.* are covered in this Conference.

This compilation contains the abstracts and extended abstracts of all papers selected for the Conference. Most of these papers reached us only in the recent days (which is not very unusual) and we have included the abstracts without much of editing due to lack of time.

Barring a few exceptions, the papers submitted for IRC 2012 are written by authors who hail from non – English speaking countries. Despite going to print without a through editing or peer review, I am sure the contents of these abstracts will create considerable excitement and interest in rubber researches everywhere.

Happy reading.

RRI India
27 October, 2012

James Jacob PhD, DIC, PhD
Director, RRI India
& General Chairman, IRC 2012

CONTENTS

INDUSTRY		29
Special talk 1	An Alternate Approach to NR Demand Estimation in India: Tyre Industry Proposal Rajiv Budhiraja	30
Special talk 2	Indian Rubber Industry - Non Tyre, An Overview Vinod Simon	30
Special talk 3	Indian Synthetic Rubber Industry - Prospective Virendra Rathod	31
Special talk 4	Global NR Prices: Retrospect and Prospect Jom Jacob	31
PLANTATION		33
Special talk 1	Rajtree Model Program For the Philippine Rubber Development Rommel A. Jalosjos	34
Special talk 2	Expanding NR Supply Chain base for Indian Rubber Industry Dr A.K.Krishna Kumar	35
Special talk 3	Strategy for R&D Driven Open Innovation (OI) Prof. V. G. Dhanakumar	36
Special talk 4	Seeding Rubber's Future Mr. Michael Fraley	37
RUBBER TECHNOLOGY		41
Invited Talk 1	Soft and Hard Nanoparticles in Natural Rubber Anil K. Bhowmick	42
Invited Talk 2	Future Challenges for Rubber Industry Vinay Sharma, Job Kuriakose and Arup K. Chandra	42
Invited Talk 3	Nonlinear Viscoelasticity of Rubber Nanocomposites Sabu Thomas	43

CROP IMPROVEMENT		45
Lead Talk 1	Genomics Assisted Crop Improvement –Applications and Potential in Tree Improvement M.S. Sheshshayee	46
O-1	Study on Performance of Clones in SSCT Derived from Hybridization among 198 ¹ <i>Hevea</i> Germplasm and Oriental Clones Nurmi-Rohayu A.H., Mohd Nasaruddin bin Mohd Aris, Zarawi bin Ab Ghani, Rasyidah binti Mohd Razar and Ong Chin Wei	48
O-2	Performances of RRIV’s Clones in Non-Traditional Rubber Growing Regions of Vietnam Le Mau Tuy, Vu Van Truong, Le Dinh Vinh, Vu Van Chien, Tran Thanh and Lai Van Lam	49
O-3	Recombination Breeding of <i>Hevea brasiliensis</i> in India: Clones Evolved From the 1983 Hybridization Programme Alice John, M.A. Nazeer, V.C. Mercykutty and Kavitha K. Mydin	50
O-4	Amazonian Accessions of Wild <i>Hevea</i> Germplasm – A Potential Source of Drought Tolerance M. A. Mercy, D.B. Nair, Meena Singh, T. Meenakumari, C.P. Reghu and Kavitha K. Mydin	51
O-5	Identification of Promising Clones of <i>Hevea</i> for Commercial Cultivation in Tripura Deepthy Antony P., P. M. Priyadarshan, Krishna Das and S. K. Dey	52
O-6	Long-Term Yield and Growth Performance of IRCA Rubber Clones (<i>Hevea brasiliensis</i>) in India C.P. Reghu., G.P.Rao and Jayashree Madhavan	54
P-1	The 2011-2015 Rubber Planting Recommendation in Vietnam Vu Van Truong, Le Mau Tuy, Le Dinh Vinh, Tran Thanh and Lai Van Lam	55
P-2	Management of Wild <i>Hevea</i> Genetic Resources in India: Yield and Growth Performance in Immature PhaseG. Prabhakara Rao, Jayashree M., and C.P. Reghu	56
P-3	Sieve Tube Characteristics in Healthy and TPD Affected Trees of <i>Hevea brasiliensis</i> Gopika Gopal and Vinoth Thomas	57

P-4	Phloic Rays in <i>Hevea brasiliensis</i> with Reference to Tapping Panel Dryness and Yield Stimulation Vinoth Thomas, S. Pramod and K.S. Rao	58
P-5	Performance of RRII 400 Series Clones in a Small Holding: A Case Study T. Gireesh and Kavitha K. Mydin	59
P-6	Growth and Yield Performance of Some Exotic Clones of <i>Hevea brasiliensis</i> in North Kerala Region Radha Lakshmanan, T. Meenakumari, T.R. Chandrasekhar and M.A. Nazeer	60
P-7	Evaluation of Rubber (<i>Hevea brasiliensis</i>) Modern Clones under Certain Stressful Climatic Conditions of Odisha B. Krishan	60
P-8	Performance of Selected Exotic and Indigenous Rubber (<i>Hevea brasiliensis</i>) Clones in Traditional Region of Kerala, India Narayanan, C., Mydin, K. K., Alice, J., Licy, J. and A. Varghese. C	61
P-9	Early Performance of some Clones of <i>Hevea brasiliensis</i> in Dry Sub humid Region of North Konkan Meena Singh, Chandrashekhar, T.R., Nazeer, M. A., Annamalainathan, K. and Kavitha K. K.	62
P-10	Timber Production and Wood Quality Parameters of High Yielding RRII 400 Series Clones of <i>Hevea Brasiliensis</i> Muell. Arg. T. Meenakumari., Jayashree, C. E , Gireesh, T., Digesh, P., Reghu, C P., Vinoth Thomas and Kavitha K Mydin	63
P-11	Promising Latex Timber Clones of <i>Hevea brasiliensis</i> Evolved Byortet Selection from a Large Estate V.C. Mercykutty, T.Meenakumari and Kavitha K. Mydin	64
P-12	Inheritance of Tapping Panel Dryness (TPD) in a Full-Sib Population of <i>Hevea brasiliensis</i> Narayanan, C. and Kavitha K.Mydin	65
P-13	The dynamic system that exists in the sieve tubes of TPD affected trees of <i>Hevea brasiliensis</i> Vinoth Thomas	67
P-14	Evaluation of Half-Sib Progenies of Canopy Mutant of <i>Hevea brasiliensis</i> T. Gireesh and Kavitha K. Mydin	67

P-15	Do Twin Stock Plants Perform Better Than Single Stock Plants in <i>Hevea</i> ? Thomson Abraham, V. C. Mercykutty and Joseph G. Marattukulam	69
P-16	Growth and Yield of New Generation Clones of <i>Hevea</i> in Sub-Himalayan West Bengal G.Das, T. Meena Kumari, R. S. Singh, S. Kheroar, S. Meti, T Gohain, S. Kumar, K. K. Mydin and D. Chaudhuri	70
P-17	Evaluation of Brazilian Wild <i>Hevea</i> Germplasm in India for Cold Resistance: 2. Variability and Character Associations in Juvenile Growth Phase G. Prabhakara Rao, Saji T. Abraham and C.P. Reghu	72
P-18	Early Evaluation of a Set of Wild <i>Hevea</i> Germplasm for Drought Tolerance in the North Konkan Region M. A. Mercy, T. Meenakumari and Meena Singh	74
	BIOTECHNOLOGY	77
O-1	Production of Mutants Affected in Hormone Signalling to Dissect Defence Mechanisms in <i>Hevea brasiliensis</i> : The Case of Ethylene Pascal Montoro, Maryannick Rio, Julie Leclercq, Florence Martin, Eve Lorenzini, Florence Dessailly	78
O-2	microRNAs: New Regulators of Biological Functions in <i>Hevea brasiliensis</i> Julie Leclercq, Virginie G�ebelin, Kuswanhadi, Tetty Chaidamsari, Cuifang Duan and Pascal Montoro	78
O-3	Genetic Transformation and Regeneration of <i>Hevea brasiliensis</i> Transgenic Plant with <i>GAI</i> Gene Ying Wang, Xiongting Chen, Kunxin Wu, Lei Hong	79
O-4	Profiling the Proteomic Alterations between the Large and the Small Rubber Particles of <i>Hevea brasiliensis</i> Using 2D-DIGE Qiulan Xiang, Kecan Xia, Longjun Dai, Guijuan Kang, Yu Li, Zhiyi Nie, Cuifang Duan, Rizhong Zeng	80
O-5	Molecular Analysis of <i>Botryodiplodia theobromae</i> Isolates from Rubber in Vietnam using rDNA ITS Sequencing and ISSR Markers Nguyen Anh Nghia, Vu Thi Quynh Chi and Nguyen Xuan Dong	81

O-6	Studies on <i>Agrobacterium</i> -mediated Genetic Transformation of Embryogenic Cell Suspension and Plant Regeneration in <i>Hevea brasiliensis</i> Zehai Jiang, Zhe Li, Quannan Zhou, Xuemei Dai, Aihua Sun, Tiandai Huang, Yanmei Ni, Huasun Huang, Weifu Lin	82
O-7	<i>In vitro</i> Micropropagation of <i>Calopogonium caeruleum</i> for Soil Cover Crop under Rubber Plantation Wittaya Promme	83
O-8	Caffeic Acid O-Methyltransferase (<i>COMT</i>) Gene of the Phenyl Propanoid Pathway Involved in Resistance to <i>Corynespora</i> Leaf Disease in Rubber (<i>Hevea brasiliensis</i>) Thakurdas Saha, Bindu Roy C, Minimol Ravindran and K.U. Thomas	83
O-9	De novo transcriptome sequencing of abiotic stress responsive transcripts of <i>Hevea brasiliensis</i> Mohamed Sathik, M.B., Molly Thomas, Lisha P. Luke, Nasnim Ebrahim, Krishnakumar, Annamalainathan and James Jacob	84
O-10	Generation of Transcriptome Resources in Rubber (<i>Hevea brasiliensis</i>) in Response to <i>Corynespora Cassiicola</i> Causing <i>Corynespora</i> Leaf Disease for Gene Discovery and Marker Identification Using Ngs Platform C. Bindu Roy and Thakurdas Saha	85
P-1	Cloning of <i>Arabidopsis WUS</i> Gene, Construction and Identification of <i>WUS-EGFP</i> Fusion Gene Plant Expression Vector Zhenghong Bi, Zhe Li, Zhiqiu Wang	86
P-2	Expression of NAC Transcription Factor under Drought Stress in <i>Hevea brasiliensis</i> Molly Thomas, Sathik, M.B.M, Saha, T., Luke, L.P., Annamalainathan, K. Krishnakumar, R. and James Jacob	87
P-3	Over-Expression of Chitinase Gene and <i>in vitro</i> Antifungal Activity of Recombinant Chitinase Protein Against <i>Corynespora cassiicola</i> Infecting Rubber (<i>Hevea brasiliensis</i>) Shaji Philip, Amith Abraham, Annakutty Joseph, Thomas K.U and Sathik M.B	88
P-4	Identification and Molecular Characterization of Multiple forms of α -1, 3-Glucanase Gene Promoter from <i>Hevea brasiliensis</i> through Inverse PCR Supriya R., Saleena A. and Thulaseedharan A	89

P-5	<i>Half Ovulo</i> Embryo Culture –An Ideal Method for Raising True-To-Type Seedlings in <i>Hevea brasiliensis</i> Rekha, K., Lincy, V., Jayashree, R., Sushamakumari, S., Sobha, S., Saha, T. and Thulaseedharan, A.	90
P-6	Chlorophyll A/B Binding Protein Gene Expression in Juvenile and Mature Leaf Explants and Its Relationship with <i>In Vitro</i> Culture Response in <i>Hevea brasiliensis</i> R.G. Kala., R. Supriya., Suni Anie Mathew., V. Anju., P. K. Jayasree., S. Sobha and A. Thulaseedharan	91
P-7	High Frequency <i>Agrobacterium</i> Mediated Genetic Transformation in Rubber Tree Via. Vacuum Infiltration Sobha S, Rekha K, Sushamakumari S, Jayashree R, Kala R. G. Jayasree, P. K, Deepa K and Thulaseedharan A.	92
P-8	Effect of Nurse Culture on Inducing Division of Isolated Pollen Protoplasts of <i>Hevea brasiliensis</i> Sushamakumari.S, Sneha Joseph, Sobha.S, Rekha. K, Jayashree. R. and Leda Pavithran	93
P-9	<i>Agrobacterium</i> Strain- A Key Factor Determining the Efficiency of Transformation in <i>Hevea brasiliensis</i> Jayashree, R., Rekha, K., Venketachalam, P., Sushamkumari, S., Sobha, S., Leda, P., Thulaseedharan, A and Nazeem, P.A.	94
P-10	Functional Characterization of a Novel <i>Hevea</i> \hat{A} -1, 3-Glucanase Gene Promoter and Its Regulatory Role in <i>Hevea</i> and Tobacco Tissues Supriya R., Saleena A., Suni A. M and Thulaseedharan A	95
P-11	Structural and Functional Characterization of a NBS-LRR Disease Resistance Gene Associated With Tolerance against <i>Corynespora cassiicola</i> Causing Leaf Fall Disease in Rubber (<i>Hevea brasiliensis</i>) Thakurdas Saha, C. Bindu Roy and Minimol Ravindran	97
P-12	Cloning and Expression of 3-Hydroxy -3-Methylglutaryl- Coa Reductase 1 (<i>Hmgr</i> 1) from <i>Hevea brasiliensis</i> P.K. Ambily, Molly Thomas, R. Krishnakumar, M.B. Mohamed Sathik and K. Annamalainathan	98
P-13	Cloning and Expression of HbACO2 of <i>Hevea brasiliensis</i> M. B. Mohamed Sathik, P. V. Raghi, P.K. Ambily, R. Krishnakumar, Molly Thomas and K. Annamalainathan	100

CROP MANAGEMENT

O-1	Rubber-Based Farming System: in a Challenging Industry Development and Expansion in Southern Philippines Roger O. Bagaforo¹ and Ernie C. Camacho²	104
O-2	Effect of High Density planting on growth and yield of selected RRIM 2000 series Zulkefly Sulaiman, Khairul Ashraf Adrutdin and Mohd. Fauzi Mohd. Yusoff	105
O-3	Cropping Pattern of Coffee as Rubber Intercrop at Farmer Level in Muara Enim and Musi Rawas Districts of South Sumatra Province, Indonesia M.J. Rosyid and Heru Suryaningtyas	106
O-4	Soil Fertility Evolution and Correlation with Leaf Nutrient Contents in Rubber Plantation in Hainan, China Hua Yuangang, Lin Zhaomu, Luo Wei, Cha Zhengzao and Chen Qiubo	107
O-5	Adaptation of Root Trainer Technology in Different Agroclimatic Environments Francisco Andicoechea, Joseph John, Dr R K Matthan	107
O-6	Nutrient Status of Soils and Leaves of Immature Rubber in the East Coast of Upper Part of Southern Thailand Saichai Suchartgul and Somsak Maneepong	108
O-7	Correlations of some Soil Chemical Properties, Leaf Chlorophyll content and Growth during Immature Phase of Rubber Tree Krissada Sangsing	109
O-8	Reducing the Immature Phase of Natural Rubber Cultivation: Role of Agromanagement Techniques Sherin George, Shankar Meti and Sabu P. Idicula	110
O-9	Effect of Weeding Frequency and Fertilizer Rates on the Growth Performance and Budding Successes of <i>Hevea</i> Rootstock Seedling in a Humid Forest Area of South Eastern Nigeria Esekhade, T.U., Okore, I.K., Ogidi, E.G.O. and Arasowan ,J.	111
P-1	Nutrient Management in Rubber Seedling Nursery- Studies in the Integrated Approach through Incorporation of Bio Inoculants Joseph, M., Joseph, K., Mathew J., Jacob J., Hareeshbabu, G. and Elias, R.S.	112

P-2	Indian Summer Monsoon Rainfall (ISMR) Over Kerala Exhibits a Temporary Shift Sailajadevi. T	113
P-3	DTPA-Extractable Micronutrients in Rubber Growing Soils of North-East India M. Choudhury, D. K. Patgiri, N. G. Barua and D. K. Borah	113
P-4	Seasonal Variation on Crop-Weather Relation in <i>Hevea</i> Grown Under the Sub-Himalayan Climate of West Bengal Gitali Das¹, Shammi Raj and Dhurjati Chaudhuri	114
P-5	Evaluation of Field Performance: Polybag Vs. Root Trainer Rubber Plants at Different Stages Sherin George, Sabu P. Idicula, Soman, T. A. and Syamala, V.K.	115
P-6	Carbon and Nutrients stock in mature rubber plantations with and without control of under flora Joshua Abraham and Phebe Joseph	116
P-7	Distribution of Potassium Forms in Soils Under Repeated Rubber Cultivation and Virgin Forest A. Ulaganathan, R.J. Gilkes, M.D. Jessy, K.K. Ambily and N. Usha Nair	117
P-8	Under-Flora in Rubber Plantations: Its Effect on Soil Properties Joshua Abraham and Phebe Joseph	118
P-9	Estimates of Nitrogen Fixation by Leguminous Cover Crops Grown in Immature Rubber Plantations Using ¹⁵ N Isotope Dilution Technique Prasannakumari.P, Joseph.M, Jessy.M.D and Philip. A	120
P-10	High Density Planting in Rubber Plantations Annie Philip, Radha Lakshmanan, Jessy, M.D. and Mary Varghese	122
P-11	Nutrient Management in Rubber Seedling Nursery: Effect of Inorganic and Biofertilizers Syamala, V.K., Sherin George, Jessy, M.D. and Kochuthressiamma Joseph	124
P-12	Secondary and Micronutrient Nutrition in Rubber Seedling Nursery Mercykutty Joseph	125
P-13	High Density Planting - An Option for Higher Productivity of Rubber in North Eastern Region of India S. K. Dey and B. Datta	127

P-14	Available Boron Status and Its Relationships with Physico-Chemical Properties of Soils under Rubber Cultivation in North-East India M. Choudhury	128
P-15	Influence of NPK Fertilizers on Growth of RR II 400 Series Clones of Rubber (<i>Hevea Brasiliensis</i>) and Soil Properties in Tripura during Immature Phase Debasis Mandal, Bhaskar Datta, Tapan Kumar Pal and Sushil Kumar Dey	129
P-16	Tillage, Super Absorbent Polymer and Direct Seeding in Polybags can Mitigate Drought Effects During Early Stage of Rubber (<i>Hevea brasiliensis</i>) Plantation Jessy M.D. and Prasannakumari, P.	130
P-17	Macro and Micronutrient Status of the Traditional Rubber Growing Regions of South India A. Ulaganathan, V.K. Syamala and M.D. Jessy	131
P-18	Observed Changes in the Climate Extremes over Tripura, Northeast India Sailajadevi T	132
P-19	Possibility of using household waste as Nutrient carrier in Rubber plantations P.M. Mathews	133
FARM MECHANISATION		135
O-1	Mechanised Land Preparation for <i>Hevea</i> Planting and Plant Growth: A Case Study Radha Lakshmanan	136
O-2	Mechanised Agronomic Practices in Rubber Plantations – Indian Scenario Phebe Joseph and M.D.Jessy.	136
O-3	Mechanization of Ground Spraying in Rubber Plantations against Diseases Jacob Mathew, Sadanand K. Mushrif, Edwin Prem, Vijayan K. and Jatin S. Patel	137
O-4	Evolutionary changes in ground spraying technology for disease management in rubber plantation – An over view Jacob Mathew and Vijayan K	138

P-1	Mechanised Land Preparation for Rubber: Risks and Benefits Phebe Joseph and M.D., Jessy.	139
PLANT HEALTH MANAGEMENT		141
O-1	Integrated Disease Management of White Root Disease on <i>Hevea</i> Rubber in Indonesia by using Trichoderma-Based Biofungicide Triko Combi Budi Setyawan, Soekirman Pawirosoemardjo and Hananto Hadi	142
O-2	New Diseases Reported and Maladies Reached Epidemic Proportions in Rubber Plantations since late 20 th Century C.K Jayasinghe and K.M.S Tennakoon	143
O-3	Identifying Pathogenicity Genes in the Rubber Tree Anthracnose Fungus <i>Colletotrichum Gloeosporioides</i> through Random Insertional Mutagenesis Huang Guixiu	143
O-4	Surveillance on Corynespora Leaf Fall Disease: Incidence and Severity on Natural Rubber (<i>Hevea brasiliensis</i>) in Certain Hot Spots Areas in Kerala Sadanand K. Mushrif and Jacob Mathew	144
O-5	Environment and Farmer Friendly Biodegradable Rubber Spray Oil for Controlling Abnormal Leaf Fall Disease P.V Joseph, Simmi Datta, Pankaj Bhatnagar, Deepak Saxena, B. Basu and R.K Malhotra	145
O-6	Relative Abundance of Mistletoe in <i>Hevea</i> Plantation in Edo State, Nigeria Orumwense, K.O., Eguavoan, O. I., Aigbodion, A.I., Anegbeh, P.O and Omorusi, V.I	146
P-1	Pink Disease of <i>Hevea</i> Rubber in Northern Part of West Bengal and North East India G. C. Mondal, H. K. Deka, Shammi Raj and Sabu P. Idicula	146
P-2	Interaction of Certain Bacterial Antagonists of Rubber Pathogens with Fungicides Used in Rubber Cultivation Kochuthresiamma Joseph, P. K. Bijitha and Roshni Susan Elias	147

P-3	Effect of Pollen Substitute on the Development of Indian Honey Bee, <i>Apis Cerana Indica</i> during Dearth Period in Rubber Plantations S.Thankamony	148
P-4	Incidence of Tapping Panel Dryness in North-East India H.K.Deka, G.C.Mondal, Jacob Mathew and C.Kuruvilla Jacob	148
P-5	Frequency of Occurrence and the Role of <i>Colletotrichum</i> Species on the Colletotrichum Leaf Fall Disease of <i>Hevea brasiliensis</i> in the Traditional Rubber Growing Regions of Kerala Annakutty Joseph and Divya Das	149
P-6	Evaluation of Bio Pesticides and Insecticides on The Control of Bark Feeding Caterpillar, <i>Aetherastis Circulata</i> Attacking Rubber Trees S.Thankamony	151
P-7	Towards Understanding <i>Colletotrichum</i> “Species Complexes” Causing Leaf Disease in Rubber C. Bindu Roy, Jacob Mathew and Thakurdas Saha	151
P-8	Reaction of the Polycross Progeny of Prepotent Clones to Two Major Leaf Diseases of Rubber (<i>Hevea brasiliensis</i>) Sadanand K. Mushrif, Kavitha K. Mydin and E.Edwin Prem	153
P-9	Management of Purple Root Disease of <i>Hevea</i> Seedlings in Nursery G. C. Mondal, H. K. Deka and Sabu P. Idicula	154
P-10	Environmental Factors Associated with Buildup of High Density <i>Aedes Albopictus</i> Vector of Chikunguniya Virus in Rubber Plantations Shammi Raj, Pradeep Kumar N., Jacob Mathew, Jose V.T., Thankamani S. and Sabesan S.	156

CROP PHYSIOLOGY/ BIOCHEMISTRY

O-1	A Study on Chlorophyll Fluorescence, Soil Acidity, Soil Moisture Contents and Plant Height in Relation to Different Polybag Size for Rubber Planting Materials During Transportation and after Field Transplanting Wan Zuraidi Sulaiman, Mohd. Nasaruddin Bin Mohd. Aris	
-----	--	--

	and Zarawi Abd. Ghani	158
O-2	Impact of Salicylic Acid Treatment to Enhance Drought Tolerance of Nursery Rubber Plants (<i>Hevea brasiliensis</i> Mull.Arg) When Introducing Them to Monaragala District in the Intermediate Zone of Sri Lanka S A Nakandala, K D N Weerasinghe, P Seneviratne, S M M Iqbal, J Jayasanka and P D Pathirana	159
O-3	Hydrogen Peroxide is Essential for Wound-Induced Secondary Laticifer Differentiation via Activating Jasmonate Biosynthesis in Rubber Tree (<i>Hevea brasiliensis</i> Müll. Arg.) Wei-Min Tian, Shu-guang Yang, Min-jing Shi, Ji-Lin Wu, Bing-Zhong Hao	160
O-4	ATP Concentration in Latex as a Biochemical Marker for Early Evaluation of Yield in <i>Hevea brasiliensis</i> S.Sreelatha, James Jacob, V.C.Mercykutty, Sheela.P.Simon, R. Krishnakumar and K. Annamalainathan	161
O-5	Impacts of Water Stress on Gas Exchange, Water Relations and Chlorophyll Content in Five <i>Hevea brasiliensis</i> Clones Noorliana Mohd Zan	162
O-6	<i>Hevea</i> B Serum Proteome Profiling Using Liquid Chromatography-Mass Spectrometry Norazreen Abd Rahman and Siti Arija Mad Arif	164
O-7	Drought Tolerance in Transgenic Mnsod <i>Hevea brasiliensis</i> in A Dry Humid Environment K.V.Sumesh, P.R.Satheesh, S.Sreelatha, S.Ravichandran, A.Thulaseedharan, R.Krishnakumar, K.Annamalainathan, Meena Singh and James Jacob	165
P-1	Supercritical Fluid Extraction of Flavonoids From <i>Hevea</i> Leaves Nurul Hainiza Abd Razak, Norazreen Abd Rahman and Siti Arija Mad Arif	166
P-2	Study on Lignin and Cellulose Total Content Iin Two Rubber Clones Planted in Different Densities Rasyidah binti Mohamad Razar and Mohd. Nasaruddin bin Mohd. Aris	166
P-3	Plant Hormones and Oxidative Stress in <i>Hevea brasiliensis</i> R. Krishnakumar, P. K. Ambily and James Jacob	168
P-4	Studies on Cold Tolerance of <i>Hevea brasiliensis</i> (Muell. Arg.) Clones under Controlled Environmental Conditions	

	J. Sarkar, B. Remya, K. Annamalainathan and R. Krishnakumar	169
P-5	Localization of Peroxidase Enzyme in the Bark of <i>Hevea brasiliensis</i> Gopika Gopal and Vinoth Thomas	170
	CROP HARVESTING AND POST HARVEST	171
O-1	Influence of age and girth at opening on rubber yield, biochemical and tapping panel dryness parameters of <i>Hevea brasiliensis</i> in determining tapping norms Obouayeba Samuel, Soumahin Francis Eric, Kouassi Kan Modeste, Coulibaly Lacina Fanlégué., Koffi Mathurin Okoma, Angelo Evariste Badou N'guessan, Kouamé Christophe, Aké Sévérin	172
O-2	Impact of The Reduction of The Tapping Frequency on the Agronomic and Physiological Parameters of Clone PB 260 of <i>Hevea brasiliensis</i> in the Centre West of Cote D'ivoire in Order to Make Up For The Shortage of Tapping Labour Eric Francis Soumahin, Guy Joël Olivier Atsin, Hilaire Tanoh Kouakou, Lancina Falengué Coulibaly, Sahabane Mohamed Traore, Joseph Yamoussou Alle, Samuel Obouayeba	173
O-3	Alternative Tapping Systems for RRIC 100 Clone From Opening Eva Herlinawati and Kuswanhadi	174
O-4	Rain Guarding is Essential for Introducing Modern Methods of Latex Harvest Technology K.R Vijayakumar	174
O-5	Influence of Various Tapping Systems on Rubber Yield of Two Clones RRIV 3 and PB 260 at South-East Region in Vietnam Kim Thi Thuy, Do Kim Thanh, Nguyen Nang, Nguyen Thi Thanh Thao and Nguyen Quoc Viet	175
O-6	Response of Several IRR Series <i>Hevea</i> Clone to Tapping System with Stimulation Kuswanhadi, Eva Herlinawati and M. Lasminingsih	176
O-7	Anaerobic Digestion of Rubber Waste for Production of Renewable Energy A. I. Aigbodion, S. O. Omorogbe, D. A. Olugbemide and E. U. Ikhuoria	176

P-1	Concept of Crop Secondary Yield and Its Application in Research and Production of <i>Hevea</i> Tree Lin Weifu	177
P-2	The Effect of Rain Guarding on Reducing Latex Loss K Thomas Wijaya	178
P-3	Aerobic Digestion through Air Diffusion for the Effective Treatment of RSS Processing Effluent Harish M and Jacob Mathew	178
P-4	Effect of Panel Changing on Long Term Yield Response of <i>Hevea brasiliensis</i> (Clone RRII 105) under Different Frequencies of Tapping and Stimulation R. Rajagopal, K.U.Thomas and K.Karunaichamy	180
P-5	Long Term Yield Response of Low Frequency Tapping and Yield Stimulation in Clone RRII 105 K. Karunaichamy, K.U. Thomas and R. Rajagopal	181
ECOSYSTEM PROCESS		183
O-1	A Study on Carbon Sequestration in Various Plant Parts of Two <i>Hevea</i> Clones Planted in Four Planting Densities Mohd. Nasaruddin bin Mohd. Aris and Rasyidah Mohamad Razar	184
O-2	Measurement of Co ₂ Flux in Rubber Plantation by Eddy Covariance Method K. Annamalinathan, P. R. Satheesh and James Jacob	185
O-3	Seasonal Changes in Xylem Sap Flow Rate in Mature Rubber Trees K. Annamalinathan, Joby Joseph, Badre Alam and James Jacob	186
P-1	Distribution of <i>Hevea brasiliensis</i> in South and Northeast India: Preliminary Results with Maximum Entropy Species Distribution Model Debabrata Ray, VS Chitale, James Jacob and MD Behera	187
GEOSPATIAL TECHNOLOGY		189
O-1	Assessing Agricultural Drought in Natural Rubber Plantations Using MODIS/Terra Satellite Data Meti S., Shebin S.M., James Jacob., Pradeep B., and Jessy, M.D.	190

O-2	Application of Light Detection and Ranging (Lidar) Technology for Field Monitoring Safiah Atan, Mohd. Shahir Shamsir, Cheong Siew Chin, Ong Chee Wei, Nor Azira Abu Bakar and Suzanna N. Azmy	190
O-3	Digital Image Recognition System for Rubber Clones Produced in Malaysia Ong Chin Wei, Shamsul Bahri Abdul Razak and Badrul Ezam Baharuddin	191
O-4	Estimation of Above Ground Woody Biomass in Natural Rubber Plantation Using Remote Sensing and GIS Technique ebabrata Ray and James Jacob	192
O-5	Retrieving of Leaf Area Index for Rubber Plantation by Using HJ-1A/1B CCD Data Bangqian Chen, Zhixiang Wu, Junming Chen, Jikun Wang, Guoyu Lan, Chuan Yang, Jianhua Cao, Zhongliang Tao, Guishui Xie	194
P- 1	Spatio - Temporal Analysis of Rubber Area Changes in Kanniyakumri District of Tamil Nadu Using Remote Sensing and GIS Meti S., Pradeep B., James Jacob and Jessy, M.D.	195
ECONOMICS		197
O-1	An Economic Analysis of the Socio-Economic Dimensions of Participatory Experimental Trials on Low Frequency Tapping (LFT) in Kerala Binni Chandy, K.U. Thomas, S. Veeraputhran, Siju, T.	198
O-2	Trends in Adoption of Planting Density in Small holdings in the Traditional Natural Rubber Growing Regions of India T. Siju, S. Veeraputhran, Joby Joseph and Tharian George K.	199
O-3	Uncertain Prices and Segmentation of Market as a Survival Strategy: The Case of Latex Processing Industry in India Joby Joseph and Tharian George K	199
P-1	Marketing Efficiency of Organized and Unorganized Rubber Growers in Tripura: A Comparative Analysis Gaurav Sharma, Tharian George K., S. Veeraputhran, Joy Joseph and S.K. Dey	200

P-2	Adoption of Intercrops across Size-classes and Regions: A Study of Traditional Rubber Growing Regions in India T. Siju, Tharian George K., S. Veeraputhran and Joby Joseph	201
P-3	Adoption of RRII 400 Series Rubber Clones by Rubber Small Growers S.Veeraputhran, Siju.T, Joby Joseph, and Tharian George. K	201
EXTENSION		203
O-1	AEC - Challenges and Opportunities for Thailand Rubber Industries Mr. Suthee Intraskul	204
O-2	The Impact of Extension Approaches for Enhancing Productivity of NR – the Indian Experience with Special Reference to Clone RRII 105 B. Rajeevan, J. Thomas, D. Anil Kumar, Ramesh B. Nair	205
O-3	Participatory Rubber Extension in Tripura-Case Study of Manimalayar Rubbers (P) Ltd. Arunabha Majumdar, L Anita Devi, Shyamal Sen, M Narayan Potti	206
O-4	Rubber Clinic: A Distance Diagnostic and Information System for the Management of Pests and Diseases of Natural Rubber Jacob Mathew, Thomson Abraham and Biju B.	207
P-1	Relevance of Productivity Enhancement in Rubber Plantations of Meghalaya- A Case Study P.K.Raghunath and K.P.Rajeev	208
P-2	Remote Plantation Management (RPM) of Karimplavelil Rubber Plantations, Punalur, Kerala: A case study Usha Rani.S., Biju C. Oommen J.Thomas	208
P-3	Yield Modelling and Analysing the Critical Factors of Productivity of Natural rubber (<i>Hevea brasiliensis</i>) Suja S Nair and Ramesh B Nair	209
GROWERS INNOVATIONS		211
GI-1	Production of Best Quality Rubber Planting Materials the Cheerakuzhy Innovations Jose Cheerakuzhy	212

GI-2	Innovative Approach towards Treatment of Powdery Skimin Centrifuge Factories and Management of Replanting large area – HML experience Santosh Kumar	213
GI-3	Harvesting and Latex Collection: My Innovations Joseph C.J.	214
RUBBER TECHNOLOGY		215
O-1	Effect of Modification Technique on Properties of Blends of Natural Rubber and Modified Tyre Crumbs D.G. Edirisinghe, W.D.M. Sampath and M.K. Mahanama	216
O-2	Preparation and Mechanical Property of the Epoxidized Natural Rubber from Field Latex Adul Na Wichian and Nuchanat Na Ranong	217
O-3	Natural Rubber Nanocomposites from Pristine and Organically Modified Layered Silicates by Melt Intercalation Process Siby Varghese, K. N. Madhusoodhanan and Rejitha Rajan	217
O-4	A Process for Preparation of Carbon Black / Silica / Nanoclay Master Batch from Fresh Natural Rubber Latex K.K. Sasidharan, Rosamma Alex and Thomas Kurian	218
O-5	Viscosity Behaviour and its Influence in Natural Rubber and Synthetic Latex Processing and Product Manufacturing Systems Joseph John, Preethy Paul, E.Ponce, G. Lottmann and R. K. Matthan	219
O-6	Use of Coconut Pith as Bio Filler for EPDM Rubber Composites P.Ranjith, K.Rajkumar, P Thavamani, Golok B Nando	219
O-7	Silica Reinforcement of Epoxidised Natural Rubber of Varying Epoxy Content Jacob K.Varkey, Sadhan K.De and K.T.Thomas	220
O-8	Stable Free Radical Assisted Peroxide Vulcanisation: Cure Characteristics and Vulcanisate Properties Benny George and Rosamma Alex	221
P-1	Processing of Nano Fillers in Rubber – A Novel Technique K.Rajkumar, P.Thavamani, P.Jeyanthi P.Pazhanisamy	221
P-2	Low Protein Natural Rubber Processed Through Gamma Ray Irradiation Technology K. N. Madhusoodanan, Rosamma Alex, John Britto and Sadeesh Babu P.S	222

P-3	Multicomponent Nylon/Coir Pith/Epoxy Composites for Particle Board Applications R.Narendar, K. Priya Dasan	222
P-4	Synthesis of ZNO Nano Particles, Their Characterization and Their Effect on Stability and Storage on LA-TZ Latex Anand. K, Siby Varghese and Thomas Kurian	223
P-5	Mechanical, Thermal, Solvent Transport and Biodegradation Behavior of Cellulose Microfibres (CMF) / Poly (Ethylene-Co-Vinyl Acetate) (EVA) Composites A. Sonia and K. Priya Dasan	223
P-6	Nano Silver Doped Water Soluble Hyperbranched Polyesters for Coating Applications C. Kavitha and K. Priya Dasan	224
P-7	Preparation and Characterization of Natural Rubber-Rice Husk Ash Composites Ayswarya E.P and Eby Thomas Thachil	224
P-8	Effect of CarbonBlack and BaSO ₄ on the Thermo-Chemical Resistance of Natural Rubber Composite Nisha Nandakumar and Philip Kurian	225
P-9	Natural Rubber Compounding- A Possible Route to Combat Environmental Littering due to Waste EPS. Renju V. S. and Eby Thomas Thachil	226
P-10	Natural Rubber Based Proton Conductive Membrane for Fuel Cell Applications: A Review Sandhya K and P.Predeep	227
P-11	Less Labour Intensive Processing System [LELIPS]- A New Approach for NR Processing C Mathew Joseph	228

SPECIAL TALKS

INDUSTRY

ST-2

An Alternate Approach to NR Demand Estimation in India: Tyre Industry Proposal

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The share of tyre sector in India's natural rubber (NR) consumption has witnessed a progressive increase from less than 50% to nearly 65% in the last one decade. This consumption growth has been driven by robust growth in GDP and the resultant impressive growth in automobile sector, spurring tyre and NR demand / consumption.

Recent years, from 2008 onwards, have also witnessed volatility in growth, especially in automobiles and tyre sectors. From spectacular double digit growth to a dip in *yoy*, Industry has experienced unparallel volatility in demand, prices, capacity creation etc.

In such a dynamic and constantly changing environment, it is important to have a fair and reliable estimation of NR demand from medium and long term policy perspective. NR demand estimation is also imperative as India moves into a phase of NR demand outstripping its domestic availability.

As a proactive and representative body of Tyre Industry and a key stakeholder in the NR value chain, Automotive Tyre Manufacturers' Association (ATMA) has evolved an alternate approach to NR Demand Estimation in India, a proposal which has since been shared with the Rubber Board of India.

This presentation will provide an insight into the proposal and its usefulness as a tool from policy planning perspective.

ST-3

Indian Non-Tyre Industry - An Overview

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India is a large market for the products it makes and the purchasing power of the average Indian is steadily on the rise. There is a sizable pool of young employable people who are skilled and productive. India manufactures a wide range of products and its technologists and engineers are some of the best in the World.

There are several disadvantages in the Indian Rubber Industry, particularly in the non tyre sector. Availability of skilled manpower is a serious drawback. Similarly availability of raw materials, especially natural rubber is assured quality and quality at reasonable price is a major concern. Since 85% of the units are in the MSME sector, latest technology is not accessible or affordable for most. Better Infrastructure is immediately required. Delayed decisions or sometimes lack of a decision from the government is another concern.

ST-4

Indian Synthetic Rubber Industry - Prospective

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Globally, India figures amongst top five consumers of Rubber. Indian rubber industry is unique in pattern of rubber consumption; Natural rubber consumption is significantly higher as compared to that of synthetic rubber. Natural and Synthetic rubber are complimentary to each other, with each kind of rubber having it's own unique property. The presentation looks into underlying reason behind higher NR consumption, which is more linked with the application rather than Indian industry's resistance to switch over to synthetic rubber. Indian rubber industry was amongst early adopters of synthetic rubber; existence of SBR plant way back in 60's and setting up of PBR plant in 70's are some good examples.

India's relative consumption of synthetic rubbers as compared to natural rubber is on rise and it is likely that the trend will continue. Indian capacity of synthetic rubber is going up by almost 400 KTA, Reliance and IOC have announced expansion plans

Global NR Prices: Retrospect and Prospect

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Natural rubber prices have scaled unprecedented peaks and downslides during the period 2007-2012. The paper attempts to analyse the global trends in rubber prices during the period from January 2007 to September 2012 with reference to the commodity's demand & supply, crude oil market, currency trends and speculative influence of relevant events. It also attempts to provide a short-term outlook of the market by taking into account the emerging trends in demand and supply and other drivers.

PLANTATION

ST-7

Rajtree Model Program For the Philippine Rubber Development

Rommel A. Jalosjos

Provincial Governor
Province of Zamboanga Sibugay, The Philippines.

In the year 2010, the insight for the rubber expansion-replanting program was developed with the common goal of promoting and implementing “environmentally” friendly way of maintaining and sustaining high quality rubber and other agricultural products in the Province of Zamboanga Sibugay.

Our mission does not focus on the expansion and production alone, it also focuses in bringing and putting into action in saving and protecting mother earth against climate change. We believe that planting rubber trees will largely contribute in reducing dangerous global warming.

The leaders and the people of Zamboanga Sibugay have a vision to act in creating programs that will open the doors for protection of the environment, global awareness, development and growth of both the industry and the people for a sustainable future.

We believe that together, sharing the same goal towards a brighter tomorrow, for the future generations, we have began a journey to make it all happen.

The RAJTREE Program is a strategic program to protect the welfare of Sibugaynons. Governor Rommel A. Jalosjos of Zamboanga Sibugay together with his Consultants and Provincial officials came up with the idea to expand and enhance the rubber industry of the province of Zamboanga Sibugay. Priority development areas are those covered by the Community-Base Forest Management areas (CBFM), Ancestral Domains, Protected areas declared under NIPAS and Socialized Integrated Forestry Management Agreement (SIFMA). The program seeks to maximize and offer great opportunities to deliver social co-benefits.

The program targets biodiversity conservation. RAJTREE Program recognizes the need for local government units (LGU) to create opportunities for our communities to engage in participatory planning and multi-stakeholder approaches as a main approach to stronger policies and to increase communication and coordination among stakeholders, agencies and other sectors with links to environmental protection, conservation and food chain security. The strategic planning, and implementation will be strengthened by convergence

of all Government sectors headed by the Provincial Local Government Unit (PLGU), Department of Agriculture (DA), Department of Environment and Natural Resources (DENR), Department of Labor and Employment (DOLE), Department of Social Welfare and Development (DSWD), Department of Education (DEPED), TESDA, Municipal, Barangay Local Government Units (BLGU), with the farmers of the province. The participation of the willing and qualified private sectors and investors to provide financial assistance to all stakeholders are welcome in the program.

Major program Components are:

- Technical and Other Infrastructure Development
- Institutional Development
- Agricultural Development
- Human Resource Development
- Production and Marketing
- Processing and Manufacturing

The paper describes in detail, various components and its implementation.

ST-8

Expanding NR Supply Chain for Indian Rubber Industry

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Development of rubber sector in India has been a success story. While the consumption of Natural Rubber (NR) maintained a steady pace of growth during the last several decades, a vibrant production system supported the industry by way of making available NR, the vital raw material, to meet almost the entire demand of the industry. The country however still continues to be a net importer of NR and future projections are of a widening gap between production and consumption. It is also a fact that the domestic production of NR cannot keep pace with projected increased consumption. Scope of increasing production beyond the current levels of growth rate is limited considering the several constraints. Moreover ensuring supply of NR through public funded social initiatives has limitations in this era of liberalisation, beyond a limit. This points to the need for effective institutional mechanisms including PPP / PCP to be adopted with the involvement of consuming industry there by creating additional production capacities and a new supply chain to

supplement the existing one to ensure availability of NR. As the most important factor for production, suitable land, is not available in large parcels in India, one has to look for countries like Africa to establish large plantations. The paper presents the potential for expansion for NR production base both within and outside the country with strong manufacturing Industry participation and the scope of aligning such programmes to Govt of India's policy on its engagement with Africa.

ST-9

Strategy for R&D Driven Open Innovation (OI)

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“Open Innovation (OI)” aims at greater R&D agility and performance and reduce risk exposure. Open innovation is based on the paradigm that opening up of the innovation funnel to enable the R&D organizations to absorb external expertise and/or to identify new paths to market for the R&D's Intellectual Property (IP). OI focuses on the active communications of intellectual property; absorption of ideas and solutions from customers; and refer to R&D-driven open innovation such as “open approaches to innovation” context. This approach sheds new light on the role of OI in cultural and behavioural changes across R&D organization. On the journey of establishing the OI capability, we need to learn quickly what skills matter and how to find people who embody them. As such, our strategic actions are geared towards: leaders interested in building an OI, capability within R&D, nurturing an OI department in identifying high potential OI scientist(s).

Although the era of open innovation has begun for many R&D organizations, we still need to understand a clear mechanisms, inside and outside of the organization, when and how to fully benefit multi-disciplinary team from the concept. The advantages of cooperation in R&D set-up and global integration are increasing in the OI era. As the focus shifted from purely internal R&D activities, the research community started emphasizing that the R&D organization should be open to outside innovation.

This session provides a broad awareness on OI and its relevance to cooperative R&D in global rubber sector, the necessary knowledge to do a critical assessment of existing approaches to innovation, to identify additional opportunity, to inspire and to plan a professional implementation of open innovation portfolio in place. The implications and trends that underpin open innovation will be discussed in terms of strategic, organizational, behavioural, knowledge, legal and commercialization perspective. To this end, an open

innovation readiness within core 3 processes viz., the outside-in, the inside-out and the coupled processes will also be discussed to have a positive effect on R&D performance. In other words, there are important implications for new and emerging methods of R&D in change management.

ST-10

Seeding Rubber's Future

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For several years PanAridus has been quietly working on identifying the major issues necessary for commercial success of guayule. The most critical aspect to economic success is the ability to produce guayule rubber that is profitable in a commodity based market. This not only requires a keen vision and understanding of agribusiness, it requires genetic superiority.

PanAridus has not only acquired the world's largest germplasm bank in guayule; its team of world renowned experts developing patented genetic strains in their Casa Grande facility; and their patent pending extraction process.

Now PanAridus breaks another industry milestone with the release of guayule rubber samples for the industry.

The vision at PanAridus has always been to bring over 100 years of history with guayule to commercialization and it begins with "seeding rubber's future".

INVITED TALKS

IT-2

Soft and Hard Nanoparticles in Natural Rubber

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Rubber requires reinforcement for useful properties. Usually fillers like carbon black, silica etc. are used for reinforcement. Filler - reinforced rubbers are called composites. If the fillers are nanoparticles, these may be called nanocomposites. In the present paper, both soft and hard nanoparticles have been chosen for preparing nanocomposites based on natural rubber. Synthesis and characterization of both these nanoparticles will be presented. Hard nanoparticles like montmorillonite, hectorite, double layer hydroxide, carbon nanoparticles, zinc oxide, silica etc. can be dispersed by using particular processing methods. Soft nanoparticles were synthesized in the laboratory. The effect of these nanoparticles on the desired properties, their dispersion and filler-polymer interaction will be described. All these nanoparticles impart superior mechanical, dynamic mechanical, thermal, wear and barrier properties to their macrocomposites even at a low loading. Their structure property relationship will be highlighted.

IT-3

Future Challenges for Rubber Industry

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Rubber industry across the globe is undergoing tremendous pressure due to ever-growing demand from customers and volatile market. Now, with the phenomenal growth of automotive and tyre industries, there is an increasing demand for rubber. The tyre industry being the largest consumer of the rubber is most affected by any fluctuations in the market, whether it is soaring natural rubber prices or crude oil prices. The challenges for tyre industry are highly fluctuating price of natural rubber and crude oil prices resulting in escalating cost of synthetic rubber, ever increasing consumption demand from growing economies like India and China, etc. Challenges faced due to decline in production of natural rubber, owing to climate change, shortage of cultivable land, high labour cost, lingering

fear of South American Leaf Blight (SALB) etc. have forced rubber manufacturers to turn towards synthetic rubber alternatives. Besides crude oil prices, synthetic rubber market is also subject to environmental regulations, etc. which has raised a question mark over its future.

There are several other challenges owing to obligations like REACH norms, tyre labelling, low rolling resistance and reduced carbon footprints. REACH requires 'downstream users' such as the tyre and rubber industry to gather information on the properties of the substances that they use, to promote their safe management and to progressively substitute the most dangerous substances with safer alternatives. Tyre labelling requires the manufacturer to display information on the fuel efficiency, wet grip and external rolling noise of tyres with an aim to increase safety, environmental and economic efficiency of road transport by promoting fuel-efficient and safe tyres with low noise levels. Efforts for the eco-friendliness of tyres are growing, though, with an emphasis for using bio-based raw materials. Tyre companies are investigating more speculative ways to use sustainable and renewable rubber formulations, including latex derived from alternative sources. Tyre industry is also keen in reducing overall CO₂ emission in the manufacturing process. In this journey, concepts like incorporation of fillers in latex stage can make a revolutionary change. Another challenge nowadays is the end life of tyre management. Tyres are banned from landfills in most of the developed countries. Various efforts are currently underway in many countries where different legal systems exist to reduce the number of tyres in landfills and waste piles and to find innovative, environment friendly uses for end of life tyre. Scientific community in tyre industry, research institutions, and academia should join hands together to meet these challenges.

IT-4

Nonlinear Viscoelasticity of Rubber Nanocomposites

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Nonlinear viscoelasticity of rubber nanocomposites (Payne effect and Mullin's effect) has received a lot attention during the last few years since rubber nanocomposites are being used in dynamic applications. The Payne effect is a very interesting feature of the dynamic behaviour of rubber nanocomposites, especially rubber compounds containing fillers such as carbon black, carbon nanotubes, silica etc. from macro to nanoscales. The Payne effect is named after the British rubber scientist A. R. Payne, who

made extensive studies of the effect (e.g. Payne 1962). The effect is sometimes also known as the Fletcher-Gent effect, after the authors of the first study of the phenomenon (Fletcher and Gent 1953). The effect is observed under cyclic loading conditions with small strain amplitudes, and is manifest as a dependence of the viscoelastic storage modulus on the amplitude of the applied strain. Above approximately 0.1% strain amplitude, the storage modulus decreases rapidly with increasing amplitude. At sufficiently large strain amplitudes (roughly 20%), the storage modulus approaches a lower bound. In that region where the storage modulus decreases the loss modulus shows a maximum. The Payne effect depends on the filler content of the material and vanishes for unfilled elastomers. Physically, the Payne effect can be attributed to deformation-induced changes in the material's microstructure, i.e. to breakage and recovery of weak physical bonds linking adjacent filler clusters. Since the Payne effect is essential for the frequency and amplitude-dependent dynamic stiffness and damping behaviour of rubber bushings, automotive tyres and other products, constitutive models to represent it have been developed in the past (e.g. Lion *et al.*, 2003). Similar to the Payne effect under small deformations is the Mullins effect that is observed under large deformations. In the present talk, the Payne effect and Mullins effect of nanocomposites will be presented. Fillers such as nanosilica, carbon black, carbon nanotubes, nanoclay etc. show strong Payne effect in rubbers. The effect of coupling agent and temperature on the Payne effect has been carefully studied. The variable density model of Goritz and Mayer has been utilised to model the behaviour of the nanocomposites. The model gave an excellent fit with the experimental.

LEAD TALK

Genomics Assisted Crop Improvement – Applications and Potential in Tree Improvement

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The ever increasing human population is expected to create an unprecedented demand on the production of food, fiber and fuel. Predictions indicate that an increase in production of agricultural crops needs to be increased by 25 to 30% in the next 15 years to remain food and nutrient secure. Besides these food grains, there is an equal need for the enhancement of productivity and total production of several commercially important tree species. These realizations have provided a tremendous impetus to the evolution of systematic investigations in tree species leading to genetic enhancement to improve productivity as well quality.

Tree improvement has traditionally been impeded due to the lack of characterized germplasm, lack of clarity on the mechanisms governing variability in growth and productivity, complexity associated with the measurement of important traits in trees, complex and large size of the genome, etc. One of the most important factors that significantly distracted tree improvement programs was the long generation times of most of these perennial plants. However, considering the importance and the projected demand for the products of tree species, there is a very urgent need to evolve mechanisms to enhance productivity of important plantations crops and to increase the breeding efficiency.

Conventional tree improvement programs have concentrated on identifying superior clones and exploiting them for either vegetative propagation or for obtaining seeds from such trees. This approach would take a painfully long time besides being unable to guarantee a success in improvement in productivity. These issues have very strongly necessitated the application of modern biotechnological methods in tree improvement programs. The significant extent of out crossing among most tree species, the available genetic diversity in every trait among the clones and ramets render the tree species a fabulous material for adopting the modern biotechnological tools for Genomics assisted crop improvement tools. With the advent of the next generation sequencing strategies, significant progress has been made in sequencing the entire genome of several important tree species. Poplar and Eucalyptus are the most extensively sequenced plantation crops and a large number of other species are presently being sequenced globally.

With the application of genomic tools becoming inevitable in tree improvement programs, it is essential to develop deeper understanding of the science and technology associated with genomics based applications in crop improvement. The most simple method

in genomics assisted crop improvement is the use of DNA based molecular markers. Traditionally, these DNA markers are defined as DNA fragments that generally do not have a regulatory influence on any plant trait. Several marker systems such as RFLP, RAPD, AFLP have been used with limited success in trees. It is now realized that co-dominant marker systems such as SSR and SNP have greater applicability in mapping the genomic regions that govern the variability in a given trait. The advent of the next generation sequencing strategies have provided a significant boost to the generation of sequencing information on transcriptomes besides the whole genome in several species. This paved way to the rapid discovery of SSR and SNP markers in tree species which was till recently, a major constraint in adopting genomics assisted crop improvement methods. These advancement in biotechnological tool was to certain extent matched by the progress made in computational and statistical methods that led to the development of precise strategies for identifying the exact genomic region that govern a trait and thus made it possible for functional validation of genic regions through functional genomics and provided options for drawing inferences from related species through comparative genomics. Though modern genomics methods have a tremendous application in tree improvement programs, these strategies would have a significant bottle neck due to the long generation time of these tree species.

Excellent progress in advancement of computational statistics combined with genomics tools is expected to significantly overcome these constraints leading to rapid progress in tree improvement. With the tremendous diversity available among tree species and the extent of out crossing render the tree species an extremely amenable candidate for QTL discovery through the application of population genetic approaches, referred to as Association mapping or LD mapping. This approach involves the extensive molecular and phenotypic characterization of a set of diverse individuals and associating the marker diversity with phenotypic diversity to identify genomic locations that govern variability in the traits interest. Conventionally, the identification of genomic regions controlling complex traits called quantitative trait loci (QTL) is achieved by generating a structured population by crossing two lines contrasting for the trait of interest. This approach, though accurate, would be extremely difficult (if not impossible) to adopt in perennial species. Thus, association mapping has been proposed and is extensively being adopted globally for QTL discovery. Now that the marker discovery is made easy through sequencing approaches, what seems to be essential is the assembly of a set of diverse germplasm accessions and enumeration and development of phenotyping strategies.

More recently, a novel method of marker assisted selection combined with association mapping, referred to as Genomic Selection, is being adopted for reducing the generation time while increasing the precision of trait improvement. This approach has been successfully adopted in improving the yield and quality of oil palm. This approach significantly reduced the time required for the generation of stabilized lines with the targeted improvement in the traits of interest.

Critical issues pertaining to the marker development, application of these genomic tools and the importance of generating genetic resources in genetic improvement of plantation crops will be di

CONTRIBUTED PAPERS

SESSION 1

CROP IMPROVEMENT

Study on Performance of Clones in SSCT Derived from Hybridization among 1981 *Hevea* Germplasm and Oriental Clones

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Over the past fifty years of rubber breeding programme, the small population introduced by Wickham was successfully increased in yield production. However, yield increases will not be expected with existing Wickham germplasm due to inbreeding depression, unless there is a new introduction of variability. In order to overcome the narrow genetic base of *Hevea* population in Malaysia, several attempts has been carried out to broaden the genetic base such as an introductions of new genetic materials. More than 10,000 genotypes of the 1981 *Hevea* germplasm were evaluated and some of the promising genotypes were incorporated in the breeding programme by crossing with high yielding oriental clones. The objective of this paper is to evaluate the growth performance and latex production of clones for selection of improved breeding materials.

A total of forty three progenies derived from 13 crosses between 1981 *Hevea* germplasm and oriental clones of the 1990 hand pollination programme were evaluated in Small Scale Clone Trial. The experimental design was 10 x 10 simple lattices with two replications. This trial was carried out with planting distance of 30' x 9'. The girth measurement of each progenies was carried out from the age of two years after planting and repeated annually. The progenies were opened for tapping at the seven years after planting with 50% of the plants reaches tappable size when the girth is at least 45 cm using the half spiral tapping cut and tapped third daily tapping system. The bark thickness measurement was carried out after three years of tapping period.

Latex production for three years of tapping period of the progenies tested ranged from the highest 74.43 g/t/t (F5/21) to 5.30 g/t/t (N30/14). Twelve progenies showed mean yield better than PB 260. However, only four genotypes; F2/51 (74.43 g/t/t), K42/14 (73.93 g/t/t), I12/21 (68.50 g/t/t) and O27/11 (64.98 g/t/t) showed significantly higher mean yield than PB 260 (44.28 g/t/t) based on least significant difference test.

Results for girth performance showed that J14/22 had the highest mean girth of 65.0 cm and 74.6 cm after five and seven years after planting respectively while J16/15 had the lowest mean girth of 36.1 cm and 43.5 cm after five and seven years of planting respectively. After five years of planting, thirty eight progenies were found to have mean girth

measurement more than 45 cm. Eight progenies showed significantly higher mean girth measurement than PB 260 when the results were subjected to least significant different test for mean comparison i.e. J14/22 (65.0 cm), K42/14 (62.9 cm), N34/14 (61.2 cm), C2/11 (61.2 cm), F7/3 (59.6 cm), J18/3 (59.1 cm), J14/8 (59.0 cm), and W39/12 (58.9 cm). Out of 32 progenies, 14 progenies showed significantly higher mean girth measurement than PB 260 after seven years of planting based on least significant difference test. However, only four progenies i.e. J14/22, N34/14, J14/8 and W39/12 were found to have significantly higher increment than PB 260. Out of 39 progenies, 17 were found to have significantly higher increment than PB 260 during tapping period.

In term of bark thickness, genotype I12/21 showed significantly higher virgin bark and renewed bark after three years of tapping than PB 260 with 9.9 mm and 7.9 mm respectively. On the other hand, genotype J16/15 showed significantly lowest virgin bark with 5.6 mm while N24/6 showed significantly lowest renewed bark with 4.9 mm.

It was reported that germplasm materials are vigorous and possesses long straight boles (logs) that suitable for timber production (Ramli et. al, 1995) however, the latex production is low (Masahuling, 2005). As the additive genetic effect controlled the growth performance and latex yield production (Simmond, 1969; Tan and Subramaniam, 1975), it is suggested to incorporate the 1981 germplasm materials into the rubber breeding programme through cross-breeding. Thus, increase both growth vigour and latex production within the hybrid, thus producing better latex timber clones in the future. In addition, the introductions of germplasm materials into the rubber breeding programme are essential in order to improve the quality of future planting material.

O-2

Performances of RRIV's Clones in Non-Traditional Rubber Growing Regions of Vietnam

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As non-traditional rubber growing regions, the Northern part of Vietnam presents various stress factors, particularly climate features such as prolong low temperature and low solar radiation in the winter, very high air temperature in the summer, strong wind etc. Acclimatization of rubber trees to non-traditional region has gained the attention of the rubber plantation industry and policy makers to meet the increasing demand for natural rubber. In this context, evolving planting material for cultivating rubber trees in such area

is one of the mandates of research. Experiments were conducted in the Northern part of Vietnam, located between 19°19N (Nghe An) and 22°33N (Lai Chau), involving several *Hevea* clones, of which the majority are RRIV's clones. These clones have been evaluated for their adaptabilities and performances in the cold climate. As the results in SSCT in Nghe An and Phu Tho provinces show, many RRIV's clones have not only showed a good adaptability to cold climate but also good yield performances in comparison with the recommended clones, RRIM 600 and GT 1. Among them, RRIV 1 and RRIV 104 have been considered as the most promising clones in these areas. In addition, various LSCT and Block planting trials have been also conducted in the northern mountainous region since 2008 with a large number of RRIV's clones. At the immature period, low temperature showed to affect the growth of these clones, however, the severity of influence varied among clones. The results indicated that the performance of the clone RRIV 124 was not only the most stable in all clonal trials but also in large planting areas due to its cold tolerance and recovery after serious cold-waves. The other promising RRIV's clones were RRIV 210 and RRIV 125.

O-3

Recombination Breeding of *Hevea brasiliensis* in India: Clones Evolved From the 1983 Hybridization Programme

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Genetic improvement of rubber in India was initiated with recombination breeding programmes which led to the development of the RRII 100, 200, 300 and 400 series of hybrids. These clones were instrumental in bringing about significant improvement in productivity of rubber in India. The present study deals with small scale evaluation of 110 hybrid clones developed by hybridization in six cross combinations among 12 parents and 5 ortets which were selections from GG1 polyclonal seedlings planted at the Central Experimental Station of RRII. The clones were evaluated over 5 years of tapping in seven small sale trials laid out in Kerala Agricultural University, Vellanikkara campus, Trichur.

There was significant clonal variability in rubber yield, timber yield, summer yield depression, girth at opening, girth increment rate at immaturity and under tapping. RRII 105 which was planted as high yielding check clone gave yield ranging from 38.97 to 52.56g/t/t with a mean yield of 45 g/t/t over 5 years of tapping across the 7 trials. The yield of hybrids ranged from 9.76 to 76.2 g/t/t while girth at opening (in the tenth year of tapping) ranged from 41.26 to 82.67 cm. Girth increment rate at immaturity ranged from 3.16 to 6.91

cm, girth increment under tapping from 0.01 to 2.21 cm and bole volume of hybrids ranged from 0.03 to 0.35 m³/tree across 7 trials.

Considering the range in variability for rubber yield and timber yield, clones better than RRII 105 with rubber yield greater than 45 g/t were considered as high latex yielders. Clones with bole volume > 0.2 m³/tree at the age of 20 years were designated as timber clones while bole volume > 0.1 m³/tree were considered as promising timber yielders. Accordingly, the clones were classified as latex timber clones (rubber yield better than RRII 105 and promising timber yield of >0.1 m³/tree), latex clones (with high rubber yield better than RRII 105 but with low timber yield), timber latex clones (with high timber yield of > 0.2 m³/tree and rubber yield on par with that of RRII 105) and timber clones which were high timber yielders with bole volume greater than 0.2 m³/tree and poor rubber yield.

A total of 23 promising clones could be identified from this population of hybrids evolved from 1983 hybridization programme. Of these 11 clones exhibited high rubber yield of which nine were dual purpose latex timber clones. (83/24, 83/35, 83/29, 83/173, 2/372, 83/191, 83/17, 83/224 and 83/234). Four of the latex timber clones were derived from the cross RRIM 600 x GI 1, two belong to the cross GT 1 x RRII 105, two were ortets selected from GG1 polycross trees and one belong to the cross PB 5/51 x RRII 105. Based on the criterion of timber yield, 11 clones had exhibited very high timber yield of > 0.2 m³/tree at the age of 20 years (83/31, 83/8, 83/12, 83/60, 83/111, 83/117, 83/120, 2/185, 83/19, 83/11 and 83/37) of which two clones had rubber yield also on par with RRII 105 and were designated as timber latex clones (83/37 and 83/11). So among 115 clones evaluated, the recovery of high rubber yielders was 10 percent and high timber yielders was 11 percent.

O-4

Amazonian Accessions of Wild *Hevea* Germplasm – A Potential Source of Drought Tolerance

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India received 4548 wild germplasm accessions of *Hevea brasiliensis* from the expedition conducted by International Rubber Research and Development Board in 1981 to the Amazon forests of Brazil, mainly concentrating in three states, namely Acre (AC), Rondonia (RO) and Mato Grosso (MT). This wild germplasm collection is conserved at Rubber Research Institute of India (RRII) and is now in various stages of evaluation. Wide variability observed in this large collection indicates that this collection has a broad genetic base, potentially

important in broadening the existing narrow genetic base of cultivated rubber. Being a likely repository of genes conferring tolerance to various biotic and abiotic stresses, this wild collection is useful in developing *Hevea* clones tolerant to stresses, so that rubber cultivation can be extended to non traditional rubber growing areas experiencing adverse climatic conditions. As soil and atmospheric drought is a serious issue now-a-days limiting rubber cultivation, developing drought tolerant clones is very important. The potential use of wild Amazonian collection towards the development of drought tolerant *Hevea* clones is described in this paper, where accessions having drought tolerance potential are identified by conducting screening of this wild gene pool using various drought related morphological, physiological, structural and biochemical parameters related to drought tolerance.

O-5

Identification of Promising Clones of *Hevea* for Commercial Cultivation in Tripura

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India stands first in productivity and fourth in production of natural rubber (*Hevea brasiliensis*). To meet the increasing demand for NR for the industry, an increase in its production is inevitable. About 93% of NR production in India is contributed by Kerala and Tamil Nadu which comprise the traditional rubber growing tracts of India. Any deviation from the latitude range of 10°S and 8°N of equator is a non traditional environment. The state of Tripura of the NE India (22°56'-24°32'N and 91°10'-92°21'E) represents non-traditional region for rubber cultivation. Tripura has a sub-tropical warm humid climate with a conspicuous winter season and strong wind limiting the growth and yield of rubber. Hence, it is crucial to identify the promising clones for this region for enhancing production and productivity of rubber. With this objective, clone trials were conducted from 1979 onwards at Regional Research Station of RRII at Agartala, for evaluation of potential clones for their adaptability. An attempt is made to identify the potential clones for commercial cultivation in Tripura, considering their performance in different evaluation trials conducted here.

Thirty two oriental clones have been evaluated so far in Large Scale trials (LST) and on-farm trials (OFT) in Tripura. The trials had partially overlapping sets of clones with RRIM 600 as local check. Yield data from these trials on performance of clones in Tripura were compiled and analysed. First trial planted in 1979 consisted of 15 clones (RRII 5, RRII 105, RRII 118, RRII 203, RRIM 600, RRIM 605, RRIM 703, PB 5/51, PB 86, PB 235, RRIC 52,

RRIC 105, GT 1, Gl 1 and Harbell). Yield data for 14 years was recorded. Second trial planted in 1987 involved 12 clones (RRII 105, RRII 118, RRII 208, RRIM 600, PB 5/51, PB 86, SCATC 83/114, SCATC 93/114, Haiken 1, PR 107, GT 1 and Gl 1). Yield data of seven years were compiled and incorporated in this paper. Third trial consisting of 10 clones (RRII 105, RRIM 600, RRIM 612, PB 217, PB 260, PB 235, PB 311, SCATC 88/13, SCATC 93/114 and Haiken 1) was planted in 1995. Yield data for seven years from this trial were compiled and analysed. Fourth trial with 13 clones (RRII 51, RRII 105, RRII 203, RRII 176, RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, PB 217, PB 235, RRIM 600 and RRIC 100) was established in 1996. Yield data for nine years was compiled and analysed.

First on farm trial at Killamura had six clones (RRII 203, RRII 208, PB 235, PB 260, RRIM 600 and Haiken 1) planted in blocks in 1997. Yield data for five years was compiled for analysis. Second OFT at TFDPC plantation in Bagafa had 8 clones (RRIM 600, RRIM 703, RRII 118, RRII 203, RRII 208, PB 235, PB 260 and Haiken 1) planted in 2000. Mean yield for two years were included.

The yield performance of the clones in each trial was analysed to see the potential of the clones for commercial cultivation in Tripura. In the first large scale trial, mean yield revealed that PB 235 (57g/t/t) recorded highest yield among the clones followed by RRII 203 (52.3 g/t/t) and RRIM 600 (47.3g/t/t). Clones RRII 105 (41g/t/t), RRII 118 (43.6 g/t/t) and RRIM 703 (44.7 g/t/t) also gave high mean yield in the trial. The steady increase in yield of RRII 203 over the years was also reported. In the second trial, clone RRII 208 gave the highest mean yield (43.7 g/t/t) followed by RRIM 600 (37.7 g/t/t). Clones PB 5/51 (33.9g/t/t), RRII 118 (32.1g/t/t) and SCATC 88/13 (30.2 g/t/t) also gave high yield. Based on pattern analysis of yield data from this trial RRII 208 and RRIM 600 were identified as clones with adaptability over environments. In the third trial, clone PB 311 gave the highest yield among the clones tested (48.9 g/t/t) followed by RRII 105 (48.1g/t/t). Clones SCATC 93/114 (46.1g/t/t), RRIM 600 (44.3 g/t/t), PB 235 (43.2 g/t/t), PB 260 (41.4 g/t/t), SCATC 88/13 (41.1 g/t/t) and PB 217 (40.5 g/t/t) also recorded high mean yield in the trial. In the fourth trial, clone RRII 422 (48.5g/t/t) recorded the highest yield among the clones evaluated followed by RRII 429 (46.3 g/t/t) and RRII 417 (42.6g/t/t). RRIM 600 (41.3 g/t/t), RRIC 100 (37.9 g/t/t), RRII 105 (39 g/t/t) and PB 235 (36.4 g/t/t) also recorded high yield in the trial.

In the OFT at Killamura, PB 235 had the highest yield (1136 kg/ha/yr) followed by RRIM 600 (1054kg/ha/yr) and RRII 208 (1021kg/ha/yr). Yield over 2 years from OFT at Bagafa showed that RRIM 600 was the highest yielder (845 kg/ha/yr) followed by PB 235 (790 kg/ha/yr). Based on a survey on existing commercial plantations planted in 1980's which predominantly had RRIM 600, RRII 105 and GT1 clones, it was earlier reported that RRIM 600 (1212 kg/ha/yr) was the highest yielder followed by RRII 105 (1058 kg/ha/yr), while GT 1 yielded 791 kg/ha/yr. Susceptibility of RRII 105 to wind damage was also reported.

The yield data revealed that the performance of the clones across trials was in agreement. Clones RRIM 600, PB 235, RRII 208 and RRII 105 consistently gave high yield in all trials in which they were tested. Apart from these, 12 clones (RRII 203, RRII 118, SCATC 88/13,

SCATC 93/114, PB 217, PB 311, RRIM 703, PB 260, RRII 417, RRII 422, RRII 429 and RRII 430) gave higher mean yield than the grand mean (36.7g/t/t). Clones PB 260 and RRII 203 gave high yield in the on farm trials as well. Although the RRII 400 series clones are promising in the LST, yield data from OFT's, which are yet to be tapped, needs to be obtained for confirming their adaptability for this region. Clone RRII 422 was observed to be susceptible to cold in the early establishment stages. Incidence of TPD is moderate in RRIM 600 and PB 235, low in RRII 203, RRII 208 and RRIM 703 and above average in RRII 400 series clones. Based on the trials conducted so far, clones RRIM 600, RRII 208, PB 235, PB 260 and RRII 105 showed better yield performance and can be considered suitable for commercial planting in Tripura. Clone RRIM 600 with attributes like high yield, stability over environments, low wind damage and mild incidence of powdery mildew has been recommended in category I for planting in this region. High yielding clones recommended in category II for planting include RRII 105, RRII 203, RRII 208, RRII 417, RRII 422, RRII 429, RRII 430, PB 235 and GT 1. Yield data from the four on-going on-farm trials will aid in further validation of yield performance of the clones in Tripura.

O-6

Long-Term Yield and Growth Performance of IRCA Rubber Clones (*Hevea Brasiliensis*) in India

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As part of a bilateral clone exchange programme, India introduced five hybrid clones of *Hevea brasiliensis* in 1991 from the Institut de Recherches sur le Caoutchouc (IRCA), Cote d'Ivoire. The field trial comprising of these clones and a popular Indian clone RRII 105 was laid out during 1992 in a randomized block design with five replications and a plot size of eight plants. The trial is located at the Central Experiment Station, Chethackal, Rubber Research Institute of India, Kerala state.

The trial was opened for regular tapping during 2001 at the age of nine years and the growth performance and monthly dry rubber yield trend were evaluated. The characters studied were (i) girth (cm) of the tree at 150 cm height from the bud union, every year from the fourth year of growth (ii) mean girth increment(cm) before the commencement of tapping and during A and B panel tapping, (iii) mean dry rubber yield (g/t/t) for a period of ten tapping years, (iv) plugging index (PI), (v) mean dry rubber content (DRC%), (vi) bole height (m), and (vii) clear bole volume (m³) at the age of 19 years.

Significant clonal differences were observed for all the seven characters except girth increment. At the time of opening, IRCA 111 and IRCA 130 were superior (55.5 and 55.3

cm respectively) to all other clones in terms of vigour, while the remaining 3 IRCA clones were on par (50.8 to 53.0 cm) with RRII 105, which was the least vigorous (49.3 cm). IRCA 111 and IRCA 130 maintained their superiority over the next 10 years of tapping too. By the 10th year of tapping, RRII 105 (69.8 cm) was on par with IRCA 130 (75.9 cm), while IRCA 111 continued to show the highest girth (77.3 cm).

Clonal differences for girth increment (GI) were not significant over the 5-year period prior to commencement of tapping, or over the next five years (A panel). However, the average GI (cm/year) for the five clones before tapping commenced was much higher (6.1 cm/year) than that after commencement of tapping (2.1 cm/year in the A panel and 1.48 cm/year in the B panel). In the B panel however, girth increment rates of the six clones became significantly different ($P < 0.05$). Over 10 years of tapping, IRCA 111 and IRCA 130 showed a growth of 2.17 and 2.07 cm/year respectively, followed by RRII 105 with 1.88 cm/year.

Clonal differences for yield were highly significant every year ($P < 0.01$). In the first year after opening the trees for tapping, clone IRCA 130 had highest yield (52.0 g/t) followed by IRCA 111 and IRCA 18 (47.2 and 39.7 g/t), while the check clone RRII 105 recorded 36.2 g/t. Mean yield over ten years was highest in the clone IRCA 130 (76.0 g/t) followed by IRCA 111 (55.9 g/t) and RRII 105 (56.8 g/t).

Yield components such as plugging index (PI) and dry rubber content (DRC %) was recorded in the 17th year of growth. Lowest PI was recorded in the clone IRCA 130 and IRCA 111. In the case of % DRC, the clones IRCA 109, IRCA 230, IRCA 18 and IRCA 111 are in the order.

Higher bole height was recorded in IRCA 130 (6.88 m), IRCA 109 (5.97 m) and IRCA 111 (5.93 m). With regard to timber volume, IRCA 130 (0.24 m³) remain in the top followed by IRCA 111 (0.20 m³), IRCA 109 (0.16 m³) and IRCA 18 (0.16 m³). These four clones had significantly higher bole volume than that of RRII 105. Two clones (IRCA 130 and IRCA 111) with good dry rubber yield and timber yield can be considered as potential latex-timber clones.

P-1

The 2011-2015 Rubber Planting Recommendation in Vietnam

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Since 1977, the Rubber Research Institute of Vietnam (RRIV) has established a network of clonal trials in major rubber growing areas throughout the country. Based on the results

obtained from the network of clonal trials, rubber planting recommendation in Vietnam has been released every three to five years to guide the elite clones for the rubber planters. Currently, the 2011-2015 Rubber Planting Recommendation categorized clones in three classes for six major rubber growing areas of Vietnam including Southeast region, Central Highlands of below 600 m a.s.l., Central Highlands of 600-700 m a.s.l., South Central Coast, North Central Coast and Northeast region. In present planting recommendation, several outstanding RRIV's clones had been included for large scale (Class I), moderate scale (Class II) as well as small scale (Class III) planting. These recommended clones have contributed to the increase in rubber production by replanting and expanding the areas under rubber cultivation throughout the country.

P-2

Management of Wild *Hevea* Genetic Resources in India: Yield and Growth Performance in Immature Phase

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Ninty nine per cent of global natural rubber (NR) requirement is met by the *Hevea brasiliensis* (Willd. ex. Adr. de Juss.) Muell. Arg. cultivars. Genetic resources of *Hevea* are fast eroding in the primary center of origin, Brazil. To broaden the genetic base of cultivated rubber, the International Rubber Research and Development Board (IRRDB) organized an expedition during 1981 in the Amazon basin, Brazil. This expedition resulted in collection of over 64,736 seeds and budwood from 194 exceptionally good trees. As an IRRDB member country India has received a share of this collection during the period between 1984 and 1990. The wild *Hevea* accessions introduced into India are being conserved in various source bush nurseries and evaluated in a phased manner. These accessions were assessed in the first five years of growth to ascertain the extent of variability present in this wild population for various growth and yield attributes for the identification of desirable genes. Highly significant clonal variability was observed for yield and all the growth characters viz., girth in the 3rd and 4th year, girth increment per year, over 3 years, number of branches, crotch height, plant height and height increment except girth in the 2nd year. The yield ranged from 0.01 g/t/t (MT 5081) to 3.52 g/t/t (MT 4788), girth ranged from 3 cm (AC 493, RO 1374, RO 2367) to 15.90 cm (AC 1218) in the 2nd year and from 10.67 cm (RO 2328) to 37.50 cm (RO 3792) in the 5th year, girth increment

from 0.67 cm (RO 309, AC 3905) to 10 cm (AC 577, AC 734), number of branches from 1.67 (RO 1754) to 13.50 (MT 1680, RO 4194) , crotch height from 1.20 m (RO 4574) to 9.60 m (RO 335), plant height from 0.85 m (MT 5136) to 7.25 m (MT 2226) in the 2nd year and from 1.50 m (RO 4574) to 7.25 m (RO 229) in the 5th year, height increment over 3 years from 0.50 m (RO 4574) to 9.80 m (AC 989). Correlations worked out among the eleven quantitative traits revealed that yield was significantly correlated with all the growth characters. Based on this study, the top 10% of the potential accessions showing early growth vigour and promising juvenile yield (MT 4788, AC 226, AC 4155, MT 1007, AC 3905, RO 326, AC 3884, RO 1524, MT 905, RO 303 and AC 760) were identified which could be of use in future crop improvement programmes.

P-3

Sieve Tube Characteristics in Healthy and TPD Affected Trees of *Hevea brasiliensis*

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Among the different tissues of the bark of *Hevea brasiliensis*, sieve tubes are greatly affected due to Tapping Panel Dryness. To study the characteristics of the sieve tubes with respect to TPD, o-dianisidine is used as a specific stain for the tissue. A comparison of sieve tube characteristics such as length, diameter, density and grouping pattern of healthy and TPD trees is described here. It has been observed that the sieve tube characteristics of healthy trees were higher than the unaffected area of TPD affected tree which is followed by the affected region of the tree. The sieve tube groups which constituted of 6-10 individual sieve tubes were recorded to be highest in both healthy and TPD affected trees. In healthy trees, sieve tubes were more or less uniform in outline throughout the soft bark where as in the affected area of TPD affected trees, outline of sieve tubes was uneven. Also in affected region of TPD trees, areas of undifferentiated mass of cells were seen along with differentiated sieve tubes which indicated the altered cambial activity especially differentiation. Following onset of TPD, the anatomical changes that occurred in the sieve tubes are also discussed.

Phloic Rays in *Hevea brasiliensis* with Reference to Tapping Panel Dryness and Yield Stimulation

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Hevea brasiliensis, the prime source of natural rubber, is collected by tapping the bark on the tree trunk. High yielding clones of *Hevea* when tapped intensively showed the symptoms of the cessation of latex flow gradually from the tapping panel called Tapping panel dryness (TPD), leading to a number of anatomical deformations in the bark which includes altered cambial activity, heavy deposition of definitive callose and p-protein in the sieve tubes, high tannin accumulation, sclereid formation etc.

The unproductive bark in the TPD trees when treated with 5% ethephon started to yield latex for a short period of time. Latex biosynthesis needs high input of photoassimilate which routed downwardly through the sieve tube is being blocked by the heavy deposition of definitive callose and p-protein in TPD trees. The study was initiated to trace the structural modifications occurred in the phloic rays as an alternative route for translocation under necessity.

Phloic rays, the main route for radial translocation of photoassimilates in the bark of *Hevea* have a non-storied alignment of oval to elongated or dumb-bell shaped multiseriate ray with 1-4 uniseriate isodiametric terminal cells on either ends of the ray. The dimensions of phloic ray differ significantly in the bark of healthy, TPD affected and stimulated trees. A decrease in length and an increase in width of phloic rays were evident in TPD affected trees over healthy trees. Average height of the ray (μm) in the bark of healthy, TPD affected, unaffected area above the TPD affected area and TPD panel applied with ethephon was 495, 259, 416 and 285 respectively. Ray height of $1000\mu\text{m}$ (3.4%) was found in healthy trees while in TPD trees maximum ray height was noted to be $500\mu\text{m}$ (1.9%). In healthy trees, 57% of the rays fall in the stratified height class of 300-500 μm but in TPD trees, 78% of the phloic rays is having a height less than 300 μm . The average width of the ray measured 56.81 and 74.25 μm respectively for healthy and TPD trees. In healthy trees 61% of the ray falls under the width strata of 40-60 μm and in TPD trees 68% is in the 60-80 and 24% in the 80-100 μm width strata.

Transformative division of the fusiform initials of cambium give rise to ray cells leads to an increase in its density, which was more pronounced in the TPD affected and stimulated bark over healthy bark. The phloic rays thus formed had an outline without uniformity in cell size or shape. In TPD affected and stimulated trees, many of the axial parenchyma

cells took part in conduction either by modified as protein storing cells or enlarged to form a uniseriate arrangement that exist among the bark tissue independently or in adherence with the phloic rays to make it wider.

Histochemical evidences revealed that after stimulation, starch deposition from the wood beneath the affected panel was completely mobilized from the respective tissue. For the production of latex from the unproductive bark of the TPD tree on stimulation, adequate quantity of nourishments is being mobilized to the site of action by strengthening the radial transport system in the affected area is discussed.

P-5

Performance of RRII 400 Series Clones in a Small Holding: A Case Study

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On-farm evaluation is the last stage of evaluation in the breeding and selection cycle of *Hevea brasiliensis*. Five potential clones (RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430) selected based on the performance in breeders trials were passed on to an on-farm evaluation (small holding) in southern Kerala in 2001 to understand regional performance along with the popular check clone RRII 105. These clones were subjected to detailed evaluation. Of the five clones tested, highest tappareability was observed from the clone RRII 417 (82%) and RRII 414 (80%) when compared to the control RRII 105 (22%) 7 years after planting. RRII 422 (mean: 74, range: 51- 97 g/t/t) registered the highest yield followed by and RRII 417 (mean: 64, range: 43- 86 g/t/t) and RRII 430 (mean: 63, range: 43- 84 g/t/t) while RRII 429 (mean: 62, range: 42- 81 g/t/t) and RRII 414 (mean: 59, range: 37 - 81 g/t/t) also showed improved yield compared to the check clone RRII 105 (mean: 48, range: 29 - 66 g/t/t). DRC of these clones varied from 33 to 37% over three years but RRII 422 and RRII 105 remained consistent across seasons. Clones exhibited expected variability in terms of growth, DRC and yield. The interim results demonstrated that the trunk growth and dry rubber yield were corroborating with the breeders data and these clones remained superior to the check variety 11 years after planting. The present study could also identify potential clones among 400 series for southern Kerala and adjoining areas. These results could be useful for growers for adoption of newly recommended clones and reiteration of superiority of RRII 400 series clones.

Growth and Yield Performance of Some Exotic Clones of *Hevea brasiliensis* in North Kerala Region

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The growth and yield performance of a set of exotic clones from Malaysia and Ivory Coast were evaluated in comparison with RRII 105, the most popular high yielding indigenous clone, for their adaptability in the Northern tract of Kerala. The region is characterised by absence of summer showers and relatively long dry spells. Analysis of growth up to 16 years after planting revealed significant clonal differences in growth, only from the 10th year of planting. Girth and girth increment were the highest for IRCA 130 and significantly superior to the control clone, followed by PB 330. Lowest girth was observed for RRIM 703. IRCA 130 also exhibited significantly high annual and summer yield followed by PB 255. Peak yield was observed in the month of September irrespective of clones. IRCA 130 showed significantly superior branching height and bole volume followed by PB 255. Incidence of TPD and pink disease in IRCA 130 was comparable to the control clone RRII 105. The suitability of the newly introduced clones for the region is discussed.

Evaluation of Rubber (*Hevea brasiliensis*) Modern Clones under Certain Stressful Climatic Conditions of Odisha

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The yield and growth performance of *Hevea brasiliensis* modern clones were evaluated in stressful climatic conditions in Odisha in eastern part of India. Prolonged high summer temperature, low rainfall and soil moisture stress are the major environmental constraints. Crop experiences severe drought in summer months. Growth performance of the clones

revealed that RRII 300 had the highest girth and girth increment. In mature phase at eleventh year after planting, clones RRII 300 (54.8 cm) followed by RRII 351, IRCA 111, RRII 208 and RRIM 600 also showed superior growth and adaptability as compared to other clones. Clone RRII 351 (30.7 g/t/t) had most superior mean annual rubber yield followed by IRCA 109, RRII 352 and RRIM 600 over other clones. Highest yield contribution for all modern clones was recorded in winter season. Yield and growth performance of modern clones along with popular clone RRIM 600 under the stressful climatic conditions are discussed.

P-8

Performance of Selected Exotic and Indigenous Rubber (*Hevea brasiliensis*) Clones in Traditional Region of Kerala, India

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Performance of ortet and hybrid clones was evaluated in the on-farm trial at Shaliackary Estate (Punalur, Kerala). Mean girth at opening ranged from 44 cm in RRII 51 to 49 cm in PB 255. Girth of check clone RRII 105 was 45 cm. Regarding tappareability at opening year, clone PB 255 had high tappareability (73%) while RRII 51 showed minimum tappareability (40%). RRII 105 showed a tappareability of 53%. Based on data on mean yield of rubber in the A panel in the experimental clones, PR 255 showed maximum yield (1301 kg/ha/yr) while PB 255 showed minimum yield of 1136 kg/ha/yr. Check clone RRII 105 recorded maximum yield (1578 kg/ha/yr). Under controlled upward tapping system, there was remarkable increase in rubber yield. While average yield of the clones in the virgin panel was 1248 kg/ha/yr, the average yield in the higher panel (under CUT system) was 2644 kg/ha/yr. RRII 176 recorded a maximum mean yield of 3782 kg/ha/yr followed by RRII 51 (3501 kg/ha/yr). The check clone RRII 105 recorded a comparable yield of 3105 kg/ha/yr. It is also worthwhile to note that RRII 176 (Mil 3/2 x PB 5/60) which yielded more than 3500 kg/ha/yr of dry rubber as compared to PB, PR and SCATC clones, also had previous records of only mild incidence of abnormal leaf fall and powdery mildew.

Early Performance of some Clones of *Hevea brasiliensis* in Dry Sub humid Region of North Konkan

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North Konkan region of west coast of India is conditionally suitable for rubber (*Hevea brasiliensis*) cultivation. The region experiences prolonged severe soil moisture deficit (5-6 month per year), high temperature (occasionally goes to 41 °C), high intensities of the solar radiation (11 hrs), very low atmospheric humidity (36%) and annual rainfall ranging from 2300 – 2800 mm (June-September) with non satisfactory contribution due to its unimodal pattern.

A study was undertaken at Regional Research Station, Dapchari (20.04° N altitude and 72.04 ° E longitude with 48 MSL) to evaluate the growth and initial yield performance of three primary clones (Ortat selection) and twelve secondary clones (derived from known parentage) evolved in India (RRII 5, RRII6, RRII 105, RRII 208, RRII 308), Indonesia (PR 255, PR 261), Malaysia (RRIC 52, RRIC 100, RRII, 102, RRII 105) under prevailing condition of dry sub humid North Konkan region of western India. The observations on girth (cm) at, immature, mature phase, MGI (mean girth increment, cm per year) in immature phase and seasonal yield (gram per tree per tapping) i.e., annual yield, peak yield, stress yield and % summer yield depression for four years after tapping found highly significant. No difference in variability were recorded for the characters i.e., juvenile girth and mature MGI. The clone RRII 105 performed better in respect of vigor and yield followed by RRII 208, RRII 6, PR 255, and RRIC 100 in initial two years of tapping. Clone RRII 208 attained a highest yield from third year after tapping. The less summer yield reduction was also noticed in higher yielder clones while maintaining high summer yield which indicates, the ability of these clones to withstand in severe drought condition of North Konkan region. Better initial survival, early tappability, wide adoptability to initial growth, higher yield, stability, better yield during annual, peak yielding and stress season of clone RRII 208, RRII 105, RRII 6 indicates their potentiality for as a parents for improving breeding and selection work and for future planting in this drought prone region.

Timber Production and Wood Quality Parameters of High Yielding RRII 400 Series Clones of *Hevea Brasiliensis* Muell. Arg.

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Worlds' supply of non renewable resources is becoming increasingly critical day by day. Wood, a renewable energy source, its availability is fast diminishing. In this context, research and technological improvements in rubber wood as a plantation timber source assumes significance in the years to come. A by-product of rubber plantations, there is a continuous supply of rubber wood in every replanting cycle of 25-30 years. In India, in the earlier years, Rubber Research Institute of India (RRII) have produced many successful clones with a multi fold increase in latex yield. With the recent interest in rubber wood, emphasis is now given to breeding and selection of fast growing clones with high latex yield and timber production.

A source of fire wood in olden days, rubber wood is today used in value added products in the form of furniture, furniture components, treated and processed wood etc. Thus the quality of timber is also a crucial factor and information on wood quality parameters of popular clones is essential to augment the rubber wood processing and product development sector for various end uses. Accordingly, development of latex-timber clones and identifying clones with good quality timber traits is a priority area of research of the RRII. Hence a comprehensive study was under taken on the clonal variability among certain newly released high yielding clones of RRII 400 series and their parents with the objective of identifying clones with high timber yield and quality in terms of physical, mechanical and anatomical properties in a holistic manner.

Five clones of the RRII 400 series viz., RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430 along with their parents RRII 105 and RRIC 100 were selected for the study. Six trees each of these seven clones, 23 years after planting were clear felled. Wood discs were prepared for physical (basic density and moisture content), mechanical (Modulus of Rupture, Modulus of Elasticity and volumetric shrinkage) and anatomical (pore size and fibre characters) properties according to standard procedures. Clonal variability was analysed following standard procedures.

Timber volume of all the 400 series clones in general, was found greater than both the parental clones viz. RRII 105 and RRIC 100. RRII 430 recorded the highest wood density (670 kg/m³ followed by RRII 417 (662 kg/m³). Among the controls, RRII 105 showed better strength properties than RRIC 100. RRII 430 followed by RRII 417 showed comparable strength properties with that of RRII 105. Volumetric shrinkage values viz., green to air dry (G-AD) and green to oven dry (G-OD) conditions of RRII 400 series clones was higher than that of the parental clones. However the shrinkage values from air dry to oven dry condition (AD-OD) were on par with that of parental clones. This is a good indication of the suitability of the wood of these clones for various value added industrial applications.

Porosity in terms of number of pores (vessel elements), total pore area and average cross sectional area of pores are certain desirable traits in terms of enhancing chemical impregnation during processing. The structural properties of test clones were comparable with that of the parents. RRII 430 exhibited relatively higher frequency of pores with lower pore area. Incidence of tylosis is yet another indicator of porosity. Clonal variability was observed for this trait also. RRII 400 series clones recorded shorter fibre length and higher fibre width compared to RRII 105, indicating their faster rate of growth. The clone RRII 422 was relatively inferior with respect to timber output as well as quality parameters. The higher timber output and quality traits comparable with that of RRII 105 make the RRII 400 series clones good candidates as Latex Timber Clones. This study is the first report on the wood features of RRII 400 series clones.

P-11

Promising Latex Timber Clones of *Hevea brasiliensis* Evolved By ortet Selection from a Large Estate

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Ortet selection is one of the earliest methods of crop improvement in *Hevea brasiliensis* which involves selection of promising trees from seedling plantations and their vegetative multiplication. Since 1988, the RRII has been carrying out considerable work on mother tree selection or ortet selection to evolve promising clones from a population with large genetic variability. On the basis of observations made on 1, 80,321 seedling trees raised from Tjir 1 seeds planted in 552 ha. during 1961 at Plantation Corporation of Kerala, Kodumon, Pattanamthitta, Kerala seventy-five elite trees were selected for the detailed study in 1990. Forty ortets were finally selected based on yield, girth and bark parameters. They were cloned and evaluated in two small scale trials with one set of 27 clones and

another set of 16 clones in a randomized block design with three replications. Clonal performance was evaluated for eight years of tapping. The criteria for evaluation were yield, yield components, girth and secondary attributes. The growth parameters included girth increment rate during the immature phase *i.e.* between the 3rd and 7th year after planting, girth at opening, girth increment rate under tapping estimated based on eight years of tapping. Timber yield was estimated at the 15th year of planting in terms of clear bole volume.

Clonal variation for the traits resulted in selection of promising rubber yielders, latex-timber clones and promising timber yielders. Among these three clones viz. OKn 39, OKn 36, OKn 49 recorded a mean yield of 55.25g/t/t, 53.38g/t/t and 55.97 g/t/t/ respectively and they were superior in yield to that of RR11 105 (50.54 g/t/t). OKn 4, OKn 36, OKn 49, OKn 41, OKn 28, OKn 54, OKn 75, OKn 39 were vigorous and showed 100% tappareability at the year of opening. Among the selected clones, the highest girth increment on tapping was recorded by the clone OKn 36 followed by OKn 39 which was significantly superior to RR11 600 a clone which is noted for high yield and high girth increment rate on tapping. OKn 50, OKn 28, OKn 54, OKn 11, OKn19 were promising timber yielders as evident from the higher clear bole volume. Three top ranking clones, OKn 39, OKn 36, OKn 49 for yield recorded above average bole volume also and appear to be promising latex- timber clones. The clones possess desirable secondary characters also. The superiority of ortets with respect to specific traits is discussed in detail with emphasis to the three promising selections based on latex timber yield.

P-12

Inheritance of Tapping Panel Dryness (TPD) in a Full-Sib Population of *Hevea brasiliensis*

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Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg. (family, Euphorbiaceae; diploid, 2n=36), the Para rubber tree, is monoecious, entomophilic and predominantly out-crossing. Laticifer cells in the bark tissue of the tree are the source of natural rubber in the entire plant kingdom.

TPD (Tapping Panel Dryness) is a symptom expressed by the tree when latex is not exuded from excised laticifer cells which in turn causes significant losses in yield in almost every rubber plantation. There are several theories about the causes of TPD but no single convincing cause has been found. Nevertheless, it is generally observed that trees which

yield precocious amount of latex may ultimately show high incidence of TPD, and the symptom of TPD may be reversible or permanent. It has also been observed that a tapping rest sometimes result in alleviation of TPD symptoms. Numerous studies have already been done on TPD, however, from breeding and yield-improvement point of view, we still need more information on TPD causing factors and ways to address this important problem affecting yield. In order to achieve appreciable level of genetic gain through breeding, it is also important to know heritability of TPD among various clones. This information would be a decisive factor in *Hevea* breeding. Earlier studies have indicated high broad sense heritability for TPD among clones.

The experimental population consisted of 11 separate families of full-sib progenies (hybridization involving 25 parental clones) and their parent clones, planted in 1993 in a small scale trial at Central Experimental Station, Chethackal (Pathanamthitta Dt., Kerala), following a replicated simple lattice design (5x5 design, four replicates, seven trees per replication). For the present study, observation was taken on all the trees on the occurrence of TPD during 2011 and 2012. The symptom of TPD was confirmed through tapping for two consecutive years. Based on data from all the replicate trees of full-sibs and their parent clones, narrow sense heritability of TPD was estimated based on parent-offspring regression. The regression was computed based on standard biometrical techniques using regression of off-spring on parent, covariance of off-spring on parents and variance of parents. For regression analysis, the mean values of parents and that of progenies were subjected to regression in which the sloping line indicates the linear regression of offspring on mid-parent. The slope of the line provides a direct estimation of narrow sense heritability (h^2) for the particular trait.

There was considerable variation for TPD in the population. In the parental clones, occurrence of TPD exhibited significant variation ranging from 3.6% in PB 5/51 to 46.4 % in PB 235. Clone RRII 33 did not show any incidence of TPD. Among full-sibs, progenies of combination PB 5/51 x RRII 208 showed minimum TPD occurrence (3.6%) while progenies of RRIM 600 x PB 235 showed maximum incidence (29.6%). Progenies of cross combination RRIM 600 x RRII 33 were devoid of any TPD symptoms. Overall, results from the above study suggested that progenies generated by cross between a non-TPD clone like RRII 33 may result in progenies with less TPD incidences. Also, the study suggested that progenies generated through hybridization between a high-TPD clones like PB 235 may possess tendency to have more TPD incidence. Although the above study indicates that TPD may be governed by heritable gene action, which could imply possible genetic gain through breeding for TPD tolerance, more such progeny data would be required for conformation.

The dynamic system that exists in the sieve tubes of TPD affected trees of *Hevea brasiliensis*

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A large number of globular objects with kinetics occur in a dynamic system and was reported recently in the sieve tubes of healthy trees of *Hevea brasiliensis* (Para rubber tree, the prime source of natural rubber). Sieve tubes recently differentiated from the cambial zone in the tree trunk of *Hevea* showed uniform distribution of globular objects in a continuous state of motion, when 40-50 μ m thick tangential longitudinal sections of both fresh and long term preserved bark were viewed under light microscope. The object measures an average diameter of 1.6 μ m dispersed with a density of 200 per 1000 μ m² in a viscous medium in which they are embedded. The speed of the video graphs of the moving objects recorded through the microscope was reduced to 5% for convenience and found that these moving objects develop spatial patterns with respect to both loci and time within the sieve tube. At a time, patterns develop at a different loci are different and are not repeating hence, across the loci the displays are chaotic. In each locus as the time proceeds, develops a linear pattern that lasts for 152 milliseconds are considered as a block. The pattern in a block remains stagnant except at the 52nd and 102nd milliseconds in which develop 2 intermittent oscillating vibration. Similar movement developing spatial pattern with respect to time was also observed in the sieve tubes of the TPD affected bark but it showed minor variation from the healthy trees. In the TPD affected bark, the characteristics of the block are different from healthy trees and are described here.

Evaluation of Half-Sib Progenies of Canopy Mutant of *Hevea brasiliensis*

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Hevea brasiliensis (Willd. ex A. Juss.) Muell. Arg., belonging to the family *Euphorbiaceae*, is cultivated mainly in South East Asian countries. Inheritance of most of the agronomic

characters in *Hevea* is quantitative in nature and transfer of complex traits needs larger breeding populations and generations. Canopy and tree architecture are important features often determining both the tree-level and stand-level productivity and its susceptibility towards wind. Compact canopy genotypes not only avoids wind damage but also helps the planters in getting enough room for optimal utilization of land. However, genetic base of crown architecture is less intensively studied and is rarely utilized in tree improvement programmes. A natural mutant of *H. brasiliensis* showing distinct morphological variation in the crown has been reported. The objective of the present study is to understand the growth and crown structural variability in the selected half-sib progenies of a natural mutant in a traditional rubber growing area.

The study was performed at the Central Experiment Station of Rubber Research Institute of India. The plant materials comprised of clones of four genotypes selected from half-sib progenies of canopy mutant. These four clones along with control (RRII 105) were brown budded onto rootstocks. The successful stumps were recovered and planted in polythene bags (55 cm x 25 cm when laid flat) filled with top soil and raised in a nursery. Six month-old plants were planted in the field site in replicated blocks adopting randomized block design with plot size of five plants in six replications, and uniform spacing of 4.9 m x 4.9 m. Recommended crop management practices were performed throughout the experiment. Growth parameters were observed from the field grown plants in the 4th year onwards. Tree height (m) and diameter of the canopy (m) also measured at the age of four years. On maturity, S/2 d3 6d/7 system of tapping was followed. Dry rubber yield was measured from each experimental tree in gram per tree per tapping (g/t/t) by coagulating the latex in collection cups. Mean dry rubber yield was recorded by taking one sample per month (12 samples per year).

Analysis of variance revealed significant variability among clones of different morphotypes in terms of growth and dry rubber yield (CV 33.4%) in the field over 17 years. Crown morphology was described as per Ford (1985) as either decurrent globose or excurrent types or its intermediate types. Girth and yield of the four morphotypes (Semi Compact: 46 cm, 17.2 g/t/t; Intermediate: 81.0 cm, 28.3 g/t/t; Normal type: 76 cm, 25 g/t/t and RRII 105: 64.2 cm, 45 g/t/t) showed significant variability. The mean canopy spread/width of the normal type and RRII 105 was more than 5 m whereas the intermediate type has a canopy spread of 3 to 3.5 m only. The intermediate crown type can be considered promising than the check clone in terms of extent of crown width though rubber yield is less. Increase in plant density could compensate for lower yield and could also reduce the damage/ tree loss in wind prone areas. This clone could be subjected to further density cum clone trials to arrive at an optimum tree stand for profitable yield.

Identification of varieties with medium to high growth and compact canopy could maximize space utilization; reduce wind damage and adoption of optimum crop management practices like intercropping. The present study could also identify potential compact crown types which can be either utilized directly for close spacing trials or could enrich present genetic resources for further breeding.

Do Twin Stock Plants Perform Better Than Single Stock Plants in *Hevea*?

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A splice approach grafting to produce a tree with double root system is commonly carried out on some fruit trees for early fruiting. The resultant tree has more than one root system for better intake of nutrients and water which in turn enhances faster growth and better yield. The present study was undertaken to compare the performance of plants having single stock (single root system) and twin stock (double root system) in terms of growth and yield in *Hevea*.

Planting materials with single root system were produced as per the standard techniques while plants with two root system were produced by twin-grafting two stock plants at one whorl stage. Clone RRII 105 was used for the study. The trial was laid out at CES Chethackal and the design was RBD with three replications having a plot size of nine plants. Seven treatments were included viz; (1) twin stocks raised in polybags, (2) single stocks raised in polybags, (3) twin stocks raised in field by seed-at-stake planting, (4) single stocks raised in field by seed-at-stake planting, (5) twin stocks raised in seedling nursery, transplanted to the field as budded stumps, (6) single stocks raised in seedling nursery, transplanted to the field as budded stumps and (7) polybag plants planted in the field, as control. To study the performance of the plants data was recorded on survival rate in the field, annual girth for 13 years and monthly dry rubber yield for a period of five years. The growth parameters included girth increment during the immature phase for seven years, girth at opening and girth increment under tapping estimated based on five years of tapping.

Survival rate of plants raised in field by seed at stake planting in both twin and single stock seedlings was significantly lower compared to respective polybag plants as well as budded stump plants. Casualty due to wind damage was comparable between the treatments.

The girth increment between plants having single stock and twin stock was found to be comparable statistically during immature phase as well as at the time of opening and after tapping for five years. Among the treatments highest girth increment during the immature phase before tapping was 30.8cm, recorded by single stocks raised in polybags and lowest of 23.6cm by twin stocks raised in field by seed-at-stake planting. At the time of opening for tapping the highest girth increment of 52.7cm was recorded by control plants and lowest of 44.9cm by twin stocks raised in field by seed-at-stake planting. After tapping for five years highest girth increment of 62.8cm was recorded by single stocks raised in polybags and lowest of 52.6cm by twin stocks raised in field by seed-at-stake planting.

The mean yield between plants having single stock and twin stock was also found to be comparable statistically after tapping for five years. The highest mean yield was recorded by control plants (61.0g/t/t) and the plants raised in field by seed-at-stake planting showed the lowest mean yield (43.6g/t/t).

The present studies showed that twin stock plants and single stock plants do not differ significantly in growth and yield and having two root system may not give the plant any added advantage in *Hevea*.

P-16

Growth and Yield of New Generation Clones of *Hevea* in Sub-Himalayan West Bengal

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Five rubber (*Hevea brasiliensis*) clones derived from crossing between RRII 105 and RRIC 100 along with five other clones viz. PB 217, RRII 176, RRII 203, RRII 105 and RRIM 600 were evaluated since 1996 in the Regional Experiment Station of Rubber Research Institute of India, Nagrakata, Jalpaiguri, West Bengal, India. All the recommended cultural practices were followed.

Clonal variation was observed in all the parameters studied viz. girth at the opening of panel (1st tapping), girth at 16th year of planting, girth increment during immature and mature period, timber volume and biomass. Significantly high girth at the panel opening time was observed in RRII 429 and RRII 203 compared to the check clone RRIM 600; however, at 16th year girth of all the clones were either at par or inferior to the check. During immature phase girth increment of RRII 429, RRII 417 and RRII 422 and during mature period, girth increment of RRII 105 were superior to the check clone. Clear bole volume of RRII 203 was significantly higher than the check clone RRIM 600.

The data on mean yield over nine years showed that only one clone ie. RRII 429 scored highest yield over the check clone. The superiority of RRII 429 on yield was reflected on the first and second virgin bark also. The pre-winter yield contribution of RRII 429 was similar to RRIM 600. The rest of the clones were either on par or lower to the check. Highest dry rubber content was found in RRII 417 compared to RRIM 600.

The mean yield over seven years was considered as the more stable data because for the first two years, the percentage of tappable of all the clones had not reached upto 70% which is the recommended percentage of plants for initiating latex harvesting. The yield data over seven years showed that yield in RRII 417 and RRII 429 was higher than the check clone. The yield improvement of RRII 417, RRII 422 and RRII 429, over the check clone, was appreciable in the 1st and 2nd panels of virgin bark also.

The monthly yield distribution of over months, the yield ten clones were divided into three groups viz. 400 series clones (average of yield of 400 series clones), other clones (average of yield of Wikham clones) and all clones (average of all ten clones). The data showed that from May till December, there was a gradual increase in yield in all the groups with an appreciable yield increase during pre-winter (October to December) period. However, the yield of 400 series clones was much higher than the other groups especially during pre-winter period indicating that clones of this group were well adapted with the winter temperature. The performance index of clones was calculated (Mydin and Mercykutty, 2007) considering different growth and yield parameters. For getting stable result, from 3rd to 9th year yield data was considered. The ranking on the basis of performance index showed that RRII 203 was the best performing clone followed by RRII 417 and RRII 429. The RRII 422 and RRII 430 opted 7th and 8th position.

A simple linear correlation between yield and growth parameters in different categories viz. RRII 400 series category and all the 10 clones showed that in RRII 400 series, a significant correlation was observed between yield and girth at opening, girth at 12th year, clear bole volume, biomass and girth increment during immature phase. In the second set, however, clear bole volume was not correlated with rubber yield. This indicated that RRII 400 series clones in particular were better adapted to the agroclimate of the region and rubber yield increased concomitantly with growth, biomass and timber volume.

Components of variance viz. genotypic and phenotypic variance, clonal repeatability over seasons and broad sense heritability were calculated. The clonal repeatability for yield was found to be 0.7 and broad sense heritability was 41.25 indicating better stability and heritability of the trait over seasons. For dry rubber content, a trait related to yield was not prominent indicating a low range of variation for the trait. For growth traits, the clonal repeatability estimates and broad sense heritability ranged from 0.16 to 0.38 and 14.7 to 27.60 respectively suggesting low response that could result from selection. From the lower percentage value of the broad sense heritability data it might be stated that phenotypic selection in terms of growth would not be effective. For yield, the estimated genotypic and phenotypic variance exhibited high value in comparison to the estimated error variance indicating higher genetic effects in its expression. The estimated genotypic and phenotypic coefficients of variance appeared to be small for girth, DRC and girth at opening of panel indicating that environmental effect (over season over years) were of great importance for these traits. Among the traits studied rubber yield is the one that can be improved by selection, as also reported earlier.

This study on performance of new generation clones in Sub-Himalayan West-Bengal revealed that RRII 429 ranked first on the basis of girth, biomass and yield. The performance of RRII 417 and RRII 203 was also superior. Therefore, RRII 429, RRII 417 and RRII 203 would be the preferred clones for the region. These clones exhibited high rubber and timber yields. Heritability estimates showed prospects of improvement in rubber yield by selection.

P-17

Evaluation of Brazilian Wild *Hevea* Germplasm in India for Cold Resistance: 2. Variability and Character Associations in Juvenile Growth Phase

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Natural rubber (*Hevea brasiliensis* Muell. Arg.) is a strategic industrial crop cultivated mainly in the Southeast Asian countries. To broaden the narrow genetic base of cultivated rubber in the Far East and also to protect the fast depleting genetic resources in the centre of origin in the Amazon rainforests of Brazil, a huge collection of wild *Hevea* germplasm was made by the International Rubber Research and Development Board (IRRDB) during 1981. The expedition covered three states in Brazil viz., Acre (AC), Rondonia (RO) and Mato Grosso (MT) and the collections distributed to the member countries including India. Around 4500 accessions are being conserved in nurseries in India, and are under different stages of evaluation for identification of desirable genes.

Rubber cultivation is expanding to non-traditional regions in India, due to limited scope of further expansion in traditional area. These areas are often confronted with various biotic and abiotic stresses especially drought and temperature extremes. Cold stress in particular, is a serious threat for growth and development of rubber plants and to the sustainability of crop yields. Various phenotypic symptoms in response to cold stress induced poor germination of seeds and stunted growth etc. Indeed, cold stress can lead to major crop losses. Latex production in *Hevea brasiliensis* is affected by environmental factors like temperature and mean temperature below 20 °C was found to inhibit growth of *Hevea*. It is reported that long durations of low temperatures affect the productivity, normal growth of *Hevea* plant.

The present study was undertaken in a cold prone region to evaluate performance in the juvenile growth phase, to ascertain the extent of variability in the population and the

character associations. The study was conducted at the Regional Experiment Station of the Rubber Research Institute of India, Nagrakata, West Bengal, sub-Himalayan region of India, where in the peak winter period, the minimum winter temperature falls below 17 °C. 18 wild accessions, 2 popular clones along with two control clones RRIM 600 and Haiken 1, were planted in a field trial in randomized block design during 2000, with three replications. The spacing adopted was 4.9 x 4.9 m with five plants per plot. Among the 18 wild accessions, five were from Acre, four from Mato Grosso and nine from Rondonia.

The genotypes exhibited highly significant clonal differences ($P < 0.01$) for all the quantitative traits. In the post winter period maximum leaves per plant was recorded in MT 900 (29.27) as compared to the control clone Haiken 1 (28.20), while the accession AC 3293 recorded very high loss in leaves. High increase in number of whorls per plant during winter period was noted in MT 1020 (2) as compared to the control Haiken 1 (0.80). Increment of plant height during winter ranged from 7.15 cm (AC 3074) to 45.01 cm (MT 1020) as compared to the control clone (40.73 cm). Girth of a plant indicated its vigorous habit in the early growth phase of the plant. Girth of the trees at the juvenile phase ranged from 5.36 cm (AC 3293) to 11.53 cm (MT 915) while the control clone Haiken 1 recorded a girth of 10.50 cm.

Correlations worked out between the eight quantitative traits revealed that girth was significantly related with other morphological traits. Girth was significantly positively correlated with plant height, number of leaves and number of whorls per plant in the in the pre and post winter period. Plant height has significant correlation with number of leaves and number of whorls per plant in the pre and post winter period.

The accessions were ranked using seven parameters- annual girth and pre and post winter growth traits, for overall performance. Rank sum values ranged from 12 to 144 with a general mean of 80. Vigorous accessions such as MT 900, RO 2908, RO 2886, RO 3197, MT 2229, MT 915, MT 1020 and AC 3810 were on the top rank. All these accessions were performing well when they were ranked in the post winter period also using the traits no of leaves, no of whorls and plant height.

The present study confirmed the presence of wide variability in the germplasm for most growth contributing traits. Based on this study the top 20% of the potential accessions showing early growth vigour with cold tolerance such as MT 1020, RO 3043, AC 3075, RO 2948 and RO 2901 was identified. These accessions will be of use in future crop improvement programmes after assessment of their mature performance. These selections have potential to broadening the genetic base of present-day cultivated rubber and also for the development of cold tolerant clones for these regions.

Early Evaluation of a Set of Wild *Hevea* Germplasm for Drought Tolerance in the North Konkan Region

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The expedition conducted by International Rubber Research and Development Board in 1981 in the Amazon forests of Brazil, resulted in a collection of 4548 wild germplasm accessions of *Hevea brasiliensis* in India. This expedition mainly concentrated in three states of Brazil, namely Acre (AC), Rondonia (RO) and Mato Grosso (MT) with different agro climatic conditions. This wild germplasm collection is conserved at Rubber Research Institute of India (RRII) and is now in various stages of evaluation. Wide variability observed in this large collection indicates that this collection has a broad genetic base, potentially important in broadening the existing narrow genetic base of cultivated rubber. Being a likely repository of genes conferring tolerance to various biotic and abiotic stresses, this wild collection is useful in developing *Hevea* clones tolerant to stresses. Early evaluation of this germplasm accessions at a drought prone area, gives an indication towards their drought tolerance potential.

One such field screening with a set of 130 wild accessions was conducted at the drought prone area of Dapchari in Maharashtra, W.India. This region experiences high temperature (exceeding 40°C in April), high light intensity and very low soil moisture during the summer months. It has a rainfall pattern limited only to four months in a year, with an average annual rain fall of 7.5mm per day and an average of 90 rainy days in a year. A potential high yielding clone RRII 105, a proven drought tolerant clone RRIM 600 and a drought susceptible clone, Tjir 1 were the check clones used. The accessions for field screening were randomly selected from the conservation nurseries maintained at Central Experiment Station at Chethackal in Kerala. They were evaluated for six consecutive years from 2003 to 2009 for assessing their growth and clonal response towards drought stress experienced from February to May, at RRS, Dapchari. The statistical design adopted was augmented RBD with five plants per plot at a spacing of 2.5 X 2.5 meters. There were nine blocks and the three check clones were repeated in each block. Standard cultural practices were followed. Data recording on growth and drought related parameters during summer and non summer periods was repeated for assessing the response of these accessions towards drought stress. Plant height, girth, number of whorls and number of leaves were the growth parameters studied and leaf senescence (%) was the drought related parameter measured. Annual girth increment was recorded continuously for three years from third to fifth year as an indicator towards attaining early tappable and summer girth increment was recorded continuously for five years from first to fifth year as an indicator towards summer stress

tolerance. Xylem characteristics and intra xylary phloem in one year old twig samples were used as drought related anatomical parameter. Recent studies have suggested the use of this trait for selection towards potential drought tolerant clones due to the possible activation of this tissue under conditions of stress. The accessions were subjected to test tapping at fourth year to know their yielding potential under drought prone condition. Finally the mortality rate among the accessions was assessed at the end of six years after exposure to six summer seasons, which give an indication towards genetic diversity among these wild accessions towards drought stress tolerance.

Wide variability was noticed among the accessions for all the characters studied. In general, MT and RO accessions showed superiority for almost all the growth characters. After experiencing first summer at Dapchari, accessions MT 1697 and RO 2387 showed good vigour and accessions MT 47 and MT 4222 showed less leaf senescence. In MT 1623 there was good foliage even after exposing to summer stress. In 12 accessions, annual girth increment was higher than all the three check clones. During the summer period, 13 accessions showed good growth in terms of girth compared to the proven drought tolerant clone RRIM 600. Quantification of intra xylary phloem showed clonal variability. Out of 130 accessions evaluated, two potential accessions RO 1769 and RO 2976 gave test tap yield higher than the check clones RRIM 105 and RRIM 600 under drought stress. Based on the overall growth performance under drought stress condition for a period of six years, test tap yield and mortality rate, seven accessions (MT 2229, MT 915, AC 173, RO 1769, RO 2976, MT 1660, MT 62) out of 130 could be selected for identifying candidate genes based on the drought tolerance attribute they possess after detailed evaluation at the drought prone area.

SESSION 2

**BIOTECHNOLOGY/
MOLECULAR BIOLOGY**

O-1

Production of Mutants Affected in Hormone Signalling to Dissect Defence Mechanisms in *Hevea brasiliensis*: The Case of Ethylene

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Production of mutants by genetic transformation is one alternative to dissect the response to hormonal treatment. Ethylene is an important plant hormone involved in latex production. Transgenic *Hevea brasiliensis* plants overexpressing an ethylene mutant receptor *etr1-1* from *Arabidopsis thaliana* were regenerated. These plants did not show any morphological response the ethephon stimulation. This plant material is a source of information to understand the role of ethylene in *Hevea brasiliensis*.

O-2

microRNAs: New Regulators of Biological Functions in *Hevea brasiliensis*

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Fine regulation of gene expression is partially ensured by microRNAs (miRNAs) in response to external stimuli. They negatively regulate gene expression by targeting the cleavage or inhibit the translation of target messenger RNAs (mRNAs). In *Hevea brasiliensis*, environmental and harvesting stresses are known to affect natural rubber production. Deep sequencing of small RNAs was carried out on plantlets, subjected to severe abiotic

stress, and on latex from trees affected or not by TPD, using the Solexa technique. By combining the LeARN pipeline, data from the Plant microRNA database (PMRD) and *Hevea* EST sequences, we identified 68 conserved miRNA families already characterized in other plant species, and 15 putatively novel miRNA families. MiRNA targets were computationally predicted and analysed. Targets involved in rubber biosynthesis, ROS-scavenging systems and ethylene perception and transduction pathway are presented.

O-3

Genetic Transformation and Regeneration of *Hevea brasiliensis* Transgenic Plant with *GAI* Gene

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Typhoon is a major natural disaster in *Hevea brasiliensis* cultivation areas in China. The degree of typhoon damage has positive correlation with height of tree. In this report, the gene coding for *GAI* was selected for the genetic transformation by microparticle bombardment method. The vector pBI121, which contains CaMV35S promoter, kanamycin resistance gene, *GUS* reporter gene and *GAI* gene, was used for the transferring of *H. brasiliensis* anther callus. Regenerated plantlets were obtained on a subculture medium supplemented with 50 mg l⁻¹kanamycin. The result showed that the embryoid induction rate can reach to 1.87% when shot at 6 cm distance from the resist net to calli, and the transformation was confirmed by histochemical staining using X-Gluc (5-bromo-4 chloro-3-indolyl b-d glucuronide) and presenting positive by analysis PCR and Southern blotting. The result demonstrated that genetic transformation of *H. brasiliensis* callus with the gene coding for *GAI* and the regeneration of transgenic plant could be done successfully by microparticle bombardment method. The transgenic plants may become dwarfing phenotype and increasing tolerance to typhoon damage.

Profiling the Proteomic Alterations between the Large and the Small Rubber Particles of *Hevea brasiliensis* Using 2D-DIGE

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Natural rubber is synthesised and stored in the rubber particles of *Hevea brasiliensis* (para rubber tree). It has been demonstrated that the small rubber particles (SRPs) has higher rubber biosynthesis ratio than the large rubber particles (LRPs), but the underlying molecular mechanism still remains unknown. In this study, LRPs and SRPs were firstly separated from the fresh latex using differential centrifugation, and two-dimensional difference in-gel electrophoresis (2D-DIGE) combined with MALDI-TOF/TOF was then applied to investigate the proteomic alterations associated with the changed rubber biosynthesis capacity between LRPs and SRPs. A total of 53 spots corresponding to 22 gene products, were significantly altered with the $|\text{ratio}| \geq 2.0$ and $T \text{ value} \leq 0.05$, among which 15 proteins were up-regulated and 7 were down-regulated in the SRPs compared with the LRPs. The 15 up-regulated proteins in the SRPs included small rubber particle protein (SRPP), 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS), phospholipase alpha (PLD α), ethylene response factor 2, eukaryotic translation initiation factor 5A isoform IV (eIF 5A-4), 70-kDa heat shock cognate protein (HSC 70), and several unknown proteins etc., whereas the 7 up-regulated proteins in the LRPs were rubber elongation factor (REF, 19.6 kDa), ASR-like protein 1, REF-like stress-related protein 1, a putative phosphoglyceride transfer family protein, α -1,3-glucanase, a putative retroelement, and a hypothetical protein. The comparative proteome data may provide useful insights into understanding the mechanism involved in rubber biosynthesis and latex coagulation in *H. brasiliensis*.

Molecular Analysis of *Botryodiplodia theobromae* Isolates from Rubber in Vietnam using rDNA ITS Sequencing and ISSR Markers

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A total of 20 isolates of *Botryodiplodia theobromae* from different *Hevea* clones growing in various rubber plantations in Vietnam was analysed using Ribosomal DNA ITS (rDNA ITS) sequencing and Inter Simple Sequence Repeat (ISSR) markers. Analysis of rDNA - ITS sequences revealed that 20 studied isolates have the same size (541 bp) and these sequences are identical. BLAST search showed that the rDNA - ITS sequences of the studied isolates was 98 - 99% similarity to that of rDNA - ITS of *B. theobromae* deposited in GenBank. The identical sequences in rDNA-ITS region implied high conservation of this region in the genome of the studied isolates. ISSR analysis using 16 primers produced a total of 214 DNA bands, of which the polymorphic DNA bands was accounted for 76.6%. The phylogenetic tree produced from UPGMA analysis based on Nei and Li coefficient divided 20 isolates into four genetic clusters. Analysis of these clusters showed the relationship among isolates from different regions and no relationship between genetic groups and the host origins (rubber clones). Some unique isolates that need further investigation.

Studies on *Agrobacterium*-mediated Genetic Transformation of Embryogenic Cell Suspension and Plant Regeneration in *Hevea brasiliensis*

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The establishment of embryogenic cell suspension culture and plant regeneration system of *Hevea brasiliensis* can provide sufficient and fine materials for studying genetic transformation. In this study, the plant expression vector pCAMBIA2301 containing *uidA* gene, *npt-II* gene and *AtCBF1* gene in the T-DNA region was transformed into *Agrobacterium tumefaciens* EHA105. The embryogenic suspension cells of Chinese rubber tree clone Reyan 8-79 were used as transformation recipients. The *Agrobacterium*-mediated genetic transformation had been carried out. The factors influencing genetic transformation were studied. The frequency of transient expression of *uidA* gene and the survival rate of suspension cells were used as indexes. The optimal conditions for *Agrobacterium*-mediated transformation of embryogenic cell suspensions of rubber tree were determined as the following: the bacterial suspension OD₆₀₀ was about 0.3 to 0.5, the pH value of co-culture medium was around 5.2, 100~150 μ M acetosyringone supplemented in co-culture media, the co-culture temperature was about 20 to 22 °C, about 4~5 d co-cultured on solid co-culture medium. Ticarcillin with concentration of 300 mg/L was used to inhibit *Agrobacterium* after co-culture, and kanamycin concentration gradient from low to high (50, 75, 100 mg/L) was used to select resistant embryogenic callus of rubber tree. Total of 10 lines of resistant embryogenic callus, 2177 putative transgenic embryoids and 165 putative transgenic plantlets were obtained.

GUS staining, PCR identification, putative transgenic plantlets PCR products sequencing analysis and Southern blotting analysis were used to identify the resistant embryogenic calli, embryoids and regenerated plants. The results showed that transgenic plantlets had been obtained. The exogenous genes such as *npt-II* had been successfully integrated into the genomic DNA of the transgenic plantlets of rubber tree. In this study, the *Agrobacterium*-mediated genetic transformation system with embryogenic suspension cells of rubber tree as recipients had been preliminarily established. It will be beneficial to gene engineering breeding and gene function studies of rubber tree.

O-7

***In Vitro* Micropropagation of *Calopogonium caeruleum* for Soil Cover Crop under Rubber Plantation**

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An efficient plant regeneration protocol was described for *Calopogonium caeruleum* using whole cotyledonary nodes as explants. Seed coat break using concentrate sulfuric acid for 5-10 min, treatment for maximum germination. Seeds were surface sterilized with 70 % alcohol for 10 min and double sterilization with 20 % (v/v) Clorox for 10 min and 10 % (v/v) Clorox for 5 min and germinated on Murashige and Skoog (MS) medium supplemented with 0.5 - 2.0 mg/l BA to germinate at dark chamber for 3-days and then in light for 4-days, treatment for maximum germination (85-100 %). Cotyledonary nodes were excised from 7 days old seedlings and were cultured on MS medium supplemented with 0.1-0.2 mg/l BA and 0.25-0.50 mg/l NAA was found to be more effective for inducing multiple shoots. For root induction, *in vitro* shoots were transferred to MS medium supplemented with 1.0 mg/l IAA or IBA. The highest rooting frequency (100 %). Regenerated young plantlets were acclimatized successfully in the field humidity conditions control for further development.

O-8

Caffeic Acid O-Methyltransferase (COMT) Gene of the Phenyl Propanoid Pathway Involved in Resistance to *Corynespora* Leaf Disease in Rubber (*Hevea brasiliensis*)

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Increase in lignification is often observed in response to pathogen attack. Since lignin is a non-degradable mechanical barrier for most microorganisms, it increases the tolerance of the host by blocking pathogen invasion. Plant O-methyltransferases (OMTs) play

important roles in secondary metabolism through phenyl propanoid pathway. The full-length *HCOMT* cDNA (1347 bp in size) encoding caffeic acid O-methyltransferase consisting of 369 amino acids was cloned and characterized from the bark tissue of a cultivated rubber clone RRII 118. Southern analysis of the genomic DNA using a partial fragment of *HCOMT* as probe revealed the presence of multiple forms of the gene. The involvement of *HCOMT* in disease tolerance was assessed through real time quantification of gene expression after challenging tolerant (GT 1) and susceptible (RRII 105) clones of rubber with *Corynespora cassiicola*. Spore suspension was sprayed on young emerging leaves of RRII 105 and GT 1 and covered with a polybag to maintain humidity essential for establishment of the pathogen on the host. Control plants were also maintained in a similar condition by spraying sterile water on to the leaves. RNA was isolated from the leaves collected at different time intervals (0, 4, 12 and 24 h) following inoculation and converted to cDNA for real time quantification of the expression using specific primer-pairs designed from the 3' end of the gene including partial coding sequence and 3'UTR. Differential expression of the gene *HCOMT* was noticed between these two clones, even in unchallenged condition and expression level was more in RRII 105 over the tolerant clone GT 1. However, at 4 hours following infection, the expression levels shot up in both the cases. At 12 hours, expression level was found to reduce significantly (12 fold compared to the expression level at 4 hours) only in case of RRII 105. Even though an increasing trend of gene expression level was noticed in RRII 105 at 24 h of infection, it couldn't reach up to the control level. Whereas in GT 1, expression shot up significantly after 12 h and eventually at 24 h it had increased by 62 fold compared to the control indicating that this form of OMT might participate in the hypersensitive reaction in GT 1 in response to *Corynespora cassiicola* infection. From this study it appears that gradual increment in expression of *HCOMT* gene in GT 1 after the infection in initial hours followed by a sudden boost during the period from 12 to 24 h when the pathogen establishes on the host could be one of the reasons for the tolerance observed in GT 1. Further work is in progress to identify other forms of OMTs and their functional role in disease tolerance in rubber.

O-9

Identifying Pathogenicity Genes in the Rubber Tree Anthracnose Fungus *Colletotrichum Gloeosporioides* through Random Insertional Mutagenesis

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To gain more insight into the molecular mechanisms of *Colletotrichum gloeosporioides* pathogenesis, an important pathogen of rubber tree, *Agrobacterium tumefaciens*-mediated transformation (ATMT) was used to identify mutants of *Cgloeosporioides* impaired in

pathogenicity. An ATMT library of 4,128 *C. gloeosporioides* transformants was generated. Transformants were screened for defects in pathogenicity with a detached copper brown leaf assay. 32 mutants showing reproducible pathogenicity defects were obtained and their infection phenotypes were characterized microscopically. The infection phenotypes of the 32 mutants were classified into five categories. Southern blot analysis showed 60.4% of the transformants had single-site T-DNA integrations. Genomic sequences flanking T-DNA were recovered from 16 mutants by thermal asymmetric interlaced PCR, and were used to isolate the tagged genes from the genome sequence of wild-type *Cgloeosporioides* by Basic Local Alignment Search Tool searches against the local genome database of the wild-type *Cgloeosporioides*. One potential pathogenicity gene encoded calcium-translocating P-type ATPase. Five potential pathogenicity genes had no known homologs in filamentous fungi and were likely to be novel fungal virulence factors. Three putative genes encoded glycosyltransferase family 28 domain-containing protein, Mov34/MPN/PAD-1 family protein, and alpha beta-propellor repeat-containing integrin, respectively. Five potential pathogenicity genes had putative function matched with putative protein of other *Colletotrichum* species. Two known *Cgloeosporioides* pathogenicity genes were also identified, the encoding *Glomerella cingulata* hard-surface induced protein and *Cgloeosporioides* regulatory subunit of protein kinase A gene involved in cAMP-dependent PKA signal transduction pathway. *Agrobacterium*-mediated insertional mutagenesis was used successfully to identify pathogenicity genes in *Cgloeosporioides*

O-10

Generation of Transcriptome Resources in Rubber (*Hevea brasiliensis*) in Response to *Corynespora Cassiicola* Causing Corynespora Leaf Disease for Gene Discovery and Marker Identification Using Ngs Platform

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Corynespora leaf disease caused by *Corynespora cassiicola* is one of the major diseases causing considerable reduction in the yield of natural rubber. Next Generation Sequencing (NGS) based RNA-Seq technology (transcriptome sequencing) and *de novo* transcriptome assembly was adopted to gain a comprehensive overview of the *Hevea brasiliensis* transcriptome in response to pathogen infection. Two clones of rubber: RRII 105 (*Corynespora*

susceptible) and GT 1 (*Corynespora*-- tolerant) were used in the study to understand the genes involved in host tolerance during *Corynespora* leaf disease development in rubber. Transcriptomes of the control (unchallenged) and treated (*Corynespora* challenged) samples obtained from Illumina HiSeq 2000 were compared. The sequencing output generated more than 134 million high quality reads in control and treated samples of both the clones, which were assembled using Velvet 1.2.07 followed by Oases 0.1.21 with optimal parameters into an average of 124137 contigs and 105405 transcripts with an average length of 440 and 1373 bases for contigs and transcripts respectively. The average N50 value for the contigs and transcripts were 665 and 2097 bases respectively. A total of 13239 and 19084 simple sequence repeats were detected in the control and treated samples, some being common and others being unique to both the clones and treatments. All types of repeat units (mono, di, tri, tetra, penta and hexa nucleotide) were observed with the majority being mononucleotide repeats (48%), followed by di nucleotide repeats (18.3%). The transcripts were annotated through homology search with *Ricinus communis*, *Populus* spp. and *Arabidopsis thaliana* against the genomic resources available at the GenBank. A comparison of the differential gene expression data revealed that an average of 397 and 649 genes were down regulated and upregulated respectively when comparing the control transcriptomes of the two clones. Upon challenge inoculation, it was observed that 58 genes were down regulated and 574 genes were up regulated in the clone RR11 105 when subjected to pathogen infection. Whereas, in the case of the tolerant clone GT 1, 5 genes were down regulated and 817 genes were upregulated following pathogen infection. Furthermore, the annotated genes were functionally classified according to the gene ontology. The data from this study provides a comprehensive transcript resource for understanding the response of rubber trees to the pathogen *C. cassiicola*, which is also a resource for gene discovery and development of functional molecular markers. This study further demonstrates effective use of Illumina HiSeq 2000 sequencing data for *de novo* transcriptome assembly for the commercially important crop *Hevea brasiliensis*.

O-11

De novo transcriptome sequencing of abiotic stress responsive transcripts of *Hevea brasiliensis*

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In order to extend the cultivation of *Hevea* to non-traditional rubber growing regions, clones with improved tolerance to extreme agro-climatic conditions like drought and cold

with productivity on par with the traditional regions need to be developed. For this purpose, genes/factors contributing for the abiotic stress tolerance have to be identified and incorporated in the molecular breeding programmes. Though various molecular level studies in *Hevea* have thrown lights on the stress responsive transcripts, a comprehensive report of stress responsive ESTs is not available. Hence, this study was initiated with an objective to identify drought, low temperature and ethylene stress responsive genes/factors through new generation sequencing technology. cDNA library of drought and cold stressed samples (leaves from clone RRIM 600) and ethephon treated latex samples (clone RRII 105) were sequenced (single/pair end) on an Illumina Hi-Seq platform followed by *de novo* assembly with *Velvet* and *Oases* softwares. While the minimum read length obtained was 200 bp, the maximum read length was 12673 with *Oases*. After quality check and filtrations, about 87-91 % of the reads were used for the assembly. The sequences were annotated against *Arabidopsis*, *Populus*, *Ricinus* and *Hevea* and the fold change in their expression level was worked out. While a total of 268 transcripts got up-regulated in the drought samples, 57 and 12 transcripts were unique to control and droughted samples, respectively. In cold treated samples, 960 transcripts got up-regulated and 50 and 216 transcripts were found unique to control and cold treated samples respectively. In the case of ethylene treated samples, while 49 genes got down-regulated, 175 genes were up-regulated and among them 24 transcripts were unique to ethephon treated samples. The details of the findings are discussed.

P-1

Cloning of *Arabidopsis WUS* Gene, Construction and Identification of *WUS-EGFP* Fusion Gene Plant Expression Vector

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Arabidopsis WUS gene was cloned, and the *WUS* gene constitutive plant expression vector and *WUS-EGFP* fusion gene inducible plant expression vector were successfully constructed. They were transformed into *Agrobacterium tumefaciens* EHA105. 35S-*GUS* fragment was cleaved from PBI121 by double enzymes digestion and was cloned into pCAMBIA2301 to form pCAMBIA2301-35S-*GUS* middle plant expression vector. After the extraction of *Arabidopsis* RNA, *WUS* gene was cloned by RT-PCR method and linked into pEASY-T1 vector, then the vector was doubly digested to create *WUS* fragment, which thus was inserted into pCAMBIA2301-35S-*GUS* instead of *GUS* fragment and formed

pCAMBIA2301-35S-*WUS* plant expression vector. On the other hand, *EGFP* gene was amplified from pCAMBIA2301-*EGFP* vector by PCR, then the fusion DNA fragment of *WUS* and *EGFP* which were amplified by gene splicing by overlap extension PCR was cloned into pER8 inducible plant expression vector. These two plant expression vectors were successfully transformed into *Agrobacterium tumefaciens* EHA105 by electroporation method. Double enzymes digestion, PCR and sequencing detection verified that the results were correct. The sequences of the cloned *WUS* gene and *WUS-EGFP* fusion gene were the same as NCBI2 s, so it suggested that these two vectors had been correctly constructed. The further *WUS* gene genetic transformation would help recalcitrant rubber tree somatic embryogenesis or organogenesis.

P-2

Expression of NAC Transcription Factor under Drought Stress in *Hevea brasiliensis*

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Cultivation of *Hevea brasiliensis*, the most important source of natural rubber is being extended to non-traditional regions where drought and warmer temperatures limit its growth and productivity. This warrants identification of clones tolerant to drought conditions in such regions. For this purpose, an attempt was made to identify genes/factors contributing for stress tolerance in *Hevea*. Significant up-regulation of NAC transcription factor only in relatively drought tolerant clones like RRIM 600, RRII 208 and RRII 430 indicates its strong association with drought tolerance. This study indicates the possibility of using NAC tf for screening for drought tolerance from the *Hevea* germplasm collection as well as in breeding programmes.

Over-Expression of Chitinase Gene and *In Vitro* Antifungal Activity of Recombinant Chitinase Protein Against *Corynespora Cassiicola* Infecting Rubber (*Hevea brasiliensis*)

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Corynespora leaf disease caused by *Corynespora cassiicola* has emerged as a major disease of rubber in South East Asia. During plant-pathogen interactions, various novel proteins called pathogenesis related (PR) proteins which play a major role in plant disease resistance mechanism are induced. The chitinase (PR3) is one of the most widely studied groups of PR proteins in plants during pathogenesis. *Corynespora cassiicola* induced chitinase from *Hevea brasiliensis* was characterized in the present study. The cDNA was developed from the RNA of *C.cassiicola* infected leaves of the clone GT 1. A 978bp chitinase gene was obtained from *H.brasiliensis* and over-expressed in the pET 32a⁺ expression system. *In vitro* studies of purified recombinant *Hevea* chitinase showed antifungal activity against *Corynespora cassiicola*.

The chitinase gene expression in *Hevea brasiliensis* during *Corynespora cassiicola* infection was quantified through qPCR and increased expression of chitinase transcripts was observed. In clone GT1, chitinase gene was induced up to 24 hours after *Corynespora cassiicola* infection and then came down, where as in RRII 105, chitinase level was lesser induced than control. The polyclonal antibody was raised with the recombinant chitinase and the induced clone GTI showed a prominent band in western blot, while a minor band was observed in RRII 105 in induced condition.

Identification and Molecular Characterization of Multiple forms of α -1, 3-Glucanase Gene Promoter from *Hevea brasiliensis* through Inverse PCR

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The first line of defense against fungal pathogens is performed by a group of pathogenesis-related proteins (PR), including α -1,3-glucanases. Often these processes are controlled by transcriptional activation of the respective genes. In *Hevea brasiliensis*, α -1,3-glucanase is playing a major role in combating abnormal leaf fall disease caused by *Phytophthora spp.* In the present study the inverse PCR technique, a method for the amplification of unknown flanking DNA sequences of a known genomic DNA sequence was optimized and isolated the promoter sequences of α -1,3-glucanase gene from *H. brasiliensis*. α -1,3-glucanase gene specific forward and reverse inverse PCR primers were designed based on a previously reported α -1,3-glucanase gene from a *H. brasiliensis*, clone RRII 105 (Thanseem *et al.* 2003 (NCBI # AY325498)). Under optimized conditions four bands of approximately 1.2, 0.8, 0.76 and 0.4 kb were amplified. Nucleotide sequencing of the cloned bands revealed the presence of 1126, 793, 765 and 418 nucleotides. BLASTn analysis of these sequences showed >95% similarity with the reported *H. brasiliensis* α -1,3-glucanase gene in the 5'UTR and the 5' coding region confirming that they are multiple forms of the α -1,3-glucanase gene promoter. Excluding the known coding region, the fragments contain 913, 582, 553 and 198 nucleotides of the promoter region upstream to the translation initiation codon, ATG. Out of these, the 198 bp promoter was exactly aligning with the reported promoter sequence by Thanseem *et al.* (2003) (AY325498). Although, the other three promoters showed similarity in the 5' UTR and immediate upstream region, they showed considerable variations in their distal regions. Analysis of the promoter sequences using "PLACE" software detected important cis-elements that are usually present in biotic/abiotic stress related gene promoters. The presence of CpG islands and nucleosome forming potentials has also been identified in the isolated promoters. The properties of the isolated promoter regions of α -1, 3- glucanase gene on the basis of the data obtained is discussed. This is the first report on the existence of multiple forms of α -1,3-glucanase gene promoters in the same clone of *H. brasiliensis*. After characterization of the pathogen inducible isoform, the same can be used for crop improvement through transgenic approaches as well as molecular breeding for disease tolerance.

Half Ovulo Embryo Culture –An Ideal Method for Raising True-To-Type Seedlings in *Hevea brasiliensis*

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Hevea brasiliensis (Para rubber tree) is the major commercial source of natural rubber because of its abundance in the latex, high quality, and convenience of harvesting. Rubber clones are propagated by bud grafting of the desired scion on to assorted root stocks grown from cross pollinated seeds. Since the seedlings are genetically divergent, this has been often implicated as the source of large tree to tree variation in growth and yield of bud grafted *Hevea* trees. Dependence on bud grafting has become inevitable for the propagation of selected clones and maintenance of clonal integrity. It has been reported that there is isozyme polymorphism between different trees of the same clone, due to the variable root stocks. Uniform rootstocks will be an effective solution to reduce intra-clonal variation due to stock-scion interaction. Consequently, cloning the root system is a major challenge in *Hevea* breeding. Tissue culture methods provide an alternative to produce own rooted plants in *Hevea*. However, the major problem with this method is the failure to produce an adequate tap root necessary for tree stability. Moreover, the recalcitrance of clonal explants will not allow the production of own rooted plants to a commercial scale. In this context the present work was initiated with the objective of developing multiple, true -to- type seedlings of *Hevea brasiliensis* via induction of zygotic polyembryony, and to assess the uniformity / heterogeneity among the seedlings.

Immature open pollinated fruits (10-12 week's old) of clone RR11 105 were collected from the field. Nitsch basal medium with different combinations of growth regulators viz. GA, Kinetin and Zeatin were experimented for the induction of polyembryony, following half ovulo embryo culture. Multiple embryos could be induced from a few of the cultured ovules in a combination of 2 mg/l GA₃, 3 mg /l Kinetin and 0.3 mg/l Zeatin. In this medium, the number of embryos developed from a single ovule could be enhanced to 60 as against 12 observed in an earlier pilot study (Rekha *et al* 2010). Embryos recovered were transferred for maturation in the same basal medium fortified with organic supplements such as banana powder, malt extract and casein hydrolysate (100 mg/l). For germination half strength MS medium along with 0.3 mg/l BA and 0.3 mg/l GA was used. Multiple embryos and seedlings were developed from three independent ovules and were hardened and field planted. These plants were subjected to RAPD analysis using the standard procedure. Molecular analysis of these plants using RAPD could prove the genetic uniformity as well as zygotic origin of the multiple seedlings developed. These multiple seedlings are ideal candidates for stock- scion interaction studies for which uniform own rooted plants are so far not

available. Moreover with further refinements, this method can be utilized for developing uniform rootstocks which will help to reduce intraclonal variation due to stock-scion interaction in rubber plantations.

P-6

Chlorophyll A/B Binding Protein Gene Expression in Juvenile and Mature Leaf Explants and Its Relationship with *In Vitro* Culture Response in *Hevea brasiliensis*

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A plant regeneration system via somatic embryogenesis was developed earlier from leaf explants of *Hevea brasiliensis*, the major commercial source of natural rubber. *In vitro* culture response of *Hevea* leaf explants collected from plants of different physiological maturity such as seedlings, somatic plants, bud grafted plants and mature trees varied. In the present study, work was carried out for the isolation and characterization of chlorophyll a/b binding protein gene (Cab), expressed mainly in green leaves, for which the expression is reported to be directly related to the juvenility of the plant tissues. The gene expression with respect to *in vitro* culture response of *Hevea* leaf explants collected from plants of different maturity was also studied. Cab gene was PCR amplified initially from the genomic as well as cDNA of *Hevea brasiliensis* using primers designed from the conserved regions of the Cab gene reported earlier from related plant species. The PCR amplified fragment (0.5 kb) was eluted, cloned and sequenced. The sequence revealed the presence of 525 bp that showed 91% homology with Cab mRNA from *Ricinus communis*. Further, full length Cab gene (0.8 kb) was amplified from genomic DNA using *Ricinus communis* specific primers. The sequence revealed the presence of a fragment of 802 bp which showed 90 % sequence homology with the reported cDNA sequence of Cab gene from *Ricinus communis*. This is the first report on isolation and characterization of Cab gene from *Hevea brasiliensis*. Differential expression of the gene was observed in RT-PCR with cDNA synthesized from RNA isolated from source plants of different physiological maturity with a higher expression from physiologically juvenile sources. Higher expression of the gene was also observed in northern analysis in physiologically juvenile plants such as seedlings and somatic plants than in mature bud grafted clonal materials and field grown trees. Differential expression was also noted in leaves of different maturity.

High Frequency *Agrobacterium* Mediated Genetic Transformation in Rubber Tree Via. Vacuum Infiltration

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Hevea brasiliensis (Para rubber tree) belonging to the family *Euphorbiaceae* is the major source of natural rubber. In a tree species like *Hevea*, genetic transformation offers a viable approach for crop improvement within a short period. After extensive optimisation experiments in the initial transformation protocol, the transformation frequency of upto 14% was obtained earlier. *Hevea* being highly recalcitrant to *in vitro* culture, an efficient transformation protocol is necessary for generating large number of transgenic plants with stable foreign gene expression. Therefore, vacuum infiltration was attempted for enhancing the transformation frequency using anther callus.

H. brasiliensis (clone RRII 105) anther callus for vacuum infiltration was generated following the protocol reported earlier. Five binary vectors harbouring different agronomically important genes were used for the study. The binary vectors contained the genes coding for 1) manganese superoxide dismutase (MnSOD) under the control of Cauliflower Mosaic virus (CaMV 35S) and 2) MnSOD gene under the control of Figwort Mosaic Virus (FMV34S) promoter, 3) sorbitol 6-phosphate dehydrogenase, 4) antisense 1-aminocyclopropane-1-carboxylate (ACC) synthase and 5) 3-hydroxy 3-methyl glutaryl CoA reductase (*hmgr1*). In the *hmgr1* gene construct hygromycin phosphotransferase (*hpt*) was used as the plant selectable marker gene and all the other binary vectors contain neomycin phosphotransferase (*npt II*) as the antibiotic marker gene.

The bacterial culture for *Agrobacterium* infection was prepared. Approximately 2.0 g of the proliferated anther callus was transferred to 30 mm sterile glass petri plates containing 2 ml of the *Agrobacterium* culture and subjected to vacuum infiltration for different periods (5-20 min) and vacuum pressure ranging from 10- 40 kilo Pascal (kPa). After three days of co-culture, the calli were transferred to selection medium containing either kanamycin (300 mg/l) or hygromycin (40 mg/l) and subcultured at three weeks intervals in the respective antibiotic medium for the emergence of transgenic cell lines. Putatively transformed cell lines were selected after subjecting GUS histo-chemical staining and the individual cell lines were proliferated in the callus proliferation medium fortified with the respective antibiotics. DNA was isolated from randomly selected cell lines, according to Doyle and Doyle (1990) and PCR was performed using gene specific primers.

Putatively transformed cell lines emerged after 40-50 days. It was observed that vacuum infiltration at 30 kPa for 10 minutes was found to be ideal for enhancing transformation frequency in *Hevea brasiliensis*. Irrespective of the gene constructs experimented, transformation frequency could be enhanced from 14 to 40%. In conclusion, a protocol for *Agrobacterium* mediated genetic transformation of *Hevea brasiliensis* via vacuum infiltration could be developed using proliferated anther callus. This is the first report on high frequency *Agrobacterium* mediated genetic transformation in *Hevea brasiliensis* aided by vacuum infiltration. This method can be effectively utilized for studying promoter efficiency via transient assay. Further, this technique can be employed for gene transfer into intact explants such as leaf disc, anther and ovule after appropriate modifications.

P-8

Effect of Nurse Culture on Inducing Division of Isolated Pollen Protoplasts of *Hevea brasiliensis*

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Haploids are of great relevance in crop improvement of *Hevea*, a highly heterozygous tree species with a long breeding cycle. The isolation and culture of pollen protoplasts may be a viable proposition for raising haploid plants/ homozygous lines in *Hevea*. The present work envisages the development of a method for the isolation and culture of pollen protoplasts of *Hevea*. Effect of different nurse cultures on the development of cultured protoplasts has been studied. Intact pollen grains were isolated from mature male flowers of *Hevea* prior to opening. Viable protoplasts in high yield could be isolated from these pollen grains when exposed to a mixture of 0.5% cellulase and 0.05% pectolyase in the presence of the osmotic stabilizers 0.6M mannitol and 0.3 M sorbitol. These protoplasts were partially purified and cultured in the nutrient medium with three different nurse cultures namely embryogenic callus from *Hevea*, tobacco and carrot. Division of the cultured protoplasts leading to the formation of a few microcolonies was observed in the medium containing 0.8 mg/l 2, 4-D and 0.5 mg/l BA and enriched with *Hevea* nurse culture. Cultures with micro colonies were dark incubated for further development. This is the first report of division of pollen protoplasts and micro colony formation in *Hevea brasiliensis*.

Agrobacterium* Strain- A Key Factor Determining the Efficiency of Transformation in *Hevea brasiliensis

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Hevea brasiliensis is a perennial tree and hence genetic improvement through conventional breeding is elaborate and time-consuming. Genetic transformation offers a viable approach to breeders supplementing the breeding programmes by adding valuable genes for specific characters in a relatively short period. The major objective of rubber breeding is to develop superior clones with improved dry rubber yield and wood quality. The HMGR (3-hydroxy 3-methylglutaryl Coenzyme A reductase) is considered as a key enzyme in the rubber biosynthetic pathway to transform the rubber plants for increased rubber yield. *Agrobacterium tumefaciens* mediated genetic transformation system is the most widely used method for *Hevea* genetic transformation because of its efficiency and convenience. The transfer of T-DNA and its integration into the plant genome is influenced by several factors. These include plant genotype, explant, plasmid vectors, bacterial strain, “vir” gene inducing phenolic compounds in the culture medium, culture media composition, suppression and elimination of *Agrobacterium tumefaciens* after co-cultivation etc. The selection of an appropriate bacterial strain is one of the key parameter required for an efficient genetic transformation system. The molecular and genetic basis for the host range of a bacterial strain is controlled by multiple factors within the bacterium and the host plant. Host range may further result from an interaction of particular Ti plasmids with certain bacterial chromosomal backgrounds. To identify a suitable bacterial strain giving efficient transformation with the *hmgr1* gene in *Hevea*, three different *Agrobacterium* strains harbouring the transgene were experimented.

Three *Agrobacterium* strains, EHA 105, LBA4404 and pGV 1301 were evaluated for their efficiency of transformation in *Hevea brasiliensis*. The strain LBA 4404, a vir-helper, is an octopine type of bacterial strain, harboring disarmed Ti plasmid p AL 4404. The other two *Agrobacterium* strains EHA 105, a derivative of EHA 101 (Hood *et al.*, 1993) and pGV 1301 which has a cured Ti plasmid, belong to the nopaline type. The binary vector contained hygromycin phosphotransferase gene (*hpt*) as the plant selectable marker (Gritz and Davies 1983). Three different target tissues, fresh callus, embryogenic callus and embryogenic suspension cultures were used for genetic transformation. The infected callus was incubated at different temperature regimes, 4°C, 20°C and 28°C in the co-culture medium. Effective selection of the transformants was carried out in the screening medium supplemented with hygromycin (30 mg l⁻¹). The transformants were selected and proliferated. The presence of the transgene was confirmed in the cell lines by PCR using gene specific primers. A clear

distinction in the efficiencies between the *Agrobacterium* strains was observed when the co-culture was performed at 20° C for 3 days. Highest transformation efficiency was observed with the strain EHA 105 (28%), irrespective of the target tissues tried for transformation. The PCR confirmed cell lines were further cultured for embryo induction and subsequent plant regeneration.

P-10

Functional Characterization of a Novel *Hevea* $\hat{\alpha}$ -1, 3-Glucanase Gene Promoter and Its Regulatory Role in *Hevea* and Tobacco Tissues

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Plant $\hat{\alpha}$ -1,3-glucanases (EC 3.2.1.39) coming under the family of PR-2 proteins, comprises of large and highly complex gene families involved in pathogen defense as well as a wide range of normal developmental processes. The diverse physiological functions might force these enzymes to occur as multiple structural isoforms that differ in their size, iso-electric point, primary structure, cellular localization and pattern of regulation. In *Hevea*, $\hat{\alpha}$ -1,3-glucanases play a major role in combating the abnormal leaf fall disease (ALF) caused by *Phytophthora* spp. Earlier studies at RRII showed a differential expression pattern of $\hat{\alpha}$ -1,3-glucanase in tolerant and susceptible clones of *Hevea* towards abnormal leaf fall disease. To learn the mechanism behind this differential regulation, understanding of the regulatory elements residing in the $\hat{\alpha}$ -1,3-glucanase gene promoter is essential.

In the present study, a 913 bp promoter region of a novel form of $\hat{\alpha}$ -1,3-glucanase gene has been isolated through inverse PCR. Inverse PCR comprises an initial restriction enzyme digestion of the total genomic DNA, circularization of the digested products *via* ligation, linearization of the circularized products and finally PCR amplification. The enzyme, *Ssp* I was chosen for digestion of the total genomic DNA and *Bgl* II for linearization of the circularized product. The inverse PCR primers were designed based on a reported sequence of $\hat{\alpha}$ -1,3-glucanase from *H. brasiliensis* (Acc. No. AY325498) (primers; 5' glucanase: TAG GAA ATT CCT ACC CTC CTT CTG; 3' glucanase: CCT GTT ATA CCA AGG CTT GCT G). Four bands were amplified in the inverse PCR ranging in size from 0.4-1.2 kb. The amplified band which showed 1.2 kb fragment size in the 1.2 % agarose gel was preferred for further

characterization. The fragment has been cloned in TOPO TA[®] cloning vector and sequenced. Upon sequencing using the M13 forward and the reverse primer, the result revealed that the sequence comprised of 913 bp promoter region of a α -1,3-glucanase upstream to the 'ATG' initiation codon. Within the 913 bp promoter, the 5'UTR region comprises 39 nucleotides (identified on alignment with the cDNA report by Chye *et al.*, 1996) extending upto the 'ATG' initiation codon. The BLASTn analysis of the obtained promoter sequence with the other reports showed that the 5'UTR region is showing a maximum identity of 97 % towards α -1,3-glucanase mRNA reported from RRIM 600 (NCBI Acc No: U22147). It is also interesting to observe that a portion of the promoter region is showing a 100 % similarity towards the Zebrafish (*Danio reiro*) DNA sequence from clone CH211-236H18 (NCBI Acc. No. CR385064). The 913 bp promoter sequence is found to contain the essential cis-elements that are usually present in a biotic / abiotic stress related gene promoters. It contains the TATA, CAAT, GATA, WRKY, W box elements along with other complex regulatory regions. The promoter region is rich in 'AT' base pairs similar to any other promoters. The profiles of nucleosome potential identified through the RECON programme showed the probability of nucleosome formation along the promoter region amplified. With the 913 bp promoter a very strong nucleosome forming potential was found in the region (5' - 3') between the nucleotides 85- 220, 399- 486 and also from 626 - 834 nucleotide (the value ranges between 0.5 - 0.9). Two CpG islands were also observed (using the EMBOSS CpG plot online software), in the region 449-521 with about 32.88 % CG and in the region 609 - 695 with 36.78 % CG.

Attempt was also made to understand whether the promoter form identified is functional so that the gene form identified will also be functional. Therefore, promoter: reporter gene fusion binary vector was constructed and the reporter gene expression was studied in the heterologous system, tobacco, through *Agrobacterium* mediated genetic transformation. Attempts were also done to understand the reporter gene expression in *Hevea* callus through transient assay. β -glucuronidase gene was selected as the reporter gene here. Total 3 binary vectors were developed such as one basic construct with 913 bps and two deletions of the 913 bp promoter with 550 and 200 bps. The vector used was pCAMBIA 1381 Z TDNA vector, a plant expression vector to check promoter efficiency. The GUS activity through transient assay showed a fair level of the reporter gene expression in the *Hevea* callus where as in the tobacco leaf discs no GUS expression was observed. The transformed ex-plants with the *Agrobacterium* harbouring the promoter-less vector (negative control) did not show any GUS activity in both *Hevea* callus and tobacco leaves. The construct with 913 bp promoter showed increased level of GUS activity within the *Hevea* callus than the two deletion constructs. The induction experiments with 0.1% salicylic acid also did not show any detectable levels of GUS activity in the regenerated transgenic tobacco plants. The *Hevea* α -1,3-glucanase gene promoter characterized here can be exploited for the pathogen induced expression of transgenes in the future crop improvement programmes in *Hevea* and in other crops.

Structural and Functional Characterization of a NBS-LRR Disease Resistance Gene Associated With Tolerance against *Corynespora Cassicola* Causing Leaf Fall Disease in Rubber (*Hevea brasiliensis*)

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Numerous disease-resistance genes have been cloned and characterized in various plant species. Only a few of these reported genes are transcriptionally induced or have enhanced transcription upon pathogen infection. A set of transcriptionally induced sequences (RT-RGAs), analogous to plant resistance genes of NBS-LRR class was characterized from *Hevea brasiliensis*, a tree crop producing latex of commercial utility. PCR-based approach was adopted using degenerated primers based on the conserved motifs of NBS domains of known plant *R*-genes. Sequence characterization of RT-RGAs from *Corynespora cassicola* challenged rubber plants of clone RRII 105 was performed and consecutively 32 transcriptionally active diverged RT-RGAs were identified. A comparison of these RT-RGAs with the earlier isolated RGAs revealed that a large group of closely related genomic RGAs had no function against *Corynespora* leaf disease. They did not show perfect homology with any of the RT-RGAs on the basis of deduced amino acid sequences. Similarly, two groups consisting of 7 and 15 RT-RGAs were found to be unique as they did not share any homology with the genomic RGAs but were functionally active against *Corynespora* infection. Nucleotide sequence information of RT-RGA13, one of the functional resistance gene analogues identified in rubber, showing significant over-expression in *Corynespora* infected rubber clones was used to derive its full-length sequence. Subsequently, a full-length resistance (R) gene from rubber (GT1) was cloned and characterized for the first time. Length of the R gene was 3284 bp and the coding sequences was 2547 bp. Conceptual translation of the coding sequences revealed a protein of 849 aminoacids, which had characteristic NB-ARC domain and leucine-rich repeat (LRR) motif present in most of the R gene from other species and maximum homology was found with nbs-lrr resistance protein of *Populus trichocarpa* (E-value 0.0). Protein structure prediction was performed with conceptual translation of the R gene through homology modeling. Domain analysis resulted in highest match with apoptotic protease activating factor. Besides above, characteristic domains viz. coiled coil, leucine-rich repeat, toll-like receptor2 were also identified in the deduced R protein.

The involvement of R gene in disease tolerance was assessed through real-time quantification of gene expression after challenging tolerant (GT 1) and susceptible (RRII 105) clones of rubber with *Corynespora cassiicola*. RNA was isolated from the leaves collected at different time intervals (0, 6, 12 and 24 hours) following inoculation and converted to cDNA for real time quantification of the expression using specific primer-pairs. Differential expression of the R gene was noticed between these two clones even in unchallenged condition (control) and expression level was significantly higher in tolerant clone GT 1. However, at 6 hours following infection, the expression levels reduced considerably in both the clones. At 12 hours, expression level of R gene was found to reduce drastically (5 fold compared to the expression level at 0 hour) in case of RRII 105, whereas expression shot up in GT 1 reaching initial level of expression at 0 hour. However, at 24 hours expression level increased in RRII 105 by 30% but decreased in GT 1 by 50% of the expression level noticed at 12 hours following infection in respective clones. From this observation it is evident that increased level of R gene expression in GT 1 (8 folds more than in RRII 105) at 12 hours when pathogen establishes infection on host, may be one of the reason for tolerance to *Corynespora cassiicola* observed in GT1.

P-12

Cloning and Expression of 3-Hydroxy -3-Methylglutaryl-Coa Reductase 1 (*Hmgr* 1) from *Hevea brasiliensis*

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Natural rubber (*cis*-1, 4-polyisoprene) an important raw material for many industrial uses is synthesized in the laticiferous tissue of *Hevea* tree. The mevalonate pathway, which initiates the synthesis of mevalonate by 3-hydroxy- 3-methyl glutaryl - CoA reductase (HMGR) provides precursors for diverse spectrum of isoprenoid compounds. This is a key regulatory enzyme in the rubber biosynthetic pathway. Earlier workers reported a significant positive correlation between rubber biosynthesis and HMG-CoA reductase activity in *Hevea*. Different members of *hmgr* gene were cloned and characterized from the *Hevea* clone RRIM 600. Earlier reports suggested that *hmgr1* is expressed more in laticifers than in leaves and is specifically involved in rubber biosynthesis. The objective of the present study was the *in vitro* synthesis of the HMGR1 protein. The protein would be used to get the antiserum with an objective to use it as a biochemical marker of yield potential in *Hevea*. This would

further enable screening of newly emerging *Hevea* clones and wild germplasm accessions to evaluate their yield potential.

Latex from trees of *Hevea* clone RRII 105 was collected and mRNA was isolated using Dynabeads (Invitrogen, USA). cDNA was synthesized using Superscript III^{RT} First strand synthesis kit (Invitrogen, USA) and PCR amplification with gene specific primers was performed under standard conditions. The PCR amplified cDNA fragment (1.8 kb fragment) was cloned in to pGEM-T vector and transformed in to *E.coli* cells (Gen Hunter cells). The selection of transformants was done according to the manufacturer's instructions. Presence of the insert was confirmed by colony PCR, restriction digestion and sequencing (Macrogen, Korea). pRSET-A expression vector (Invitrogen) was used for *in vitro* protein synthesis in BL21 (DE3) pLysS cells. The clone of pRSET-A/*hmgr-1* was inoculated into SOB containing ampicillin (50 µg/ml) and chloramphenicol (35 µg/ml). The overnight culture was further inoculated in to SOB for protein expression by inducing with 1mM IPTG. The protein from the culture was subjected to SDS polyacrylamide gel electrophoresis (PAGE) analysis and visualized using Coomassie blue stain.

A PCR based approach was employed to isolate *hmgr1* gene from *Hevea*. PCR resulted in the amplification of a single 1.8 kb fragment corresponding to the length of *hmgr1*. The PCR amplified cDNA fragment was cloned in to pGEM-T vector. The colony PCR and restriction digestion analyses confirmed the presence of cloned DNA inserts in pGEM-T vector. Sequencing results showed a 100% homology of our clone to earlier submitted sequence (Acc. No. X54659). Restriction digestion analysis and sequencing confirmed the DNA inserts in pRSET-A expression vector. Orientation of the insert in the expression vector was confirmed by the sequencing data.

A protein with a size of 64.6 kDa (including His Tag protein) corresponding to HMGR1 was observed in the IPTG induced culture samples of the recombinant clone. The purification of the protein is in progress. The purified protein would further be employed to develop specific antiserum that could be utilized for quantification of HMG-CoA reductase in different *Hevea* clones and wild germplasm accessions through immuno techniques. The initial steps which involved PCR amplification of specific gene, cloning into the expression vector and *in vitro* protein synthesis could be achieved successfully.

Cloning and Expression of HbACO2 of *Hevea brasiliensis*

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Ethylene has been known to stimulate latex production in *Hevea* and is being widely used for increasing the crop productivity. Ethephon (chloro-2-ethyl phosphonic acid) which is an ethylene releaser is commonly used as a yield stimulant. Ethylene stimulation in *Hevea* has been found to enhance the expression of genes involved in ethylene biosynthesis such as ACC synthase and ACC oxidases, which are also responsive to wounding. Cloning of ACC synthase (ACS) and ACC oxidase (ACO) revealed that they belong to a multigene family and are regulated by a complex network signals as a response to both internal and external stimuli. Existence of three types of ACO (HbACO1, HbACO2 and HbACO3) have been reported with open reading frames encoding polypeptides of 312, 318 and 318 amino acids, (1115, 1174 and 1074 bp long) respectively. It was also reported that HbACO1 may be responsible for basal levels of ethylene production while HbACO2 and HbACO3 are up-regulated in response to external factors such as wounding. Further investigations are needed to identify which of these ACOs are involved directly in increasing the ethylene levels in response to ethephon stimulation. Hence, this study was initiated with an objective to understand the role of ACO2 in ethylene biosynthesis in *Hevea*. For this purpose, the ACO2 has been cloned into an expression vector for getting the protein with an aim to develop antiserum which could be utilized for the screening ACO levels through ELISA technique.

Bark samples from *Hevea brasiliensis* (clone RR11 105) was collected from which mRNA was isolated using Dynabeads (Invitrogen, USA) and cDNA was synthesized using Superscript III RT First strand synthesis kit (Invitrogen, USA). ACO2 specific primers flanked with suitable restriction sites (to enable cloning into specific restriction sites in the expression vector) were synthesized and employed to PCR amplify the coding region of ACO2 from *Hevea*. The PCR amplified cDNA fragment was cloned in to pGEM-T vector and transformed in to *E. coli* cells (GenHunter, USA). The selection of transformants was done by blue white screening after which the clones were further confirmed by colony PCR and by restriction digestion using *Bam*H I and *Pst* I restriction enzymes.

The coding region of ACO2 cloned in pGEMT vector was digested with *Bam*H I and *Pst* I restriction enzymes and ligated into the appropriate sites of pRSET-A vector. This ligated mix was successfully transformed into *E.coli* (BL 21 (DE3) PLYsS) cells and the

transformants were selected based on colony PCR and restriction digestion analysis. The above results indicate the successful cloning of HbACO2 gene in pRSET-A expression vector. The selected clone was inoculated into Super Optimal Broth (SOB) containing ampicillin (50µg/ml) and chloramphenicol (35 µg/ml). The overnight culture was further inoculated in to SOB for protein expression by inducing with 1mM IPTG. The protein was subjected to SDS polyacrylamide gel electrophoresis (PAGE) analysis and was visualized using Coomassie blue stain. A band of recombinant protein (with a size of approximately 36 KDa) was observed in the IPTG induced culture samples of the recombinant clone. The protein after purification would further be employed to develop specific antiserum that could be utilized for quantification of HB ACO2 in different *Hevea* clones and wild germplasm accessions through ELISA.

SESSION 3

RUBBER TECHNOLOGY

Effect of Modification Technique on Properties of Blends of Natural Rubber and Modified Tyre Crumbs

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Tyre waste is recycled by mechanical means to produce tyre crumbs or ground rubber tyre (GRT). This low cost recycled material is used as a filler in the manufacture of rubber articles. Composites of rubber with GRT exhibit poor properties owing to poor adhesion between the rubber matrix and filler. Hence GRT is modified by various techniques to produce reclaimed rubber, which enhance the properties of their blends with virgin rubber. The objectives of this study were to develop an environmentally friendly modification technique for GRT and to evaluate the properties of blends of virgin natural rubber (NR) and modified tyre crumbs in comparison to the control, virgin NR compound.

GRT was modified according to three different techniques using the juice of a natural product. Thereafter, 85/30 virgin NR/modified GRT blend compounds and vulcanisates were produced and cure characteristics, physico-mechanical properties and swelling behaviour of the same were evaluated and compared with those of the control compound or vulcanisate. Results revealed that processing safety of the three virgin NR / modified GRT blend compounds was almost similar. However, the blend compound of virgin NR and modified GRT prepared according to technique C was slower curing than the other two blend compounds. Hardness, modulus at 300% elongation, abrasion resistance and swelling resistance of the blend of virgin NR and GRT modified according to technique B were superior to that of the virgin NR vulcanisate. Cure rate and tensile strength of the former was comparable to the latter. As most of the physico-mechanical properties of the 85/30 blend compound of virgin NR and GRT modified according to technique B were at a level acceptable for tyre treads, it could be a suitable replacement for the virgin NR compound in tyre treads.

O-2

Preparation and Mechanical Property of the Epoxidized Natural Rubber from Field Latex

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Epoxidized natural rubber (ENR) is the modified natural rubber for oil resistant which can use benefits and increase value. The researcher had studied the preparation and physical property of the epoxidized natural rubber from field latex by compared with rubber STR 5L. In this research field latex was used epoxidation with hydrogen peroxide and formic acid by control the temperature and the duration of time during 60 and 360 minutes, It will get the epoxidized natural rubber which contained the epoxide groups about 25 and 50 percent mol respectively, and it has a traction resistance property less than rubber STR 5L, however, it has a better scorch time and cure time. Furthermore, the rubber ENR-50 is resistant to ASTM No.1 and IRM 903 oil that is better than rubber ENR-25 and STR 5L, respectively.

O-3

Natural Rubber Nanocomposites from Pristine and Organically Modified Layered Silicates by Melt Intercalation Process

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Natural rubber nanocomposites were produced by melt mixing of natural rubber (NR) with organically modified silicates. For comparison a pristine layered silicate and a non-layered version were also included in the study. The layered silicate used was sodium bentonite and organoclays used were octadecylamine (MMT-ODA) and methyltallow bis-2-hydroxyethyl ammonium modified montmorillonite (MMT-TMDA). Accelerated sulphur system was used for the vulcanization of the nanocomposites. The dispersion of these silicates was studied by X-ray diffraction (XRD) and transmission electron microscopy (TEM). The organoclays incorporated composites exhibited faster curing and improved mechanical properties. The improvement in the mechanical properties of the composites followed the order MMT-ODA > MMT-TMDA > EIC > bentonite. The property

improvement was attributed to the intercalation/exfoliation of the organically modified silicates due their high initial interlayer distance.

O-4

A Process for Preparation of Carbon Black / Silica / Nanoclay Master Batch from Fresh Natural Rubber Latex

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A process for production of carbon black/silica/nanoclay ternary filler masterbatch from fresh natural rubber (NR) latex was standardized. The filler, nano clay at doses varying from 3-10 parts per hundred rubber (phr) along with carbon black and silica at a dose of 25/25 phr was incorporated in fresh NR latex by a modified coagulation process. The coagulum, containing fillers was dried at 70°C in an air oven to get latex filler masterbatch which was processed further in the conventional way. The master batch compounds containing only silica/carbon black showed higher level of vulcanization as compared to the corresponding dry mixes. The vulcanization level of nanoclay incorporated mixes increased as the content of nanoclay increased. The mechanical properties like tensile strength, modulus, tear strength, abrasion resistance and hardness increased with proportion of nanoclay in the mixes up to 5 phr and with further amount the change was only marginal. The heat build-up values for the tri-filler masterbatches that contained 5 to 10 phr nanoclay was similar to the 25/25 carbon black/silica master batch. Lower tan delta values were observed for all the master batches containing nanoclay in the ranges 3-10 phr compared to the control dry mix containing 25/25 carbon black/silica, showing better polymer filler interaction. The tan delta values at 60°C are lower for all the master batches than the dry rubber mix containing 25/25 carbon black/silica. The tri-filler system containing 5 phr nanoclay showed the lowest tan delta at 60°C and the highest storage modulus and loss modulus within the different ternary filler masterbatches. The improvement in mechanical properties and dynamic properties shown by the silica/carbon black/ nanoclay master batches over the conventional mill mixed compounds was attributed to factors related to filler dispersion and higher level of vulcanization. Better filler dispersion was evidenced from the result of filler dispersion data obtained using Dispersion Analyzer and nanoclay exfoliation observed from X-Ray diffractograms.

O-5

Viscosity Behaviour and its Influence in Natural Rubber and Synthetic Latex Processing and Product Manufacturing Systems

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The viscosities of natural and synthetic latices vary over a wide range, both in their natural and compounded states. Methodologies used to measure viscosity are found to provide non-corroborating results when compared against the standards. This paper examines some changes of viscosity due to seasonal variations, concentrations, stabilisations and storage of natural and some common synthetic latices in their pure and compounded state. Particle size distribution and the uniformity of modulus and tensile properties are examined. The influence of viscosity and its role in the formation, shaping and consolidation of the latex end product, with its effect on processing parameters such as dip pick up, interlayer adhesion, ZOV, ZST/ZHST and crosslink densities are also reviewed. The control of rheological behaviour characteristics of latices as a means of manufacturing more consistent latex based products is discussed.

O-6

Use of Coconut Pith as Bio Filler for EPDM Rubber Composites

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Natural fillers find good application in composite preparation because of various reasons. Low cost, low density, biodegradability, nonabrasive nature etc. are some of the major peculiarities of natural fillers which make them ideal for composite preparation and end use applications. In the current study, coconut pith – a by-product from coconut fiber processing industries is used as value added filler in rubber formulations. EPDM-coconut pith filler composites were prepared using varying filler doses. Physico-mechanical properties and electrical resistance properties of EPDM rubber composites containing coconut pith fillers were studied. coconut pith as being cheap and biodegradable filler, the composites are ecofriendly and biodegradable with an added advantage of lower cost.

Silica Reinforcement of Epoxidised Natural Rubber of Varying Epoxy Content

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Natural rubber (NR) is a renewable material that combines excellent mechanical and dynamic properties. Tyre is one of the major areas of application of natural rubber. Replacement of carbon black by silica is growing in the tyre industry on ecological and technological grounds. Silica as a reinforcing filler in tyre contributes to lower rolling resistance and improved wet traction. However, silica also causes some processing difficulties. Polar silica surface results in high filler-filler interaction and hence forms agglomerates in the rubber matrix, especially in non polar hydrocarbon rubbers such as natural rubber leading to poor filler dispersion. The silanol groups on the surface of silica particles also causes adsorption of curatives which results in cure retardation. Unlike carbon black, the poor reinforcement of silica in natural rubber compounds is attributed to the poor filler dispersion and ineffective coupling of silica to natural rubber. Use of silane coupling agents in silica reinforced rubbers can improve rubber-silica interaction and hence better dispersion. Epoxidised natural rubber (ENR) is a chemically modified form of NR and is more polar than NR. Silica exhibits better reinforcement in ENR than in NR. Silica filled ENR exhibits low rolling resistance and high wet grip in tyres. Present study reports silica reinforcement of ENR of varying epoxy content. Silane modified silica filled NR, ENR 10, ENR 17.5, ENR 25 and ENR 50 and silica filled NR and ENR 50 were compared for their cure characteristics, stress-strain properties and technological properties viz; tear strength, hardness, heat build-up, resilience, compression set, abrasion loss and Demattia flex failure. Cure characteristics of silane modified silica filled NR and ENR of varying epoxy content and that of silica filled ENR were comparable. Silica filled NR without silane showed less scorch time, delayed cure and higher cure torque. Stress-strain properties of silane modified samples showed a regular variation with level of epoxidation. Modulus showed an increase with extent of epoxidation. Silica filled ENR 10 and 17.5 exhibited optimum modulus, strength and medium elongation. Hardness, heat build-up, compression set and abrasion loss showed an increasing pattern whereas tear strength, resilience and flex resistance showed a decreasing trend with level of epoxidation. Atomic force microscopy studies showed that dispersion of silica was better in ENR based samples.

Stable Free Radical Assisted Peroxide Vulcanisation: Cure Characteristics and Vulcanisate Properties

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The paper demonstrates the capability of a nitroxide stable free radical, TEMPO, to induce sulphur/accelerator like scorch control in peroxide vulcanisation of EPDM. Two commercially used peroxides, butyl (4,4-di(tert-butylperoxy) valerate (Trignox 17-40B) and Di(tert-butyl peroxyisopropyl)benzene (Perkadox14-40B) with typical cure temperatures of 160°C and 175°C respectively were used for vulcanisation. The addition of TEMPO induced scorch in both systems and the cure curves resembling typical sulphur/ accelerator cure curves were obtained. The reduction in cross link density with the addition of TEMPO was significantly less in Perkadox14-40B system. Addition of 4 phr of trimethylolpropane trimethacrylate (SR 350) could regain the loss of cross links due to the scavenging action of TEMPO. The vulcanisate properties of the EPDM/Perkadox14-40B/TEMPO/SR350 system were determined and compared with a control EPDM compound. The mechanism of the scorch control by the stable free radical is also discussed.

Processing of Nano Fillers in Rubber – A Novel Technique

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Dispersion of various nano fillers like nano silica, nano clay, nano TiO₂, etc. has been attempted in NBR / HNBR rubbers using a new technique of dispersion. Liquid NBR is used for dispersion of nano fillers in polymer matrix and polymer nanocomposites were characterized by Rubber Process Analyzer [RPA-2000, USA]. The dispersion of the nano fillers were investigated by XRD, SEM-EDS. The effect of increasing nano- fillers on

mechanical properties like tensile strength, modulus and elongation at break were studied. Hot air ageing of polymer nanocomposites was also studied.

P-2

Low Protein Natural Rubber Processed Through Gamma Ray Irradiation Technology

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An attempt was made to produce low protein natural rubber (LPNR) using gamma ray irradiation. Fresh latex was exposed to suitable dose of gamma radiation, creamed and then coagulated to get low protein rubber. Processing and technological properties of LPNR was assessed. During exposure to suitable dose of gamma radiation the proteins got hydrolysed to low molecular weight fractions that could be removed by the creaming process. It was observed that the LPNR prepared by this process had very good mechanical and dynamic properties due to the low level of protein.

P-3

Multicomponent Nylon/Coir Pith/Epoxy Composites for Particle Board Applications

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Multilayered coir pith/epoxy based particle boards were prepared by lay up technique. Coir pith was subjected to chemical treatment before processing. The effect of treatment was analyzed by X-ray diffraction and frontier transform infrared spectroscopy. The number of layers, board thickness, and the component ratios were varied for the composites. The boards thus prepared were evaluated for mechanical and dimensional stability and dielectric studies. The component ratio was found to have a huge impact on the overall performance of the boards. The treated and untreated raw materials were giving different property parameters and were also found to influence the performance of the particle boards.

P-4

Synthesis of ZNO Nano Particles, Their Characterization and Their Effect on Stability and Storage on LA-TZ Latex.

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Zinc oxide is an essential additive both in dry rubber and latex technology. Being an activator of vulcanization, it enhances the crosslinking efficiency in latex products. In this paper, we report a simple method for the preparation of hexagonal ZnO nano particles with the assistance of the surfactant Cetyltrimethylammonium bromide (CTAB) and its effect on NR latex stability. Particle size characterization of nano ZnO was performed through Dynamic Light Scattering technique (DLS) and HRTEM analysis. Latex preservation using this nano ZnO was attempted. The different latex stability parameters, including Mechanical Stability Time (MST), Volatile fatty acid and KOH numbers were determined in comparison with conventional ZnO dispersion.

P-5

Mechanical, Thermal, Solvent Transport and Biodegradation Behavior of Cellulose Microfibres (CMF) / Poly (Ethylene-Co-Vinyl Acetate) (EVA) Composites

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CMF/EVA composites were prepared by reinforcing EVA with different percentage of CMF by melt extrusion. CMF were extracted from *Hibiscus sabdariffa* by steam explosion technique. Structural and surface analysis of these microfibres were analysed in detail. The chemical composition of fibres showed increase in α -cellulose content and decrease in lignin and hemicelluloses for the microfibres. These factors were further confirmed by XRD, SEM and FTIR results. The mechanical and thermal properties of the composites were found to

be enhanced with filler loading. The composites were analysed for the biodegradability as per three different methods – natural burial, using *Aspergillus niger* and earthworms. The degradability of the composites was analyzed by studying the weight loss and mechanical properties of the composites before and after subjecting them to degradation.

P-6

Nano Silver Doped Water Soluble Hyperbranched Polyesters for Coating Applications

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Water soluble hyperbranched polyesters coatings have gained lots of popularity as an environment friendly product. The HBPE were synthesized by polycondensation of 2, 2', 2" nitrioltriethanol as a core molecule and butanedioic acid as a chain extender with *p*-Toluene sulphonic acid (P-TSA) as acid catalyst. HBPE of different generations such as G₁, G₂ and G₅ were prepared and the G₅ stage was coupled with acrylic acid to modify the end groups. Silver nanoparticles were prepared in the HBPE matrix by using reductive technique. The synthesised hyperbranched polyester and silver nanoparticles were characterised by different spectroscopic and analytical techniques such as FTIR, ¹H NMR, UV, XRD, solution viscosity and MALDI. The antimicrobial activity of silver nanoparticle/HBPE was tested against *E. coli* and *S. aureus*.

P-7

Preparation and Characterization of Natural Rubber-Rice Husk Ash Composites

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Rice husk is a waste product of the rice processing industry. Rice husk ash (RHA) is the residue left after the combustion of rice husk. In this work, rice husk ash is used as filler

in natural rubber (NR) compounding along with other vulcanizing ingredients. The compounding was done in a two roll mill. Different concentrations of RHA (0, 0.5, 1, 1.5, 2 and 2.5) were used. In order to enhance interfacial adhesion between NR and RHA, maleic anhydride grafted NR (MA-g-NR) was employed as a compatibilizer. Characterization of RHA was done by scanning electron microscopy (SEM), Fourier Transform infrared spectroscopy (FTIR), inductively coupled plasma atomic spectroscopy (ICPAES) and particle size analysis. Both Uncompatibilized and compatibilized NR-RHA composites were characterized by SEM and FTIR. Mechanical and thermal properties of these composites were also studied. Compatibilized NR-RHA composites showed a substantial increase in mechanical properties compared to uncompatibilized NR-RHA composites. Thermal properties of compatibilized and uncompatibilized NR-RHA composites also showed improvement by the presence of RHA.

P-8

Effect of Carbon Black and BaSO₄ on the Thermo-Chemical Resistance of Natural Rubber Composite

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Novel rubber nanocomposites based on natural rubber (NR), BaSO₄ nanoparticles (BN) and carbon black (CB) were prepared by dry mixing. The morphology, mechanical properties and thermal properties of these nanocomposites were investigated. Test results revealed that BaSO₄ nanoparticles modified with sodium stearate exhibited outstanding reinforcement due to its homogenous dispersion in the NR matrix and shows stronger polymer–filler interactions. Significant increase in tensile strength (26%) and tear strength indicated the reinforcement capability of BaSO₄ nanoparticles. Thermal studies indicated that retention in tensile strength and modulus at 300% elongation are well improved in the presence of 5BN along with 30CB. Thermo-oxidative ageing and TGA traces showed that incorporation of 5BN into black filled matrix shifts the onset of degradation from 319.5 to 326 °C, whereas maximum degradation temperature was unaffected. The degradation in acid was found to decrease with the addition of nano BaSO₄ in carbon black filled NR composites. The weight change measurements and studies on adhesion of compound to mild steel plates support the enhancement in properties. SEM micrographs show additional evidence for the better dispersion of nano BaSO₄ and carbon black in natural rubber matrix by the incorporation surface modifiers.

Natural Rubber Compounding- A Possible Route to Combat Environmental Littering due to Waste EPS

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Expanded polystyrene (EPS) is a major class of thermoplastics. The recycling of EPS involves much technological challenges stemming mainly from its very low bulk density (15-50 kg/m³). Its light weight, while being a valuable attribute, makes the material transportation cost high and limits recycling to nearby localities. In this study, this waste material has been utilized for blending with silica-reinforced natural rubber (NR). Partial replacement of NR by small percentage of waste EPS is the main focus in the present study. In view of the rising prices of NR, replacement of NR even by a small percentage of a waste material can have considerable impact on the economic viability of NR processing. The NR/EPS blends were prepared by melt mixing in a Brabender Plasticorder at 140°C for 8 min. Since NR and EPS are incompatible and immiscible a method has been devised to improve compatibility. For this, EPS and NR were initially grafted with maleic anhydride (MA) using the initiator dicumyl peroxide (DCP) to give a graft copolymer. Various graft copolymers were prepared by changing the NR/EPS ratios as 35/5, 30/10, 25/15 and 20/20. For all copolymers, MA and DCP concentrations were maintained at 1phr and 0.15 phr respectively. Grafting was confirmed by Fourier Transform infrared spectroscopy (FTIR). The grafted blend was subsequently blended with more of NR during mill compounding giving blend compositions with ratios of 95/5, 90/10, 85/15 and 80/20 between NR and EPS. A sulphur curing system was employed for the compounds. Silica reinforced NR compounds were also prepared employing the same NR/EPS blends. Morphological studies using scanning electron microscopy (SEM) showed better dispersion of EPS in the compatibilized blend. By this technique, the tensile strength, elongation at break, modulus and tear strength of most compositions were found to be either at par with or better than that of virgin silica filled NR compound. It is also noted that the thermal properties of the blends are equivalent to that of virgin NR. The study establishes the potential of this method for replacing of NR by waste EPS to some extent. While this addresses to some extent the environmental problems arising from waste EPS this technique has the potential to make NR processing more economical.

Natural Rubber Based Proton Conductive Membrane for Fuel Cell Applications: A Review

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The main energy crisis is that the world is at a point at which global energy demand begins outstripping supply. This led to the growing demand for renewable and alternative energy generation techniques like fuel cell and solar cells. Proton exchange membrane fuel cells (PEMFCs) are often regarded as an ecofriendly energy conversion device due to their high efficiency, more electricity produced per unit cell of fuel, low CO₂ emission, low operating temperature and quick start up. Nafion, a per fluorinated polymer substituted by sulfonic acid groups, is the most commonly used polymer for the fabrication in proton exchange fuel cell membranes. They are relatively expensive and difficult to synthesize and process. This review paper focuses on the synthesis of proton exchange membranes from locally available material, natural rubber. Polymer electrolyte based on modified natural rubber is prepared by incorporating salt to enhance their ionic conductivity at room temperature and the conductivity was found to increase with the weight percentage of PEO-ENR50 and LiCF₃SO₃ salt. Proton exchange membranes were synthesized by sulphonation of polystyrene butadiene rubber using chlorosulphonic acid as the sulphonating agent. Result of various analysis obtained revealed that the synthesized membrane possess better quality (solvent uptake, methanol permeability and thermal stability) in comparison to the Nafion 112 which is a commercially available membrane. The synthesized membrane was blended with carbon nanoballs which indicated 50% increase in proton conductivity. This paper suggests future work to be carried out to improve the membrane performance such as proton conductivity and tensile strength by performing chemical modification which converts the carbon-carbon double bond to carbon-carbon single bond.

Less Labour Intensive Processing System [LELIPS] – A New Approach for NR Processing

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Nearly 71% of the marketable form of Natural Rubber (NR) in India is processed into Ribbed Smoked Sheets (RSS). Sheet processing continues to be a labour intensive, drudgery involved, time consuming and more infrastructure requiring age old way without any modernisation. For the modernization of rubber sheet processing, designed and developed two devices by significantly modifying the conventional system. 1. Innovation of a Coagulation Tank with horizontal partitions suitable for bulk preparation (60 sheets at a stretch) of sheets, eliminating defects old model coagulation tanks with vertical partitions. Trials conducted with the new coagulation tank proved its advantages in terms of time, labour and space saving compared to the conventional dish coagulation. Apart from drastic reduction in the time required for preparation of coagulum, the new coagulation tank helps to improve quality of the product also. 2. A new design multi storied trolley smoke house has changed the basic concept of a smoke house as a dark walled hot and sooty room, quite unpleasant to work inside; to a work place with comfortable ambience by removing the drudgery of work inside smoke room. The new design has enabled to shift all the items of work done inside smoke room to trolley yard and also brought substantial reduction in labour, time and fuel consumption in rubber sheet drying process. The two inventions have the potential to provide a new face-lift to the NR processing sector.

SESSION 4

**CROP HARVESTING
AND POST HARVEST**

Influence of age and girth at opening on rubber yield, biochemical and tapping panel dryness parameters of *Hevea brasiliensis* in determining tapping norms

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The incidence of several girths or tapping start period on the parameters of rubber yield, the physiological profile and the sensitivity to tapping panel dryness of *Hevea brasiliensis* have been studied for a decade in southeastern rubber growing area of Côte d'Ivoire. The study was conducted from so called early (tapping start at 40, 45 cm girth) and/or late (opening at 55, 60 and 65 cm girth) tapping applied to clones GT 1, PB 217 and PB 235. This study shows that starting tapping clone GT 1 at 40 cm girth and clones PB 217 and PB 235 at 50 cm are the best treatments in terms of rubber yield, physiological parameters and tapping panel dryness. These girths at the opening were reached at approximately 6 years after planting, regardless of the clone. The satisfactory results recorded demonstrate the preponderance of the notion of "opening age" over that of circumference of tapping start. Thus, the age of 6 years after planting seems to be the best period to start tapping rubber trees, because it is a good benchmark of physiological maturity in *Hevea brasiliensis*. Despite this precedence of the age over the girth, the different results indicate the need to use both criteria to take into account a delay in the growth and especially a difference in the vegetative growth of the clones planted.

Impact of The Reduction of The Tapping Frequency on the Agronomic and Physiological Parameters of Clone PB 260 of *Hevea brasiliensis* in the Centre West of Cote D'ivoire in Order to Make Up For The Shortage of Tapping Labour

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Rubber cultivation is still faced with the problem of scarcity of skilled tapping labour despite a significant reduction in the tapping intensity due to improved latex harvesting technologies. Thus, it is essential to optimize the latex harvesting technologies by a greater reduction in the need for tapping labour so as to improve his productivity. This strategy is based on the reduction of the tapping intensity, offset by an intensification of hormonal stimulation. In that respect, a study was conducted with clone PB 260 of *Hevea Brasiliensis*, in Gagnoa in the centre-west of Côte d'Ivoire. Low frequencies tapping of latex harvesting technologies (S/2 d4, S/2 d5 and S/2 d6) which are low consumers of tapping labour were compared to standard or traditional latex harvesting technology (S/2 d3) which is a high consumer of tapping labour. The results showed that low frequencies tapping give yields per tree inferior or identical to that of the traditional tapping. Furthermore, these low frequencies tapping have no negative impact on the physiological profile, the radial vegetative growth and the sensitivity to tapping panel dryness of the trees. While reducing the need for tappers down to 50%, these systems provide a substantial increase in the income of tappers and farmers. Low frequencies tapping are the best alternative to the traditional tapping to solve the problem of scarcity of tapping labour in rubber cultivation.

Alternative Tapping Systems for RRIC 100 Clone From Opening

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Ethephon or gas stimulation is a common practice in rubber cultivation especially in commercial estates. The use of stimulation can be combined with reduction in the frequency of tapping or by reduction of tapping cut length. The purpose of this study was to compare and to evaluate the effect of alternative tapping system from opening. The results showed that the alternative tapping system of S/2 d3 ET2.5% Ga1 12/y (m) or MC20 U d3 ETG 12/y (m) were suitable. The advantage of MC20 U d3 ETG 12/y (m) compared to S/2 d3 ET2.5% Ga1 12/y (m) was saving of bark consumption extending the economic life span of rubber trees. However, the application of gas stimulation is considered as risky for the tapping panel dryness occurrence. Short tapping cut (microcut) was preferable with gas stimulation than ethephon.

Rain Guarding is Essential for Introducing Modern Methods of Latex Harvest Technology

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Though well distributed moderate rainfall is essential for good growth and production of natural rubber by rubber trees (*Hevea brasiliensis*), depending on the intensity, spells and diurnal pattern rainfall obstructs latex harvesting to different extent. In the heavy rainfall region of India, crop loss is up to 900 Kg dry rubber per 400 trees. In the low rainfall regions corresponding loss is around than 500 Kg/400 trees. Yield stimulation can make only partial recovery of the loss. Rain guarding is essential for modernizing latex harvest technology. There are two groups of rain guards, panel guards and channel guards. Panel guards which protect both tapping channel from becoming wet and the cup out are popular in India and Sri Lanka. Channel guards are used in China, Vietnam and Malaysia. Channel guards protect

from washout due to stem flow. Tapping shade that is popular in India for the base panel is more suitable for controlled upward tapping of quarter spiral cut in the high panel. Rain guarding is essential to increase production of natural rubber, income of farmers, economic life of trees and to reduce labor and incidence of tapping panel dryness.

O-5

Response of Several IRR Series *Hevea* Clone to Tapping System with Stimulation

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Response of every *Hevea* clone to latex stimulant is very different according to their physiological characters. This character will be used as a base in determining the latex harvesting system with stimulation to obtain an optimum yield for a clone. The research was aimed to obtain harvesting system suitable for a clone to obtain an optimum yield.

The results showed that for IRR 39 and IRR 44, frequency stimulation of 10 and 20 times per year respectively were enough, whilst for the other clones (IRR 32, IRR 41 and IRR 107) required a frequency of 20/y or even more, considering the sucrose content, inorganic P and TPD intensity.

O-6

Anaerobic Digestion of Rubber Waste for Production of Renewable Energy

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Anaerobic digestion of rubber leaves at three different feeding rates (FR) of 35, 50 and 65 g L⁻¹ was investigated. The experiment was carried out at ambient temperature for a retention time of 24 days. Chemical composition of the samples was determined before and after anaerobic digestion. The fractions of the biogas produced were determined by

Gas Chromatography (GC). The carbon/nitrogen ratio of the substrate of about 27 obtained in this study was within the range of optimal anaerobic digestion. Cumulative total biogas production of 4520ml, 2120ml and 290ml were found for 35, 50 and 65 g L⁻¹ FR respectively (i.e. as the FR increases the total cumulative biogas produced decreases). Extent of biodegradability of the substrate in each FR was determined by calculating reduction in total and volatile solids. The reduction in total solids were respectively 39.43%, 28.24% and 7.67% for 35, 50 and 65 g L⁻¹ FR; while reduction in volatile solids were 43.38%, 13.91% and 6.47% for 35, 50 and 65 g L⁻¹ FR respectively. These results show that 35 g L⁻¹ FR gave the highest yield of biogas. Generally, lower FR tends to favour biogas production. GC analysis of the biogas produced shows that methane constitute 52.33% of the biogas produced; others were, N₂ (4.35%), CO₂ (34.6%), and other biogas constituents (8.72%)

P-1

Concept of Crop Secondary Yield and Its Application in Research and Production of *Hevea* Tree

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The concept of crop secondary yield is brought up to improve the efficiency of research activities and production management of some nontraditional crops. Generally crop yield, the quantity of targeted plant organs of most crops harvested once for a crop, is derived from the common crop production, and the yield of these crops may be called “primary yield”. However, other crops can be harvested repeatedly for a crop from their targeted plant organs or secondary metabolites, and the yield may be termed as “secondary yield”, which is not only connected with crop heredity, microenvironment, etc., but also closely related to harvest technologies and harvest frequencies, which are a man-made stress, temporarily denominated “production stress”. Therefore crop secondary yield is evidently different in yield traits from primary yield. Rubber yield is a typical crop secondary yield, which is affected by tapping system obviously, besides clones, soil fertility, climate and so on. It is suggested that the concept of common crop yield used for formulating the strategy of research activity and production management of rubber tree might be switched to that of crop secondary yield to conquer the puzzle of early yield prediction and clone yield assessment of rubber tree and to improve the tapping system and economic life span of rubber tree.

Influence of Various Tapping Systems on Rubber Yield of Two Clones RRIV 3 and PB 260 at South-East Region in Vietnam

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A study which includes two trials was carried out to examine influence of various tapping systems on yield of two rubber clones RRIV 3 and PB 260 in the South East region of Vietnam. Two trials were established for each clone which had 8 treatments involving tapping frequencies of d3 and d4 in combination with 0, 4, 6, 8 stimulations (the non-stimulated treatments were control for each of tapping frequency). Recorded data from 2008 to 2011 showed that stimulated treatments produced higher yield (g/t/t: gram/tree/tapping) than non-stimulated treatment. Individual yields (g/t/t) of d4 treatments were higher than those of d3 treatments on both clones. However, the mean yield (kg/ha/year) over 4 tapping years of d4 of clone PB 260 was lower than those of d3 because of lesser number of tappings. The highest production (kg/ha/year) was obtained from the treatment of d3 tapping frequency combined with 8 rounds of stimulation per year on PB 260. Conversely, there was a positive response to production of low tapping frequency (d4) on RRIV 3; as a result, yield (kg/ha/year) of d4 treatments was higher than those of d3 treatments, in which the treatment of d4 with 6 stimulations per year gave highest production. Latex physiological parameters were recorded from all treatments of these trials. The results showed that, latex physiological parameters expressed positive activity of the laticiferous system after four years under low tapping frequency and stimulation. Tapping panel dryness was lower than 5 percent on all treatments of two clones.

The Effect of Rain Guarding on Reducing Latex Loss

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Rubber productivity depends on genetic and environmental factors. Rainfall especially is very important environmental resource for rubber growth. However, rainfall may also be a limiting factor for tapping activity. Rain may wash the latex dripping, delay tapping or completely cause tapping day loss.

An experiment was conducted at Sembawa Research Centre, Palembang, Indonesia. The treatment consisted of 3 tapping tasks with rain guard and the other 3 tapping task without rain guard. Observation was made on the effectiveness of rain guard preventing rain disturb and rubber yield.

Rain guard was made from bonnet, and natural gum was used to seal the gap between rain guard and bark of rubber tree. The effectiveness of rain guard was observed by measuring rain water coming into latex cup during the rain. Observation showed that rain guard may reduce the rain water coming into latex cup where water entered into latex cup was only 20% compared with that of control. Also bark become drier faster with rain guard after the rainfall was ceased. The yield difference between task with rain guard application and control was 8.2 gram/tree/tapping during the rainy days.

Aerobic Digestion through Air Diffusion for the Effective Treatment of RSS Processing Effluent

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Various types of anaerobic digesters are available to treat Ribbed Smoked Sheet (RSS) processing effluent which causes environmental pollution because of the organic

constituents present in it. The anaerobic digesters developed for this have efficiencies ranging from 80-95% in BOD and COD reduction. The water coming out after treatment in these anaerobic digesters still contains degradable matter and therefore pollution parameters are not within the limit specified for safe disposal. Therefore, it warrants further treatment. At present in RSS processing industry this is being performed aerobically in oxidation ponds. Major limitations of this kind of treatment are slow process and requirement of large area. Activated sludge process could reduce the pollution load in a shorter period of time which needs lesser space. Even though the conventional activated sludge treatment using fixed or floating aerator is very quick and efficient, it is expensive due to high electricity consumption. Therefore diffused aeration, an effective activated sludge processing mean which produces fine air bubbles that give greater oxygen transfer was experimented.

In the present study, an aerobic activated sludge system with diffused aeration technology was designed, developed and evaluated. The system consisted of a rectangular tank with 9 disc type diffusers having a diameter of 9 inch placed in the bottom of the tank at a distance of 60 cm from each other. The diffuser is made of EPDM with a rubber diaphragm at top having large number of fine holes. Air is pumped in to the diffuser using one HP Twin Lobe Rotary Air blower. Air is released through the fine holes in the diaphragm of the diffuser as fine bubbles. This oxygenation method is much more efficient in transferring air because this creates a larger transfer surface area per unit volume of added air. The diffuser system is highly resistant to clogging and corrosion and fit for wide range of air flow. These fine bubble membrane diffusers can reduce the power required to transfer oxygen by up to 50%. The pressurised movement of air bubbles provides a water stirring effect also.

In the study, the partially digested effluent from anaerobic digester was fed to the aeration tank at a controlled rate to maintain HRT of 24 hrs and subjected to oxygenation by diffused aeration. The treated water from the aeration unit was then subjected to sedimentation to remove settleable solids. The water coming out after aeration and sedimentation was analysed periodically for various pollution parameters like BOD, COD, total solids dissolved solids and pH as per standard procedures. The combined action of the aeration and sedimentation reduced the COD, BOD, TS and DS of the feed effluent by 58.5%, 88.6%, 10.3% and 2.7% respectively. The treatment could bring down the major pollution parameters vis. pH, COD and BOD to the specified safe limit. This proves that the post anaerobic treatment of RSS processing effluent using diffused aeration technology is an effective and efficient methodology for the treatment of RSS processing waste water.

Effect of Panel Changing on Long Term Yield Response of *Hevea brasiliensis* (Clone RR11 105) under Different Frequencies of Tapping and Stimulation

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Panel changing is attempted in some plantations under the assumption that it helps to manage tapping panel dryness and increase yield. It is also considered to be useful for reducing the physiological stress generated in the panel due high frequency tapping. In a Randomised Block Design experiment, effect of panel change on long term yield response of *Hevea brasiliensis* (clone RR11 105) under different frequencies of tapping and stimulation was studied over a period of eleven years. There were eight treatments comprising of d2, d3 and d4 frequencies of tapping of half spiral cuts with and with out panel change and different levels of stimulation. Considerable yield variation was observed between various treatments over the years. Effect of panel change on yield increase was more prominent in the initial five years. No significant beneficial impact of panel change on yield increase was observed under different systems of tapping. Higher yield could be obtained under d2 and d3 frequencies of tapping with upper panel change (CUT). Comparable yield could be obtained under various frequencies of tapping. Cumulative yield observed within similar systems of tapping with or with out panel change were also comparable. Significantly high yield (gram/tree/tap and per tap yield) was noticed under d4 frequency of tapping. Highest cumulative yield was observed under d2 frequency of tapping which was however observed to be at par with d3 frequency of tapping with or without panel change. Panel change results in higher TPD under d/2 frequency of tapping compared to lower frequency of tapping. Moreover, benefit of panel change is reflected only in the initial five years. Panel management after first five years of tapping is difficult. Hence, continuous panel change cannot be considered for managing TPD or to get sustainable high yield over long term.

Long Term Yield Response of Low Frequency Tapping and Yield Stimulation in Clone RR11 105

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Among the rubber producing countries, India ranks the first position in natural rubber (NR) productivity. In India, cost of production is also high due to undulating topography, agroclimate and other cultural practices. Cost of tapping is the single major item in cost of production of natural rubber. In some countries, tapping alone accounts for more than 70% of the cost of production of NR. India and other rubber producing countries are facing severe shortage of skilled tappers. Under these circumstances, grower has to pay more wages to attract the tapper or leave the trees untapped due to unavailability of skilled tapper. Increasing labour productivity by adopting low frequency tapping with mild yield stimulation (ethephon) is one of the approaches to reduce the cost of production and to overcome tapper shortage. Use of ethephon under low frequency tapping is recommended for high yielding clones like RR11 105. There is no adverse effect if yield stimulation is judiciously done on high yielding clones tapped under low frequency tapping (d3, d4 and d6)

An experiment was laid out in the Experimental Farm Unit (EFU) of Rubber Research institute of India located at Kottayam, Kerala (9°32'N; 76°36'E) to compare the long term yield response of clone RR11 105 under low frequency tapping. Average stand per hectare was 450 trees. There were ten treatments comprising d2 (alternate daily tapping without stimulation as control), d3, d4 and d6 frequencies of tapping with different frequencies of stimulation. The experimental design was RBD with five replications comprising 25 trees per replication. Yield stimulant ethephon @2.5% was applied on the panel. Cultural operations were as per the standard package of practices. Trees were rainguarded and tapped throughout the year without giving annual rest. Yield from each replicate was recorded as latex and scrap separately on all the tapping days. Dry rubber yield was calculated by converting latex weight proportionate to the drc, and scrap weight based on 60 per cent drc. Tapping panel dryness (TPD) was recorded as complete drying of tapping cut. 15 years comprehensive data on yield and other parameters are presented.

Annual dry rubber yield (Kg/ha) for fifteen years (1997 – 2012) under different frequencies of tapping and stimulation were statistically analyzed. Yield under third daily tapping, fourth daily and once a week frequencies were comparable to that of alternate daily tapping. In all frequencies, there was yield reduction when the tapping panel was close to the bud union. Similarly, considerably high yield was obtained during the first year of tapping on BO-2 panel in all frequencies.

Cumulative dry rubber yield over fifteen years under d3, d4 and d6 frequencies at the recommended level of yield stimulation was comparable to unstimulated alternate daily tapping (d2). Mean annual tapping days during the study period under d2, d3, d4 and d6 frequencies of tapping were 144, 98, and 74 and 51 days, respectively. Thus by adopting d3, d4 and d6 frequencies of tapping, requirement of tapper can be reduced by 32%, 49% and 65% respectively, compared to alternate daily tapping.

Cumulative yield from BO-1 and BO-2 panel under d3, d4 and d6 frequencies were higher than that of d2. Rubber yield per unit area (kg/cm) of bark consumed in both panels were also higher under LFT. Since the use of ethephon under low frequency tapping at recommended level was only to compensate the potential loss of yield through reduced tapping days, the incidence of panel dryness under LFT was less.

Present study clearly indicated that tapping under d3, d4 and d6 along with appropriate stimulation can result in comparable yield to that of alternate daily tapping. Sustainable yield can be achieved under LFT with yield stimulation. Fixing of rainguard during monsoon is essential to ensure regular tapping and success of LFT. Benefits of adopting LFT are sustainable high yield, low incidence of panel dryness and extended period of tapping on the same panel leading to longer economic life of the trees. In India, low frequency tapping systems have been successfully extended to estates and medium holdings. It is envisaged that the current skilled tapper shortage can be resolved by adopting LFT. By practicing low frequency tapping, grower can benefit from reduction in cost of production and increased economic life of rubber trees. Adoption of LFT is also expected to reduce the impact of scarcity of skilled tapper in rubber plantation sector.

SESSION 5
CROP MANAGEMENT

Rubber-Based Farming System: in a Challenging Industry Development and Expansion in Southern Philippines

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Natural rubber expansion in Southern Philippines in the past two decades was terribly slow owing to the longer gestation period of the crop before a farming household could get harvests. In Zamboanga Peninsula region where majority of the rubber plantations of the country are located, expansion to new areas was only one percent (1%) until 2001. This was because 70% of the farms are small-holdings (average of 3-5 ha.), and therefore farmers preferred to grow crops that can provide immediate source of food and income. In 2003, the rubber-based farming system (RBFS) has been promoted in the region through participatory action research. These rubber intercropping practices was proven in providing improved farm productivity and profitability. People's organizations were tapped in the implementation process which facilitated the technology diffusion, sustainability, and the operationalization of other rubber-based community livelihood projects.

Intercropping of other high value crops gave significant production and income during the 1st five years of the plantation. Results showed that intercropped rubber farms grew faster and harvested a year earlier than monocrop farms or the conventional farmer's practice. Harvesting started on the 5th year in RBFS, while monocrop farms harvesting started on the 6th year. Average yield of RBFS is 2,269.2kg/ha/yr dry rubber while 2,084kg/ha dry rubber in monocrop farms. Farmers' net income reached to Php393, 547.00 (US\$9,152) with Return on Capital (ROC) of 299.88% while monocrop rubber is only Php249, 996.00 (US\$5,813.9) and an ROC of 150.61%. RBFS obtained excellent Marginal Benefit Cost Ratio (MBCR) of 2.99.

As of 2011, there were 278 farmers in the locality and adjacent areas expanding rubber plantation adopting the technology. These farmers adopted the full package of rubber-based farming system; availed good quality planting materials produced from the farm project and acquired rubber budsticks utilizing the unwanted branches of the rubber trees. Family labor was primarily utilized in various farm activities, while three additional jobs per hectare per year are hired to complement activities on production, plantation maintenance and harvesting.

Effect of High Density Planting on Growth and Yield of Selected RRIM 2000 Series

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Rubber smallholders sectors play a significant role in the growth and development of the Malaysian rubber industry with contributed 94.6% of the total rubber production in Malaysia. Although there is an important contribution by the rubber smallholders sector, the majority of them to be plagued by persistent low productivity and income largely due to uneconomic size of holding, planting of non-recommended clones, low adoption of technology, capital deficiency and unstable of rubber price in the past. With the low yield of rubber (average 1480 kg per hectare, 2010) from the rubber smallholders, several approaches have been developed by Malaysian Rubber Board to increase land productivity and income. One of the approaches is through adopting high density planting.

A study on the effect of high density planting of rubber on growth and yield of rubber was conducted at FELDA Jenderak Utara, Temerloh, Pahang, Malaysia, on Durian and Gajah Mati soil series, started from February 2000. The treatments study were (i) Planting densities, i.e. 500 trees/ha (4m x 5m), 700 trees/ha (4m x 3.6m), and 1000 trees/ha (4m x 2.5m) and (ii) rubber clones i.e. RRIM 2025, RRIM 2016 and RRIM 2001. The treatments were arranged according to the Split Randomized Complete Block Design (RCBD) with three replications. The results found there were significant effects of planting density and clone on growth (girth) of rubber, 63 months after planting. Densities of 500 trees and 700 trees/ha had higher growth (girth) compared to density of 1000 trees/ha with mean of 47.4 cm = 47.2 cm > 44.1 cm, respectively, 63 months after planting. Clone RRIM 2025 had higher growth (girth) compared to RRIM 2016 and RRIM 2001 with mean of 48.3 cm > 46 cm = 44.6 cm, respectively. The densities of 500 and 700 trees/ha produced higher tree productivity (g/t/t) at the second year of tapping compared to the density of 1000 trees/ha with means of 64.9 g/t/t = 64.2 g/t/t > 52.9 g/t/t, respectively with tapping system of 1/2S D3 SEN(8x/year). However, there was no significant effect of clone on tree productivity during the same period of tapping (RRIM 2025 = RRIM 2001 = RRIM 2016 with mean of 64.7 g/t/t = 60.3 g/t/t = 59.0 g/t/t, respectively). Survey on bark thickness was conducted, 84 months after planting. The result found, the density of 500 and 700 trees/ha had thicker bark thickness compared to the density of 1000 trees/ha with means of 8.6 mm > 8.1 mm > 7.0 mm, respectively. Clone of RRIM 2025 had thicker bark thickness compared to the clone RRIM 2016 and RRIM 2001 with means of 8.6 mm > 7.6 mm > 7.1 mm, respectively. Density of 1000 trees/ha had higher clear bole volume (m³/tree) compared to the density of 700 and 500 trees/ha with means of 0.107 m³/tree > 0.077 m³/

tree = 0.076 m³/tree, respectively, 84 months after planting. Clone of RRIM 2025 had higher clear bole volume compared to the clone RRIM 2016 and RRIM 2001 with means of 0.098 m³/tree > 0.080 m³/tree = 0.078 m³/tree, respectively, 84 months after planting.

O-3

Cropping Pattern of Coffee as Rubber Intercrop at Farmer Level in Muara Enim and Musi Rawas Districts of South Sumatra Province, Indonesia

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Coffee is one of the important commodities in South Sumatra Province, Indonesia. It has become the main source of income for farmers and has been cultivated for long time in their farming system. Therefore, several areas such as Muara Enim and Musi Rawas Districts in South Sumatra Province have been known as the centres of coffee production, and the coffee is called as “Palembang coffee”. Due to high fluctuation of coffee price during the last 10 years, i.e. ranging from US\$ 0.46 – US\$ 2.65, coffee cannot be solely commodity to support livelihood of farmers. In the coffee production centre area in and/or nearby rubber production centre area, several farmers have tried to cultivate coffee in integration with rubber. The reason was that the current rubber price is good and tends to increase (US\$ 0.73 – US\$ 3.08). The arising problem was that how the performance of growth and production for both coffee and rubber would be, when the two commodities are cultivated under poly culture system. This has been of interest to be studied, so that the cropping system can be further developed and more profitable. The study was done in Muara Enim and Musi Rawas Districts, South Sumatra Province. The research was conducted by using survey method and field observation. The study revealed that coffee could be cultivated as the intercrop in rubber, without having negative impact on rubber growth. Under cropping system, the coffee crop was still productive until the seedling rubber plants were 10 years old – the coffee productivity was on average of 325 kg/ha/yr (Muara Enim 300 kg/ha/yr and Musi Rawas 350 kg/ha/yr). Nevertheless, there is still a need of further detail analysis, especially with regard to the total output in cropping system. Based on Land Equivalent Ratio (LER) which was calculated as the percentage decrease in crop production between both coffee and rubber, the value of LER was > 1.0, LER of coffee as rubber intercrop for the District of Muara Enim was 1.23 and for Musi Rawas was 1.35. This means that coffee as rubber intercrop was more profitable by 23% and 35% compared to the cultivation of coffee and rubber monoculture, respectively.

O-4

Soil Fertility Evolution and Correlation with Leaf Nutrient Contents in Rubber Plantation in Hainan, China

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After more than 50 years of rubber planting, the soil fertility of tropical soils under rubber trees showed great changes. The contents of organic matter, total nitrogen, and available potassium in the plantation soils under consideration decreased markedly. However, the content of the available phosphorus presented a tendency either increased or decreased in different degrees. On the whole, widespread rubber soil fertility drop has been noticed in Hainan. In the early years of rubber planting, there was a very significant correlation between nutrient contents in rubber leaves and those in rubber plantation soils, in particular at the regions of low soil fertility. Unfortunately, the correlation has dwindled away with time. Because of the localized fertilizer application and many contour terraces constructed, the typical representative soil samples in rubber plantations were difficult to collect. Thus, it is suggested to use leaf nutrients as the indicator of rubber tree nutrition status and fertilizer requirements, and soil nutrients just as a reference indicator.

O-5

Adaptation of Root Trainer Technology in Different Agroclimatic Environments

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Root Trainer Technology for improved Nursery Development of Natural Rubber Plants has had good acceptance in the Indian NR production Scenario in the past 5 years. The advantages have been well documented in the published RRII literature. The large scale adoption of this technology in the traditional and non traditional NR Producing Areas in Guatemala is presented here based on experience in probably the largest scale of adoption of this technology in two nurseries with over 200000 plants. The process of

adoption has necessitated changes in local nursery practices of initial planting material, fertilizing, budding technique, hardening as well as exposed limitations which are being addressed to ensure a more productive end result. However the efficacy of root trainers is graphically demonstrated at the primary stages. Possible extension of RT Technology to enhance the robustness and resistance of the plants in hostile environments is discussed.

O-6

Nutrient Status of Soils and Leaves of Immature Rubber in the East Coast of Upper Part of Southern Thailand

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Major rubber plantation in Thailand is in the southern part of the country, in which soils are highly weathered and acid. Most of the plantations are replanted both from rubber and other crops repeatedly for more than 40 years. Therefore, soil fertility is declined. This study was carried out to examine nutrient status of rubber growing-soils and rubber leaves. Forty three samples of soils and leaves were collected from 4 year-old rubber plantations during June to July 2009 in the east coast of upper part of southern Thailand. The samples then were analyzed following standard procedures. The nutrient status was done by comparing nutrients with the diagnostic criteria. From the criterion of rubber soils, the results showed that CEC and exchangeable K were low in every samples (0.7 to 5.3 cmol(+)/kg) and 18 to 113 mg/kg, respectively). Most of the soils had medium organic matter and exchangeable Ca (0.6 to 2.3 % and 18 to 576 mg/kg, respectively), but low in available P and exchangeable Mg (0.3 to 28.0 mg/kg and 3 to 144 mg/kg, respectively). When comparing with the criterion of general soils, the results showed that CEC, OM, exchangeable K, Ca, Mg were low, and extractable Cu and B were low (0.03 to 1.48 mg/kg and 0.14 to 0.61 mg/kg, respectively). Extractable Zn was low to medium (0.14 to 1.40 mg/kg). Extractable Mn was medium to high in almost samples (0.5 to 110.2 mg/kg), and all of them had high available S and extractable Fe (17 to 38 mg/kg and 13.5 to 97.5 mg/kg, respectively). The results of leaf analysis showed that most of the samples had low N and K (2.20 to 3.51 % and 0.72 to 1.46 %, respectively). P and S were in a wide range of low to high (0.16 to 0.29 % and 0.11 to 0.37 %, respectively). Mg was high to very high (0.18 to 0.40 %). Fe and Cu were medium to high in almost samples (64 to 151 mg/kg and 8 to 14 mg/kg, respectively).

And most of them had high Ca, Zn and B (0.56 to 1.94 %, 19 to 61 mg/kg and 11 to 85 mg/kg, respectively). Mn was very high to toxic in almost samples (142 to 574 mg/kg). It could be concluded that the present status of soils in immature rubber was generally acidic, low in CEC, available P, exchangeable K, Cu and B, low to medium in OM, exchangeable Ca, exchangeable Mg and Zn, medium to high in Mn, and high in available S and Fe. The nutrient status of leaves was low in N and K, medium to high in Fe, low to high in P, S and Cu, medium to very high in Mg, high in Ca, Zn and B, and high to toxic in Mn.

O-7

Correlations of some Soil Chemical Properties, Leaf Chlorophyll content and Growth during Immature Phase of Rubber Tree

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Some soil chemical properties, leaf chlorophyll content and growth during immature phase of rubber tree were studied in the non-traditional area of Thailand during 2004-2009. The objectives of this study were (1) to assess rubber plant growth (2) to establish the correlations between some soil chemical properties and leaf chlorophyll content with their growth and (3) to use the data for suitable fertilizer applying in each specific area immediately. Non-probabilities sampling of forty-six smallholders farming whose have RRIM 600 clone and plant age during 2-4 years were chosen. All sampling area located at 16.84 to 21.12 °N latitude and 21- 522 m. altitude above sea level.

Wide variations of soil chemical property comparing among samplings area were found. Soil pH, organic matter, available phosphorus, exchangeable potassium, calcium and magnesium levels ranged from 4.2 to 7.9, 0.27-3.47 %, 2.5-69.5 ppm, 20-399 ppm, 62-4325 ppm and 6-718 ppm, respectively. For leaf chlorophyll content level, it ranged from 3.80 to 6.51 mg/dm².

Positive correlations of monthly girth increment (MGI) with relative growth rate (RGR) and total chlorophyll content were found. On the contrary, negative correlations of MGI with soil pH, exchangeable potassium, calcium and magnesium levels were found. However, it didn't be to find the correlations between MGI with soil organic matter and available phosphorus due to insufficiently in soil sampling.

O-8

Effect of Weeding Frequency and Fertilizer Rates on the Growth Performance and Budding Successes of *Hevea* Rootstock Seedling in a Humid Forest Area of South Eastern Nigeria

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The effect of weeding frequency and fertilizer (NPKMg 20:10:10:5) rates on the growth and budding successes of *Hevea* rootstock seedlings was evaluated at the nursery field of Rubber Research Institute of Nigeria, Akwete Substation. The weeding frequencies consisted of 24, 12, 8 and 4 times weeding per year, while the fertilizer rates were 200, 100 and 0 kg/ha. Thus having a 4x3 factorial experiment fitted into a randomized complete block design with three replications. The result showed that seedlings in field weeded 12 times/yr had a significantly taller seedlings with wider stem girths compared to the plots with weeded 6 or 4 times/yr. The fertilizer rate effect indicated no significant difference in growth performance and budding successes of seedlings that received 200kg/ha and those that had 100kg/ha NPKMg. The interaction between weeding frequencies and fertilizer rates was significant with seedlings that received 100kg/ha NPKMg 20:10:10:5 and 12 times weeding per year being more robust and with a mean budding success of 76.2% relative to other treatment combination; hence could be said to be a more suitable fertilizer rate and weeding frequency for *Hevea* seedling root stock mainly in a humid forest region.

O-9

Reducing the Immature Phase of Natural Rubber Cultivation: Role of Agromanagement Techniques

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Hevea brasiliensis (natural rubber) forms the basis of one of the most important agricultural industries in the Indian economy. The lengthy gestation period of rubber is a

major deterrent among the rubber growers to new planting and replanting. The extent of gestation period of natural rubber is mainly governed by the inherent clonal characteristics, type and quality of planting materials used edaphic and environmental factors, agro-management practices adopted and biotic and abiotic stresses. This paper is focused on the results of a field experiment conducted with an objective to develop an agronomic package to reduce the immaturity period of rubber.

The effect of different agro-management practices on enhancing the growth and reducing the immaturity period of rubber (clone RR11 105) was evaluated individually and in combination (integrated management) in a field experiment conducted at Malankara estate, Thodupuzha, representing the traditional rubber growing region in India. The treatments comprised of current recommended practice; irrigation, *i.e.* irrigating the plants during summer months at 50% of the water requirement for the initial three years; enhanced nutrient application *i.e.* pit manuring @ 500 g bone meal per plant, application of 1.5 times the recommended dose of fertilizers in four splits and 10 kg FYM, 500 g bone meal and 500 g groundnut cake as top dressing for the initial four years; selective manuring *i.e.* application of an additional dose of 1.5 times the recommended dosage of fertilizers in three splits to the weak plants for the initial four years; conservation oriented tillage *i.e.* forking the plant basin followed by heavy mulching and taking conservation pits @ 250 per ha; irrigation + enhanced nutrient application; integrated management which included irrigation, enhanced nutrient application, selective manuring, and conservation -oriented tillage. The individual plot size was 24 plants with a net plot size of six. Observations on growth, soil moisture storage, leaf area index and soil and leaf nutrient status were recorded and disease assessment was done periodically.

The results of the experiment indicated that growth of plants under integrated management was significantly superior to others throughout the experimental phase followed by the treatments irrigation + enhanced nutrient application and irrigation which were on par. The effect of different agromanagement practices was reflected in the leaf area index. The mean LAI recorded at the centre of four plants at different times was the highest for integrated management, followed by irrigation + enhanced nutrient application and irrigation which were comparable. Soil moisture storage during summer also followed a similar trend. A significantly higher soil moisture content was retained by the treatments integrated management, irrigation + enhanced nutrient application and irrigation. The soil and leaf nutrient status did not vary significantly among treatments. The incidence of pink disease was moderate. However, treatmental variations in pink incidence were not observed.

The individual effect of mulching, tillage, conservation pit and organic manure addition on improving soil moisture storage and enhancing growth of the plants is well documented. Mulching is an important agronomic practice in rubber plantations to conserve soil moisture, protect the soil around the base of plants from direct impact of heavy rains and sunlight causing soil degradation. Silt pits are one of the efficient runoff management techniques wherein a part of the runoff is conserved and reused for crop production in a sustainable manner. Earlier studies showed that split application of fertilizers and the use of increased rate and frequency of fertilizer application also contributed towards enhanced growth and thereby reducing immaturity period. Irrigation during summer also enhances the growth of the plants.

All these good agricultural practices contributed towards the increased growth of the plants under integrated management and 72 per cent of the trees under integrated management achieved tappable girth in a period of five years and nine months compared to the normal gestation period of seven years in the traditional belt. The results of the experiment clearly indicate the feasibility of substantially reducing the immaturity period through the adoption of improved agro-management techniques.

P-1

Nutrient Management in Rubber Seedling Nursery- Studies in the Integrated Approach through Incorporation of Bio Inoculants

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In rubber (*Hevea brasiliensis*), seedling plants are raised either in the ground nursery or in polythene bags for raising good quality stock plants for bud grafting. Field experiment in rubber seedling nursery was conducted during 2009-2010 and 2010-11 planting seasons to study the possibility of reducing the dose of N and P fertilizers by incorporating bio inoculants. The treatments were control, standard practice (SP), standard practice (SP) + bio inoculants, 50 per cent N and P and recommended doses of K and Mg + bio inoculants and bio inoculants alone. The consortia of bio inoculants were one isolate of nitrogen fixing bacteria (*Azotobacter* sp.), two strains of phosphorus solubilizing bacteria (*Bacillus* spp.), two strains of plant growth promoting rhizobacteria (*Pseudomonas* spp.), all isolated from the rubber growing soils and multiplied at RRII and Arbuscular mycorrhizal fungi (AMF) from The Energy and Resources Institute (TERI), New Delhi.

Growth of plants with 50 per cent reduced level of N and P fertilizer in combination with bioinoculants was on par with the standard recommended dose of chemical fertilizers either alone or in combination with bioinoculants indicating the possibility of reducing the dose of N and P fertilizers. Any additional beneficial effects of application of bio inoculants on plant growth or on soil properties could not be elucidated through the present study probably due to the already prevailing high organic carbon and available P status of the soil. *Pseudomonas* population was significantly high in BI alone, SP+BI and 50% N and P +full K and Mg + BI treatments. AMF infection per cent in the root indicated 70 to 80 per cent infection in all the plants. The results from the study need to be reconfirmed through on farm trials in different locations with varying soil fertility status.

Indian Summer Monsoon Rainfall (ISMR) Over Kerala Exhibits a Temporary Shift

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When the precipitation time series of the State of Kerala during monsoon for the period 1871-2011 were analyzed for trends and variability, a major shift in the rainfall pattern towards the end of the century was noticed, as seasonal rainfall during SW monsoon exhibited a downward trend and NE monsoon, an upward trend. When the performances of individual months were studied a declining trend was noticed for June and July towards the end of the century. Rainfall during August did not exhibit any trend and an insignificant positive trend was observed for September, October and November rainfall.

Regression analysis showed that SW and total monsoon was highly influenced by June and July rainfall. Cyclic patterns of different wavelengths were observed for individual months during monsoon when the precipitation time series were subjected to running means of different time bases and epochs of these below normal and above normal rainfall events seem likely natural variability.

DTPA-Extractable Micronutrients in Rubber Growing Soils of North-East India

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Soil samples were collected from the rubber growing areas of Assam, Meghalaya and Tripura. DTPA extractable cations were estimated and their relations with different physico-chemical properties of soils were studied. Soils varied from sandy loam to clay loam in texture, extreme to moderately acidic in reaction (pH: 3.9 to 6.1) and medium to high organic carbon content (5.50 to 22.50 mg kg⁻¹). Cation exchange capacity of the soil ranged from 4.52 to 17.45 cmol (p+) kg⁻¹. DTPA extractable Fe, Mn, Zn and Cu content of the soil ranged from 41 to 308 (mean 160.5), 6.1 to 72.0 (mean 29.1), 0.13 to 2.86 (mean 0.84) and 0.50 to 2.46

(1.47) mg kg⁻¹, respectively. Based on the prescribed critical limit, all the samples were sufficient in DTPA-Fe and Mn. Cu deficiency was observed in 4.11% of the total soil samples collected. About 29% soil samples were deficient in DTPA-Zn. Hill Zone of Assam recorded the highest (33.6%) and Garo Hills of Meghalaya recorded the lowest (21.65%) number of Zn-deficient samples. Organic carbon and clay contents of the soils were positively and significantly correlated with the DTPA-extractable micronutrients and contributed significant variations towards their availability.

P-4

Seasonal Variation on Crop-Weather Relation in *Hevea* Grown Under the Sub-Himalayan Climate of West Bengal

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Contribution of antecedent meteorological variables was studied in relation to yield of rubber (*Hevea brasiliensis*) in the hot perhumid region of Nagrakata, Jalpaiguri, north of W. Bengal. Day to day fluctuation in meteorological variables were higher during post-monsoon (October to December) compared to that of monsoon period (June to September). This was also reflected in the yielding pattern of the rubber clones. Weather parameters regressed better with yield during the post-monsoon period. Clonal variation in yield was observed in crop-weather relations. In general, over the study period, the mean relative humidity of the day prior to tapping showed an influence on the next day's yield. However, during monsoon it was the minimum temperature of two days prior to tapping and in post-monsoon period it was mostly the previous day soil temperature at 5cm depth that was related to the yield. The previous day's weather attributes significantly contributed to yield in most of the cases during the study period as well as during post-monsoon; in monsoon, weather attributes of 2 days prior to tapping were mostly important for almost all the clones. The present study showed that it is during the post-monsoon season that the rubber yield reflected better association with weather in the Sub-Himalayan climate of Nagrakata.

Evaluation of Field Performance: Polybag Vs. Root Trainer Rubber Plants at Different Stages

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An experiment investigating the comparative field performance of polybag and root trainer rubber plants at different growth stages was initiated at the Central Experiment Station, Chethackal of the Rubber Research Institute of India representing the traditional rubber growing region in India during 2008 with clone RRII 105. The treatments comprised of combinations of two types of planting material viz., direct-seeded green- budded polybag plants and root trainer plants (raised by planting budded stumps) at three growth stages viz., one- whorl, two -whorl and three- whorl. Observations on growth were recorded for a period of four years. Success in establishment was hundred per cent in the field irrespective of the planting material and its stages. The variability in girth quantified using CV for polybag one- whorl, two- whorl, three- whorl and root trainer one- whorl, two- whorl and three- whorl plants were 12.72, 12.82, 12.38, 12.35, 12.67 and 11.05 per cent, respectively. The significant difference observed in the girth of the plants among the types of planting material and its stages during the initial years progressively became less apparent and by two and a half years, only three- whorl polybag plants remained significantly superior to others. The same trend continued till four years of growth in the field. The performance of all other planting materials viz., polybag – one- whorl, two- whorl and root trainer – one- whorl, two- whorl and three- whorl were comparable. However, among these, considering the practical convenience and cost involved, root trainer one- whorl plants appear to be the ideal planting material for commercial planting of *Hevea*. The constraints and advantages of different planting materials while planting are also discussed.

Carbon and Nutrients stock in mature rubber plantations with and without control of under flora

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Though rubber (*Hevea brasiliensis*) has its origin in the Amazonian forests, its plantations worldwide are monoculture in nature. The under flora is rigorously managed in the immature phases of rubber cultivation. A case study was carried out to compare the stock of carbon and nutrients in the under flora of mature rubber plantations where its growth was controlled and not controlled. The study was carried out in Pathampuzha village near Palai in Kerala. Eight fields in each category viz., with and without control of under flora were selected and stock of carbon and nutrients in soils (0-45 cm) and in above ground flora were estimated. RRII 105 was the clone in all the fields and planting was carried out during 1990-1996 period. The cover crop *Mucuna bracteata* was established in all the fields during the initial stages. As the canopy of rubber trees crossed over, the cover crop faded out and other weeds dominated. In eight of the sixteen fields studied, weeding was regularly carried out to control the under flora while in the other eight fields weeding was restricted to narrow strips in the platform, for the tapping personnel to move around. Soil samples were collected from three depths viz., 0-15, 15-30 and 15-45 cm using soil cores and gravel (> 2mm) and fine earths (< 2mm) were estimated. Five sets of samples were collected from each depth in each field. The stocks of organic carbon (OC) and nutrients viz., total nitrogen (TN), available forms of potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn) and copper (Cu) in soils (0-45 cm) were estimated. Above ground biomass of the under flora in each field was estimated by physically removing the above ground portions of the under flora from five sites, each of 1 m² area from each field. The samples were estimated for C, TN, P, K, Ca, Mg, Zn and Cu contents and the amount of each nutrient locked in the above ground flora was calculated. It was noticed that in fields where under flora was not controlled for about ten years, there was significant improvement in the stock of OC, TN, K and Mg in soil. The above ground biomass was obviously much more in fields where under flora was not controlled. In these fields significantly higher amounts of C, N, P, K, Ca, Mg, Zn and Cu were also noticed in the above ground biomass of under flora. A management practice by which the C and nutrients build up in soil as well as in above ground under flora in mature rubber plantations is of great significance environmentally and agriculturally.

Distribution of Potassium Forms in Soils Under Repeated Rubber Cultivation and Virgin Forest

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Among the major nutrients, uptake of potassium is considered to be next to that of nitrogen for most crops which leads to its removal in substantial quantities through economic produce and biomass. Successive cultivation of the same crop has been reported to deplete soil potassium and change the distribution of K forms. This will have profound influence on the K availability to crops and should be taken in to consideration for potassium management, particularly in the subsequent cycles of cropping.

Major rubber growing soils are under Ultisols and in general, Ultisols are highly weathered with relatively low exchangeable bases. The total potassium of rubber growing soils of Kerala ranged 0.48-1.22 percent and that of Assam 1.33 per cent. NBSS and LUP surveyed rubber growing soils in the traditional region and reported that 62 percent is low in available K. In soil, K exists as solution, exchangeable and non exchangeable forms and they are in dynamic equilibrium with each other. Application of fertilizers increases solution and exchangeable K and subsequent crop removal reduce them. When the solution and exchangeable K are depleted from soil then the non exchangeable K slowly becomes exchangeable form.

Natural rubber cultivation started in India about a century ago. Most of the rubber plantations in traditional rubber growing tract are in the 3rd cycle. The removal of potassium through economic produce is limited, but at the time of felling of trees at the end of a plantation cycle, substantial quantities are removed through biomass. The objectives of the present study were to determine the effect of repeated cultivation of rubber on the K content of soil and to compare the distribution of different forms of K in soils under repeated rubber cultivation and adjacent virgin forest. We also attempted to correlate forms of potassium with other soil properties.

The study site was located at 9° 31' E and 76° 52' N in the midland of traditional rubber growing tract at Mundakayam, Kerala, India. Eight soil samples (0-30 cm) each were collected from 2nd and 3rd cycle rubber plantations and from adjacent forest. The samples were air dried at room temperature and sieved through 2 mm sieve and subjected chemical analysis. The samples were analysed for water soluble, exchangeable, and non-exchangeable, clay potassium and total potassium. The water soluble potassium was estimated by shaking the soil with distilled water for 24 hours. Exchangeable K was determined by extraction with 1M ammonium acetate. Non exchangeable K was extracted by 1 M HNO₃ digestion at 113°C for 25 minutes. Total K and Clay potassium were estimated

by X - Ray Fluorescence spectroscopy. Mineral K was calculated by subtracting non exchangeable from total K. To estimate total and clay potassium by XRF, 0.7000 g of finely ground soil and 7.000 g of flux (mixture of 35.3% lithium-borate and 64.7% lithium- meta borate) were taken in a plastic vial. The vials were rotated by hand to get the proper mixing and homogeneity. The samples were fused at 1050° C in platinum crucibles in a pre heated muffle furnace for about 40 minutes and then poured the molted liquids into platinum molts to get glass discs. After cooling, these discs were used for total elemental analysis using wave length dispersive X- ray fluorescence spectroscopy. Clay samples were prepared in the similar manner after separation of clay from soil. Soil pH, OC, CEC, available phosphorus, Calcium and Magnesium were estimated by standard methods.

Soil organic carbon, CEC and pH showed a declining trend due to rubber cultivation, however, there was no significant. The water soluble K ranged from 4.10 ppm in 3rd cycle rubber to 5.70 ppm in forest and there was no significant difference between treatments. Exchangeable K ranged from 55.9 ppm in third cycle rubber to 69 ppm in forest and they were comparable. Exchangeable K status gives an indication of the possible crop response to K application and hence is important for K management. The non exchangeable K (fixed K) was significantly lower (242.94 ppm) in 3rd cycle rubber. Mineral K was not significantly different in different systems. The total K ranged from 1.28 in 2nd cycle rubber to 0.96 percent in 3rd cycle rubber. The total K was 1.13 per cent in forest. The data indicate the presence of considerable K reserve in the soil studied even in the third cycle. Replenishment of K through fertilizers might be the reason for the maintenance of water soluble and exchangeable K forms in rubber soils. Significant positive correlation was observed between water soluble K and soil pH, organic carbon status and CEC. Exchangeable K was positively correlated with CEC. Total K was also significantly correlated with non-exchangeable and mineral K.

The study shows the presence of considerable K reserve in rubber plantations. The distribution of various K forms was comparable in different cycles of rubber cultivation and adjacent virgin forest.

P-8

Under-Flora in Rubber Plantations: Its Effect on Soil Properties

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A case study was carried out to compare the health of soils in mature rubber plantations where growth of under flora was controlled and not controlled. The location of the study fields were scattered around a small region in the traditional rubber growing belt in Kerala,

in Pathampuzha village, near Palai. Altogether sixteen fields were selected for the study, of which eight were with the regular practice of weed control and in the other eight fields; control of under flora was restricted to very minimal areas in the platform line, to allow free movement of the tapper. Rubber plants in all these sixteen fields were of same clone (RRII 105) and planted during 1990-1994 period. During the initial stages, cover crop *Mucuna* existed in all the fields and other management operations were also similar. However, in the later stages, *Mucuna* faded out when the tree canopy covered and gradually other weed and shrub species dominated. The growth of these under flora was regularly managed in eight of the sixteen fields while in the others it was not. This practice had resulted in the establishment of thick under flora in the eight fields while in the other eight, ground cover was very much restricted, obviously with less number of species. Chemical fertilizer and Cu-fungicide input were regular in fields where the under flora was controlled while those practice were not followed in the others.

Nutrients stock, and above ground biomass of these fields was assessed and are reported here. Soil samples were collected from all the studied fields, at three depths 0-15, 15-30 and 30-45 cm. Total C and N and available nutrients such as K, Ca, Mg and P were determined and stock of each nutrients in the three soil layers of each field calculated. Above ground biomass in all the fields were estimated. Moisture status was determined once in two fields in each situation, during the summer period. From ten sites in each field, samples were taken at two depths, viz., 0-15 and 15-45 cm and compared. *In situ* soil respiration was also determined once, in two fields each, in situations where under flora was controlled and not controlled.

In the top layer of soil viz., 0-15 cm, the stock content of total C and N and all the available nutrients except P and Zn were significantly higher in the fields where under flora was allowed to grow freely. However, stock content of Av. Cu was more in the top layer of soils under the situation where weeds were controlled and Cu fungicides regularly applied. In the middle layer of soil (15-30 cm), the stock of nutrient / micro nutrient contents except Mg and Cu were not significantly different in the two situations, viz., with and without control of under flora. Av. Mg was more in fields where under flora was not controlled while it was *vice versa* in the case of Av. Cu. In the bottom layer of soil (30-45 cm), Stock content of C, Av. K, and Mg were significantly higher in situation where under flora was not controlled.

In situ soil respiration was significantly higher in fields where no control of under flora indicating higher microbial activity. It was observed that the soil respiration and soil Cu content were negatively correlated. However, the reason for lower soil respiration rates in fields where weeds were controlled can not be fully attributed to the Cu content, as other factors such as organic matter also might have influenced the rate of soil respiration. Though, with one time observation on soil moisture and above ground biomass, definite conclusions are difficult, the data indicate possibility of higher moisture retention and more above ground biomass in fields where under flora was not restricted. Gravimetric moisture content in soil was found to be 12.24 and 14.65 % at depths 0-15 and 15-45 cm respectively in fields where under flora was not controlled while the corresponding values were 10.13

and 11.97 in fields with control of under flora. Soil respiration rate was 7.43 and 2.38 $\mu\text{mol}/\text{m}^2/\text{s}$ in fields where no control and control of weed flora was there, respectively.

The yield data collected had its own limitations for comparison between the two situations because of the variation in tapping systems. However, it could be noticed that in fields where under flora was not controlled, average annual tapping days were 63 and the average dry rubber yield per tree per annum was 5.27 kg, while the corresponding values in the fields where under flora was controlled were 100 days and 5.57 kg. Evidently, the yield in terms of g/tree/tap was more in fields where weeds were not controlled. Though such data can not be interpreted for arriving conclusions on yield variations, all what can be inferred is allowing under flora in rubber plantations might not reduce rubber yield.

The study indicates, allowing growth of under flora in mature rubber plantations improves soil properties. Improvement in Carbon and Nitrogen content in soils upon allowing under flora is of specific relevance not only due to agronomic reasons, but also due to environment concerns. Other nutrients such as K, Ca and Mg also tend to increase by which the fertility of the soil increases in such cases. Allowing under flora also has reflected in higher above ground biomass and higher soil respiration rate. This case study indicates the possibility of improving soil health upon allowing under flora in mature rubber plantations.

P-9

Estimates of Nitrogen Fixation by Leguminous Cover Crops Grown in Immature Rubber Plantations Using ^{15}N Isotope Dilution Technique

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The beneficial effects of leguminous ground covers on growth and yield of rubber and improving soil properties have been well documented. Leguminous cover crops fix atmospheric nitrogen, enrich soil with organic matter, improve soil fertility, reduce soil erosion and suppress weed growth. Legume covers help in enhancing the growth of rubber during immature phase and reduce the immaturity period. *Pueraria phaseoloies* and *Mucuna bracteata* are the two common cover crops grown in rubber plantations in India.

Major advantage of growing legumes is their ability to fix atmospheric nitrogen in the root nodules in association with a group of bacteria belonging to the genus *Bradyrhizobium*. Legume cover crops fix substantial quantity of atmospheric nitrogen and thus help in reducing the use of costly nitrogenous fertilizers.

The actual quantity of nitrogen fixed by these two legumes in the traditional rubber growing tract of India has not been estimated. The ^{15}N Isotope dilution method in which labeled N is added to soil has been widely used to provide reliable estimates of N_2 fixed by legumes in the field. In the present work, ^{15}N Isotope dilution method has been used to estimate the amounts of N_2 fixed by *Pueraria phaseoloides* and *Mucuna bracteata* grown in the inter-rows of immature rubber plantation in the traditional rubber growing tract of South India, using *Ipomea batatas* as the reference plant.

A field experiment was conducted in the RRII farm during the active growth period of cover crops, ie from June to November, during the year 2009, in an area planted with *Hevea brasiliensis*, clone RRII 105. The soil of the experimental area was sandy clay loam, with pH – 4.4, organic carbon-1.86 per cent, available P-3.5 mg/kg and available K-26.6 mg/kg. *Pueraria phaseoloides* and *Mucuna bracteata* and *Ipomea batatas* were established in the inter-rows of rubber plants in separate blocks in the same field. The seeds were not inoculated with *Bradyrhizobium* prior to sowing. Uniform cuttings of *Ipomea batatas*, (10 cm length), collected from Central Tuber Crops Research Institute, Trivandrum, were planted, at the rate of three cuttings per planting point, spaced 15 cm apart. Recommended doses of NPK fertilizers were applied to both cover crops and reference plant.

For labeled N application, micro-plots (1 m x 1m) were erected using GI sheets, inserted 6 cm into the soil surface in between two rows of rubber plants. There were seven replicate micro-plots for each legume and the reference plant. ^{15}N labeled urea, 5 atom % ^{15}N excess, @ 5 kg/ha was applied in each micro-plot, after the cover crop and reference plant established well. The labeled fertilizer was dissolved in 1 liter water and then poured with a watering can. The area outside the micro-plot was fertilized with equivalent amounts of non-labeled urea. The micro-plot experiment with labeled urea application was repeated again during August-September and also during October-November.

The above ground biomass from the labeled N applied micro-plots were harvested 45 days after the application. Fresh biomass was weighed at the site and sub samples were collected for estimating dry matter and N content. The plant samples were dried at 70°C in an air draught oven, weighed for dry matter determination, powdered and analyzed for tissue N and atom % ^{15}N . Total N in plant tissue was determined by micro-Kjeldahl method and N isotope ratio by Mass Spectrometry.

The percentages of plant N derived from the atmosphere (% *Ndfa*) and from labeled N (% *Ndfa*) were calculated. The data was subjected to analysis of variance.

Dry matter accumulation, N content in dry matter and N accumulation in above ground biomass were comparable for the two legumes, while these parameters were significantly lower for the reference plant *Ipomea batatas* during all the three measurement periods. ^{15}N enrichment in plant tissue was significantly lower for the legumes compared to the reference plant *Ipomea batatas*, indicating a dilution of the legume plant N by non-labeled N from the atmosphere, which is an indication of nitrogen fixation by the legumes.

The estimate of per cent N derived from fixation (%*Ndfa*) for *P. phaseoloides* ranged from 71.07 per cent of total plant N during June-July, to 42.96 per cent during Oct-Nov.

period. Corresponding estimates of N_2 -fixation for *P. phaseoloides* ranged from 79.82 kg N/ha during June-July to 39.32 kg N/ha during Oct.-Nov. and averaged 58.1 kg N/ha/year. %Ndfa for *M. bracteata* ranged from 73.27 per cent of total plant N during Jun-July to 60.13 per cent during Oct.-Nov. and corresponding estimates of N_2 fixation ranged from 83.95 kg N/ha during June-July to 57.03 kg N/ha during Oct.-Nov., and averaged 69.7 kg N/ha/year.

In our study, N_2 fixation by of *M. bracteata*, was higher than that of *P. phaseoloides*, and the difference was significant during the Oct.-Nov. period. It has been previously observed that the population of *Bradyrhizobium* which could nodulate *P. phaseoloides* in acid soils was low, and the effectiveness of nodules produced was also found to be low. N_2 fixation by *P. phaseoloides* observed in this study, ie, 58.1 kg N/ha is comparable with the estimate of 49.0 kg N/ha reported earlier in a Malaysian oxisol, estimated by ^{15}N Isotope dilution method using *Ipomea batatas* as reference plant. Isotopic estimates of N_2 fixation by *M. bracteata* are not reported, but significantly higher N-accumulation in biomass compared to *P. phaseoloides* of the same age has been reported.

The study showed that both the cover crops fixed considerable quantities of atmospheric nitrogen and that N_2 fixation by *Mucuna bracteata* was higher than that of *Pueraria phaseoloides*.

P-10

High Density Planting in Rubber Plantations

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In India 89 percent of the area under rubber cultivation is in the small holding sector. Small holder is attracted to high density planting to increase the productivity of his land. However, increasing planting density beyond an optimum may lead to poor growth and yield. An ideal density is one which gives enough above and below ground space for all the trees to grow without competition. Density determines the yield per tree and per unit area also. It also influences the planting and maintenance cost and tapping task. Hence planting density is an important parameter which influences the growth and yield of trees and profitability. The present recommended planting density for rubber in India is 420-500 trees/ha.

A field experiment was initiated at Central Experiment Station, Chethackal during 1994 with clone RR11 105 in split plot design with five densities as main plot treatment and two fertilizer quantities as sub plot treatment replicated four times to study the effect of density of planting on growth and yield of rubber. The five densities in the main plot

comprises 420 trees/ha (4.9mx4.9m), 479 trees/ha (4.6mx 4.6m), 549 trees/ha (4.3mx4.3m), 638 trees/ha (4mx4m) and 749 trees/ha (3.7mx3.7m) and the two subplot treatments are recommended dose of fertilizer (RDF) on unit area basis and RDF on per plant basis. The plot size ranged from 25 to 49 plants according to the density. Girth of plants at a height of 150 cm from bud union was recorded annually. Bark thickness and bole height were measured 18 years after planting. Tapping was initiated in 2003 adopting 1/2sd/3 system. The yield of individual trees was recorded at fortnightly intervals following cup coagulation method.

It was observed that girth of plants was not significantly influenced by the different densities up to eight years after planting. However plants in the higher densities of 549 trees/ha and 638 trees/ha recorded higher girth than that in other densities till the sixth year. In the later years plants in the lowest density of 420 trees/ha recorded highest girth and from 9th year onwards growth of plants in this lowest density was significantly higher than all other planting densities. Lowest girth was recorded in the highest density plots. It was also noticed that fertilizer treatments had no significant effect on growth of plants under different planting densities.

Plants in the lowest density had significantly higher bark thickness among the various densities and it was comparable with that in the density at 549 trees/ha.

In the first year of tapping, per tree yield was not significantly influenced by the different densities. However in the subsequent years plants in the lowest density (420 trees/ha) recorded significantly higher per tree yield (g/t/t) than all other densities. The annual yield per hectare showed a different trend. It increased with the planting density. The seven year yield data showed that in four years it was significantly higher in the highest density and it was comparable with that of the density 549 trees/ha. In the remaining three years it was highest in the density 549 trees/ha. It was also observed that the yield was not affected by the fertilizer treatments. The mean yield of seven years was also highest in the highest density and it was on par with that of 549 trees/ha.

Bole height increased with increase in planting density. It was significantly higher in the highest density. Bole height of trees in other four lower planting densities were comparable. However, it was observed that bole volume (18 year after planting) was not significantly influenced by the different densities.

Net income, considering the cost of cultivation and tapping and income from latex yield of 7 years and timber yield (18th year) was highest for the density of 549 trees/ha.

Increasing planting density significantly retarded the growth of rubber and reduced the per tree yield. Bole volume was not affected by the planting density. The yield per unit area was increased with the planting density and the optimum planting density of clone RRII 105 was 549 trees/ha.

Nutrient Management in Rubber Seedling Nursery: Effect of Inorganic and Biofertilizers

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Seedling nurseries of rubber are established for producing healthy stock seedlings for bud grafting. The beneficial use of fertilizers at different stages of growth has been reported earlier. Adequate and timely applications of fertilizers help to achieve early buddable girth. The possibility also has been reported using plant growth promoting rhizobacteria (PGPR), a mixture of phosphate solubilising bacteria, nitrogen fixing bacteria (*Azotobacter*) for improving the growth of rubber seedlings in the nursery also has been reported. Earlier study indicated that the fertilizer requirement in seedling nursery is considerably lower than the present recommendation. Hence experiments were conducted at different locations with varying soil nutrient status to find out the optimum dose of fertilizers.

First seedling nursery experiment was conducted at the Central Experiment Station, Chethackal of the Rubber Research Institute of India. Soil of the study area was acidic in reaction, high in organic carbon, available P and Mg while low in available K status during 2009-2010 seasons. The treatments included no fertilizer control, standard practice, soluble form of N and P@ 150 kg/ha and 250 kg/ha as ammophos (20-20) and PGPR in combination with N and P @ 150 kg/ha as ammophos (20-20) in randomized block (RBD) design with six replications with a plot size of 48 plants/plot. Diameter of plants and green and brown budability were analysed statistically.

The second study was conducted during 2011-2012 season, at Central Nursery, Karikkattoor with three treatments viz; standard practice and N and P@ 250 with sources of N and P as urea and rock phosphate and as ammophos (20-20) in RBD design with 12 replications with a plot size of 60 plants/plot. The experiment field was high in organic carbon, available P, K and Mg and the soil was acidic in reaction.

The third experiment was laid out at Regional Nursery, Kanhikulam, and Palakad during 2011-2012 seasons in a field with pH in acidic range, medium in organic carbon and available Mg, high in available P while low in available K status with six treatments and six replication in RBD design with a plot size of 60 plants/plot. Treatments were standard practice (NPK @ 500:250:100kg/ha) and N and P@ 250 kg/ha with sources of N and P as urea and rock phosphate and as ammophos (20-20) and each treatments in combination Mg.

Diameter and green buddability of the plants were statistically analyzed.

Application of inorganic fertilizer alone or integrating with PGPR significantly increased the diameter and buddability of rubber seedlings in the experiment at the Central

Experiment Station, Chethackal. Among the treatments, highest green buddability was noticed with application of N and P@ 150 kg/ha as ammophos (20-20) in combination with PGPR and it was on par with standard practice and N and P @150kg/ha as ammophos four months after planting. Ten months after planting, highest brown buddability was noticed in the treatment where N and P@ 250 kg/ha was applied as ammophos (20-20) and it was on par with standard practice and N and P@ 150 kg/ha as ammophos (20-20) in combination with PGPR.

No significant difference was observed between the treatments viz; standard practice and N and P @ 250 kg/ha in diameter and green buddability of plants at Central Nursery, Karikkattoor and Regional Nursery, Kanhikulam.

The results of seedling nursery studies clearly showed that healthy and vigorous seedlings can be generated by the application of a lower dose of fertilizers compared to the standard practice. All these studies confirm the possibility of reducing the present fertilizer recommendation of seedling nursery.

P-12

Secondary and Micronutrient Nutrition in Rubber Seedling Nursery

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The soils under rubber cultivation are highly weathered acid soils. Under the prevailing humid tropical climate with very high intensity rainfall, the fertility of the soil declines through the removal of surface soil by runoff and erosion, through leaching and removal of nutrients especially cations, in addition to the removal through crop uptake. Decline in the status of secondary and micronutrients is an emerging soil fertility constraint in these soils, as in general, these nutrients are not replenished through fertilizer supplements.

In rubber (*Hevea brasiliensis*), seedling plants are raised either in the ground nursery or in polythene bags for producing good quality root stock plants. On to this stock plants, bud from elite clone is grafted to produce the planting material of choice. In permanent nursery sites this process is repeated every year. The current recommended dose of chemical fertilizers to the seedling nursery is 500 kg N, 250 kg P₂O₅, 100 kg K₂O and 37.5 kg MgO / ha. Magnesium application is recommended only in the central part of Kerala where the soil is reported to be deficient in Mg status.

Field experiment in rubber seedling nursery was conducted during 2011-2012 planting season at the Central Nursery of the Rubber Board where the soil is extremely acidic with pH 4.2 and low in available potassium (K), magnesium (Mg), Zinc (Zn), Boron (B), extremely deficient in available calcium (Ca) and high in available phosphorus (P). The objective of the study was to find out the effect of application of secondary and micronutrients on the growth of seedling plants in the nursery where the soil was extremely acidic and deficient in Ca, Mg, Zn and B. The fertilizer treatments were standard recommended dose of N, P and K (SP), SP+ Ca (T₂), SP+ Mg (T₃), SP+ Zn (T₄), SP+ B (T₅), SP+ combination of Ca+ Mg+ Zn +B (T₆) and soil test based fertilizer recommendation (T₇) where Ca+ Mg + Zn and B was applied in combination with half the recommended level of N and P and 25 per cent extra dose of K. Nitrogen, P and K were supplied through urea, rajphos and muriate of potash, respectively. Calcium was supplied through powdered shell lime @ 750 kg/ha (7.5 kg/100 Sq.M). Magnesium was supplied through magnesium sulphate @ of 50 kg/ha (0.5 kg/100Sq.M). Zinc was supplied as zinc sulphate @25kg/ha (2.5 kg/100 Sq.M) and B as Borax @12.5 kg/ha (1.25 kg/100 Sq.M). Sprouted seeds were planted in the nursery bed as per the general guidelines of the standard nursery practice. Growth of the plants (diameter) was recorded during December 2011 and January 2012 and the plants were used for green budding during the first week of February. Leaf samples were collected during March 2012 and analyzed for the total nutrient concentration. Similarly, soil samples were also collected during March 2012 and nutrient availability was assessed.

Statistical significance between treatments was recorded in the growth of the plants. During the first observation recorded in December (four month old plants), the highest diameter was recorded in treatments T₆ (SP+ Ca+ Mg+ Zn+ B) followed by T₇ (soil test based fertilizer recommendation) which were on par with SP + Mg and SP + Zn treatments and were significantly superior to the standard practice. The diameter during January 2012 also recorded statistical significance between treatments. The highest growth was recorded by the treatment T₇ (soil test based fertilizer recommendation) and this was on par with all the other treatments, except the standard practice indicating clearly the beneficial effect of application of secondary and micronutrients, either individually or in combination.

In the plots where boron was not supplied, its status was only in traces. But in the SP+ B treated plots, the available B was improved and was brought to the critical level. Calcium application through lime significantly improved the calcium availability in the soil but pH of the soil, remained in the extremely acidic range.

Soil test based balanced fertilizer application was found to be the best treatment in improving the growth of the plants and in maintaining the fertility of the soil. Similarly, saving in fertilizer cost is also achieved by reducing the dose of N and P fertilizer to half the recommended dose. The present study clearly indicated the importance of secondary and micronutrient supplements in achieving good growth of plants and in maintaining the fertility and productivity of the soil.

High Density Planting - An Option for Higher Productivity of Rubber in North Eastern Region of India

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The effect of planting density in rubber (*Hevea brasiliensis*) was studied in an experiment conducted with three densities viz. 420, 620, 824 trees/ha. It was observed that lower density had higher percentage of trees ready for tapping during initial years, due to higher growth. However higher density achieved required girth in subsequent years. In spite of decrease in plant number over the years, the highest density had always lower girth even 24 years after planting. Higher density also had higher percentage of runs. The higher plant densities produced taller plants, increased crotch height and decreased the number of branches. Plant density affected yield per tree and yield per unit area. Yield per hectare increased with increase in plant density during initial years, however this declined later. High yield per tree per tap was observed in the lowest density with lower yield per unit area. Yield increased in all densities with application of stimulant. Percentage of yield increase due to application of stimulant was higher (40%) in medium density (620 trees/ha) compared to other densities. Percentage of wind damage was lower in higher density during initial years. The volumetric yield of *Hevea* logs varied with density. Total timber volume per hectare was high in high planting density and lower per tree volume of log compared to lower density. Maintaining a density of 600 trees per hectare appears to be most suitable for North Eastern region of India.

Available Boron Status and Its Relationships with Physico-Chemical Properties of Soils under Rubber Cultivation in North-East India

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Rubber (*Hevea brasiliensis*) is fast attaining the most important status as a commercial plantation crop in the north-east India where and it occupies an area of about one hundred thousand hectares at present. The yield level in the region is, however, considerably below the national average. Besides the limiting agro-climatic factors, inadequate and imbalanced nutrition seems to be one of the major factors for such low yield. Boron, an essential micronutrient for all vascular plants, plays an important role in pollination and fruit setting. The supply of soil B to plants largely depends upon the physico-chemical properties of soils and it is therefore desirable to study its relationship with soil characteristics. However, no information on Boron status in the rubber growing soils of the region is available. The present study was, therefore, undertaken to determine the B status and its relationship with various physico-chemical characteristics of soils under rubber plantations in the region. Thirty surface (0-30cm) soil samples each from rubber plantations of Lower Brahmaputra valley, hill zones and Barak Valley of the state of Assam, North Tripura, South Tripura and West Tripura districts of state of Tripura and Garo Hills of Meghalaya were collected. Different physico-chemical properties and hot water soluble Boron were estimated using standard methodologies. Prescribed critical limit was used to find out the percentage of soil samples as deficient and sufficient in HWS-B. Correlation coefficients of HWS-B with different physico-chemical properties were worked out. Step down regression analysis was carried out to find out the factors affecting the variability of HWS-B in soil. Results showed that the HWS-B content in the rubber growing soils of north-east India varied from 0.22 to 1.24 mg kg⁻¹ with an average value of 0.73 mg kg⁻¹. The highest mean HWS-B content was observed in the Barak Valley of Assam (0.79 mg kg⁻¹) and the lowest in the Garo hills of Meghalaya (0.64 mg kg⁻¹). Based on the content of HWS-B < 0.5 mg kg⁻¹ as B deficiency, various rubber growing regions of the north-east was in the order as follows: South Tripura (47.38%) > North Tripura (46.52%) > Garo Hills of Meghalaya (42.76%) > West Tripura (41.89) > Lower Brahmaputra Valley of Assam (41.52%) > Hill Zone of Assam (39.18%) > Barak Valley of Assam (36.74%). On an average, about 42 per cent samples was

found to be deficient in HWS-B. Heavy rainfall, undulating land surface with steep slopes, inadequate ground cover etc. are the major factors contributing to the severe leaching of Boron which in turn resulted in its deficiency at such high magnitude. It was significantly and positively correlated with clay ($r = 0.305^{**}$) and organic carbon ($r = 0.392^{**}$) of the soil. It did not show any significant relationship with sand, silt, pH and CEC of the soil. Moreover, stepwise regression analysis revealed that organic carbon and clay together contributed 21.3 per cent variability of HWS-B in the rubber growing soils of north-east India. Addition of other factors considered in this study did not bring any noticeable changes in the variability of HWS-B. The findings warrant adequate attention to the judicious application of B to overcome its deficiency and its maintenance through addition of sufficient amount of organic matter with adequate ground cover in the rubber plantations of the north-east.

P-15

Influence of NPK Fertilizers on Growth of RRII 400 Series Clones of Rubber (*Hevea Brasiliensis*) and Soil Properties in Tripura during Immature Phase

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Field experiment on growth performance of high yielding RRII 400 series clones of rubber (*Hevea brasiliensis*) viz. RRII 417, RRII 429 and RRII 430 with RRIM 600 as check clone, was studied during 2004-2011 under the agro climatic conditions of Tripura. Influence of higher doses of NPK fertilizers on growth of these clones during immature phase of plantation was also investigated. At the end of seventh year of plantation, the clone RRII 429 registered highest girth (51.5 cm) followed by RRIM 600 (49.6 cm) and the clone RRII 417 (47 cm) as least. Highest tappareability was observed for the clone RRII 429 (67.1%), whereas tappareability of other three clones ranged from 47.7-60.1 percent only. The clone RRII 429 showed significant response towards application of NPK fertilizers. At the end of six year of plantating, the clone RRII 429 registered a mean girth of 50.6 cm and 73.3% plants attained tappable girth upon application of 150% NPK. The other three clones required at least seven years or more to register a mean girth of 50 cm and to attain 70% tappareability. The

result showed that immaturity period of RRII 429 could be reduced at least by six months to one year by applying higher doses of NPK (150% of RDF). Application of higher doses of NPK fertilizers in soils showed a significant improvement for organic carbon (OC) and available phosphorus, particularly at surface layer; however a gradual decline in available potassium-balance was recorded in rubber soils inspite of regular application of potassic fertilizer indicating potassium vulnerability of soils under rubber in Tripura.

P-16

Tillage, Super Absorbent Polymer and Direct Seeding in Polybags can Mitigate Drought Effects During Early Stage of Rubber (*Hevea brasiliensis*) Plantation

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In India, attempts are being made to expand rubber cultivation to agro-climatically less favourable regions, where drought is a major constraint. Climate uncertainty and increasing drought are adversely affecting the establishment and growth of plants even in traditional regions. Objective of the present study was to develop a viable technology for mitigating adverse effects of drought in young rubber plants in dry areas. We tried super absorbent polymer, tillage and K supplement at Puthukkad estate, Trichur, a drought prone area during 2010-12. Three types of planting materials viz., polybag plants raised from green budded stumps, polybag plants raised through direct seeding and root trainer plants were also evaluated. Observations on chlorophyll content index (CCI), soil moisture and growth were recorded. Plants which received super absorbent polymer, tillage and K supplement retained significantly higher CCI compared to control during dry season. Soil moisture status during dry season was significantly higher in the treatments with super absorbent polymer and tillage. Tillage and super absorbent polymer improved growth of plants also during 2012. Polybag plants raised through direct seeding was significantly superior to polybag plants raised from budded stumps with respect to CCI and growth and during both years and was superior to root trainer plants after two years. The results show that tillage and super absorbent polymer are effective in mitigating adverse effects of drought in young rubber plants and polybag plants raised through direct seeding have a better performance in dry areas compared to the other two planting materials. Dipping in super absorbent polymers improved the viability period of green budded stumps also.

Macro and Micronutrient Status of the Traditional Rubber Growing Regions of South India

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Large scale rubber cultivation commenced in India about a century ago and currently most of the plantations are in second and largely in third cycles. Assessment of secondary and micronutrient status of replanting fields of large rubber estates of Kerala and Tamil Nadu, traditional rubber growing regions was carried out during 2011. The soil samples (0-30 cm) were collected from 98 replanting fields of 19 estates in different regions viz., north, north central, central and south region. The soil samples were analyzed for Ph, organic carbon, available P, K, Ca, Mg, S, Fe, Cu, Mn, Zn and B. Results showed that 84 per cent of the replanting fields were low in available ca, 95 percent in available mg and 63 per cent in available s status compared with the general critical limit (300 ppm, 120 ppm and 5 ppm for Ca, Mg and S respectively). In the case of available Mg, when compared with the critical level followed for rubber (10- 25 ppm) 21 per cent of the fields were with low status. Among the micronutrients, Fe, Cu and Mn were above the critical level in all the fields, while 68 per cent of the fields were low in Zn and b status. 58 per cent of the fields were extremely acidic ($\text{pH} < 4.5$). Regional differences in secondary and micronutrient status was also observed. The current fertilizer management practice for rubber focus on major nutrients, N, P and K and mg is recommended in selected regions during the initial years only. Among the micronutrients, Zn is recommended in immature field for first two years. The low status of secondary and micronutrients observed in this study indicate the need for generating base line data on these nutrients through more detailed field survey and careful monitoring to evolve appropriate management strategies.

Observed Changes in the Climate Extremes over Tripura, Northeast India

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Extreme climate events today pose a serious threat to many populated and urbanized areas world wide. An accurate estimate of frequency and distribution of these events can significantly aid in policy planning. Extremes in the temperature are characterized by daily temperature levels exceeding the tolerable limits, and changes in their frequency, duration and amplitude. During the last two decades more attention has been given to study the extremes in daily temperatures and their variability.

Objective of the study was to characterize the climate extremes in a nontraditional rubber growing area i.e. Agartala, Tripura (23° 53'N, 91°15'E). Climate extreme analysis was performed annually and seasonally for Agartala, West Tripura using the tools defined by the World Meteorology Organization (WMO) Expert Team on Climate Change Detection, Monitoring and Indices. Database (1984-2009) from the agrometeorological observatory, Agartala has been employed for analysis.

Seasonal analysis showed that number of hot days (annual count of days with Tmax > 35°C) increased (0.09 days/year) during SW monsoon. Number of days/nights exceeding the 90th percentile threshold was identified as warm days/warm nights and number of days/nights remaining less than 10th percentile was termed as cool days/nights. Increase in the number of warm days (0.3 days/year) was noticed during SW monsoon and winter followed by NE monsoon (0.2days/year). Annually number of warm days and cool days were increased at a rate of 0.6 and 0.5 days/year respectively.

Increase in the number of very hot nights (Tmin > 25° C) were observed at a rate of 1.8 days/year during SW monsoon and a positive trend was noticed during NE monsoon and summer. Number of cool nights (Tmin < 10th percentile) were declined during SW monsoon followed by NE monsoon. Upward trend in the number of warm nights was observed during SW monsoon followed by NE monsoon and winter.

Number of cold spells (annual count of days with 4 consecutive days with daily Tmin < 10th percentile) declined over the years though not significantly. Number of days where Tmax exceeded the temperature thresholds defined as mean (31.9 °C), leaf equivalent temperature 33°C and mean plus 16 (33.8° C) was increased at a rate of 0.8° C, 0.6 °C and 0.5°C respectively over the years during SW Monsoon.. Over a span of 25 years, number of warm spells (three continuous days with Tmax > 33° C) increased at a rate of 0.16 spells per year.

Analyzing the temperature regime, it was noticed that rate of warming was more pronounced in minimum temperature than maximum temperature. In general, minimum temperature in Agartala shows a higher rate of increase and higher coefficients of determination (r^2) than maximum temperature. The reason for such a trend in minimum temperature can be attributed to the effect of suburban modification which is well manifested in minimum temperature than maximum temperature. The result is in agreement with earlier studies that reported that rates of change of temperature and r^2 were higher for suburban stations compared to urban stations, an indication of the increasing urban sprawl.

Precipitation extremes were assessed. According to climate extreme analysis, wet days, heavy precipitation days and very heavy precipitation days were defined as daily rainfall > 1mm, 10 mm and 30 mm, respectively. Number of wet days per years declined in all the seasons except NE monsoon. Number of heavy precipitation days did not decrease/increase in other seasons except during winter where a slight downward trend was noticed. Number of very high precipitation days did exhibit a positive trend only during SW monsoon. Simple daily intensity (annual total precipitation/ number of wet days) increased during summer (0.3mm/year) and SW monsoon (0.1mm/year). Maximum number of consecutive wet days declined and maximum number of consecutive dry days increased in Agartala, though not significantly. Rainy days during SW Monsoon declined at a rate of 0.4 days per year. Extreme rainfall events during the season increased at a rate of 0.15 days per year over a span of 25 years.

It was observed that the lower limit of the upper extreme (10 %) values of Tmin were >25.5 ° C for summer, >26.2 ° C for SW monsoon >24.3 ° C for NE monsoon. The study identifies the temperature range at which the maximum warming occurs and also whether the warming has been confined to the upper extreme temperature only or whether it has its influence at the lower ranges too. Result indicates that frequency of occurrence of warm nights is increasing in Agartala than days which could be adverse for natural rubber yield in other seasons except winter. However, a wider meteorology database is essential to quantify the findings.

P-19

Possibility of using household waste as Nutrient carrier in Rubber plantations

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Hevea brasiliensis, the prime source of natural rubber was introduced into India in 1873 and has been commercially cultivated since 1902 with the aim of establishing an assured

source of important industrial raw material. In the beginning rubber plantations were raised mostly in newly cleared forests rich in plant nutrients. Most of the plantations in India are now in the second or third replanting cycle. To ensure optimum growth and yield and to protect sustainability of the system, maintenance of soil fertility through regular application of fertilizers is very important. Rubber is cultivated mainly in ultisols. They have high aluminum, low pH, iron and manganese toxicity, high P fixation, low molybdenum availability, low base saturation, extensive soil erosion and low microbial activity. Application of organic manures can greatly help in minimizing these productivity constraints. Keeping this in view a field experiment was initiated at our Central experimental station at Chethackal. The study comprised of four treatments viz. T1 (control), T2 (Rubber Board's recommendations), T3 (household compost @6 Kg /plant and T4 (compost @ 3Kg/plant plus high analysis fertilizer). There was no significant difference between compost and straight fertilizer treatments on the girth of trees when the two were used as nutrient carriers. Application of composted household waste have proved to enrich the soil as evident from significant increase in organic carbon, phosphorus, available calcium, available iron and available zinc. The Girth of the trees were significantly correlated with soil solution Mn^{2+} , Zn^{2+} and Cu^{2+} . The average soil solution activity were 3.59×10^{-19} , 1.44×10^{-5} , 5.3×10^{-3} moles L^{-1} for Zn^{2+} , Cu^{2+} and Mn^{2+} respectively. The concentration of Zn^{2+} was under saturated with respect to all the major zinc bearing minerals namely, smithsonite ($ZnCO_3$), Zincite (ZnO), Willeminite (Zn_2SiO_4) and Frankinite ($ZnFe_2O_4$). Speciation studies with Zn prove the occurrence of $ZnSO_4^0$, $ZnNO_3^+$, $ZnH_2PO_4^+$, $ZnCl^+$, $ZnCl_4^{2-}$ in soil solution. These solution can further reduce the activity of Zn^{2+} and its subsequent availability to plants. Cu^{2+} was under saturated with respect to common minerals like Azurite ($Cu_3(OH)_2(CO_3)_2$), Tenorite (CuO), Malachite ($Cu_2(OH)_2CO_3$) and Cupric ferrite ($CuFe_2O_4$). Hence these important Cu bearing minerals would have been lost by the process of weathering under high temperature and high rainfall conditions existing in the soils supporting *Heavea brasiliensis*. The aqueous activity of Cu^{2+} was estimated and was above that of $Cu(OH)_2^0$, $CuOH^+$, $Cu_2(OH)_2^{2+}$, $Cu(OH)^{3-}$ and $Cu(OH)_4^{2-}$. Mn^{2+} was oversaturated with respect to minerals Pyrolusite ($\hat{\alpha}$ - MnO_2), Manganite (\prime - $MnOOH$), bixbyite (Mn_2O_3), Birnessite (W $MnO_{1.8}$) and Nustite (\leftarrow - $MnOOH$). The Mn^{2+} ion activity was above all other species ($MnCl^+$, $MnHCO_3^+$, $MnCO_3^0$, $MnCl_2^0$) These chemical equalibria in soil shows compost treatments improved the bioavailable forms of Zinc and Copper in soil solution because the mineral forms were unstable and already leached out of the soil, nevertheless there was sufficient manganese from inorganic phase of the soil. Therefore addition of organic manure play a significant role in supplying copper and zinc nutrients in highly weathered soils in which *Hevea brasiliensis* is grown. About 1.15 lakh tones of urban wastes are produced each day in India of which 55% is biodegradable and can be used for land application.

SESSION 6

FARM MECHANISATION

O-1

Mechanised Land Preparation for *Hevea* Planting and Plant Growth: A Case Study

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Establishment and early growth performance of rubber plants (*Hevea brasiliensis*) studied under a mechanised system of pitting/terracing with JCB was compared with the conventional manual system of land preparation, one year after field planting. Survival and establishment of plants were not effected by the different systems of pre-planting operations. Growth of plants in terms of girth, height and number of whorls was significantly higher under the mechanised system of cultivation. No marked variations in bulk density was seen between the systems followed. Cover crop establishment was observed to be better with mechanised terracing system. The per hectare total cost of land preparation involving pitting, filling up of the pits, terracing and planting worked out to be less in mechanised land preparation using JCB. Mechanised land preparation is ideally suited for speedy completion of planting operations.

O-2

Mechanised Agronomic Practices in Rubber Plantations – Indian Scenario

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Adoption of mechanisation in agricultural operations has led to significant improvement in agricultural productivity and sustainability. The impacts of mechanised land preparation on soil properties and mechanised weed control on regeneration of weeds in rubber plantations were examined in field experiments. The land preparation methods evaluated were (a) pitting, terracing and tilling inter rows by *Hitachi* (b) pitting and terracing by *Hitachi* (c) pitting by tractor mounded hole digger and manual terracing and (d) Standard practice - manual pitting and terracing. The trial was laid out in a randomised block design with 4 replications. Soil samples were collected from the inter row area of individual plots to determine the mechanical properties, bulk density, porosity and soil organic carbon

content before and after the treatment imposition. The results of the study indicated that pitting, terracing and tilling inter rows by *Hitachi* lead to significantly higher porosity, lower bulk density and soil organic carbon compared to untilled treatments. The rate of soil erosion in pitting, terracing and tilling inter rows by *Hitachi* was 28 per cent more than the no tilled treatments. Comparative cost analysis revealed that pitting and terracing by *Hitachi* saves around 50 per cent of cost of cultivation compared to the standard method (manual) of land preparation.

In the evaluation of weed control methods, the treatments included were slashing of weeds with sickle (manual weeding), spraying of herbicide (glyphosate 2L/ha) and mechanical weeding by weed cutters. The results showed that weed control was most effective in herbicide applied plots and regeneration of weeds occurred only 60 days after treatment imposition. In manual and mechanical weed control methods, the weeds attained 100 percent of regeneration 75 and 90 days after treatment imposition, respectively. The economics of different weed control methods showed that the weeding cost can be reduced by 65 and 75 percent by chemical and mechanical weed control methods compared to manual method.

O-3

Evolutionary changes in ground spraying technology for disease management in rubber plantation – An over view

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Pest and disease incidence existed in rubber plantations from the beginning of its commercial cultivation. Timely management of these biotic stresses that can either retardation of growth or reduction in yield is an important step towards the sustainable production and productivity of natural rubber. Development and introduction of various equipments and devices for the application of chemicals to control pests and diseases in rubber plantation also have a history that is as old on the history of rubber cultivation itself. Both high volume and low volume spraying devices are being used either for prophylactic or curative application of pesticides in rubber plantations. Significant developments in terms of improvements and modification have taken place in the spraying technology in rubber cultivation. The spraying devices used, their performance, upgradation, modifications etc. from the beginning of commercial cultivation of natural rubber in India from 1902 are discussed here.

Napsack and backpack sprayers are used for nursery and immature plants to manage various disease. These sprayer working under hydraulic pressure have been modified to

electrically operated back pack sprayer, due to which manual labour could be reduced considerably. Rocker sprayers which were manually operated are also modified to electrically or mechanically driven systems, which have considerably reduced the labour.

The use of low volume sprayers especially mistblowers in rubber plantations commenced for managing abnormal leaf fall disease caused *Phytophthora spp* during 1957 to 1960. During this period micron 420 with BSA engine was used. This machine having a weight of 120 kg. required 8 workers for operation. But later through various modifications of engine and blowers the efficiency was improved considerably, reducing the man power requirement as well. Due to the paucity of helicopters for aerial spraying, tractor mounted mistblowers were also developed in due course which could deliver the spray fluid to above 90 feet. Considerable man power saving could be achieved by this technology also.

Development of Mistblowers-cum-duster operated by one person is another significant achievement in the plant health management recently. Progressive developments and changes taken place in the spraying technology in rubber cultivation during the last few decades are discussed in this article.

O-4

Mechanization of Ground Spraying in Rubber Plantations against Diseases

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Prophylactic ground spraying of copper oxychloride in agriculture spray oil using mist blowers is a general practice to manage abnormal leaf fall (ALF) disease caused by *Phytophthora spp*. However, this practice has become less attractive due to paucity of labour. In view of this, various alternative ground spraying systems were attempted. In the present study, the blower assembly of the conventional mist blower was attached to a mini tractor. The power for operating the sprayer was provided by the power take off (PTO) shaft of the tractor. Field studies revealed no significant difference with respect to leaf retention between the conventional mist blower and the tractor mounted mist blower. Therefore, the conventional atomizer was replaced with a modified one and field tested. The delivery height obtained with the new system was comparatively more. However, certain defects like slip of propeller shaft while moving in the uneven terrain and power output incompatibility between tractor and the mist blower were noticed in the system during the field operations and due to which the expected discharge height was not attained. In order

to overcome the defects, the spraying attachment was connected directly to the PTO shaft of the tractor using a single wheeled gear box assembly and a blower assembly having 12 hp capacity. The modified system was field tested again and a delivery height of 26.8 m was obtained. The new system resulted in leaf retention of 50% while the conventional mist blower gave only 30% on the most susceptible clone RRIM 600. The improved mini tractor mounted mist blower was found very effective for the plant protection operations in the rubber plantations of India, due to its less labour requirement and more area covered at unit time.

P-1

Mechanised Land Preparation for Rubber: Risks and Benefits

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Cultivation of rubber is a long term investment, which requires good agro management and soil conservation practices for sustainable production. In Kerala, severe labour shortage is affecting the timely completion of agricultural operations in the rubber plantations. The increasing labour wages also prompt the growers to look for alternative methods like labour substituting with machinery.

The high intensity of rain fall and undulating topography of rubber growing areas increase the vulnerability of soil erosion. Traditionally no tillage was practiced in rubber plantations and it helped to conserve soil and maintain soil quality. However, now there is a large scale shift to mechanized land preparation and mechanization is adopted to varying levels depending on terrain, availability of machineries, intercropping practices etc. Though mechanization reduce cost of cultivation considerably and labour dependence, indiscriminate use of heavy duty machines can increase tillage induced soil erosion which is considered to be a factor contributing to the degradation of soil (Lewis and Nyamulinda, 1996). Concerns of soil degradation and sustainable agriculture are often related to the loss of top soil and soil organic carbon (D’Haene *et al.*, 2009). With this back ground, an experiment was initiated to assess the effect of mechanized land preparation on soil erosion and physical properties of rubber growing soils.

A field experiment was initiated at Mundakayam estate, Mundakayam, Kottayam district, Kerala during the year 2010. The trial was laid out in a randomised block design with 4 replications. The four land preparation methods evaluated were (a) pitting, terracing and tilling inter rows by *Hitachi* (b) pitting and terracing by *Hitachi* (c) pitting by tractor

mounded hole digger and manual terracing and (d) Standard practice - manual pitting and terracing. Soil samples were collected from individual plots at 0-15, 15-30, 30-45 and 45-60cm depths and determined physical properties of soil such as bulk density, porosity, aggregate stability, hydraulic conductivity and gravel content, before and after the treatment imposition. The quantity of soil eroded was estimated by trench method and soil organic carbon as a critical indicator of soil quality was estimated using CN analyser.

The results of the study indicated that the rate of soil erosion in different land preparation methods was significantly different. One and a half years after treatment imposition the highest rate of erosion (11.6 t/ha) was recorded from the plot where pitting, terracing and tilling inter rows were done by *Hitachi*. Soil erosion from manually pitted and terraced plots showed significantly lower rate of erosion and was comparable with other two treatments (pitting and terracing by *Hitachi* and pitting by tractor mounded hole digger and manual terracing).

Bulk density of the soil was significantly lower in plots where pitting, terracing and tilling operations were done by *Hitachi* at all the depths. Bulk density was lower in the surface layer and higher in the lower layers in all treatments. Higher retention of organic matter in the surface layer might have contributed to the lower bulk density. Porosity showed reverse trend, it decreased with soil depth. Comparison between treatments showed that porosity increased by around 10 percent in tilled plots (pitting, terracing and tilling by *Hitachi*) compared to zero tilled plots at all soil depths. Tilling depth of soil by *Hitachi* is around 60-75cm and during the tillage operations loosening of soil take place, leading to higher porosity. Consequently hydraulic conductivity was also higher in tilled plots than in zero tilled soils. Soil aggregate stability was not significantly affected by land preparation methods.

Observations on gravel content of the soil indicated that the treatments with pitting, terracing and tilling by *Hitachi* showed significantly lower percent of gravel content in the upper 0-15 cm of soil and higher percent in the 45-60 cm, than other treatments which is due to the inversion of soil. In zero tilled area, soil organic carbon percent was significantly higher in the surface layers and decreased with depths. In the tilled soil, the soil organic carbon content was comparatively lower in the top layers due to the inversion of soil and burial of crop residues to the lower depths and loss of carbon through erosion.

Comparative cost analysis of different land preparation methods indicated that pitting and terracing by *Hitachi* involved the cheapest followed by pitting by hole digger. A cost saving of around 45-50 per cent was achieved through pitting and terracing by *Hitachi* compared to standard method (manual) of land preparation.

The study indicated that substantial quantity of soil is lost through erosion when pitting, terracing and tilling is done by *Hitachi*. Pitting and terracing by *Hitachi* was the most economic and sustainable method and is the most appropriate method of land preparation in rubber plantations, if care is taken to prevent erosion.

SESSION 7

**PLANT HEALTH
MANAGEMENT**

O-1

New Diseases Reported and Maladies Reached Epidemic Proportions in Rubber Plantations since late 20th Century

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More than hundred pathogens have been identified as capable of attacking the rubber tree since its domestication. Economic threat of each disease varies from one country to another depending on various factors. Today, there is a changing scene in the disease scenario and traditional diseases like Oidium leaf fall, Gloeosporium leaf fall, Phytophthora leaf fall and Phytophthora bark rot have become less significant in several rubber growing countries due to the planting of disease resistant clones. However, several new diseases spread in rubber plantations during recent past and *Corynespora* leaf fall is the most threatening disease among them. Other diseases include Papaya mealy bug infestation, Target leaf spot, Sclerotium collar rot and Foot canker and sudden wilt. Furthermore, several minor pests and diseases such as Cockchafer grub attack and Basal rot reached the epidemic proportions during the new millennium. Another set of maladies creating unrest among the growers was unusual attacks of White root disease in immature clearings and abnormalities shown in new breeds as genetical characteristics.

O-2

Integrated Disease Management of White Root Disease on *Hevea* Rubber in Indonesia by using Trichoderma-Based Biofungicide Triko Combi

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White root disease caused by *Rigidoporus microporus* is one of important rubber diseases in Indonesia. The disease occurs in many rubber plantations, such as estates and smallholders. The financial losses due to this disease is very high especially in

smallholder plantation. The appropriate method to control white root disease is integrated disease management. The integrated disease management should be done through land clearing (uprooting), cover crop planting, using healthy planting material, growing antagonistic plant, biofungicide application and well application of chemical fungicide. There are many technical problems affecting field implementation with the result that farmer gets unsatisfactory result. The problem usually appears because of an expensive and difficult application method. As the method in the integrated disease management, biological control is one of the potential methods to overcome the lack of application. The biological control using *Trichoderma* spp. is effective as preventive tactic, cheap, easy to apply and environmentally safe. Triko Combi is another semi-commercial biofungicide from Getas Research Center that contains four strains of antagonistic fungi. Active ingredients of the product are *Trichoderma viride*, *T. koningii*, *T. harzianum* and one local strain of *Trichoderma* sp. The research of *R. microsporus* infection artificially showed a potential result than the polibag seedling without treatment (control). Significant result also achieved by pressing *R. microsporus* mycelium growth through the research of in-vitro.

O-3

Surveillance on *Corynespora* Leaf Fall Disease: Incidence and Severity on Natural Rubber (*Hevea brasiliensis*) in Certain Hot Spots Areas in Kerala

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Survey on *corynespora* leaf fall (CLF) disease was undertaken in the hot spot areas in Thodupuzha and Kothamangalam regions of Idukki and Ernakulam districts, respectively in Kerala for 2 years from 2011 to 2012. The study revealed that in 2011, Nalpathu Acre of Neyassery village in Thodupuzha region registered maximum disease incidence (85%) and disease severity (47.2%) with disease severity ranging from 0-75% while in Kothamangalam region, Varapetty and Kanjiravelli villages registered 100% disease incidence. However, maximum disease severity was observed in Pinavoor area (38.0%) with disease severity ranging from 0 - 90%. In 2012, the disease incidence was on rise as reflected by the more number of units infected in Nalpathu Acre (90%) in Thodupuzha region and Kadavoor, Varapetty and Kanjiravelli villages of Kothamangalam region registering 100% disease

incidence. However, there was sharp decline in disease severity in 2012 as maximum disease severity of 13.9 % was registered in Nalpathu Acre with disease severity ranging from 0-30 %, whereas it was 22.5 % in Varapetty village of Kothamangalam with disease severity ranging from 0 to 50%. These two regions where the disease was prevalent are surrounded by forests which may be contributing to the disease development. The immature phase of most popular clone RRII 105 was very much prone to the disease while in other clones like PB 260, RRII 414 and RRII 430 the disease was negligible to less. The disease was severe mainly in February/March months and it declined thereafter. The probable reason for the decline in disease in 2012 is immediate plant protection measures taken up by the growers as a result of the experience of 2011 and proactive action taken up by Rubber Board.

O-4

Environment and Farmer Friendly Biodegradable Rubber Spray Oil for Controlling Abnormal Leaf Fall Disease

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Petroleum derived oil fractions of different viscosity and hydrocarbon composition are being used for controlling pests in agriculture industry. In rubber plantations, petroleum oil is being used as carrier oil for spraying fungicide (copper oxy chloride). This is being used for controlling the abnormal leaf fall disease. The conventional rubber spray oil is light distillate having diesel type boiling range. The residue of this petroleum products remains in the soil and water as it has lower biodegradability leading to pollution of the environment.

In order to address this issue, the authors' organizations have carried out a joint R & D and detailed evaluation of biodegradable rubber spray oil for rubber plantation for three consecutive season was done. Systematic field trials were conducted against conventional rubber spray oil. The present paper provides the details of the developmental of oil, the field trials and their outcome. New oil was found to be superior in retention of fungicide on leaves for longer period of time and reduced the abnormal leaf fall substantially. These superior results are attributed to increased spread ability and wettability of new biodegradable rubber spray oil.

Relative Abundance of Mistletoe in *Hevea* Plantation in Edo State, Nigeria

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An investigation was conducted at the 40 hectare polyclonal garden of Rubber Research Institute of Nigeria (RRIN), Iyanomo, Benin City, Nigeria for incidences of mistletoe. 6 *Hevea* clones comprising of NIG 800, 801,803, RRIM707, PR 107, GT 1 were identified and assessed based on infection category from: 0 (no infection) to 3 (serve infection) with intermediates indicating light and moderate infections . The rate of parasitism was observed to vary with clone and location in the polyclonal garden, the disease infection index calculated. Susceptibility to infection was aided by open vegetation and wide canopy base. Impact of the mistletoe infestation on *Hevea* host in this study was severe but if allowed to continue could become a serious threat to the survival of the *Hevea* plantation.

Pink Disease of *Hevea* Rubber in Northern Part of West Bengal and North East India

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Survey was carried out in 180 locations in northern part of West Bengal and North East India from August to November during the 1990-2011 for occurrence of pink disease of *Hevea* rubber caused by *Corticium salmonicolor* (Berk. and Br.). The incidence of pink disease on *Hevea* rubber was very high in northern part of West Bengal (33.4%) as compared to Maghalaya (13.7%) and Assam (2.9%). High incidence of pink disease was observed on five to nine years old rubber plants at Rango (15%) followed by Jiti rubber estate (7%) and RES, Nagrakata (1.8%) in northern part of West Bengal. Maximum infection of pink disease

was observed on the main trunk of five years old rubber plants at Rango rubber estate (7.5%) followed by Jiti (3%) during September, 2001 for the first time in northern part of West Bengal and caused a total loss of the affected stands. Weather factors like monthly total rainfall (mm), number of rainy days, maximum temperature (oC) and relative humidity (%) from July to September during 1996-2011 are also reported in this paper. The rainfall and number of rainy days were the major predisposing factors influencing the development of pink disease

P-2

Interaction of Certain Bacterial Antagonists of Rubber Pathogens with Fungicides Used in Rubber Cultivation

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The antagonistic activity of 77 bacterial isolates collected from the rhizosphere and non rhizosphere soil and tissues of different clones of rubber were tested against the five major pathogens of rubber, viz., *Phytophthora meadii*, *Corynespora cassiicola*, *Colletotrichum acutatum*, *Corticium salmonicolor* and *Phellinus noxious* by dual inoculation and selected five isolates showing more zone of inhibition of each of the pathogens. *Pseudomonas* spp. and *Bacillus* spp. were the main antagonistic bacterial groups which included the endophytes and rhizosphere colonizers. They produced various antipathogenic and plant growth promoting metabolites. The compatibility of the antagonists with four systemic fungicides viz., tridemorph (Calixin), hexaconazole (Contaf), propiconazole (Tilt) and carbendazim (Bavistin) and two contact fungicides viz., mancozeb (Indofil M-45) and wettable sulphur (Sulfex) commonly used in rubber plantations was studied at different concentrations under *in vitro* conditions. Carbendazim was the only systemic fungicide tested which was safe to all the isolates studied. The contact fungicide mancozeb was inhibiting the growth of all the antagonists even at the lowest level studied while wettable sulphur was not harmful to most of the isolates even at double the recommended level. *Pseudomonas* spp. in this study were more tolerant than *Bacillus* spp., to the fungicides used in rubber cultivation.

Effect of Pollen Substitute on the Development of Indian Honey Bee, *Apis Cerana Indica* during Dearth Period in Rubber Plantations

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The effect of pollen substitute on the total bee population and brood rearing activity of Indian honey bee, *Apis cerana indica* during dearth period in rubber plantations was evaluated. The highest percentage increase in population (55.09 %) was recorded in colonies fed with both sugar syrup and pollen substitute (SPS) followed by 47.88 and 37.88 percent increase in sugar fed (SF) and pollen substitute (PS) fed colonies respectively. Similar trend was observed in the case of comb and brood area development. SPS colonies had maximum percentage increase of comb (33.26 sq cm) and brood area (52.58sq.cm) after 45 days followed by those in sugar fed colonies (20.09sq.cm and 43.50sq.cm). Minimum percentage increase in the brood development and bee population was noticed in pollen substitute alone fed colonies. Pollen substitute along with sugar syrup prevents absconding or loss of colonies during dearth period in rubber plantations. Influence of external pollen substitute to honey bee colonies for the successful maintenance of apiaries in rubber plantation is discussed in this paper.

Incidence of Tapping Panel Dryness in North-East India

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The occurrence of TPD in different panels under exploitation has been studied in the states of Assam and Northern part of Tripura state in Northeast India. The clone RRIM 600

which is more popular in the region has been considered for the TPD study. The results showed the percentage of trees affected by TPD has increased with the progression of tapping but it varied seasonally. No definite trend on the incidence of TPD was observed. High incidence of TPD was observed in C (BI) panel as compared to A (BO1) and B (BO2) panels. In A (BO1) panel only 1.10%, B (BO2) panel 2.33% and in C (BI) panel 9.89% trees were recorded as TPD affected during 2007 where >76% of the total panel dried and ceased latex flow which rose to 1.34%, 13.67% and 14.17% respectively during the year 2009. The percentage of trees with very high TPD incidence was much higher in C (BI) panel (19.0%) in Kokrajar as compared to the same panel in Bongaigaon district. An increase in TPD plants from summer season through pre winter and showed more incidence in older trees in C (BI) panel as compared to younger plants with A (BO1) panel. But some variations in incidence of TPD in different panels were also observed. The present study indicated the severity of TPD incidence in the North eastern states of India and the precautions to be taken to check the disorder.

P-5

Frequency of Occurrence and the Role of *Colletotrichum* Species on the Colletotrichum Leaf Fall Disease of *Hevea brasiliensis* in the Traditional Rubber Growing Regions of Kerala

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Colletotrichum leaf disease (CLD) is a serious problem causing considerable damage to the rubber plants in the nursery and immature plantations in the field. Tender leaves produced soon after bud burst during rainy season are affected and in severe cases the leaves become distorted, turn black, shrivel and fall off resulting in die-back of shoots. In high rainfall areas the shoot tips become devoid of leaves giving a pencil tip appearance through out the rainy season from June-November. Two species, *C. acutatum* and *C. gloeosporioides* are known to cause the disease. Hence an attempt was made to examine the occurrence and frequency of Colletotrichum spp. and the major cause of the Colletotrichum leaf fall disease of *Hevea brasiliensis* in the traditional rubber growing regions of Kerala.

Diseased specimens were collected from different clones viz. RRIM 600, PB 260, RRII 105, RRII414 and RRII 430 and about 140 locations of Kerala during 2010 and 2011 disease

season. Leaf tissues showing different symptoms like typical raised spots, dark brown or black spots with yellow halo and dried margins were taken for isolation of the pathogen. Thirty to forty leaf bits with disease symptoms were taken from every location and the pathogen was isolated on potato dextrose agar. The emerging colonies from each bit were initially characterised based on the conidial shape. The isolates were further characterised by colony colour, growth rate, perithecial production and sensitivity to carbendazim (2 ppm) by poisoned food technique. Pathogenicity of *Glomerella* sp was studied under *in vivo* condition by spraying the ascospores on the most susceptible clone RRII 105. Aggressiveness of two species was studied by estimating the toxin production. The toxic effect was tested by leaf puncture bioassay and leaf wilt bioassay using the crude culture filtrate of pathogens.

On the basis of conidial morphology more than 65 % of the colonies were identified as *C.acutatum* in both the years. There were very few locations where either *C.acutatum* or *C.gloeosporioides* alone was recovered from the leaf bits. Both the species could be isolated from all types of symptoms. Isolates producing perithecia in culture were obtained from 31% of the sampling locations. Among the fast growing *Colletotrichum* isolates 24% produced the *Glomerella cingulata* teleomorph. None of the isolates identified as *C. acutaum* produced the perithecial or the ascigerous stage.

Conidia of the *C. acutatum* was hyaline and elliptic to fusiform in shape, being tapered at one or both ends. Conidia of the all isolates of *C. gloeosporioides* and *Glomerella* sp. were oblong in shape, being obtuse or rounded at both end. Colony colour of the *C. acutatum* ranged from pale orange to cottony grey or grayish white with pink tinge on the upper surface and the reverse side of the colony was pink to salmon pink or creamy white in colour. Colony color of *C.gloeosporioides* was light grey to dark grey in the upper side and reverse was grey to dark grey, black or olive green to dark green. The isolates *Glomerella* sp and *C.gloeosporioides* grew faster and were more sensitive to carbendazim than isolates of *C.acutatum*. There was no significant differences in growth rate between the isolates *Glomerella* sp. and *C.gloeosporioides*. Both slow growth rate and less sensitivity to carbendazim differentiated *C.acutatum* from *Glomerella* sp. and *C.gloeosporioides*. However these characters may not be useful to distinguish between the isolates of *Glomerella* sp. and *C.gloeosporioides*. Pathogenicity studies showed that perithecial isolates were equally pathogenic as the non-perithecial isolates of *C.gloeosporioides*. The lesions produced by *C.acutatum* was larger when compared to *C.gloeosporioides* and the perithecial strain. Studies on the impact of toxin production by two species revealed that *C.acutatum* as more aggressive with the large lesions and more wilting of the *Hevea* leaves.

The results of the present study confirm that *C.acutatum* is more predominant in the rubber growing regions of Kerala. The aggressiveness and the high frequency of occurrence indicate that *C. acutatum* is the main cause of the disease.

P-6

Evaluation of Bio Pesticides and Insecticides on The Control of Bark Feeding Caterpillar, *Aetherastis Circulata* Attacking Rubber Trees

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Effectiveness of bio pesticides viz; *Beauveria bassiana*, *Azadirachtin* 0.03%, neem oil 0.5% and combination of fenvalerate 0.02% and carbaryl 0.01% was evaluated against bark feeding caterpillar, *Aetherastis circulata* Meyr feeding on the bark of *Hevea brasiliensis*. Among the bio pesticides, neem oil 0.5% gave 79.31 per cent mortality of caterpillars followed by *Azadirachtin* 0.03% (65.55%) and *B.bassiana* (17.65%). The insecticides showed 96% control of bark feeding caterpillars. The possibility of using neem formulations for the control of *A.circulata* attacking rubber is discussed in this paper.

P-7

Towards Understanding *Colletotrichum* “Species Complexes” Causing Leaf Disease in Rubber

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Colletotrichum leaf diseases observed as raised spots, anthracnose and circular papery lesions on rubber leaves have been reported to be caused by *Colletotrichum acutatum* and *C. gloeosporioides* in India since 2002. Earlier, this disease was reported as a minor disease affecting nursery plants. However, during the past few years, the occurrence of this diseases has been on the increase particularly in young rubber plantations wherein dieback and extension of immaturity period of the rubber trees were noticed, making it one of the major diseases of rubber plantations. Therefore, understanding the different species involved in causing the disease is essential for developing efficient disease management strategies.

Till date seventy two species of *Colletotrichum* are known to affect different commercially important and ornamental crops across the world. To understand the *Colletotrichum* “species

complex”, details of the available internal transcribed spacer (ITS) sequences from type cultures were retrieved from GenBank and used for phylogenetic analysis. The phylogenetic tree revealed existence of nine different species complexes of which two were: *C. acutatum* and *C. gloeosporioides*. The species included within the *C. acutatum* complex were: *C. acutatum*, *C. fiorinae* and *C. simmondsii*. However, the *C. gloeosporioides* complex was a larger complex consisting of fifteen species: *C. asianum*, *C. cordylinicola*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides*, *C. horii*, *C. hymenocallidis*, *C. ignotum*, *C. jasmini-sambac*, *C. kahawae*, *C. musae*, *C. siamense*, *C. theobromicola*, *C. tropicale* and *C. xanthorrhoeae*.

As *C. acutatum* and *C. gloeosporioides* are already reported as the two species involved in causing Colletotrichum leaf disease in rubber, effort was taken to understand in detail about the phylogenetic relationship and the possible involvement of the different species within these two complexes. To begin with *C. acutatum* species complex consisting of *C. fiorinae*, *C. acutatum* and *C. simmondsii* was investigated. The ITS and beta-tubulin sequences of type strains of these three species were analyzed both independently as separate genes as well as in a concatenated form by including both these gene regions together in the dataset. Interestingly, *C. acutatum* isolate from rubber (GenBank Acc. No. AF488778; IMI383015; 2002) was found to fall within the *C. simmondsii* clade suggesting that the rubber *C. acutatum* is a *C. simmondsii*. Contrary to the *C. acutatum* complex which consisted of 3 species, the *C. gloeosporioides* complex contained fifteen species within the complex. The ITS sequences of the fifteen type strains along with the rubber *C. gloeosporioides* isolate (GenBank Acc. No. AF488777; IMI383016; 2002) was analyzed. It was observed that the *C. gloeosporioides* of rubber showed maximum similarity with the *C. siamense* and the type strain of *C. gloeosporioides* was found to be with greater evolutionary distance from that of the rubber *C. gloeosporioides* suggesting that the rubber *C. gloeosporioides* is possibly a *C. siamense*.

Results of the present phylogenetic analysis based on the sequences of two nuclear DNA regions (rDNA-ITS region and β -tubulin gene) indicate a phylogenetic framework for interpreting the evolutionary history of the *Colletotrichum acutatum* and *C. gloeosporioides* “species complexes”. With the availability of better barcode regions to distinguish pathogens at the species level, the conserved sequence information suggests that the *C. acutatum* of rubber is not *C. acutatum* but is *C. simmondsii* and the *C. gloeosporioides* of rubber is not *C. gloeosporioides* but is *C. siamense*. The phylogeny for the *C. acutatum* and *C. gloeosporioides* “species complex” presented here represents advancement in our knowledge on *Colletotrichum* spp. affecting rubber and needs further detailed investigation with maximum number of both these isolates collected from rubber. The data from these loci as well as from additional loci will provide an indication about the phylogenetic framework for interpreting evolutionary history of the genus. Although rDNA has been widely used in phylogenetic studies, it was found that the evolution of one gene may not represent the evolution of the entire genome. Therefore, efforts are in progress to separately sample as many additional independent genes as possible and compare the phylogenies derived from these genes to see whether they support or contradict each other to systematically characterize and understand the *Colletotrichum* “species complex” existing in rubber.

Reaction of the Polycross Progeny of Prepotent Clones to Two Major Leaf Diseases of Rubber (*Hevea brasiliensis*)

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Abnormal leaf fall (ALF) disease caused by *Phytophthora* spp. and powdery mildew caused by *Oidium Heveae* are the major leaf diseases of rubber in India. A yield loss of 38 to 56% due to ALF was reported when the trees are left unsprayed for one disease season whereas, the yield loss reported to be 8.1% due to powdery mildew in the clone PB 5/51 in Malaysia. Since protection using fungicides against the diseases is costly and likely to cause environmental hazards being toxic to animals and human beings, the alternative and more permanent approach is to develop tolerant/resistant cultivars. With this aim, a three-year study was undertaken to evaluate the polycross progeny of prepotent clones for their tolerance/susceptibility for ALF and powdery mildew diseases.

Evaluation was conducted in a compact family block design with three replications and 4-5 trees per plot at a spacing of 4.9 x 4.9 m. A total of 150 progeny clones were evaluated in two field trials, one trial (Trial I) with 10 clones per progeny and the other (Trial II), with five clones per progeny. The field trials were laid out at the Central Experiment Station of the RRII at Chethackal in Ranni. The clones were evaluated for ALF and powdery mildew diseases in the 11th to 13th year after planting.

Powdery mildew disease was assessed during February/ March of each season. For this purpose, five leaves collected from each tree from the terminal whorls of 4 branches were observed for disease severity. The leaves were graded according to the intensity of infection on 0-5 scale where 0 = no disease; 1 = 1-10 % disease severity; 2 = 11-25 % disease severity; 3 = 26-50 % disease severity; 4 = 51-75 % disease severity and 5 = > 75 % disease severity.

For ALF disease, the assessment on per cent leaf retention was carried out during September - October period after the disease season. The data obtained for three consecutive years were analysed and computed familywise and clonewise.

The results on powdery mildew indicated that the disease severity among the families ranged from 23 - 59%, 35 - 55% and 47 - 83% in 1st, 2nd and 3rd year of studies, respectively whereas the per cent retention of leaves in case of ALF disease ranged from 51- 76%, 47 - 67% and 15 - 60% in 1st, 2nd and 3rd years, respectively in both the trials. This highlights of the fact that the intensity of both the disease was higher in the 3rd year than in 1st and 2nd years of studies.

Interestingly, results of the pooled data analysis on the performance of individual clones within a family against powdery mildew disease indicated that two clones viz., 104 and 54 of the family PB 5/76 as the female parent showed disease severity of less than 25% even when the disease intensity was high in 3rd year.

It is observed from the results on the pooled data analysis obtained from three years on the evaluation of individual clones within family against ALF disease that the clone 128 of the family AVT 73 showed highest leaf retention of 76% followed by the clones 65 of the family AVT 73, 75 of PB 217 and 47 of PB 215 with leaf retention of 71%. In trial II, the clones 132 and 69 of the family PB 252 showed leaf retention of 73% over a period of 3 year assessment. The individual yearwise assessment of the 3rd year wherein the disease intensity was more, has shown that with the exception of clone 73 with leaf retention of 58%, all other clones performed well against ALF disease with per cent leaf retention ranging from 65% to 76%. Altogether, 73 clones in Trial I and 34 clones from Trial II showed per cent leaf retention ranging from 50 to 75%.

The girth, timber and rubber yield of these clones have already been reported. Among the clones found promising in terms of reaction to the ALF disease, clone 132 evolved from the parent PB 252 is reported to be latex timber clone with > 80% improvement in yield over the high yielding clone RR11 105 coupled with high timber yield. Clone 132 in this study found to be consistently tolerant to ALF disease and thus found to be a promising clone for future. Clone 69 also derived from PB 252 and was found tolerant to ALF disease is reported to be a timber latex clone with promising rubber yield and high timber yield. Clone 128 evolved from the parent AVT 73 found to be tolerant to ALF disease in this study is reported to be timber clones with high timber yield though rubber yield is less.

P-9

Management of Purple Root Disease of *Hevea* Seedlings in Nursery

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A field trial was conducted on one-year-old seedlings of *Hevea* in nursery during 2004-06 at District Development Centre, Rubber Board, Jengittchakgre, Tura under West Garo Hills District of Meghalaya. The seedling nursery for this experiment was raised in a

susceptible pocket at Jenggitchakgre from where the mortality of *Hevea* seedlings due to purple root disease caused by *Helicobasidium compactum* Boedijn was above 45% during 1998-2000. The main objective of this experiment was to identify effective and economic control measures for the disease. Efficacy of three systemic fungicides viz. Tridemorph, Propiconazole and Carbendazim was evaluated. In addition to that Bioflora Natural and Fallow Bed for one and two years were also included in this experiment for comparison. Twelve treatments viz. T1 (Untreated control), T2 (Propiconazole 0.1%), T3 (Propiconazole 0.2%), T4 (Propiconazole 0.1% alternate with Tridemorph 0.15%), T5 (Tridemorph 0.15%), T6 (Tridemorph 0.3%), T7 (Tridemorph 0.15% alternate with Carbendazim 0.15%), T8 (Carbendazim 0.15%), T9 (Carbendazim 0.3%), T10 (Carbendazim 0.15% alternate with Propiconazole 0.1%), T11 (Bioflora Natural 20 g/bed) and T12 (Fallow Bed for one/two years) were imposed on one-year-old seedling plants in a randomized block design with three replications per treatment in nursery for the management of purple root disease. Out of three systemic fungicides, the efficacy of Propiconazole and Tridemorph was always superior to Carbendazim even at lower concentrations (0.1 to 0.15%) tested for the management of purple root disease in seedling nursery. The treatments with Carbendazim (T8 and T9) reduced the infection of purple root disease to 35.5% and 67.9%, respectively over control indicating that Carbendazim may require some higher strength than the tested concentrations (0.15 and 0.3%) for effective control of the disease. The treatment with Bioflora Natural (T11) and Fallow Beds for one/two years (T12) showed cent per cent reduction of purple root disease over control indicating that these two treatments were very effective in the management of purple root disease of *Hevea* seedlings in nursery. The cost of all the fungicides and Bioflora Natural at the doses tested is reported in this paper. Tridemorph (0.15%) is the cheapest systemic fungicide (Rs.1.54 per litre fungicide solution or Rs.2.31 per bed) followed by Bioflora Natural (Rs.4.80 per bed) in the management of purple root disease.

Environmental Factors Associated with Buildup of High Density *Aedes Albopictus* Vector of Chikunguniya Virus in Rubber Plantations

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Rubber production suffered a major setback in Kerala during 2006-'07 due to absenteeism of workers afflicted by outbreak of the Chikunguniya disease. The primary vector was *identified* as *Aedes albopictus*, a mosquito that breeds in domestic and peridomestic habitats. Meteorological factors being important drivers of mosquito-borne disease transmission, a study was attempted at delineating favourable environmental factors influencing the build-up of the vector *Aealbopictus* population in selected rubber growing districts of Kerala. Temperature, rainfall and humidity being known to influence the build-up of this vector, were studied with the field collected data from 2008 to 2010 from Chethackal, Aimkombu, Malankara and Kumarakam. The seasons chosen for the study are pre-monsoon (March to May), monsoon (June to September), post-monsoon (October to December) and winter (January to February). Vector population density in PMD (per man hour density) was studied with antecedent atmospheric variables by calculating lag period correlations of up to 14 days. Analysis of variance of PMD between seasons and locations were calculated. It was found that in Malankara, the daily average temperature and relative humidity 8 days prior to the collected vector population density showed a significant correlation of 0.45 and 0.52, respectively. The 10 day mean temperature with vector density showed significant correlations of 0.50 and 0.52 during the monsoon and post-monsoon seasons respectively. Days of heavy rainfall are not very conducive for sustaining vector density in rubber plantations. The seasonal vector population density between Malankara and Chethackal were similar and also between the monsoon and post-monsoon seasons. Malankara and Chethackal also showed similar patterns with vector build-up with climatic variables. Regression equations were made for the different seasonal vector population based on the meteorological variables. Conditions influencing the build-up of population density of *Aealbopictus* are mainly concentrated between 7 to 12 days antecedent weather conditions.

SESSION 8
ECONOMICS

An Economic Analysis of the Socio-Economic Dimensions of Participatory Experimental Trials on Low Frequency Tapping (LFT) in Kerala

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The study is a socio-economic evaluation of the demonstration plots having S/2 d3 tapping system with stimulation. The demonstration plots were established in the rubber smallholdings in different locations to popularize low frequency tapping (LFT) system by participatory monitoring and evaluation. The main objectives of the study were to analyse the socio-economic profile of the participating smallholdings and to identify the contributory factors/ barriers for the adoption/non-adoption of LFT by the growers. The database consisted of a sample survey covering 48 participating rubber small growers and the tappers attached to the holdings. The analysis showed that the average size of demonstration plots (0.89 ha) is higher than the average size of rubber smallholdings in Kerala (0.50 ha) indicating that holding size is one of the factors prompting the adoption of LFT. The dependence on hired labour for tapping is higher and there exists an in-built flexibility for adopting new tapping technique in the case of growers dependent on family labour. It is also observed that the average size of holding (4.45 ha), average size of rubber area (3.30 ha) and average size of demonstration plot (0.91 ha) are higher for the growers dependent on hired labour. Hence, the size of holding (number of trees) emerges as the key factor facilitating the adoption of LFT in the case of holdings dependent on hired labour. There is a steady shift towards multiple grower dependence from the historically observed pattern of single grower dependence. The resistance from tappers to start LFT was observed only in the smaller holdings with lower number of trees and the resistance was mainly due to: (i) loss of tapping days; and (ii) increase in work load due to higher yield from unit area. The growers overcome the resistance by (i) resorting to self tapping; (ii) by assuring employment to tappers in other grower's holdings; and (iii) by offering incentives for extra production. Despite the positive signals emerging from the scheme the scale neutrality of LFT remains suspect in the unique regional context of Kerala with smaller size of the holdings and higher dependence on hired labour. The study highlighted the point that size of holding is a major factor determining the adoption of S/2 d3 tapping with stimulation. Availability of family labour is a key factor influencing the adoption of LFT in smaller size groups with less than 1 ha area under rubber. Hence, institutional arrangements to overcome the in-built deficiencies of size and rigidities of the labour market are crucial for the effective implementation of LFT from a long-term perspective.

Trends in Adoption of Planting Density in Small holdings in the Traditional Natural Rubber Growing Regions of India

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The study revealed that the planting density adopted by growers has increased significantly since the latter half of the 2000s coinciding with release of RRII 400 series. The analysis has also revealed that in the traditional belt, except for North Kerala, the planting density for new planting was higher than replanting. However, except for South Kerala and North Kerala, no significant difference between densities adopted for RRII105 and RRII 400 series was evident. Analysis of holding size class –wise density revealed the existence of an inverse relationship between the size of holdings and planting density.

Uncertain Prices and Segmentation of Market as a Survival Strategy: The Case of Latex Processing Industry in India

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Historically, price movements of all forms of natural rubber in India have been either directly or indirectly linked to the trends in the price of RSS 4 grade. Under the protected price policy regime, the arrangement has ensured comparative stability and margins at various stages. The price movements of all forms of processed/unprocessed rubber including preserved field latex (PFL) and centrifuged latex (CL) have also been based on the price movements of RSS 4. However, instability in the RSS 4 prices consequent to the trade policy reforms since the early 1990s led to important structural adjustments in the domestic market. As a survival strategy, the latex processing industry has gradually de-

linked PFL price from RSS 4 and linked to CL price in order to protect the margins since the mid 2000s. The preliminary observations of the study highlighted the crucial role of the growing narrowness of the domestic market due to the significant growth in the import of latex based value added products rather than the import of CL for the segmentation of the latex market. Hence, a two pronged strategy of rehabilitating the latex based manufacturing segment so as to ensure commensurate rate of growth in latex consumption and rejuvenating the latex processing industry to reinforce the focus on exports is suggested.

P-1

Marketing Efficiency of Organized and Unorganized Rubber Growers in Tripura: A Comparative Analysis

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The study has examined the marketing efficiency of organized small rubber growers under the Block planting Scheme (BPS) - Rubber Producers Societies (RPS) network and unorganized growers in the case of ribbed smoked sheets (RSS) in Tripura. The study is based on primary data collected from 305 small rubber growers and 39 market functionaries. The better marketing efficiency of growers organized under the BPS-RPS network over the unorganized growers has been reflected in terms of lower price-spread, higher efficiency index and increased producers' share. The study suggests appropriate institutional interventions for more coverage of the small rubber growers.

P-2

Adoption of Intercrops across Size-classes and Regions: A Study of Traditional Rubber Growing Regions in India

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Among the various intercrops, banana is the most popular intercrop in the traditional region spread over five agro-climatic zones. However, the results of the study showed that there are notable differences in the extent of adoption of intercropping, choice of crops and size-class-wise preferences. The highest level of adoption of intercrops was observed in Kanyakumari region (72.85 %) followed by Central Kerala (72.20 %) and South Kerala (68.18 %) during the seven year period under study (2004-10). Adoption of intercrops was found to be lowest in North Kerala (36.20 %). The analysis revealed that pineapple replaced banana as the choicest intercrop in Central Kerala. The size of holding was found to be a key determinant in the selection of intercrops. A positive relationship was observed between the size of holding and adoption of commercial crops such as banana and pineapple whereas subsistence crops such as tapioca, amorphophallus and colocasia are preferred in the smallest size-class.

P-3

Adoption of RR II 400 Series Rubber Clones by Rubber Small Growers

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The paper examines the response of small growers to the recommendation of multi-clonal planting in the context of release of RR II 400 series clones for commercial cultivation since 2005. The data pertaining to 57369.7 ha. under 130658 RPD permits which availed subsidy from the Rubber Board during the seven year period from 2004 to 2010, were gathered from 26 Regional Offices of the Rubber Board located in the traditional rubber growing region. The study revealed that, RR II 105 had a mono-clonal status in clone adoption for 25 years till 2005 as it occupied 91 per cent of the planted area. This was mainly due to the absence of alternative clones. But the mono-clonal planting of RR II 105 started declining since 2006. This was mainly due to the introduction of RR II 400 series

clones. It is evident that the share of RRII 400 series clones increased from 7.1 per cent in 2004 to 28 per cent in 2010 in the total planted area. But trends in adoption of individual clones did not exhibit a unique pattern across size-classes and regions over the period. It is in sharp contrast to the experience of RRII 105 since its release in 1980. Adoption of multi-clonal planting was positively associated with the size of holdings during the period under review. But the strength of this relationship has been dependent on region-specific factors. Therefore, the study brings out the need for evolving a long term policy of region-specific clone recommendations based on life-cycle commercial yield performance.

SESSION 9

**CROP PHYSIOLOGY /
BIOCHEMISTRY**

A Study on Chlorophyll Fluorescence, Soil Acidity, Soil Moisture Contents and Plant Height in Relation to Different Polybag Size for Rubber Planting Materials During Transportation and after Field Transplanting[#]

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The study was carried out with the aim to assess and evaluate the effects of polybag size to the rubber planting materials with emphasis on chlorophyll fluorescence, soil moisture contents, soil acidity and plant height during transportation stage and after field transplanting. The survivality rate was also observed after the plants were transplanted in the field. Overall results showed no significant difference between all the parameters measured during transportation process at three and six hours for young budding of clone RRIM 928 and RRIM 2025. For the bare-root budded stump, clone RRIM 2025 showed a significant difference for chlorophyll fluorescence at three hours based on analysis of variance at $p < 0.05$. The chlorophyll fluorescence was found to be differed significantly between plants in polybag size 7"x15" – 6"x13" and 6"x13" – 5"x10" when the results were subjected to least significant different test for means comparison ($p < 0.05$). In term of survivality rate, generally, without concerning the clone, it was found that young budding in polybag size 5"x10" showed the highest mortality at 108 days (24%) compared to young budding and bare-root budded stump in polybag size 7"x15" (0%). At 190 days, young budding plants in polybag size 5"x10" showed another 10.5% mortality, followed by 5.9% mortality each for bare-root budded stump in polybag size 5"x10" and 6"x13" and 3.6% for young budding in polybag size 6"x13". Both plants in polybag size 7"x15" showed no mortality even after that time period. Further measurement of all the parameters were also carried out at 108 days and 190 days after field transplanting. It was clearly showed that young budding of RRIM 928 planted in polybag size 7"x15" exhibited the highest plant height and differed significantly at 190 days after transplanting based on analysis of variance at $p < 0.05$. Polybag size was found differed significantly between 7"x15" – 6"x13" and 7"x15" – 5"x10" when the results were subjected to least significant different test for means comparison. The results indicated that bigger polybag size played an important factor and could influence the plant height after transplanting. For the soil acidity, it was found that soil in polybag size 5"x10" were more acidic compared to other larger polybag size. Further analysis of variance using polybag size as source of variation showed a significant difference for the soil acidity ($p < 0.001$) at 190 days after transplanting. Mean comparison for soil acidity were found to

be differed significantly between polybag size 7"x15", 6"x13" and 5"x10" subjected to least significant difference test at $p < 0.001$. The results indicate that polybag size contributed to different level of soil acidic which is resulted from different soil volume and presumably related to the concentration of the fertilizer when the soil moisture were losses in smaller polybag. Based on the result obtained, it can be concluded that the use of bigger polybag size such as 7"x15" and 6"x13" are very important to ensure that plants growth not affected during transportation and after field transplanting compared to smaller polybag size of 5"x10". Therefore, a minimum polybag size for production of two whorled hardened leaves of rubber planting materials should be 6"x13" which gives a comparable survivality rate, vigorous growth and better tolerance to uncertainty temperature. The findings also in line with the current revision of recommendation for rubber clonal planting materials specification in the Malaysian Standard.

O-2

Impact of Salicylic Acid Treatment to Enhance Drought Tolerance of Nursery Rubber Plants (*Hevea brasiliensis* Mull.Arg) When Introducing Them to Monaragala District in the Intermediate Zone of Sri Lanka

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Present experiment was conducted in Monaragala District, in the Intermediate zone of Sri Lanka (Average rainfall <1623 mm, with distinct dry period during June-August) to examine if treatment of rubber nursery plants with Salicylic acid (SA), would elevate the drought tolerance of the nursery plants. Five SA concentrations (0mM, 0.05mM, 0.1mM, 0.5mM, 1mM) were tested with soil drench and foliar application to determine the concentration of Salicylic acid which could improve the drought tolerance of rubber nursery plants at Monaragala sub station, Rubber Research Institute of Sri Lanka during the dry months of June – August, 2011. Design of the experiment was factorial randomized complete block design (RCBD).

Soil physical properties, permanent wilting point (PWP) of rubber nursery plants and the depletion level of available soil water content that could affect on the plant growth were tested prior to apply imp SA. Subsequently soils were saturated with water and kept at the field capacity was reached. Plants were treated with 100 ml of SA per plant with the above concentrations and subjected to water stress. Stress tolerance was monitored by observing the wilting patterns of the plants through out the stress cycle of 21 days until, plants reached their permanent wilting point.

Soil physical properties of bulk density, true density and porosity of the potting media of nursery plants were 1.32 g/cm³, 2.5 g/cm³ and 48% respectively. PWP of the rubber seedlings was 4.3%.

Out of all treatments, rubber nursery plants treated with 0.5 mM SA as soil drench, showed a significant enhanced tolerance to the drought. Furthermore avenues are opened to elevate the depletion level up to 70% by using salicylic acid 0.5 mM concentration for the better growth and development of rubber nursery plant while reducing the irrigation need of the plants. Salicylic acid treatment with irrigation was found to be a promising and a feasible physiological approach to increase the drought tolerance of rubber nursery plants.

O-3

Hydrogen Peroxide is Essential for Wound-Induced Secondary Laticifer Differentiation via Activating Jasmonate Biosynthesis in Rubber Tree (*Hevea brasiliensis* Müll. Arg.)

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Although phytohormones are among the most important factors that are involved in regulating vascular tissue differentiation, the signal pathway in the differentiation of a special tissue within vascular tissues is still largely unknown. Secondary laticifers in the secondary phloem of rubber tree are differentiated from vascular cambia and easy to trace by histochemical staining of the rubber inclusions. The signal pathway in the secondary laticifer differentiation induced by mechanical wounding was revealed by using techniques

of physiology, experimental morphology and pharmacology. Water deficit triggered a burst of hydrogen peroxide and jasmonates, and induced the secondary laticifer differentiation in the secondary phloem of the wounded bark tissues. The secondary laticifer differentiation was also induced by application of exogenous hydrogen peroxide and methyl jasmonate, but inhibited by abscisic acid. Diphenyleneiodonium chloride (DPI), a specific inhibitor of NADPH oxidase, was effective on inhibiting the increase in the level of hydrogen peroxide as well as of jasmonates. It blocked the wound-induced, but not the methyl jasmonate-induced secondary laticifer differentiation. Taken together, we postulate a signal pathway mediating wound-induced secondary laticifer differentiation in rubber tree.

O-4

ATP Concentration in Latex as a Biochemical Marker for Early Evaluation of Yield in *Hevea brasiliensis*

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Availability of ATP (Adenosine 5' triphosphate) in the laticiferous tissues of *Hevea brasiliensis* plays a major role in rubber biosynthesis through its direct involvement in metabolic pathways and indirectly through H⁺ ATPase activity. A strong positive correlation was observed between dry rubber yield and latex ATP when ATP was measured in the latex of ten *Hevea* clones with different yield potentials such as low, medium and high. In general, significantly high latex (ATP) was observed in high yielding clones compared to medium and low yielding clones. The correlation between same day latex yield and ATP during the peak yielding season (September-November) also showed direct relationship. This study was extended to immature plants of the same clones to confirm the practical application of this finding in crop improvement programme. Latex (ATP) was analysed in two year old young plants and continued for five years to correlate with rubber yield of the mature tree. A positive correlation was noticed between (ATP) in young plants and mature tree rubber yield. Young plants of high yielding clones always showed higher latex (ATP) concentration. In view of the direct relationship with yield, significant differences between clones and its seasonal insensitivity, latex (ATP) could be used as a marker for early prediction of high yield in *Hevea*.

Impacts of Water Stress on Gas Exchange, Water Relations and Chlorophyll Content in Five *Hevea brasiliensis* Clones

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Five *Hevea brasiliensis* clones were used that is RRIM 929, RRIM 2002, RRIM 2008, RRIM 2014, RRIM 3001 in the first and second experiment when they were 3 months old or at two whorls hardened of leaves. Leaf gas exchange were measurement used a closed system of infra-red gas analyzer LICOR 6400 Portable Photosynthesis System (IRGA :LICOR Inc. Nebraska, USA). Fully expanded leaves were used to record net photosynthesis rate (A_{net}), stomata conductance (g_s), transpiration rate (E) and intercellular CO₂ concentration (C_i). Instantaneous water use efficiency (WUE) was calculated by dividing the net photosynthesis rate (A) by transpiration rate (E). The leaf used for stomatal conductance measurement was chosen for leaf water potential (pL) determination using the pressure chamber (Skye, plant moisture vessel, SKPM 1400 Series, UK) and Chlorophyll content was estimated following the method of witham *et. Al.*, (1986) to get the chlorophyll a, chlorophyll b and total chlorophyll measurement. The soil moisture was measured by Moisture Meter type HH2 (Delta-T Devices with Theta Probe sensor).

In the well watered treatment, the soil remained 39.9% throughout the experiment suggesting that plants did not suffer any moisture stress. The soil moisture in the water stress treatment given progressively decreased with days of withholding water. After seven days of no watering, the soil moisture in the water stress treatment was significantly lower than that in the well watered or irrigated treatment. Leaf water potential (pL), stomata conductance (g_s), net photosynthesis (A), transpiration rate (E) and water use efficiency (WUE) of well watered or irrigated plants remained between 0.74 to 0.79 Mpa, 0.74 to 0.79 molH₂O/m²/s, 2.6 to 2.8 μmolCO₂/m²/s, 2 to 2.5 mmol/m²/s and 1.2 to 1.0 μmol(CO₂) mmol⁻¹ (H₂O), respectively throughout the drying period. However, the pL, g_s, A, E and WUE of stress treatment decreased rapidly as the duration of withholding water increased. By the seventh and fourteenth day of withholding water, the pL, g_s, A, E and WUE of stress plants dropped which significantly lower than that of the well watered or irrigated plants.

Relationship between soil moisture content (SMC) among the variables during the whole water stress period of the five *Hevea* clones. There was a polynomial function relationship between SMC (%) with A and gs in all clones. SMC explained 67%, 84%, 83%, 72% and 74% of the variability in A in RRIM 929, RRIM 2002, RRIM 2008, RRIM 2014 and RRIM 3001, respectively and SMC explained 55%, 67%, 60%, 57% and 55% of the variability in gs in RRIM 929, RRIM 2002, RRIM 2008, RRIM 2014 and RRIM 3001, respectively. There was strong linear relationship between SMC with E and pL in all five clones. The SMC explained 88%, 88%, 86%, 78% and 84% of the variability in E in RRIM 929, RRIM 2002, RRIM 2008, RRIM 2014 and RRIM 3001, respectively and SMC explained 90%, 84%, 91%, 77% and 83% of variability in pL in RRIM 929, RRIM 2002, RRIM 2008, RRIM 2014 and RRIM 3001, respectively and there was a relationship between pL with A, gs and E explain by a polynomial function in all clones.

Exposure of five *Hevea* clones to water stress treatment led to lower chlorophyll a, chlorophyll b and total chlorophyll content. Significant reductions in this variables for stressed *Hevea* were observed in comparison to the control. These reduction after 28th days of stressed were 59%, 60%, 69%, 64% and 59% in RRIM 929, RRIM 2002, RRIM 2008, RRIM 2014 and RRIM 3001, respectively for chlorophyll a. The reduction showed the same trend on chlorophyll b and total chlorophyll content.

The pL, A, gs and E values observed under stressed conditions were lower than the control value in all five clones which could be due to clonal differences, especially in the pL finding. pL in RRIM 929 and RRIM 3001 response to a slight decline, compare to RRIM 2014 showed a rapid decline starting on the fourteen day of water stress. However, relationship between pL and gs showed RRIM 2002, RRIM 2008 and RRIM 3001 with a moderate decline indicates a water conservation (dehydration postponement) mechanism employed by the clones which help them to maintain the plant water status during water stress. On the other hand, slow stomata response under water stress in RRIM 2002, RRIM 2008 and RRIM 3001 suggest the low contribution of stomatal closure to drought avoidance of the clones compare to the other 2 clones studied. The trigger for stomatal closure under period of water stress is believed to be associated with root to shoot communication via abscisic acid (ABA) translocation in many crop (Davies *et. Al.*, 1990; Davies and Zhang, 1991; Blum and Johnson, 1993). Furthermore, high WUE had shown RRIM 3001, RRIM 2002 and RRIM 929 were significantly high compare to RRIM 2008 and RRIM 2014 is mainly result of reduced transpiration rate by reduce the stomata opening with low but positive net photosynthesis activity during water stress period. However, the maintenance of low level of A at very low pL in RRIM 2002, RRIM 2008 and RRIM 929 during two whorls harden leaves development may be critical in providing the energy required to maintain translocation of assimilates from the source (root) to the developing sink, the leaves (Lepord *et. Al.*, 1998). The decrease in chlorophyll at decreasing leaf water potentials can be attributed to the sensitivity of this pigment to increasing environmental stresses, especially to water stress and salinity, which has been reported by several researchers (Moran *et. Al.*, 1994; Younis *et. Al.*, 2000). Despite the total chlorophyll content in RRIM 2008 and RRIM 2014,

this clones showed a greater reduction of this parameter under water stress compare to RRIM 2002, RRIM 3001 and RRIM 929.

The data presented showed some distinct differences in the response of the five clones to water stress impose during two whorls harden leaves. Although the clones have similar responses under well watered conditions, they do differ in stomatal regulation and maintenance of plant water status, photosynthesis and transpiration rates under declining soil moisture content during their immature stage. RRIM 3001, RRIM 2002 and RRIM 929 maintain photosynthesis under water deficit by maintaining high plant water status (pL). The response of RRIM 3001, RRIM 2002 and RRIM 929 are similar for most of the parameters measured indicating closer performance of the clones under dry environments. The variations observed in plant water status, gas exchange and chlorophyll content among the clones and the different parameters determined in this study could be used to selection of the clones suit for dry environments.

O-6

***Hevea* B Serum Proteome Profiling Using Liquid Chromatography-Mass Spectrometry**

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B serum is the fluid content of luteoids that consists various proteins. The main aim of this study is to apply nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS) in profiling B serum proteome and to assess its output. Purified B serum proteins extracted from centrifuged latex using freeze-thaw method were subjected

to dialysis to remove salts and other impurities, and finally followed by fractionation using spin column ultrafiltration. In this study, only the soluble proteins in the supernatant were analysed by nano-LC-MS/MS. A total of 13 proteins were identified and the detailed analysis of these proteins is presented here.

Drought Tolerance in Transgenic Mnsod *Hevea brasiliensis* in A Dry Humid Environment

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Abiotic stresses like drought, temperature and light extremes cause production of reactive oxygen species through different mechanisms creating oxidative stress, which causes significant crop loss. Genetic engineering appears to be an alternative approach towards imparting stress tolerance in crop plants. One year old plants of MnSOD transgenic *Hevea* lines (L1 and L2) and an untransformed line of clone RRII 105 were used in the present study to evaluate their physiological performance in a dry humid environment by withholding irrigation for six days and to see the recovery after re-watering for three days. The dry matter partitioning was relatively more towards the root in transgenic lines (55% and 60% in L1 and L2, respectively) while, it was less in the untransformed RRII 105 (43%). After six days of moisture stress in polybags, pre-dawn leaf water potential and relative water content declined in all the lines, however, L1 showed higher tissue water content throughout the drought and following recovery periods. Chlorophyll content and effective quantum yield ($\dot{O}PSII$) during drought period did not show a significant reduction. Net photosynthetic rate (P_N) declined rapidly and by the 3rd day of drought treatment, reached near zero, barring L1, which showed slower decline in P_N . The decline in stomatal conductance (g_s) was more rapid than P_N in all the lines. On re-watering, recovery in both P_N and g_s was better in the transgenic lines than untransformed RRII 105, which did not recover fully from the drought impact. Antioxidant enzymes namely, superoxide dismutase and peroxidase did not show any consistency in their activities in the three lines. SOD activity was higher in transgenic line L2 whereas the other lines had lower activity under well watered and drought conditions. Lipid peroxidation was more in the transgenic lines. The present study did not give a clear picture on the antioxidant enzyme activity and membrane damage in the transgenic lines. However, it was found that line L1 had better drought tolerant capacity under the drought conditions in the North Konkan region of India and the transgenic lines also showed better recovery upon new watering.

P-1

Supercritical Fluid Extraction of Flavonoids From *Hevea* Leaves

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We describe the usage of Supercritical Fluid Extraction (SFE) system for the extraction of antioxidants from *Hevea* leaves. The phytochemical screening and HPLC analysis of *Hevea* leaves extracts by SFE revealed the presence of flavonoids. Flavonoids are polyphenolic compounds that are most commonly known for their antioxidant activity. The present study describes the effects of pressure (100, 200 and 300 bar) and temperature (40, 50 and 60 °C) on total extraction yield and antioxidant activity of SFE extracts of *Hevea* leaves. The results showed that extraction pressure and temperature had significant effect ($P < 0.05$) on the total extraction yield and antioxidant activity. These findings suggest that temperature at 50 °C is more convenient to be selected for the SFE extraction of antioxidant compound such as flavonoids from *Hevea* leaves. Further investigation would be required for a detailed characterization of the flavonoid compounds in *Hevea* leaves.

P-2

Study on Lignin and Cellulose Total Content in Two Rubber Clones Planted in Different Densities¹

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Lignocellulosic materials extracted from rubber tree serves as potential raw material for biofuel. Four types of planting density; 500, 1000, 1500 and 2000 plants/ha have been used in Rubber Forest Planting Density Trial in Rubber Research Institute Mini Stations in Tok Dor, Terengganu in April, 2000. Two types of rubber clones i.e. RRIM 2020 and RRIM 2025, with three replications, were planted to investigate the effect of planting density on tree growth. Biomass weight and moisture content were obtained for these eleven-year old plants on ten different plant parts, which are leaf, petiole, twig, small branch, large branch, bole at tree bottom, bole at 150 cm from ground, bole at tree first branching, tree bark and root. Analyses of lignin and cellulose concentration were carried out for each plant parts of

RRIM 2020 that was planted in 500 plants/ha density. The data was used as a projection for total content of lignin and cellulose for all trees in the trial. The purpose of making such projection was to estimate total content of lignin and cellulose that were able to be extracted from eleven-year old rubber tree, particularly from plant parts that were discarded from tree felling activity during replanting programme, for the production of biofuel in the form of ethanol. Example of the plant parts are leaf, petiole, twig, small branch and root.

The benefits of biofuel production from renewable sources include reducing the pressure on the usage of fossil fuel, producing green fuel that emits zero carbon into the atmosphere and reducing the possible attack of root diseases in rubber plantation from the unused plant parts that are normally left on the ground after replanting program which later became nutrient source for pathogen. The different planting densities investigated in the study shall answer the question of which densities could give rise to the most total content of lignocellulosic material and therefore shall be applied in the future planting for the purpose of biofuel production.

Summary of biomass weight showed that the highest mass was observed mostly in large branch for 500 and 1000 plants/ha densities. As the density goes higher as in 1500 and 2000 plants/ha, the trend shifted for bole. This explains the bigger tree crown in 500 and 1000 plant/ha densities, and the taller trunk in 1500 and 2000 plants/ha densities. The smallest biomass weight was all observed in petiole. The highest moisture content was observed mostly in petiole while the lowest moisture content was observed in bole. Analyses of extractives, lignin, holocellulose and alphacellulose concentration were conducted in plant parts of RRIM 2020 planted in 500 plants/ha density, of which the data was used for calculation of projection of total lignin and holocellulose. The highest concentration of extractives was found in leaf. Extractives are organic extraneous materials that include tannins and other polyphenolics, colouring matters, gum starch and simple metabolic intermediates and these explained how the amount was observed to be the highest in leaf as compared to other plant parts. The highest concentration of lignin was also observed in leaf. For concentration of holocellulose the highest value was seen in bole at first branching while the highest concentration of alphacellulose was seen in large branch. The projected amount of total lignin and holocellulose for RRIM 2020 and RRIM 2025 were the highest in large branch for 500 plants/ha density, while for 1000, 1500 and 2000 plants/ha densities the highest value was observed in bole. The smallest value was seen in petiole for both clones planted in all densities. Analysis of variation for total content of lignin and holocellulose was conducted with clone and density as source of variation. There was no significant difference for total lignin and holocellulose in difference clones, except for leaf and root. For different densities mostly there was significant difference in total lignin and holocellulose in plant parts, except for petiole, bole and bark. Mean comparison using Least Significant Difference test revealed that the highest total lignin was found in 500 plants/ha for leaf, twig, small branch, large branch and root. The different with other densities was also found to be significance which means that planting in this density would result in significantly higher biomass weight and subsequently bigger total lignin can be extracted. Mean comparison for total holocellulose also showed the highest value in 500 plants/ha

density for leaf, twig, small branch, large branch and root. This observation suggested that 500 plants/ha density shall be taken as the most ideal planting density for the purpose of biofuel production, particularly from unused plant parts such as leaf, petiole, twig, small branch and root. The expected amount of total lignin and holocellulose that can be extracted from unused plant parts of RRIM 2025 planted in 500 plants/ha are 40.51 kg/tree and 92.70 kg/tree respectively. The projected amount of total lignin and holocellulose per hectare with expected 10% tree loss at eleventh year of planting are 18,230 kg/ha and 41,715 kg/ha respectively. The amount of this lignocellulosic materials shall be able to produce a considerably high output of ethanol. Based on average 42% cellulose and 21% hemicelluloses in wood, the maximum theoretical yield of ethanol can be calculated to be 0.32 grams of ethanol per gram of wood or per 0.63 gram of pure holocellulose. This calculation is based on a full conversion of cellulose and hemicelluloses to sugars, and conversion of sugars to ethanol at the theoretical yield of 0.51 g/g. Therefore the calculated total amount of ethanol production for eleven years of planting is 21,189 kg/ha. Lignin on the other hand is not directly converted to ethanol. Since heating values for lignin is 28 MJ/kg, which is similar to that of coal, lignin is often used to produce heat that is required in the ethanol production process. This material is frequently utilized as an energy source for power generation because there are a few efficient chemical conversion processes available that can convert lignin into liquid fuels or higher value chemical substrates. However higher technological advancement should be able to convert lignin to biofuels by using various pathways. In a nutshell lignocellulosic materials are highly potential to be applied in the production of biofuel, where the materials could be extracted from unused plant parts of rubber tree during rubber replanting programme.

P-3

Plant Hormones and Oxidative Stress in *Hevea brasiliensis*

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Plant hormones are naturally occurring organic substances that influence in various metabolic processes in plants. These signal molecules produced within the plant in low concentrations and regulate growth and metabolism of the plant. High intensity tapping and over-harvesting of latex from rubber trees have direct effect on the development of oxidative stress, which ultimately lead to several physiological disorders including tapping panel dryness (TPD). Under such complex physiological state the endogenous levels of

stress hormones like ethylene and ABA and growth hormones like IAA, GA₃ and zeatin in the soft bark tissues of *Hevea* were studied along with other stress component like hydrogen peroxide (H₂O₂) and peroxidase. During oxidative stress, the stress hormones level increased and the growth hormones level decreased in the bark tissue. Both ethylene and ABA concentrations were high in the tree as a whole when the trees were exposed to oxidative stress. The levels of H₂O₂ and its scavenging enzyme, peroxidase, present in healthy trees seem to be normal and appear to be capable of scavenging the H₂O₂ molecule produced in the healthy tissue. But the amount of peroxidase produced in the bark tissue was inadequate to detoxify H₂O₂ produced under elevated stress hormone levels and thus leading to oxidative stress in trees under stress. The low growth hormones and excess stress hormones levels in the tissue during stress that limit the cellular differentiation and metabolism in the *Hevea* trees are discussed.

P-4

Studies on Cold Tolerance of *Hevea brasiliensis* (Muell. Arg.) Clones under Controlled Environmental Conditions

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Low temperature during winter season is a major constraint for the extension of rubber cultivation in sub-tropical environments that are prevailing in north-eastern India. Cold stress strongly affects the growth and development of rubber plants as well as production of latex. In the present study four *Hevea brasiliensis* clones viz., RRII 105, RRIC 100, SCATC 88/13 and Haiken 1 were exposed to cold stress under controlled growth chamber conditions. Morphological symptoms such as yellowing and drying of leaves were prominently noticed in RRIC 100 followed by RRII 105 and less prominent in SCATC 88/13 and Haiken 1. The photobleaching of the photosynthetic pigments, chlorophyll and carotenoids were comparatively lesser in Haiken 1 and SCATC 88/13. Maximum photochemical efficiency of PSII (Fv/Fm) and effective quantum yield of PSII (ÖPSII) were stable in SCATC 88/13 followed by Haiken 1 under low temperature conditions. The rate of lipid peroxidation was severe in RRII 105 and RRIC 100, indicating that these two clones succumbed to cold mediated oxidative stress. Two stress proteins were found in the chloroplast protein profile of Haiken 1 and SCATC 88/13 that may probably be involved in conferring cold tolerance to these two clones.

Localization of Peroxidase Enzyme in the Bark of *Hevea brasiliensis*

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Hevea brasiliensis, the Para rubber tree, is the prime source of natural rubber, exploited by means of controlled wounding called tapping to obtain latex from the tree trunk. It is a deciduous tropical tree which exhibits seasonal activity of tissues that leads to alter the anatomy of bark periodically. Anatomically, the bark of *Hevea* can be demarcated into inner soft bark and outer hard bark among which functionally active tissues like sieve tubes, phloic rays, etc. are confined to the soft bark and those tissues in the hard bark region are less functional or shriveled. Localization of peroxidase enzyme with respect to seasons has been carried out in the bark of mature trees of *Hevea brasiliensis* using guaiacol and hydrogen peroxide as the substrate. Cell walls of sieve tubes including sieve plates, and cytoplasm of phloic rays, both in the soft and hard bark region of the bark showed positive indication for peroxidase activity with reddish brown coloration. Phloic rays exhibit seasonal variation for this enzyme while the activity is localized throughout the year in the sieve tubes. Sieve tubes differentiated recently from the derivatives of cambium also showed high enzyme activity. Companion cells are not giving any indication on peroxidase activity but it is localized in the intercellular spaces of axial parenchyma cells in the soft bark.

The cell walls of the phloic rays remain passive for peroxidase activity throughout the year whereas the stained cytoplasm appears to be granulated and is in a state of streaming motion. Bark samples collected in the month of January and April showed deep coloration for phloic rays extending from the phellogen to the periphery of the cambial zone. In the month of June, the partially differentiated phloic rays in the cambial zone stained for peroxidase and subsequently differentiated ones are completely unstained. The phloic rays in the month of September remain unstained in the soft bark while it gave a brown coloration in the hard bark region. In the December samples, phloic rays are unstained throughout its extent in the bark. The deciduous nature of the tree is thus evident for peroxidase activity too, which is observed in the cytoplasm of the phloic rays but not for sieve tubes.

SESSION 10

ECOSYSTEM PROCESSES

A Study on Carbon Sequestration in Various Plant Parts of Two *Hevea* Clones Planted in Four Planting Densities¹

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Since carbon dioxide is a primary greenhouse gas, many scientists argue that the increase in atmospheric carbon dioxide from human activities has resulted in an enhanced greenhouse effect and could result in corresponding changes in global climate, including higher global temperatures. Carbon sequestration, a process that removes carbon dioxide from the atmosphere by locking it transiently could be the only solution to tackle the problem. As far as rubber plantation is concerned, carbon could be sequestered in plant parts, products, litters, debris and soil. In this study total carbon was measured in different plant parts of RRIM 2020 and RRIM 2025, planted in four planting densities; 500, 1000, 1500 and 2000 plants/hectare. The objective were to evaluate which planting densities could give rise to higher total carbon and which plant parts sequester the most carbon. A summary on total fresh weight (TFW), total dry weight (TDW) and total carbon (TC) gives a general overview on biomass information and pattern of carbon disposition. At 500 plants/ha density, mostly both clones have the highest TFW, TDW and TC in large branches. As density goes larger, the pattern shifted towards clear bole. This gives indication that the tree crown grew bigger in less dense planting in order to maximize the photosynthesis activity, while in more dense planting the competition among trees increases and forces them to grow taller in order to reach for more light source and hence the clear bole mass becomes greater. When plant parts' total carbon are subjected to the calculation of variance ratio, it was found that the type of clones used in the study did not show any differences in total carbon sequestered in their plant parts. However when densities were made as a source of variance in the analysis, majority of the plant parts were shown to be affected by it. Total carbon in plant parts was shown to be the highest in 500 plants/ha, this is followed by 1000, 1500 and finally 2000 plants/ha. Different pattern was seen only for petiole where the highest total carbon was observed in 1000 plants/ha, followed by 1500, 500 and 2000 plant/ha. Calculation of variance ratio for total carbon in plant parts of each clones and densities showed significant difference, this means total carbon sequestered in different plant parts was significantly different in the amount. For RRIM 2020 in 500 plants/ha density, the highest TC was seen in large branch, followed by bole, root, small branch,

bark, twig, leaf and the lowest in petiole. For 1000 and 1500 plants/ha densities, the highest TC were shown in bole, followed by root, large branch, small branch, bark, twig, leaf and the lowest in petiole. For 2000 plants/ha density, the highest TC was found in bole, followed by root, bark, twig, small branch, large branch, leaf and the lowest in petiole. Different pattern was documented for RRIM 2025; in 500 plants/ha density, the highest TC was seen in large branch, followed by bole, root, small branch, bark, twig, leaf and the lowest in petiole. For 1000 plants/ha density, the highest TC was seen in bole, followed by large branch, root, small branch, bark, twig, leaf and the lowest in petiole. For 1500 plants/ha density, the highest TC was shown in bole, followed by root, large branch, small branch, bark, twig, leaf and the lowest in petiole. For 2000 plants/ha density, the highest TC was observed in bole, followed by large branch, root, bark, small branch, twig, leaf and the lowest in petiole. Clearer view in the pattern of total carbon sequestered in different densities can be shown through orthogonal polynomial trend analysis calculation, where densities are incremented equally. For clone RRIM 2020, there was no significant trend model for leaf and bole. For petiole the trend was cubic, while for twig, small branch, large branch, bole and root they were all linear model. For clone RRIM 2025, there was no significant trend model for petiole, bole and bark. The pattern was cubic for leaf, while the rest of the plant parts showed linear model. In conclusion densities played big role in determining the amount of carbon that can be sequestered in plant parts of rubber tree, where 500 plants/ha density shall give the highest amount of total carbon sequestration. Rubber plantation is indeed an excellent option to reduce the pressure of carbon dioxide effects to the environment by locking the atmospheric carbon into biomass form.

O-2

Measurement of CO_2 Flux in Rubber Plantation by Eddy Covariance Method

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Natural rubber plantation has an economic life of 25-30 years during which it sequesters significant quantities of CO_2 from the atmosphere into biomass. There are several methods to study CO_2 sequestration potential of a perennial plantation crop like natural rubber. Eddy covariance (EC) method is a state-of-the-art technology in which the fluxes of CO_2 and water vapour in any ecosystem can be measured in real time. In the present study, we describe measurements of carbon and water flux continuously for two years in a five-six year old immature natural rubber plantation. From the EC data, the net ecosystem exchanges (NEE) of CO_2 and water vapour were calculated. Mostly, day time recorded net CO_2 influx

(Aeco) in to the plantation whereas night time witnessed efflux of CO₂ through ecosystem respiration (Reco). The net CO₂ exchange is the difference between photosynthetic assimilation by the canopy and the total respiratory efflux from the foliage, roots and soil. Sunny days with good soil moisture favoured better CO₂ sequestration by the rubber plants in this tropical ecosystem. On an average, the NEE was 12g CO₂/m²/day which is equivalent to 43.8 t CO₂/ha/year. The annual mean NEE, an indicator of C-sequestration rate, increased year by year when the plant growth increased. The ET values calculated from the latent heat of vapourization (LE) indicated a 3-4 mm net evaporative water loss from the immature rubber plantation per day.

The current rate of build-up of CO₂ in the atmosphere is about 2 ppm/year. Taking a life cycle average rate of 30 tCO₂/ha/year, the world's 10.5 million ha of natural rubber plantations help to offset the current rate of build-up of CO₂ in the atmosphere to the tune of 1.9%. This is a significant ecosystem service provided by the natural rubber plantation.

O-3

Seasonal Changes in Xylem Sap Flow Rate in Mature Rubber Trees

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Sap flow measurement is a precise technique to study the tree water relations and to quantify whole-plant water use. The water usage of grown up rubber tree is not thoroughly studied so far. Sap flow measurements can provide an accurate method for determining the transpiration water loss from any plantation crop. The quantum of water loss in a tree depends on canopy size, physiological condition and soil and atmospheric factors. There is also an increasing interest in water and carbon fluxes at the canopy- atmosphere interface in the confert of global climate.

The experimental site was located at Rubber Research Institute of India (RRII), Kottayam, Kerala at an altitude of 72 MSL, longitude 76° 34' E and latitude 9° 32' N. Xylem sap-flow was measured in two rubber clones, namely RRII 5 and PR 255 of 19 years age with a mean girth of 78 and 82 cm, respectively at 150 cm above bud union. The terrain was mainly with gentle slope and the soil was laterite. Granier's TDP probes with 80 mm length were inserted in main trunk at the breast height and the probes were connected to data logger. The data were downloaded every week and the mV signal was converted to flow velocity (V). Rate of flow of xylem sap through the trunk was recorded round the clock continuously for two years.

The diurnal and seasonal differences in the trend of sap flow rate were very evident which responded to the ambient weather conditions (such as intensity of sunlight temperature, rain fall etc.). In the morning hours, as the sun light intensity increased there was a corresponding sharp increase in sap flow rate which attained maximum level around mid day. In the evening as the light intensity declined, the sap flow rate also declined. However, the rate of decline in the sap flow with respect to reduction in sunlight intensity in the late afternoon was lower than the rate of increase in sap flow with increase in the light intensity in the morning hours. Even during the mid day, when there was a sudden reduction in sunlight intensity due to a passing cloud, sap flow rate did not show any immediate concomitant reduction.

The wintering phenomenon in *Hevea* generally starts from later part of December and completes by January. During defoliation period the sap flow rate declined drastically (from 40 litre/day/tree to 7 litre/day/tree). New foliage was started appearing from third week of January onwards. The sap flow rate slowly increased concomitant with the onset of new flush of leaves and attained maximum rate of flow after second week of February. The maximum and minimum rate of sap flow per day was observed during December and February, respectively. Sap flow rate during summer season was smaller than post-monsoon season. Significant positive relationship existed between T_{max} and sap flow rate during pre-monsoon periods with saturated soil moisture.

Taking a mean water consumption of 22 l/day/tree and assuming there are 400 trees/ha, the water consumption through transpiratory pull works out to be around 1.5 mm/day.. Taking the long-term average rain fall in the region (3000 mm/year), it can be seen that the water loss due to transpiration by the trees amounted only to 12% of the annual rainfall.

SESSION 11

**GEOSPATIAL
TECHNOLOGY**

O-1

Assessing Agricultural Drought in Natural Rubber Plantations Using MODIS/Terra Satellite Data

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Drought assessment and monitoring are important in the management of a crop particularly in the context of global warming and climate change. Rubber being a perennial crop goes through periods of moisture deficits during different phases of its growth. Real or near real time drought monitoring is required for adopting proper management strategies to mitigate the adverse effects of drought. Satellite-based remote sensing techniques are increasingly becoming handy in assessing and monitoring drought in many crops. Severe drought during the summer season results in appreciable reduction in growth and yield in natural rubber. In the present study, an attempt has been made to identify the spatial extent of agricultural drought stress in natural rubber plantations of Kerala and Kanyakumari district of Tamil Nadu using satellite based remote sensing data and GIS. By combining remotely sensed land surface reflectance and thermal properties from Terra MODIS, changes in land surface temperature (LST) and normalized differential vegetation index (NDVI) over a region is estimated. Vegetation temperature condition index (VTCI), derived from the association between NDVI and LST gives an estimate of the temperature status of the vegetation for a given NDVI which is an indirect reflection of the soil moisture status. Our results indicate that VTCI is a powerful proxy estimate of the drought stress rubber plantations experience during peak summer season in Kerala and Kanyakumari district of Tamil Nadu.

O-2

Application of Light Detection and Ranging (Lidar) Technology for Field Monitoring

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Light detection and ranging (LIDAR) technology was used as tool to measure the girth of 3,320 *Hevea brasiliensis* trees in the Malaysian Rubber Board (MRB) Experimental Station, Kota Tinggi, Johor as a pilot project in January 2012. LIDAR is an optical remote sensing technology that can measure the distance to, or other properties of a target by illuminating the target with light or laser. This technology uses a 3D laser scan to measure the tree's diameter at breast height (DBH). LIDAR was used along with conventional measuring tool to measure the DBH of rubber trees. The data from both measuring tools showed that the measuring accuracy were comparable with means of girth for LIDAR (0.3835 m) and mean of girth for caliper (0.3434 m), p-value= 0.004. Even though the results show that measuring accuracy of both methods was comparable, the LIDAR technology was less labour intensive as it was able to measure DBH at a wider area in a short amount of time. Meanwhile the DBH of every single tree had to be measured when using the conventional measuring tool. LIDAR was also found to be cost effective in completing the field data collection as well as analysis of data. Unfortunately the accuracy of the LIDAR is dependent on the condition of the area to be measured.

O-3

Digital Image Recognition System for Rubber Clones Produced in Malaysia

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Clone inspection on *Hevea brasiliensis* throughout Malaysia is currently being carried out by limited number of LGM qualified clone inspectors. The process of clone inspection and verification is very critical because it must guarantee the supply of genuine rubber clones recommended by Malaysian Rubber Board (MRB) to the growers, thus making sure they will harvest the maximum yield in future.

At the moment, clone inspection is done by MRB clone inspectors using visual method. However, the number of experienced clone inspectors is dwindling and newly trained clone inspectors are not in the position to take over the task yet. It takes years of experience, effort and the right prodigy to become a good clone inspector. This process is considerably quite lengthy, time consuming and prone to individual biasas this is based solely on different level of one's many years of field experience. With estimated more than 200 *Hevea* nurseries nationwide to be inspected and verified, this is an immense job for our limited personnel to handle.

Recently, MRB has initiated a digital system where this innovative approach will identify *Hevea* clones. An artificial intelligent system (AI) as a backbone system for mobile devices is successfully developed and incorporated into Android-based smartphones. In order to standardize and also to become a reference point during sampling, reference materials of which comprised of 6 selected *Hevea* clones- RRIM 928, RRIM 3001, RRIM 2001, RRIM 2024, PB 350 and PB 260 has been established at Rubber Research Experimental Station, Sg. Buloh (RRIES).

Field tests had been carried out to verify the system in various private nurseries throughout Peninsular Malaysia with mixed results. A comprehensive dataset to further validate the leaf morphological characteristic of clones and to normalize the vast variations and discrepancies between reference materials and clones in the private nurseries have been dealt with and incorporated into the system.

O-4

Distribution of *Hevea brasiliensis* in South and Northeast India: Preliminary Results with Maximum Entropy Species Distribution Model

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Prediction of present and future distribution of natural rubber, *Hevea brasiliensis*, was attempted using the Maximum Entropy species distribution model in South and Northeast India. Preliminary results of model simulation on present distribution of natural rubber in Tripura state in Northeast India was validated with the satellite image based current spatial rubber map and found to be highly correlative. Future distribution of natural rubber was predicted for South and Northeast India for the year 2050 using the bioclimatic variables of SRES-A1B scenario generated by Intergovernmental Panels for Climate Change (IPCC). Present distribution of natural rubber predicted by the model indicates that South-western aspects of the southern parts of Western Ghat in the states of Kerala, and Kanyakumari district of Tamil Nadu are the dominant areas under natural rubber. In NE India, natural rubber distribution was more predominant in the West and South Tripura districts, Northern Mizoram, Western Meghalaya and lower Assam. The model predicts that natural rubber expand more towards Northern parts of Western Ghat along the Malabar coast, upper Assam, Northern Tripura and parts of Meghalaya. The model performance was assessed

using receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) for training data and test data of natural rubber were 0.983 and 0.990 respectively indicating that omission of training and test data by the classifier is less than 30% and 10% respectively. The jackknife plot of AUC and regularized training gain indicates that Bio7 (annual range of temperature) is the most effective single variable followed by diurnal range of temperature, isothermality and temperature seasonality that determine the future distribution of natural rubber in India. Relevance of the present findings is discussed in the context of global climate change and the increasing demand for natural rubber, both domestically and globally.

O-5

Retrieving of Leaf Area Index for Rubber Plantation by Using HJ-1A/1B CCD Data

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Leaf area index (LAI) is a key variable characterizing many biological and physical processes such as photosynthesis and carbon cycle, and it could, more importantly, serve as a practical indicator for rubber trees (*Hevea brasiliensis*) in yield prediction and post-disaster evaluation after severe typhoon disturbances. Although numerous studies have been performed to derive forest and crop LAI over the past few decades, few have dealt with rubber tree, especially by using remote sensing technology. In this study, both the temporal variation of LAI in rubber plantation and potential use of 30 m resolution satellite data from China HJ-1A/1B of the Environment and Disasters Monitoring Microsatellite Constellation Charge-Coupled Device (CCD) cameras for LAI estimation were investigated.

In situ measurement of LAI in 25 rubber plantations including clones of CATAS7-33-97, PR107, RRIM600, and CATAS7-20-59 at the experimental farm of Chinese Academy of Tropical Agricultural Sciences (CATAS) was made by using the Plant Canopy Analyzer (LAI-2000, LI-COR) optical instrument. The stand age of sampled plantations was 15 years by average, and ranging from 7 to 29 years. The experiment started in March 2012, and was repeated once a month. Five serial HJ-1A/1B CCD images, which were acquired on 23/03/2012, 24/04/2012, 01/05/2012, 03/05/2012, and 11/07/2012, respectively, and close to the date of field LAI measurement, were used to explore their relationship to the field LAI through bivariate correlation method. Spectral bands and three vegetation indices

including Normalized Difference Vegetation Index (NDVI), Simple Ratio (SR) and Soil Adjusted Vegetation Index (SAVI), derived from raw digital numbers (DN), radiance and atmospherically corrected reflectance were examined.

The field data shows LAI booms in March (2.20 ± 0.63) and April (2.98 ± 0.73) after synchronized defoliation in late February, and keeps increasing with the rainy season. By the end of August, the LAI is 4.05 ± 0.95 by average with a maximum of 6.17. The temporal variation of field LAI is quite consistent with phenological behavior of rubber tree in leaf development in Hainan Island. About 70% of total leaves are produced during the first flush period (March to April), and the rest 30% come from the subsequent two flushes during the next five months. The LAI also varies with stand age: the young plantations (≤ 10 years) have the highest LAI, followed by the old plantations (≥ 20 years) and the mid-aged plantations which have the lowest value.

The field LAI is negatively correlated with visible bands, and highly significantly correlated with near-infrared (NIR) band, NDVI, SR, and SAVI. In late spring, the NIR spectral band has the highest Pearson's correlation coefficients ($r=0.81$) with the field LAI. However, SAVI is more promising ($r=0.60-0.71$) than NIR, NDVI, and SR in summer. Both radiometric calibration and FLAASH atmospheric correction could improve the correlation strength between the field LAI and the vegetation indices and the spectral bands than using raw DN value, and atmospheric correction is preferred. The correlation relationship derived from HJ-1A/1B data was quite consistent with the results extracted from two contemporary multispectral images, Landsat ETM+ and China Satellite Surveying and Mapping Application Center (SBSM) ZY-3 images, which were acquired on the same days with HJ-1A/1B CCD images and analyzed by using the same methods.

It is also found that the degree of understory canopy was an important factor that limits the accuracy in estimating LAI of rubber plantation by remote sensing. The plantations with thick understory vegetation cover were very weakly correlated with both spectral bands and vegetation indices, while their counterparts obviously possessed the highest correlation coefficients of 0.89 and 0.87 respectively for late spring and early summer. However, with the gradual increase of LAI, the effect of understory vegetation cover tends to be weakened. Finally, two statistically significant regression models were derived based on NIR ($R^2=0.66$, $RMSE=0.37$) and SAVI ($R^2=0.61$, $RMSE=0.47$), respectively for late spring and early summer. The HJ-1A/B data show great potential use in estimating rubber canopy parameters in the tropical area due to their good fitness in the regression models and the strong image acquisition capabilities that benefit from 2-days revisit period.

Spatio - Temporal Analysis of Rubber Area Changes in Kanniyakumri District of Tamil Nadu Using Remote Sensing and GIS

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Reliable up to date information on agricultural land and its changes are important for agriculture planning and management. In this article we attempt to quantify the spatio-temporal changes of natural rubber areas in Kanniyakumari district of Tamil Nadu over a period of 27 years (1980-2007) using temporal satellite data along with soil management unit (SMU) map derived from soil survey conducted during 1996-98 by NBSS and LUP. Area under rubber in 1980 was 10693.3 ha and it has increased to 15886.7ha in 1992 and then to 20781.7 ha in 2007. Overlay analysis of rubber area during 1980 -2007 has showed that 8891.5 ha have been newly planted with rubber during this period. Majority of this newly planted area has come up in good to moderate soil with slope moderate to gently sloping. Study has revealed that substantial rubber area expanded to new lands during 1980-2007 period and calls for updating the soil information. Present study also revealed that rubber area is skewed towards old plantation as indicated by 52 per cent of area remaining not replanted during 1980-2007. The study assumes significance because so far no spatio-temporal rubber area mapping has been carried out in the study area. The study has thrown light on the extension of rubber cultivation in relation to soil and topographic condition as well as identifying old rubber areas which will be useful for the planters and extension personnel.

Estimation of Above Ground Woody Biomass in Natural Rubber Plantation Using Remote Sensing and GIS Technique

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Forests and plantations contain around 85% of terrestrial carbon pool and thereby they play an important role in global carbon cycle. Therefore, the estimation of the above ground biomass (AGB) accumulated in trees either inside forests or plantations becomes important. In recent years, Natural Rubber (NR) plantations have become an important component in the ecosystem of northeast India. The greatest uncertainty in the role of forest and plantation in carbon sequestration potential is attributed to accurate estimation of AGB. There is constant research effort being made all over the world to refine the methodologies for estimation of AGB in various ecosystems. In the same direction, estimation of AGB in NR plantations is also relevant to understand its role in ecosystem level carbon cycling.

There are many empirical methods to estimate AGB such as field inventory and remote estimation to arrive at equation based AGB estimation. With the advancement of remote sensing and GIS technique, it becomes easy to estimate the AGB indirectly at a larger scale and resolution. In the present study, an attempt was made in NR plantation of the Taranagar research farm of Rubber Research Institute of India at Agartala using IRS P6-LISS IV satellite imageries. The Taranagar farm area was identified visually through bright red tone of NR patch and its associated land use patterns in the LISS IV satellite images of 10th April 2010. The study area was sub-set from the image tile by ERDAS-Imagine 2011 software. The reflectance values were converted to Digital number (DN) values using a prescribed formula.

The field data on primary attributes towards the AGB in tree species, the girth at breast height (gbh), was recorded in five different sample plots representing different age groups of trees in the entire study area. Each sample plot was of 100 square meter comprising of around 40 trees. The AGB was estimated using an equation previously generated in NR [Shoot weight in Kgs(w) = 0.0202 G^{2.249}, G is the girth in cm at 150cm height]. The AGB per pixel (23.5m x23.5m for LISS IV sensor) for each sample site was calculated and then correlated with corresponding DN values of NIR band of the multispectral image of the LISS IV image of the study area and linear fixed-effect model (Y=381.42x-5469.9) was developed taking the DN values as the independent variable (x) and field-AGB values (Y) as the dependant variable. The R² value for the model was 0.73. A plantation biomass

map was generated using this model with the help of Modeler module of ERDAS-Imagine 2011 software. The pixel DN value of the biomass map is indicative of AGB per pixel of the Taranagar farm. The plantation biomass map of Taranagar farm reveals that each pixel will have around 760kgs of biomass and it can be extrapolated to 225tons/ha.

Such studies using remote sensing and GIS application will always pose the greatest limitation of ground validation of the modeled results. However, this present study could overcome this limitation due to availability of field data recorded during the felling of mature trees for replanting. At the time of felling trees, it was recorded that 147 cubic meters (cum) of merchantable wood was obtained from 647 trees, which weighs around 220.5 tons (1cum fresh rubber wood weighs 1.5 tons under normal conditions). Considering the density of planting as 450 trees/ha, the total AGB is estimated to be 143.4 tons/ha. The estimate of AGB of that particular plot through remote sensing technique was found to be 245.9 tons which includes the weight of leaves and non-merchantable wood. As per the already published data, 60% of total biomass goes to trunk wood in case of NR trees under Indian climatic conditions. Therefore, merchantable wood weight estimated through remote sensing would be 147.5 tons/ha (60% of 245.9 tons), which tallies well with actual field data (143.4 tons/ha).

This study conducted to estimate the AGB in NR plantation is preliminary and is subject to further refinement. However, accuracy assessment of AGB estimation using this allometric equation was made with the limited ground data available and the accuracy (RMSE, Root Mean Square Error) was 2.36 which is well within the limit of observed values in similar studies.

SESSION 12
EXTENSION

AEC - Challenges and Opportunities for Thailand Rubber Industries

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Thailand became the world's largest producer and exporter of natural rubber (NR) since 1991. Up to present, Thailand still ranks the biggest NR producer with the total production of 3.569 million tons accounting 33.35 percent of the world total NR production. For the country earning in 2011, it is without any surprise that rubber and rubber products including rubber wood and furniture have altogether push rubber sector income to the level high of 678,943 million baht compared to 489,242 million baht in the year earlier or equivalent to the increase of 38.7 per cent. This amount of total earning from rubber sector has proved to be its high potentiality and growth for not only the wealth of 6 million people involved in the rubber industry but the security for the rubber smallholder's likelihood and welfare as well.

Taking into consideration on the ASEAN Economic Community (AEC) to be commenced in the year 2015, Thailand rubber industry has likely to get an advantage on the expansion of the single market of nearly 600 million population for its rubber products especially the auto and auto parts. Those two categories are considered as the main business consuming nearly 70 percent of natural rubber as raw material and surely with booth rubber economic with substantial growth rate. However, in case of rubber production, since the country has already accelerate its new planting area up to the saturated capacity of 18.7 million rais whereby the land cost and labor wage are of its advantage compared to the neighboring countries like Myanmar, Laos and Cambodia. It is also suggested that Thailand should take an attempt to enter into other new promising alternative area outside the country to invest in rubber plantation for NR raw material supply for the country.

The Impact of Extension Approaches for Enhancing Productivity of NR – the Indian Experience with Special Reference to Clone RRII 105

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India occupies the first position in productivity among NR producing countries in the world, and the fourth position in production and fifth position in area. This high productivity has been achieved due to the nearly 100% coverage of High Yielding Clones (HYC) on one hand and adoption of productivity enhancement agronomic measures on the other. The introduction of high yielding clone RRII 105 during 1980 has contributed significantly to the productivity enhancement from 788 kg./ha to 1935 kg/ha in 2011 in the traditional rubber growing areas of Kerala and Tamil Nadu. The present study critically analyses the crucial role of the extension-delivery system of Rubber Board in Transfer of Technology (TOT) from lab to land. The various variables of extension tools/mechanisms responsible for the adoption of HYC RRII105 and the consequent productivity enhancement were studied in detail. The growing demand to cater to over 1.19 million smallholder farmers with an average area of 0.54 ha has been a huge challenge with hardly a couple of hundred extension officers. Periodic field visits combined with strict norms of eligibility criteria on reimbursement of cultivation incentives had an important role in the successful adoption process. The impact of on-farm advisory services during field visit to units covered under Rubber Plantation Development (RPD) Scheme was found to be highly significant statistically. The impact of other extension tools like generation and supply of planting materials, group meetings, trainings, procurement and supply of critical inputs at concessional rates etc. when considered individually was found insignificant, but, the cumulative impact was found highly significant statistically while analyzing the trend. The effective extension tool of group approach through cluster development was initiated in the rubber sector with the advent of Rubber Producers' Societies (RPS) in 1985.

Participatory Rubber Extension in Tripura- Case Study of Manimalayar Rubbers (P) Ltd.

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The branch of Manimalayar Rubbers (P) Ltd., a joint venture of Rubber Producers' Societies and the Rubber Board, at Agartala has become a major player in rubber and estate input business in Tripura. The acceptance of the company as the best service provider is obvious from the remarkable quantitative improvements in rubber procurement from 28.74 MT to 2690.45 MT, annual turnover from Rs 6.63 Lakh Rs. 56.72 Crore and sale of inputs worth Rs. 1.93 Lakh to Rs. 3.17 Crore from 1997 to 2011. The market intervention by the Company could change the buyers' market to the 'seller's market' and facilitated the farmers to realize the highest farm gate price. Manimalayar Rubbers serves as a facilitator to the producers, as Rubber Board's priority has been for immature area. The company operates its own designer module of disseminating knowledge base on tapping, processing, smoke house construction, pest management, chemical stimulation etc. based on Board's scientific stipulations. Field visits are conducted for training on quality sheet making and filling of ammoniated field latex. The company is the single largest destination of graded sheet rubber in the entire state. The company also has key role in maintaining integrity and transparency in the RPS sector by providing fair deal and fair price. The intimate contact that prevails round the year also protects the societies from disintegration and ensures better performance. The company supports its stakeholders by means of credit sale, advance payment. Materialistic and financial supports are also given to the clusters of the producers at the time of commencement of tapping-processing at resource poor units. Dividends for transacting with the company are also given as marketing assistance to the RPSs. Workshops, seminars, social service activities etc. are also arranged by RPSs in the patronage of the company. Under its corporate social responsibility, Company had contributed Rs. 5 lakh to the state's Chief Minister's Relief Fund. Providing need-based service to the stakeholders in purchases, sales and after sales' services etc. the strategy. With the initiative of the Company, a new processing/manufacturing company (Tripura Latex) could be floated involving the restyled primitive and nomadic tribal 'jhumias' as shareholders representing rubber producers. Concerted efforts of the Company, Rubber Board and the stakeholders proves the success of a PPP for achieving inclusive development of remote villages in Tripura.

Remote Plantation Management (RPM) of Karimplavelil Rubber Plantations, Punalur, Kerala: A case study

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One of the major social factors limiting scientific cultivation of Natural Rubber in South India is the growing presence of absentee ownership. In all absentee ownership plantations, the inability for proper labour management is leading to escalating cost of cultivation, low productivity, low quality etc. resulting in low price realization. Having experienced severe loss from the plantation because of absenteeism, the management of Karimplavelil estate, Punalur on Narickal route in Kollam District of Kerala State, South India, introduced a novel Remote Plantation Management (RPM) model for remote supervision utilizing ICT facilities through a simple DVR system since 2007 by Sri. Biju. C. Oommen, who stays in Trivandrum, 80 km away from his plantation. The primary data collected from the Karimplavelil estate is presented as a case study with the objective to (i) familiarize a Digital Video Recording System implemented for effective Remote Plantation Management (RPM) employed on the plantation (ii) to analyse the qualitative impact of RPM (iii) invite suggestions for further improvement of RPM.

Major Components of a DVR system for Remote Viewing designed for remote management in Karimplavelil estate is a very simple system which include (i) Cameras (ii) Digital Video Recorder (DVR) (iii) Broadband Connection (Modem) (iv) Client PC with remote view software and broadband connection. The owner sitting at home gets a view of the processes done in the estate in his PC/laptop/Mobile phone. Total expenditure of the investment for Remote Supervision DVR system came to around Rs.45000/- during 2007. Though economically not quantified, the less investment on manpower for supervision for around 250 weeks and the travel cost saved amounted to around a minimum of Rs.3 lakhs, the higher price realization due to increased quality and quantity, amounting to a minimum of 20% increase from what the owner could have realized otherwise, are all gains of the RPM. The scope of RPM using advanced ICT devices for futuristic plantation management is discussed.

Rubber Clinic: A Distance Diagnostic and Information System for the Management of Pests and Diseases of Natural Rubber

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Accurate and timely diagnosis of diseases/pests and adoption of suitable control measures are essential to sustain and improve the productivity of natural rubber. Though suitable control/management strategies are available for each pest and disease, in many cases, growers find it difficult to diagnose the problem in time. A Distance Diagnostic and Information System (DDIS) – ‘Rubber Clinic’ was designed and developed at the Rubber Research Institute of India (RRII) which is an interactive portal with the web address ‘<http://clinic.rubberboard.org.in>’. It is a rapid diagnosis and identification portal of pests and diseases of natural rubber. The Clinic can be accessed round the clock and thus the response time is reduced from days or even sometimes weeks to hours compared to the traditional methods of diagnosis. Diagnosis can be made through two methods. Through ‘Self Diagnosis’ mode, farmers can compare the images/photographs of the disease symptoms along with the descriptions uploaded in the web-pages, to diagnose the problems on their own and adopt appropriate control strategies provided in the clinic. If the grower finds difficulty to diagnose the problem through ‘Self Diagnosis’ mode, digital images of the problems in the field could be uploaded on the site along with a brief description of the symptoms. Experts in RRII will in turn identify the problem and appropriate recommendations will be provided to the grower through ‘Assisted Diagnosis’ mode. Each user can create a login user account through which he can communicate/interact with experts and receive the recommendations. There is provision for online live chatting with scientists/experts from 2 to 3 pm (IST) Monday - Friday. The Rubber Clinic will periodically organize group chatting on contemporary issues such as disease outbreaks etc. which will be advertised in the Clinic web pages well in advance. Researchers can also benefit from the archived DDIS database of the Clinic as resources for research programmes. Apart from disease and pest problems, users can also ask questions on any topic related to rubber cultivation/processing which is examined by experts in the respective fields and recommendations are given through the Rubber Clinic. ‘Rubber Clinic’ is a NET/MS SQL based three-tier application using object database technologies. This was launched in January 2010 and until April 2012 there have been 16600 hits. Among the growers, 61% were able to diagnose the problems through the ‘Self Diagnosis’ mode and 39% resorted to ‘Assisted Diagnosis’. Leaf diseases were more reported than all the other diseases. The system creates a digital image library that could also be used in educational programmes and diagnosis.

P-1

Relevance of Productivity Enhancement in Rubber Plantations of Meghalaya- A Case Study

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Rubber is now cultivated in the Northeastern (NE) states of India with proper research and development support to meet the ever increasing demand of natural rubber in future. Rubber plantations in NE region experience various stress conditions like low temperature during the winter, storms, hail accompanied by high velocity wind etc. that affect the growth and yield adversely. Meghalaya state receives a fairly good rainfall. Productivity of the rubber plantations is low on account of less tapping days, inadequate chemical fertilizer application, disease control measures and lack of rainguarding, non-availability of fertilizer, chemicals and materials coupled with lack of awareness among the growers as rubber is a new plantation crop to this region. A field trial conducted in the model plantation of District Development Centre, Jenggitchakgre, Meghalaya, revealed the significance of rainguarding, yield stimulation under low frequency tapping, discriminatory fertilizer application and disease control measures for productivity enhancement in the NE region. Rubber Board may undertake the creation of awareness among the growers and supply of inputs for a certain period.

P-3

Yield Modelling and Analysing the Critical Factors of Productivity of Natural rubber (*Hevea brasiliensis*)

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A Study was conducted in the 26 small holdings in the adjacent three villages of Kottayam district which is one of the best rubber tracks in India to find out the most significant variants of productivity and to develop a suitable model for yield prediction using the variants. The plots selected were homogenous with respect to

soil,clone(RRII105),Panel tapped and rain guarding.The variables studied were Stand/ha, tapping quality,tapping days, other trees,Planting material quality, Immature and mature maintenance and girth of trees. Of these eight exogenous variables, tapping quality emerged as the most significant variable affecting productivity which needs to be given utmost attention followed by stand/ha,immature maintenance and girth of trees. Using the variables, a model was worked out and this model estimated the productivity with 6.5% error only

GROWERS' INNOVATIONS

Production of Best Quality Rubber Planting Materials the Cheerakuzhy Innovations

Jose Cheerakuzhy

Cheerakuzhy Young Bud Root Trainer Rubber Nursery
Kottappuram post, Sreekrshnapouram via
Palakkad district, Pin -679513, Kerala, India

India initially lagged behind other NR producing countries in the development and promotion of new methods of vegetative propagation. Till 1955 India was depending on imported R&D inputs and planting materials. In recent years, there have been remarkable developments in techniques aimed at producing good quality planting material which has a key role in ensuring high productivity. Cheerakuzhy Rubber Nursery, established in 1979 has played an important role, in association with Rubber Board in the development of rubber nursery industry in India.

From producing green/ brown bud stumps, polybag plants and modified young budding technique in the past, today we focus on young budding in root trainer system developed by the Rubber Research Institute of India. Initial trials to develop Young Budded Root Trainer rubber plants on large- scale failed. We faced lot of problems such as blackening of roots, growth retardation of roots, budding failure, die back after cutting back and attack of pests and termites in root medium (coco peat). After continuous observations and trials we could rectify these problems. Most of the problems were related to the lignin and tannin contents in the coir pith (coco peat). Finally, we could standardize a unique system to produce Young Bud Root Trainer plants which have several advantages compared to conventional polybag plants.

Experiment with young bud root trainer system were started in our nursery in 2007 and the technique was standardized in 2009 in collaboration with the Rubber research Institute of India. Since then, we have distributed such plants all over India in large-scale. In our experience young bud root trainer plants have shown better growth rate than brown bud polybag plants.

The initial investment to start a root trainer nursery is high due the requirement of large number of iron stands to harden the plants compared to an ordinary rubber nursery. Also, the quality of the plants can be increased, if we produce the plants under modern green house/poly house condition which is also expensive. Green houses/polyhouses are not compulsory, but to ensure better quality of planting materials they are recommended. Once the initial investment is realized, the running cost would be considerably reduced because of the reuse of Root Trainer cups and ability to run the nursery in a factory mode even on a shift basis.

As the weight of a root trainer plant is less than half a kilogram, the farmer can save much money towards planting and transporting cost. For the planting of root trainer plants, the labour requirement is very less which is very significant in an era of labour shortage. In smallholdings, the planting can be done even by kids and family members.

The main advantages of a root trainer nursery are that effective management techniques can be used. Even shift system can be adopted in a modern root trainer nursery.

To facilitate the work in the production process of root trainer plants, and as a part of farm mechanization and quality upgradation, we have attempted on modernizing the infrastructure facilities with the construction of polyhouses and also developed, and have been using some special tools and equipment in root trainer nursery which are discussed in this paper.

GI-2

Innovative Approach towards Treatment of Powdery Skim in Centrifuge Factories and Management of Replanting large area – HML experience

Santosh Kumar

Harrisons Malayalam Ltd. (RPSG GROUP), India

Latex processing Industry in India is dependent on latex collected from a large number of growers spread over large geographies over varying periods of time. The latex is stored in barrels for prolonged periods and this effects the quality of latex .The seasonality of latex also effects its qualities and many times the coagulated skim generated by processing this latex is powdery, unstable and not amenable to rolling. This is a real problem which gets accentuated in the high cropping months wherein the factories are already burdened with higher crops. Various methods are employed by processors which include stacking and storing for long periods of time, applying mechanical pressure etc but these are not are time energy consuming and cumbersome.

Team Mooply Headed by Mr Jayakrishnan ,Manager Moopy estate assisted by Mr Vijneesh and team have effectively cracked this problem by using preheating of skim before processing .This simple innovation has not only helped in reduction of backlog of unprocessed skim but improved skim recovery,reduced process losses and improved quality of processed skim.

The process is detailed in the presentation and shown in the Video enclosed.

HML has embarked on an extensive replanting programme aimed at modernising its fields .An area of around 3000 hectares has been planted in the last 5 years in the company. This has resulted in a very large area under modern clones. HML can claim to have the largest and the most extensive collection of the most modern clones released by RR11. Managing Large replantings has its own challenges .Challenges in large scale planting relate to plant preparation, ensuring uniform and accelerated growth of plants under varying conditions. This paper tries to put forward few approaches and initiatives being undertaken by the company to reach these objectives.

GI-3

Harvesting and Latex Collection: My Innovations

Joseph C.J, Chettiyil, Areacode.P.O, Malappuram Dist, Kerala, India

In mature rubber plantations under harvesting, the conventional rainguards come in contact with tapping panel and lead to loss of latex. Under high wind, the plastic sheet may even force the collection cups to tilt and spill the latex. During rainy season, loss or damage to latex is common. With the simple innovation of a Velcro strip attached to conventional rainguard and its counter part on rubber tree, along with collection of latex through specially designed device into collection bottle containing ammonia solution, one can over come these problems. The attempted devices are simple, cheap and efficient..

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- Convener : Dr. Tharian George, Jt. Director (Economics)
Co-Convener : Mr. M.N. Gopinath, Jt. RPC (Development)
Co-Convener : Dr. D. Chaudhuri, Project Co-Ordinator

2. Registration

- Convener : Dr. Kavitha K. Mydin , Jt. Director (CI)
Co-Convener : Dr. M.D. Jessy, Dy. Director (Agronomy)

3. Hall Arrangements

- Convener : Dr. Thomas Baby, Dy Director (IE)
Co-Convener : Mr. B. Biju, A.D. System
Co-Convener : Mr. M.R. Anilkumar, Instrm. Officer
Co-Convener : Mr. V. Vijayappan, Estate Officer
Co-Convener : Mr. K.N. Madhusoodanan, Scientist (RT)

4. Programme

- Convener : Dr. Jacob Mathew, Dy. Director (Pathology)
Co-Convener : Dr. Rosamma Alex , Dy. Director (RT)
Co-Convener : Mr. D. Anil Kumar, Jt.RPC, (Extension)

5. Exhibition

- Convener : Dr. Siby Varghese, Dy. Director (TC)
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7. Transport / Local Trips/ Field trip

- Convener : Dr. K.U. Thomas, Jt. Director (LHT)
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8. Posters

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