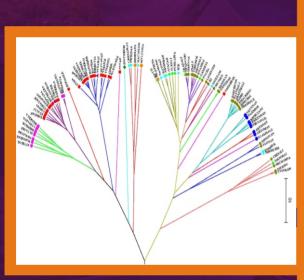
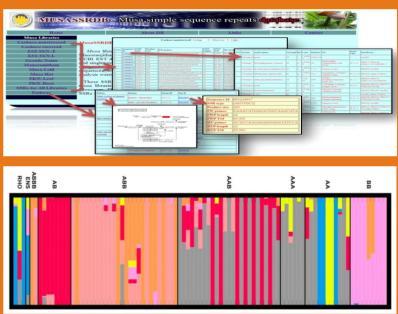


**ANNUAL REPORT 2013 - '14** वार्षिक प्रतिवेदन २०१३ **- '**१४





# NATIONAL RESEARCH CENTRE FOR BANANA

राष्ट्रीय केला अनुसंधान केंद्र

# ANNUAL REPORT 2013 - '14 वार्षिक प्रतिवेदन २०१३ - '१४

# राष्ट्रीय केला अनुसंधान केंद्र



भारतीय कृषि उनुसंधान परिषद तायनूर पोस्ठ तोगमलै रोड तिरुच्चिरापिक ६२० १०२ए तमिल नाडु

# NATIONAL RESEARCH CENTRE FOR BANANA

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# **PREFACE**

Banana and plantain forms the staple food and nutritious fruit to more than 130 countries in the tropical and subtropical world population. The National Research Centre for Banana, Tiruchirapalli completed two decades of its presence and contributed greatly in research findings to improve productivity and to mitigate the production constraints posed by major biotic and abiotic stresses. Now entering into the third decade and in this endevour, research, education and extension activities have been reoriented by the scientists with more focus on the new advanced methods utilizing the latest facilities. I take great pride in presenting the outcome of research activities in the areas of Improvement, Production and Protection in the Annual Report 2013'-14.

In the research year of 2013 - '14 several milestone research activities were carried out and completed. In Improvement, two selections *viz.*, NRCB 8 and NRCB 10 (Namwa Khom), were superior over their checks, Monthan and Karpuravalli under multilocational testing. The population structure analysis using the multi-locus genotype data obtained through DArT assay clearly identified the presence of seven distinct banana populations. Universal primer mat K was more sensitive than rbC L primer for barcoding of banana genotypes belonging to *Musa* and related genus *Ensete*. Tissue culture protocols with low cost alternatives like sago/isabgol as medium have been developed for three commercial varieties of banana *viz.*, Udhayam, Rasthali and Grand Naine.

In production section, which includes production, physiology,biochemistry and post-harvest technology, significant achievements were made which include application of 300g N and 400g  $\rm K_2O$  in the ratio of 7:2:1 N and 4:3:3  $\rm K_2O$  in seven splits during different stages advanced the fruit maturity and recorded the highest bunch weight with fruit TSS of 31.2°B. The fertilizer tailoring equations/ready reckoner developed for cv. Grand Naine were validated by which the fertilizer can be minimized based on the soil levels and also depending on the targeted yield. Soil moisture stress on Grand Naine plants at 3<sup>rd</sup> and 5<sup>th</sup> month resulted in 27-45 days delay in flowering and reduction in number of fruits and bunch weight. Priming the plants with 0.1 mM salicylic acid as foliar spray before imposing soil water deficit stress could alleviate the stress condition and improved the plant growth and yield. Poovan, Karpuravalli and Udhayam leaves had six days shelf-life comparable with wild species like Elavazhai and Phirima. Treatment of banana leaves at 20 °C for 30 min enhanced the shelf-life by five days.

In protection, identified stem weevil attractive volatile components from cvs. Poovan and Nendran. Developed consortium of biocontrol agents and botanical for the suppression of Fusarium wilt disease in the field. Endo and epiphytic fungal and bacterial bio-control agents had comparable effect with fungicides in controlling *Mycosphaerella eumusae* pathogen. Zimmu leaf extract at 50% conc. resulted in complete inhibition of *Erwinia* spp. Complete genome of a new BSV species spontaneously expressed from Virupakshi (syn: Hill Banana) has been amplified using RCA method

and cloned and a full length genome of BSV species infecting cvs. Rasthali and Poovan were cloned into pUC18 vector.

In the HRD area, three scientists of the Centre have undergone programme on leadership development. Two scientists got trainings on statistics and food processing respectively and two other scientists underwent trainings on bioinformatics and tissue culture protocol in overseas laboratories during the year. Participated in four 'Banana Festival" organized in collaboration with Confederation of Indian Industries (CII) Tamil Nadu chapter and Banana growers Federation to motivate the farmers in banana cultivation. A farmer, Shri Loganathan has created a world record in banana production by harvesting 165 tons/ha in Chinnamanur area by adopting the improved production technologies developed by NRC Banana.

I compliment and also thank Dr. B. Padmanaban, Chairman and Dr. M. Mayil Vaganan, Dr. C. Anuradha and Mr. P. Ravichamy, members of the Publication Committee for their good works in compiling, editing and bringing out this report of the Centre in time.

I express my sincere gratitude to Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR for his valuable guidance and Dr. N.K. Krishna Kumar, Dy. Director General (Horticulture Science), ICAR for his constant inspiration and encouragement.

(M.M. Mustaffa)
Director



# 2 EXECUTIVE SUMMARY

Forty eight plantlets were regenerated through embryo culture from the seeds of wild M. Acuminata collected from Andaman and Nicobar islands and seeds of Ensete superbum collected from Western Ghats of Kerala were also regenerated. NRCB selections viz., 8 and 10 (NamwaKhom), were proved to be superior over their checks, Monthan and Karpuravalli under multilocational testing. Morphotaxonomic characters ofthree introduced banana accessions viz., Pisang Berangan (AA), Guineo (AAAh) and Blue Torres Straight Island (ABB) were documented and their identity has been confirmed. Diversity analysis of 91 banana accessions including 87 Musa genotypes using Diversity array Technology markers, which clearly delineated the accessions according to their genomic groups and subgroups, status. The population structure analysis using the multi-locus genotype data obtained through DArT assay clearly identified the presence of seven distinct banana populations. Universal primer mat K was more sensitive than rbC L primer for barcoding of banana genotypes belonging to Musa and related genus Ensete. Earliest greening with establishment and highest shoot proliferation were observed with MS medium with BAP, IAA and CW as additive and with BAP/TDZ + IAA in development of variety-specific tissue culture protocol for Ney Poovan. Seven genes were identified to understand the expression of genes in banana during various developmental stages under in vitro plant regeneration. Tissue culture protocols with low cost alternatives like sago/isabgol as medium have been developed for three commercial varieties of banana viz., Udhayam, Rasthali and Grand Naine.

Twenty five differentially expressed proteins were identified by proteomic analysis with respect to somatic embryogenesis in banana. Germination of 14,292 hybrid seeds under *in vitro* condition obtained from 130

bunches involving different cross combinations showed highest germination in ABB x AA cross combination and successfully regenerated 270 hybrid plantlets were field planted. One parthenocarpic diploid progeny (No. 115) and one hybrid progeny (No. 134) were found to be more promising for further breeding against Fusarium wilt, nematodes and leaf spot disease. Seed priming protocol has been standardized for germination and regeneration of Ensete superbum seeds through embryo culture. Nine putative Rasthali mutants, eight with disease score one and one with disease score two against Fusarium wilt, were identified and were initiated in-vitro for further multiplication. Among six parents and their 10 progenies screened for reaction to Fusarium wilt, only Namaran × Pisang Lilin hybrid was symptoms free with a disease score one and followed by Sennachenkadali × Lairak hybrid with score of 1.3.

Proved by cloning study that chitinase could be one of the factors imparting resistance against nematodes by using purified Musa chitinase protein against nematode eggs which resulted in several abnormalities like vacuole formation inside the eggs, change in shape of the eggs (from oval to round) and also matured eggs failing to hatch. Musa EST-SSR database has been upgraded with assigning putative function to all the SSR containing ESTs, which will be useful for development of candidate gene markers for a specific trait. The usefulness of genome specific markers namely chitinase and SSR 4 for identification of genomic combination in the progenies were confirmed employing the progenies of Karpuravalli (ABB) x Pisang Jajee (AA). Through genome and transcriptome wide analysis of chitinase isoforms (CIs), it was found that out of three classes, the one class I CI located in chromosome 9 was more specific to nematode resistance mechanism with 10.495-fold over expression.

In the second ration crop of Udhayam, application of 300g N and 400g K<sub>2</sub>O in the



ratio of 7:2:1 N and 4:3:3 K<sub>2</sub>O in seven splits during differentstages advanced the fruit maturity with 110.6 days and recorded the highest bunch weight of 27.6kgwith fruit TSS of 31.2°B). Among different planting densities, planting of single sucker per pit at a spacing of 2.4 X 2.4m with 1736 plants/ha recorded the earliest fruit maturity with 112.3 days. In the second ratoon crop of Poovan, application of 20 kg FYM, 0.9 kg neem cake, 2.0 kg vermicompost and 0.9kg groundnut cake recorded the highest bunch weight of 15.9 kg with 12.1 hands and 188.5 fingers/bunch and better fruit quality with highest TSS (26.4°B), higher pulp: peel ratio (5.46) and the lowest acidity (0.35%) were also recorded in the treatment. The fertilizer tailoring equations/ ready reckoner developed for cv. Grand Naine were validated in Pazhur and Sirugambur villages of Tiruchirapalli district, Tamil Nadu. Based on the initial soil NPK contents of both places, the NPK requirements (in g per plant) were worked out for different yield targets from 75 to 150 tons per hectare. The fertilizer tailoring equations holds good up to the target 105 and 120 t/ha Pazhur and Sirugambur respectively, if the actual yield is considered while it was suitable up to the target of 135t/ ha, if the return per unit investment due to fertilizer is the concern. The nutrient dynamics studies under different regimes recommended dose of fertilizers for Ney Poovan and Rasthali bananas in various developmental stages have been taken during the year.

Imposition of soil moisture stress on Grand Naine plants at 3<sup>rd</sup> and 5<sup>th</sup> month resulted in 27-45 days delay in flowering and bunch malformation, reduction in number of fruits and bunch weight, which could be alleviated by priming the plants with 0.1mM salicylic acid as foliar spray before imposing soil water deficit stress. Soil moisture stressed (75-80% available soil moisture) Grand Naine plants produced 8.9% more ABA than irrigated while the stress tolerant banana cultivars like Saba, Karpuravalli, Ney Poovan and Jwaribale

exhibited higher antioxidative enzymes activity. Priming of Grand Naine plants with 20 mM glycine betaine prior to imposing soil moisture stress sustained photosynthesis, stomatal conductance and transpiration by recording 5 to 13-fold more over unprimed stress imposed plants. Phenotyping of banana genotypes for salt stress tolerance indicated Saba and Karpuravalli recorded tolerance to NaCl stress by recording less reduction in photosynthesis (< 34%), stomatal conductance (25%) and transpiration (45.68%) under moderate 50 mM NaCl stress. Priming of banana plants with beta amino butyric acid than salicylic acid and subsequent imposition of salt stress with 50 mm NaCl was more effective in alleviating NaCl stress.

Forty three differentially expressed proteins have been identified and their biological functions annotated vis-à-vis salt (NaCl) stress tolerance and based on the annotated proteins, a generic model for transduction of signals in the cells in response to NaCl stress in bananas constructed showing their involvement of salt overlay sensitive (SOS), mitogen activated protein (MAP) kinase cascades, H<sub>2</sub>O<sub>2</sub> signalling, Na ions extrusion, lignification of vascular bundles and root growth retardation pathways of plant. Around 30 differentially expressed proteins with more than two-fold changes due to the root lesion nematode infection in roots of Anaikomban and Nendran have been identified and their biological functions annotated and these proteins were mainly involved in transcription of PR genes, cell wall remodelling and secondary metabolism. Methods for identification of chlorophylls and their catabolites from the banana peel tissues by liquid chromatography and 2-DE proteomic analysis of banana peel and pulp tissues with around 650 proteins reproducibility have been standardised. Preliminary study indicated the effectiveness of 1-methylcylcopropene in enhancing the shelf-life of preclimacteric Cavendish (Grand Naine) and Indian (Poovan) bananas. Poovan, Karpuravalli and Udhayam leaves had six days



shelf-life comparable with wild species like Elavazhai and Phirima and a survey revealed Poovan is the main cultivar grown for leaf purpose in Tiruchirappalli district. Treatment of banana leaves at 20 °C for 30 min enhanced the shelf-life by five days. Making of flower baskets from course fibre of banana pseudostem sheath and preparation of banana figs in different shapes have been standardised.

Stem weevi1 attractive volatile components belong to alkene, acetate, aldehyde, ketone alcohol and fatty acids from the leaf sheath extracts of cvs. Poovan and Nendran were identified. Protease, chitinase and lipase (cuticle degrading enzymes) from 25 isolates of Beauveria bassiana and 39 isolates of Metarhizium anisopliae were analyzed to develop an effective fungal consortium for corm weevil management. Developed a consortium of biocontrol agents and botanical for the suppression of Fusarium wilt disease in pre-infected Rasthali banana plants. Endo and epiphytic fungal and bacterial bio-control agents had comparable effect with that of standard control of Propiconazole (0.1%) + mineral oil (1%) in controlling Mycosphaerella eumusae pathogen. Zimmu leaf extract at 50% conc. resulted in complete inhibition of Erwinia spp.

Complete genome of a new BSV species spontaneously expressed from Virupakshi (syn: Hill Banana) has been amplified using RCA method and cloned and a full length genome of BSV species infecting cvs. Rasthali and Poovan were cloned into pUC18 vector. DAC-ELISA using recombinant antiserum of viral associated protein and IC-PCR were standardized for detection of episomal BSMYV. Thirty proteins with two-fold differences in intensity were identified in the BSV infected Poovan and these proteins were found to be involved in defense, signal transduction, cell structure and function, photosynthesis and energy, plant growth, protein designation/storage and transcription/ translation. Thirty nine differentially expressed proteins were identified in BBTV-infected and healthy Hill banana leaf tissues with important proteins being Mitogen-Activated Protein Kinase, Calmodulin motif containing protein, ABC transporter, Cinnamyl alcohol dehydrogenase. Validation of select differentially expressed proteins by semi quantitaive-PCR corroborated with the protein results.

## **Transfer of Technology**

In 2013-14, four radio talks in All India Radio, Tiruchirapalli and seventy five lectures were delivered in different aspects of banana cultivation including postharvest management by Scientist. Participated/ organized eight exhibitions at regional/ national levels. Ten offcampus trainings on production and postharvest technology of banana were conducted. Seventeen research papers, ten book chapters, seventeen popular articles and seven technical bulletins/ extension folders were published by scientists of the Centre. Fifteen research papers/ posters were presented by the Scientists during the National and International Conferences/ Symposia/ Seminars/ Workshop/ Meetings. Totally 75 Conferences/ Seminars/ Symposia/ Workshops/ Meetings were attended by the Scientists at Regional/ National/ International.

As many as 16 VIPs and about 4800 banana farmers, Agricultural & Horticultural officers, self help groups and students visited and were appraised the activities of the Centre on their visit. Technologies on post harvest handling, packing and storage were transferred to four entrepreneurs. Mother cultures of tissue culture banana plants received from DBT recognised tissue culture production units were tested for banana viral diseases and fidelity.

## **Linkages and Collaboration**

The Centre has developed good linkages with international institutes *viz.*, Bioversity



International, France and QUT, Australia. Collaborated with different National Research Institutions for different activities viz., NBPGR, New Delhi; BARC and CIRCOT, Mumbai; IIHR, Bangalore; Coffee Board, Bengaluru; NHB, DST and DBT New Delhi; NCL Pune, all SAUs and Bharathidasan University, Tiruchirapalli, Tamil Nadu. Tissue culture industries involved in banana mass propagation, farmers, exporters, banana federations, State Horticulture and Agriculture departments and self-help groups are linked with the Centre for various research and developmental activities. NRCB also coordinates with AICRP (Tropical Fruits) Centres working on banana. The Centre has collaborated CTCRI. with Thiruvananthapuram and CPRI, Shimla for development of extruded product by blending banana, cassava and potato flours and with CIAE, Regional Station, Coimbatore for Developing banana central core stem slicer, juice extractor and Developing postharvest mechanization package for banana central core.

## **HRD** and Education

Three scientists of the Centre have undergone Management Development Programme on leadership development. Two scientists got trainings on statistics and food processing respectively and two other scientists underwent trainings on bioinformatics and tissue culture protocol standardisation and somaclonal variant selection in overseas laboratories during the year. A total of 17 research papers (10 national and 7 international) have been published by the scientists of Centre and another 13 research papers abstracts were presented in various national and international seminars/ conferences/ symposia, etc. Fifteen M. Sc. and B. Tech. (Biochemistry, Biotechnology and Microbiology) students of various colleges/ universities of TamilNadu were guided by the scientists for project/thesis works on different aspects of banana.

#### **Revenue Generation**

A total of Rs. 53.20 lakhs was generated as revenue during the year 2013-'14 by the Centre.



# 3 INTRODUCTION

The National Research Centre for Banana was established on 21st August 1993 at Tiruchirapalli, Tamil Nadu by the ICAR, New Delhi with an aim to increase the production and productivity of bananas and plantains through mission mode basic and strategic research approaches. This Centre is located at 11.50° N latitude and 74.50° E longitude, 90 m above MSL and receives 800 mm rain annually. The climate is warm and humid and the average minimum and maximum temperature are 25 and 35 °C respectively. The Centre has a research farm with 36 ha land and infrastructure facilities like library, meeting and exhibition halls, green house, quarantine lab and net house facilities.

The Centre works on four major thrust areas of research *viz.*, Improvement, Production, Postharvest Management and Protection. It has well-equipped research laboratories for tissue culture, biotechnology, soil science, nutrient management, physiology and biochemistry, entomology, nematology, fungal, bacterial, viral pathology and postharvest technology.

The NRC for banana has been identified as the National Repository for banana. It has a field gene bank consisting of 360 banana germplasm accessions from the North -Eastern region, Western Ghats and Andaman and Nicobar Islands and also exotic banana accessions from International Transit Centre (ITC), Belgium through NBPGR, New Delhi. The Centre has completed seven in-house research projects and 15 are in progress. In addition to Centre's in-house projects, 16 externally funded projects funded by DBT, NHB and INIBAP were completed. The Perspective Plan and 'Vision 2050' document on the research priorities and also reports by QRT and RAC were published. The Centre conducts two meetings of Institute Research Council to review the on-going research projects and also monitor the progress made on the of RAC and QRT recommendations. The vision of the Centre is to increase the production and productivity of bananas and plantains to meet the growing need in India. Research Advisory committee under the Chairmanship of Dr. G.L. Kaul monitor the research activities of the Centre and reviewed the progress.

## The mandates of the Centre

- ◆ To undertake the basic and strategic research for developing technologies to enhance the productivity and utilization of banana
- ◆ To develop improved cultivars through traditional and biotechnological methods and conserve the diversity
- ◆ To serve as national repository of germplasm and information related to banana and plantain and also to disseminate the knowledge to improve the production and productivity
- ◆ To provide leadership and coordinate the network research for generating location specific variety technology and for solving specific constraints on banana and plantain production
- ◆ To collaborate with relevant national and international agencies in achieving the above objectives

#### **Salient Achievements**

## **Improvement**

A field gene bank with 360 core accessions have been assembled from both indigenous and exotic sources, maintained in the Centre's field gene bank repository at Tiruchirapalli. Among the collections, 92 accessions are highly resistant and 25 are resistant to Sigatoka leaf spot disease. NRCB released variety Udhayam, which belongs to Pisang Awak sub group, is a high yielder. Embryogenic cell suspensions (ECS) for five



different commercial varieties viz., Rasthali, Nendran, Ney Poovan, Robusta and Grand Naine have been developed and regeneration protocol from ECS for Nendran and Rasthali has been standardized. NRCB has developed a DNA Bank for Musa germplasm with 225 accessions. A farmers' friendly method of mass production of banana planting material by 'Macro propagation' technique to meet the need of small and marginal farmers in multiplication of disease free traditional varieties of banana locally. A short duration and high yielding Red Banana (AAA) and a ash coated Monthan (ABB) high yielder were identified from farmers' field and Formosona (a high yielding Cavendish banana resistant to Fusarium wilt (race-4) from Taiwan Banana Research Institute (TBRI) Taiwan were added. NRCB selection - 08 proved its superiority in respect to high yield and less crop duration in all the AICRP Centers tested. Cultivar Saba based progeny (No. 183) was found promising in terms of fruit qualities like firm pulp, good cooking quality and suitability to chips making. Namwa khom (Pisang Awak, ABB) a dwarf exotic introduction was found promising and was suitable for high density planting. NRCB selection viz., 10 (Namwa Khom), was found to be superior over their check, Karpuravalli under two locational testing. The population structure analysis using the multi-locus genotype data obtained through DArT assay clearly identified the presence of seven distinct banana populations.

Universal primer 'mat K' was more sensitive than 'rbC L' primer for barcoding of banana genotypes belonging to *Musa* and related genus *Ensete*. Cloning study indicated chitinase could be one of the factors imparting resistance against nematodes. *Musa* EST-SSR database has been upgraded with assigning putative function to all the SSR containing ESTs.

## **Production**

Poovan plants supplied with 20 litre water/day/plant with 75% N (150 g/plant) as

fertigation increased the yield by 20% and recorded maximum net profit with a benefit ratio of 1.96. A combination of distillery sludge 2.5 kg + 1 kg vermicompost + 1 kg neem cake + 2.5 kg poultry manure per plant recorded the maximum growth and yield parameters in Rasthali and Karpuravalli bananas. Application of gypsum 2 kg/ plant + FYM 15 kg/ plant + 120% recommended K in saline sodic soil increased the yield by 51 % over control in Nendran and Rasthali bananas. Paired row planting system with 4,500 - 5,200 plants/ha, increased the productivity and fruit quality with 75% of recommended fertilizers dose as fertigation in Robusta, Grand Naine and Red Banana. In the second ration crop, application of 20 kg FYM, 0.9 kg Neem cake, 2.0 kg vermicompost and 0.9 kg groundnut cake recorded the highest bunch weight of 15.9 kg with more number of hands (12.1) and fingers/bunch (188.5). Application of 20 kg FYM + 0.9 kg Neem cake + 2.0 kg vermicompost + 0.9 kg groundnut cake as well as other organic treatments significantly improved the porosity (45.5 %) as well as particle density (44.3%) as against a porosity of 40.2% with 100% inorganic that was on par with 125% inorganic fertilization.

Application of 15 kg rice husk ash + 25g VAM + 80% recommended NPK gave an additional profit of Rs. 32,500/ha. Soil application of Fe and B along with foliar spray of Zn under high pH soil condition, increased the bunch weight of Ney Poovan banana by 43.5% over control, resulting in an additional net profit of Rs. 38,000/ha. In cv. Ney Poovan under high pH soil condition indicated a application of sulphur 20g/plant reduced the soil pH from 8.6 to 7.8 in the rhizosphere soil and increased the plant growth (up to 12.5%) and yield parameters (up to 14%) significantly over the control. In the first ration crop of cv. Udhayam, application of recommended dose of NK fertilizers (RDF) (300:400g N&K plant 1) in ratio of 7:2:1 N and 4:3:3 K,O at vegetative, flowering and bunch development



stages recorded the earliest fruit maturity. Fertilizer adjustment equations for Poovan and Karpuravalli bananas were developed by following the Soil. The fertilizer adjustment equations developed at NRCB were validated at different banana growing areas in Tamil Nadu, West Bengal, Kerala and Karnataka through AICRP Centres. Impact of source reduction (leaf pruning) on flowering and fruit yield studies indicated in cvs. Poovan, Nev Poovan and Karpuravalli, reduction in source area increased more photosynthesis as a compensation mechanism. Eight drought tolerant accessions were identified based on leaf water retention capacity. The leaf longevity was more in Robusta than Nendran, Rasthali and Ney Poovan bananas. Saba, Karpuravalli and Ney Poovan have been identified as tolerant cultivars to salt stress. Drought tolerant Saba and Karpuravalli cultivars maintained higher (>200) K/Na ratio in leaf (lamina and midrib) than susceptible Ney Poovan, Nendran and Robusta cultivars (4.52 to 17.56) at shooting and fruit maturity. Imposition of soil moisture stress on Grand Naine plants at 3<sup>rd</sup> and 5<sup>th</sup> month resulted in 27-45 days delay in flowering and bunch malformation, reduction in number of fruits and bunch weight, which could be alleviated by priming the plants with 0.1mM salicylic acid as foliar spray before imposing soil water deficit stress. Priming of Grand Naine plants with 20 mM glycine betaine prior to imposing soil moisture stress sustained photosynthesis, stomatal conductance and transpiration by recording 5 to 13-fold more over unprimed stress imposed plants. Phenotyping of banana genotypes for salt stress tolerance indicated Saba and Karpuravalli recorded tolerance to NaCl stress by recording less reduction in photosynthesis. Imposition of soil moisture stress on Grand Naine plants at 3<sup>rd</sup> and 5<sup>th</sup> month resulted in 27-45 days delay in flowering and bunch malformation, reduction in number of fruits and bunch weight, which could be alleviated by priming the plants with 0.1mM salicylic acid as foliar spray before imposing

soil water deficit stress. Priming of Grand Naine plants with 20 mM glycine betaine prior to imposing soil moisture stress sustained photosynthesis, stomatal conductance and transpiration by recording 5 to 13-fold more over unprimed stress imposed plants. Phenotyping of banana genotypes for salt stress tolerance indicated Saba and Karpuravalli recorded tolerance to NaCl stress by recording less reduction in photosynthesis. A study on biochemical mechanism of resistance of bananas to Pratylenchus coffeae generally indicated that the activity of phenol oxidizing enzymes, stress related enzymes and the level of total phenols, lignin and tannins were higher even at 30 days in resistant than in susceptible cultivars. The induction of these above said enzymes were more in the nematode challenge inoculated plants than in the unchallenged plants. The phenol-ammonium acetate protocol yielded the highest protein concentration than other protocols tested for protein extraction from root tissues for proteomics study. Proteomics analysis of P. coffeae infected bananas indicated 60 differentially regulated proteins and biological functions of 19 proteins were annotated. Around 30 differentially expressed proteins with more than two-fold changes due to the root lesion nematode infection in roots of Anaikomban and Nendran have been identified.

## **Postharvest Technology**

Pre-packaging in 400 gauge LDPE bags, low temperature storage, use of ethylene absorbents and pre-storage treatments have resulted in extension of shelf life up to 3 months in Robusta, Grand Naine, Rasthali and Ney Poovan bananas. Several value added products like flower *thokku*, peel *thokku*, fruit pickle, fig, biscuits, jam, ready to serve beverages and functional foods like *chapathi*, bread and health drink have been developed. Banana and Jamun juice blend was the best among the blends made with other fruit juices rich in antioxidants. A recipe for banana flower



based ready to make soup has been standardized. A new product, banana pulp based 'Sip-up' was prepared without pasteurization. The product can be stored up to 15 days at 0°. The post-harvest storage treatments to extend the shelf-life of Red Banana, the hands of 80% mature fruits packed in KMnO<sub>4</sub> impregnated polybag and stored at 13.5°C with RH 95% increased the shelf-life of fruits up to 145 days with better pre-harvest quality and organoleptic characters. Banana pulp based ready-to-drink beverage of cv. Robusta, the dilution of pulp juice at 1:4 revealed high TSS and moderate acidity with acceptance (7.41 Hedonic scale), which was on par with banana RTS beverage. Poovan, Karpuravalli and Udhayam leaves had six days shelf-life comparable with wild species like Elavazhai and Phirima and a survey revealed Treatment of banana leaves at 20 °C for 30 min enhanced the shelf-life by five days.

#### **Protection**

production technique Paecilomyces lilacinus, (an egg parasite of rootknot nematode) using banana petiole and pseudostem has been developed. Maximum reduction of 90% nematode population with 50% increase in plant growth and bunch weight was recorded in plants treated with *P. linacinus* + P. flourescens + Neem cake + Marigold as intercrop. The combined application of Bacillus subtilis and B. cereus in cv. Ney Poovan resulted in 60% increase in plant growth with 90% reduction of root lesion nematode populations than individual treatments. The screening of Musa germplasms against major nematodes resulted in the identification of 5 diploids and 8 triploids resistant to both root-lesion and root-knot nematodes. Swabbing 0.06% Chlorpyrifos 20 EC on the pseudostem of 1.2 m height during 5 to 8 months completely controlled BSW incidence. Treating suckers with Monocrotophos 36 EC (14 ml/liter) followed by soil application of Carbofuran 3G at the rate of 30 g per plant at 4<sup>th</sup> and 7<sup>th</sup> month after planting found effective against corm weevil. Pseudostem split trap swabbed with chaffy grain formulation of Beauueria bassiana trapped the stem and corm weevils better than traps swabbed with maize flour formulation. Of the 37 semiochemicals tested, maximum olfactory response by corm weevil was recorded to bisabol-ol, which found effective for banana corm weevil monitoring under field conditions. Field evaluation conducted using funnel trap in a weevil (O. longicollis) endemic areas of Theni and Dindigul districts of Tamil Nadu showed that the weevil attraction was maximum (80%) in the treatment of Semiochemical No. 1 + host plant volatile extract obtained from cv. Nendran. The GC/ MS profile of leaf sheath of cv. Karpuravalli indicated 23 volatile components having RT values ranging from 2.621 to 21.372. The volatile components include Alkanes-10, Alcohol-1, Aldehyde-1, Ketone-1, Phenol-1, Fatty acids-6. Stem weevil attractive volatile components from cvs. Poovan and Nendran were identified. Cross reaction between race 1 and race 2 of Foc has been observed in VCG analysis. Diversity of Foc isolates has been studied using RAPD, PCR-RFLP analysis of IGS region and the sequence analysis of rDNA-ITS region. Use of Carbendazim (0.1 %) for dipping the suckers before planting followed by soil drenching in root zone (@ 1-2 lit at 2,4 & 6 month after planting) and stem injection(@2ml at 2,4 &6 MAP) effectively controlled the Fusarium wilt disease in Ney Poovan cultivar under field conditions. The evaluation of different VCGs in cv. Grand Naine under in vitro screening indicated VCG 0124 only caused wilt disease in India. The combined application of rhizospheric and endophytic fungal antagonists along with or without fungicide application under field condition significantly increased the bunch weight (up to 74.8%) and suppressed the Fusarium wilt disease. The biopriming of banana plants with the combined application of Pseudomonas putida + Alpinia, Pseudomonas putida + Hibiscus sp., Pseudomonas putida + Zimmu, Bacillus sp. + Zimmu combinations



resulted in complete control (100 %) and significantly increased the plant growth parameters. Developed a consortium of biocontrol agents and botanical for the suppression of Fusarium wilt disease in preinfected Rasthali banana plants. Microscopic examination and molecular analysis of 96 isolates of Mycospharella spp. isolated from different cultivars of banana grown in different regions of India revealed the presence of M. eumusae indicating that the leaf spot in India is caused by M. eumusae. Endo and epiphytic fungal and bacterial bio-control agents had comparable effect with that of standard control of Propiconazole (0.1%) + mineral oil (1%) in controlling Mycosphaerella eumusae pathogen. Zimmu leaf extract at 50% conc. resulted in complete inhibition of *Erwinia* spp.

Soil application of increased dose of fertilizer (150% of RDF) in cv. Poovan has compensated the yield loss due to BBrMV. Polyclonal antiserum to BBTV was produced and ELISA technique has been standardized for detection. NA probe and PCR based diagnostic techniques have been developed for all the banana viruses. A multiplex PCR technique has been developed for detecting three banana viruses simultaneously. Complete genome of BSV infecting Poovan has been cloned and sequenced. Promoter sequences from BBTV were cloned and sequenced. Duplex PCR for all the four viruses CMV, BBrMV, BBTV and BSV has been standardized. Real Time-PCR technique for simultaneous detection of banana viruses was standardized. Rolling circle amplification (RCA) approach which uses bacteriophage Phi29 DNA polymerase has been standardized to detect episomal virus of BSMysV in Poovan and BBTV in Hill banana. Primers and probe have been designed for rep gene of BBTV and assessed the quantity of its transcripts in latent and severely infected plant susing real time-PCR. A Loop mediated Isothermal Amplification (LAMP) based highly sensitive method for non-symptomatic samples was

standardized for the detection of BBTV. Proteomics analysis of BBTV infected Hill banana indicated 40 differentially regulated proteins and biological functions of 32 proteins were annotated. Complete genome of a new BSV species spontaneously expressed from Virupakshi (syn: Hill Banana) has been amplified using RCA method and cloned and a full length genome of BSV species infecting cvs. Rasthali and Poovan were cloned into pUC18 vector. Thirty proteins with two-fold differences in intensity were identified in the BSV infected Poovan and these proteins were found to be involved in defence, signal transduction, cell structure and function, photosynthesis and energy, plant growth, protein designation/storage and transcription/ translation. Thirty nine differentially expressed proteins were identified in BBTV-infected and healthy Hill banana leaf tissues.

## Transfer of Technology

In this year, four radio talks through All India Radio and seventy five lectures were delivered in different aspects of banana cultivation including postharvest management. Totally 75 seminars/ conferences/ symposia/ workshops/ meetings were attended by the Scientists at regional/ national/ international. Participated/ organized eight exhibitions at regional/ national levels. Ten off-campus trainings on production and postharvest technology of banana were conducted.

As many as 16 VIPs and about 4800 banana farmers, Agricultural & Horticultural officers, self help groups and students visited. Technologies on post harvest handling, packing and storage were transferred to four entrepreneurs. Mother cultures of tissue cultuture banana plants received from DBT recognised tissue culture production units were tested for banana viral diseases and fidelity.

# Linkages and Collaboration

The Centre has developed good linkages with the following international institutes *viz.*,



Bioversity International, France and QUT, Australia. Collaborated with different national research institutions for different activities viz., NBPGR, New Delhi; BARC and CIRCOT, Mumbai; IIHR, Bangalore; Coffee Board, Bengaluru; NHB, DST and DBT New Delhi; NCL Pune, all SAUs and Bharthidasan University, Tiruchirapalli, Tamil Nadu. NRCB also co-ordinates with AICRP (Tropical Fruits) Centres working on banana and ICAR institutes like CTCRI, CPRI and CIAE.

## **Revenue Generation**

A total of Rs. 53.20 lakhs was realized as revenue generation by the Centre during the year 2013-'14.

Budget details (Revised Estimate) for the year 2013-14

		Rs	. In Lakhs
S.No.	Head of account	PLAN	NON-PLAN
1	Estt. Charges	0.00	346.63
2	Overtime Allowance	0.00	0.04
3	Travelling Allowance	5.23	4.00
4	Contingencies	123.59	86.69
5	HRD	1.00	0.48
6	Equipments	70.66	24.00
7	Furniture & Fixtures	0.00	0.00
8	Library & Journals	8.51	0.00
9	Vehicle	1.29	10.00
10	Pension & Retirement Benefits	0.00	27.88
	Total	210.28	499.72



# 4 RESEARCH ACHIEVEMENTS

#### 4.1 CROP IMPROVEMENT

## 4.1.1 Genetic Resource Management

## **Collection**

Survey was conducted in southern districts of Tamil Nadu and Trivandrum areas of Kerala and collected 17 banana accessions which include landraces and farmers' varieties. Four accessions of Grand Naine from Israel were introduced from TERI Biotech as rooted plantlets. Short duration and high yielding clones of Red banana (AAA) and Ash Monthan (ABB) were collected from Bhavani area of Tamil Nadu and established in the field genebank. Wild M. acuminata from Baramurah hills, Behula and Batheeshwar from Nagicherra, Tripura were collected and established in the field genebank. Formosona, a Foc race 4 resistant Cavendish variety was introduced as proliferating cultures from TBRI Taiwan. Wild M. acuminata. ssp. seeds were collected from Andaman and Nicobar Islands from which 48 plantlets have been developed through embryo culture. Seeds of Ensete superbum from Western Ghats of Kerala were regenerated into plants through embryo culture and planted in satellite genebank at Agali.

#### Characterization

During this period, three introduced banana accessions viz. Pisang Berangan (AA),





Fig. 1: Bunch & ripened hand of Guineo (AAAh)

Guineo (AAAh) and Blue Torres Straight Island (ABB) were morpho-taxonomically characterized using IPGRI Banana descriptor and their identity were confirmed. Guineo produced a bunch of 11.5 kg with 5 hands and 12 fruits per hand. Fruits are very bold weighing 150-200g and tasted similar to Cavendish fruits (Fig. 1).

## Diversity analysis using DArT markers

Diversity array technology (DArT) is a high throughput microarray hybridization based technique using 4499 DArT markers with a call rate of 99.6%. Analyses were performed using Dendra UPGMA software. Tree was constructed using weighted Neighbor – Joining (NJ) algoritham. Diversity among 91 banana accessions which consisted of one member from Ensete, three Rhodochlamys and 87 Musa genotypes (9 BB, 11 AA wild, 6 AAA, 8 AB, 25 AAB, 27 ABB and 2 ABBB) were studied. DArT markers clearly delineated the accessions according to genomic groups and subgroups and more interestingly, grouping of accessions based on their geographical origin. Ensete superbum exhibited closeness to selected members of wild M. acuminata and Rhodochlamys sections. Boothibale remained unique accession among Pisang Awaks (ABB), Peyan and Cuba among ABB's, Pagar Banana among AAB's. Mutants and synonyms have also been recognized (Fig.2).

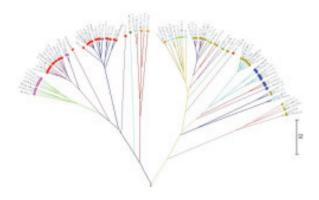


Fig. 2: Circular tree representing 91 Musa accessions



# Population STRUCTURE analysis of *Musa* mini core collection

The multi-locus genotype data obtained through DArT assay was used to analyze the Musa population structure STRUCTURE software. The program STRUCTURE (version 2.3.4) was used to estimate the number of hypothetical subpopulations (K) and to estimate the membership probability of each genotype to the sub-populations using Bayesian clustering mode. Hypothesis of one to ten subpopulations was set and a Markov Chain Monte Carlo (MCMC) of 9,999 burn-in phases followed by 9,999 iterations was run independently 8 times using an admixture

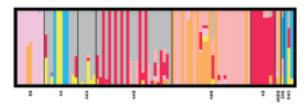


Fig. 3: Diversity structure of 91 *Musa* accessions based on 4499 loci derived from DArT assay generated by Structure program

model. The Delta K peaked at a K-value (population number) of seven (7). It clearly identified the presence of 7 distinct populations, assigned the individuals to distinct populations, possible migrants and admixed individuals in the core collection maintained at NRCB (Fig.3).

# Diversity among BB accessions using ISSR markers

Phylogenetic relationship and diversity of wild BB clones were analyzed using ISSR markers. From the eleven ISSR markers that produced discrete, repeatable amplicons, a total of 162 alleles were identified with a mean of 14.7 alleles per primer based upon the presence (1) or absence (0) of alleles. The average PIC observed in the present study was 0.28 (Table 1). The NTSYS software derived dendrogram for 24 accessions resulted in the formation of two major clusters with all BBs' in one cluster and AA and bispecific clones in another cluster (Fig.4). The clustering of the test clones is based on their geographical origin indicating that the interaction between the genetic makeup and geographical origin is stronger than

Table 1. ISSR diversity of M.balbisiana clones

S.No	Primer	TNB	NPB	Polymorphism %	PIC
1.	807	24	24	100.00	0.27
2.	808	14	14	100.00	0.33
3.	811	16	16	100.00	0.30
4.	834	10	9	90.00	0.20
5.	840	14	1	78.57	0.24
6.	818	14	13	92.85	0.26
7.	841	17	17	100.00	0.31
8.	812	19	19	100.00	0.25
9.	868	10	9	90.00	0.30
10.	842	8	8	100.00	0.37
11.	836	16	16	100.00	0.26
Total		162	156	1051.42	3.09
Average		14.7	14.18	95.58	0.28



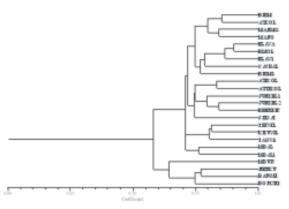


Fig. 4: Dendrogram showing the phylogenetic relationship among the *M.balbisiana* clones

that of phenotypic appearance. However, the present study has confirmed the uniqueness and robustness of the *M. balbisiana* core set with no synonyms. The rich diversity within *M. balbisiana* could be an asset for the identification of genes conferring resistance to various biotic and abiotic stresses and also to study their interaction with BSV.

#### Evaluation of elite clones

Elite clones of Neypoovan were collected from clonal plantations in Bhavani based on

their high yield ranging from 15-18 kgs. About 45 clones were planted in the farmer's field for evaluation purpose. Recommended package of practices were adopted and regular observations on vegetative and reproductive parameters were recorded (Table 2).

Eight clones with nos. 1, 4, 8, 22, 26, 27, 29 and 39 yielded higher than other clones and control. The crop duration of all these high yielding clones was less than 305 days except clone 39 (310 days).

# Studies on gene specific primers in differentiating Musa spp. of indigenous origin

DNA barcoding was attempted using universal primers namely rbC L and mat K currently recommended by the Consortium for the Barcode of Life (CBOL) exclusively for plant kingdom. The discriminating potential of the chloroplast genes was initially tested in seven germplasm accessions belonging to various sections of *Musa* and related genus *Ensete*. The test accessions were Matti and Sannachenkadali (AA) (indigenous land races), Calcutta 4 (AA), Attikol (BB), *M. laterita*, *M.* 

Table 2. Growth and yield parameters recorded in elite Clones of cv. Neypoovan

Plant No.	Plant height (cm)	Girth (cm)	No. of leaves (cm)	Petiole length (cm)		Days taken for shooting	Bunch Weight (kg)		No. of fingers	Crop Duration (days)
1	330	69	8	73	16200	194	16	11	220	301
4	310	64	7	67	15454	194	20	13	253	301
8	330	62	7	69	15525	192	13.5	10	204	303
22	335	72	8	68	14960	196	21	13	261	303
26	330	68	7	70	14080	194	18	13	240	301
27	330	68	7	73	14080	196	18.5	14	239	303
29	330	69	7	72	14520	197	18	13	235	304
39	300	70	7	73	15142	203	18.5	14	247	310
Control	320	66	8	70	13660	260	14.8	12	216	380
T-value	1.36	0.99	0.25	0.86	2.40	24.01	2.42	0.69	2.04	6.56
Level of significan	NS nce	NS	NS	NS	Signifi cant	Signifi cant	Signifi cant	NS	Signifi cant	Signifi cant



ornata and Ensete superbum. The minimum identity of 27% was observed between M. laterita and M. ornata in mat K as against 97% in rbC L. Similarly the identity between Matti and Sannachenkadali was only 31% in mat K as against 89% in rbC L indicating that mat K could be a better gene which could be used for differentiating spp. as well as varieties of the same species (Table 3 and 4). The data developed in the form of sequences have been submitted in the NCBI database with the accession numbers assigned from KJ506055 - KJ506068. These sequences despite their use in varietal identification, they are further considered valuable for the detection of genetic structure of Musa germplasm and identification of diverse parental combinations to develop segregating progenies with maximum genetic variability for selection and improvement.

## Evaluation of germplasm

ITC plants Akpakpak (ITC 0217, AAB) and Orishele (ITC 1325, AAB) have been regenerated and taken for primary hardening while secondary hardened ITC plants Pisang Madu (AA), Pisang Berangan (AAA), Pisang Rajah - South Johnstone (AAB), Khai Thong Runag (ITC 0662) have been field planted for evaluation.

## **Evaluation of NRCB selections**

## NRCB selection - 10

Multilocation testing of Selection-10 belonging to Pisang Awak subgroup at NRCB, Trichy and BRS, Kannara along with local check AICRP (TF) was studied and the results are presented in Table 5.

Table 3. mat K identity matrix

Species name	Sannachenkada	li <i>E.superbum</i>	M.laterita	M.ornata	Matti	Attikol	Calcutta 4
Sannachenkada	li ID	0.387	0.952	0.267	0.306	0.301	0.898
E.superbum	0.387	ID	0.389	0.289	0.285	0.340	0.347
M.laterita	0.952	0.389	ID	0.265	0.299	0.300	0.906
M.ornata	0.267	0.289	0.265	ID	0.347	0.323	0.272
Matti	0.306	0.285	0.299	0.347	ID	0.337	0.293
Attikol	0.301	0.340	0.300	0.323	0.337	ID	0.299
Calcutta 4	0.898	0.347	0.906	0.272	0.293	0.299	ID

Table 4. rbC L identity matrix

Species name	Sannachenkadali	Attikol	Calcutta 4	E.superbum	M.laterita	M.ornata	Matti
Sannachenkada	ali ID	0.299	0.240	0.953	0.295	0.296	0.892
Attikol	0.299	ID	0.294	0.301	0.937	0.932	0.361
Calcutta 4	0.240	0.294	ID	0.222	0.285	0.295	0.236
E.superbum	0.953	0.301	0.222	ID	0.287	0.285	0.888
M.laterita	0.295	0.937	0.285	0.287	ID	0.967	0.356
M.ornata	0.296	0.932	0.295	0.285	0.967	ID	0.354
Matti	0.892	0.361	0.236	0.888	0.356	0.354	ID



Table 5. Evaluation of NRCB selection - 10 with local Karpuravalli (ABB) at different locations.

		Tric	chy	Kaı	Kannara			
Traits	Sel -10	Local Karpuravalli		Level of significance				Level of significance
Plant height (cm)	233.2	416	179.2	**	276.2	393.6	22.51	**
Girth (cm)	93.2	85	6.8	**	80.8	73.4	6.04	**
Bunch weight (kg)	17.4	15.8	2.92	**	17	18.4	2.74	*
No of hands	12.2	14.6	7.58	**	12.2	12	0.27	NS
No of fruits/hand	17.6	16.4	3.46	**	18.2	16.2	7.07	**
Duration	364.4	486.1	43.95	**	325.8	386.8	3.08	**
TSS of Pulp (Brix)	26	26.5	0.28	NS	25	25.5	0.38	NS
Acidity	0.28	0.27	1.25	**	0.3	0.31	1.24	**
Fruit weight (g)	117.5	112.2	6.28	**	110.4	108.9	4.6	**

Evaluation of ratoon crop of NRCB sel-10 (Namwa Khom) with Karpuravalli as local check indicated its superiority for dwarf stature and earliness. Preliminary evaluation has confirmed that Selection -10 has the potential

to produce 18-20 kg bunch with 12-13 hands and 16 fruits per hand (Fig. 5) and harvested in an average of 367 days. The average plant height of 2.30-2.60m promised its utility under high density planting and earliness confirms its adaption to annual cropping system. A new



evaluation block with 50 Fig. 5: Bunch of plants each has been raised NRCB selection 10 under two spacings for confirmatory trials.

## Evaluation of NRCB selection -8

NRCB selection -8 has proved its superiority over local Monthan (ABB) in terms of both bunch weight and duration under multilocation trials at NRCB, Trichy, TNAU, Coimbatore and BRS, Kannara. Bunch yield across the locations was non-significant ranging

between 26.2 to 27.6 kgs. Similarly all other yield parameters were non-significant. The crop duration significantly varied from 388.4 to 439.6 days (Table 6).

# **4.1.2** Study on different plant regeneration systems

# Development of direct regeneration protocols for commercial banana varieties

Fifty immature male flower buds of three varieties namely Rasthali, Udhayam and Ney Poovan were initiated *in vitro* in seven different treatments for the standardization of direct regeneration protocol. In case of cv. Udhayam, BAP was required for greening and TDZ for formation of meristem clumps and vice versa in case of cv. Rasthali while both BAP and TDZ were required for greening of floral meristems in cv. Neypoovan indicating that the hormonal requirement is variety and stage dependent (Fig. 6).

# Development of variety specific tissue culture protocol for cv. Ney Poovan

Attempts were made to determine the appropriate type and optimum level of



Table 6. Evaluation of NRCB selction-8 with Local Monthan in three different places

Traits		Tri	ichy			Loca Coimb				Kan	nara	
	Local	Sel-8	t-test	Level of significance	Local	Sel-8	t-test	Level of significance	Local	Sel-8	t-test	Level of signifi cance
Plant height (cm)	367	371.4	1.91	**	369.2	365.4	1.55	NS	346.6	275.6	8.15	**
Girth (cm)	74.2	86	24.08	**	85.8	77.6	10.5	**	58.2	79.8	40.82	**
Bunch weight (kg)	19.8	27	14.6	**	21.2	26.2	7.2	**	15.4	27.6	26.01	**
No of hands	27	9.2	5.87	**	7.6	12.6	10.6	**	6.8	12.6	9.17	**
No of fruits/ hand	12.8	13.6	1.78	NS	12.4	13	1.5	NS	6.8	12.6	1.63	NS
Duration	442.6	385.4	26.9	**	432	439.6	10.5	**	343.6	388.4	56.89	**
TSS of Pulp (Brix)	24	20	4.47	**	24.3	22.6	11.89	**	24	22.7	2.21	NS
Acidity	0.42	0.27	15	**	0.34	0.24	4.2	**	0.35	0.28	4.85	**
Fruit weight (g)	292	270	19.2	**	206.8	115.2	92.5	**	217.2	187.5	22.21	**

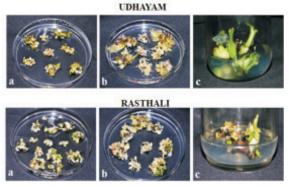


Fig. 6: Stages of growth and development of male flower bud regeneration into shoots

- a-Initial establishment; b-Meristem clump formation;
- c-Shoot regeneration

cytokinin required to achieve maximum shoot proliferation in cv. Ney Poovan. Three types of cytokinin namely BAP, KIN and TDZ in different combinations and at different concentrations with additives like coconut water, adenine sulphate and bavistin were also supplemented in the medium. Among the various combinations of growth regulators tried for the initial establishment and shoot proliferation, earlier greening establishment (5.0 days) was observed in MS medium containing BAP, IAA and CW while shoot proliferation was maximum (6.1 - 6.5 shoots) in MS medium supplemented with TDZ + IAA. The response of cv. Ney Poovan for both initial establishment and shoot proliferation was poor in low cost medium as against the normal MS medium fortified with BAP and IAA.

# Development of macropropagation protocol for cv. Udhayam

Among the two substrates namely sand and saw dust and two growth regulators namely



BA and Ethrel tried for macropropagation of Udhayam, saw dust + ethrel (300 ppm) was found better as it resulted in an average of 2.2 buds within a time span of 34.0 days as against 1.5 buds in 49.4 days in the same saw dust substrate with BA.

In another trial of macropropagation, the substrate used was only vermicompost and the corms were kept tightly closed in fertilizer bags after decortication and treated with growth regulators. Instead of Ethrel, growth regulators were tried in various combinations on two different explants namely whole corm and corm bits. Initial results indicated that three months old whole corm showed better response when NAA and BA were combined in terms of per cent response and number of buds sprouted. During the course of one month period, only one primary bud was observed in case of bits irrespective of the growth regulator treatment. The experiment is in progress.

# Expression profiling of genes during developmental stages of banana under various *in-vitro* and *in-vivo* regeneration systems of cvs. Rasthali and Nendran

Basic research was done to study the complex genetic pathways involved in various aspects of plant morphogenesis, including somatic embryogenesis and direct regeneration pathways leading to *in-vitro* plant regeneration.

A number of genes are being identified that can be expressed transgenically to enhance or even to initiate plant regeneration. Preliminary studies have been conducted to understand the expression of genes in banana during developmental stages under various *in vitro* and *in-vivo* regeneration systems like shoot tip culture, macropropagation, male flower culture, embryogenic cell suspension culture of two contrasting cvs. Rasthali and Nendran (Table 7).

Seven genes were selected based on literature survey which are involved in developmental stages, of which four are involved in somatic embryogenesis namely LEC1 (Leafy Cotyledon1), LEC2 (Leafy Cotyledon2), SHR (Short Root) and WUS (Wuschel). While others in direct organogenesis viz., ARF (Auxin Response Factor), CUC (Cup Shaped Cotyledon) and ESR1 (Enhancer of Shoot Regeneration). With respect to macropropagation, genes which were found to be upregulated in all stages irrespective of cultivars are CUC, LEC2, SHR, WUS, ARF and irregular expression was observed from two genes are ESR1 and LEC1.

In shoot tip culture method, ARF, ESR1 and SHR genes were upregulated in cv. Rasthali and others were down regulated while in cv. Nendran, all genes were upregulated, except ESR1 and WUS. In cv. Rasthali, WUS and

Table 7. Genes used for expression profiling during developmental stages of banana under various in- vitro and *in vivo* regeneration systems of cvs. Rasthali and Nendran

	Genes	used for this study	Function
1	WUS	Wuschel	Vegetative to embryogenic transition
2	LEC1	Leafy cotyledon1	Initiate ectopic somatic embryogenesis
3	CUC	Cup shaped cotyledon	Required for meristem development
4	SHR	Short root	Establishes ground tissue via assymetric cell division
5	LEC2	Leafy cotyledon2	Initiate ectopic somatic embryogenesis
6	ARF	Auxin response factor	Regulate auxin signaling
7	ESR1	Enhancer of shoot regeneration	Enhance shoot regeneration

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ARF which are two genes were found to be up-regulated in all stages; ESR1 was found to be up-regulated in all stages except S1 (initial explant) and others showed their expression in a zigzag model. But in cv. Nendran, CUC and LEC1 was upregulated in all stages except S1; WUS expressed positively at S2 (shoot induction) and S3 (shoot multiplication); others were negatively regulated in male flower culture methods.

In case of embryogenic cell suspension, cv. Rasthali CUC was found to be upregulated in all stages except S4 (single shoot); SHR and LEC1 showed its positive expression in S1, S2 but contrasting expression was observed by WUS gene, others expressed negatively in all stages. Contrasting results were observed in cv. Nendran with respect to all genes were upregulated in stages S2 and S3 except WUS. But WUS showed negative regulation in all stages.

# Quantification of plant growth hormones at different developmental stages of banana cvs. Rasthali and Nendran

A preliminary study has been carried out to know the status of phytohormones (Zeatin, Gibberellic acid, Indole acetic acid and Abscisic acid) at various developmental stages of banana under *in-vitro* and *in-vivo* conditions. HPLC analysis was carried out to study the hormonal profile of various explants at different developmental stages for four different regeneration methods (macropropagation, shoot tip culture, somatic embryogenesis, male flower bud culture) for cys.Rasthali and Nendran.

Zeatin was found to be high in all four stages of *in-vitro* development of cv. Rasthali corroborating its ease of *in-vitro* multiplication over cv. Nendran, except for different stages of proliferation through macropropagation.

Elevated level of gibberellic acid was exhibited by cv. Rasthali at all stages of invitro proliferation, like direct regeneration through male flower buds and shoot tip culture while embryogenic calli and macropropgation stages recorded low levels gibberellic acid suggesting its possible negative role. Similar trend was also observed for IAA (Fig. 7).

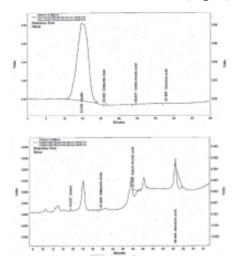


Fig. 7: Hormone concentrations a) friable embryogenic and b) non- embryogenic calli of cv. Rasthali

Abscisic acid concentration was found to be below detection level in all samples of cv. Rasthali except friable embryogenic stage but in cv. Nendran the same was found to be in very low concentration (0.005 mg/g).

# Use of low cost alternatives in banana tissue culture

Low cost tissue culture protocols have been developed for three commercial varieties of banana viz., Udhayam, Rasthali and Grand Naine. Irrespective of the treatment, reverse osmosis water and table sugar were used as the water and carbon sources respectively. The study included six different treatments i.e., Sago alone, Isabgol alone, Sago + Agar, Isabgol + Agar, Sago + Isabgol including control where double distilled water, sucrose and agar were used for media preparation. The analysis of economics of low cost medium used indicated that the return on investment is 105.5% in low cost medium as against 86% in normal medium. They were tested for their genetic fidelity using five ISSR markers. Results



indicated that the genetic stability was high in cvs. Grand Naine and Udhayam as against cv. Rasthali where the dissimilarity varied from 0 – 17.5% and the maximum changes were observed in plants derived from sago and isabgol + agar (Fig. 8).

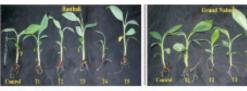




Fig. 8: Effect of low cost media on plant growth and development in three different commercial varieties Control-Agar; T1-Sago; T2-Isubgol; T3-Sago + Agar; T4-Isubgol + Agar; T5-Sago+Isubgol

# 4.1.3 Proteomic analysis of somatic embryogenesis in banana (*Musa* spp.)

Embryogenic calli was obtained from 8th to 16th position of immature floral hands of male flower buds (Rasthali and Grand Naine cv.) after 3-6 months of initiation on callus induction medium. But the explant of cv. Udhayam failed to produce any embryogenic calli. The embryogenic and non embryogenic calli were harvested on the 6th month from the single plate for protein extraction (Phenol – ammonium acetate method). Iso Electric Focusing (IEF) condition was standardized for the callus protein by having 4500V as a maximum volt. Two Dimensional gel (2D) electrophoresis was carried out for the embryogenic and the non embryogenic calli of Grand Naine cultivar (Fig. 9a&b) and found that more than 25 protein spots were qualitatively differentially expressed (Table 8).

Table 8. List of proteins associated with somatic embryogenesis in cv. Grand Naine identified by 2- dimensional gel electrophoresis combined with MALDI-TOF analysis

Spot No.		Name of the Protein	Accession No.	EC/ NEC	Database	Score	Query coverge
1.	10280	Ribosomal Protein	gi   302749045	EC	NCBInr	82/75	27%
2.	7600	Probable protein phosphatase	P2C33_ORYSJ	EC	SwissProt	51/48	38%
3.	7450	Cytochrome P450	gi   460387155	EC	NCBInr	78/75	28%
4.	10278	Nudix hydrolase	gi 359484618	EC	NCBInr	80/75	50%
5.	6737	Alpha subunit of RNA Polymerase	gi 484759827	EC	NCBInr	77/75	30%
6.	6829	2S sulfur-rich seed storage protein	2SS1_BEREX	EC	SwissProt	61/58	51%
7.	10311	TIR-NBS-LRR class disease resistance protein	gi   15242338	EC	NCBInr	79/75	20%
8.	10312	MYB Transcripion Factor	gi 51572282	EC	NCBInr	78/75	53%
9.	10281	DEAD-box ATP-dependent RNA helicase	RH28_ORYSJ	EC	SwissProt	63/58	18%
10.	5248	Heat shock 70 kDa protein	gi 357503377	EC	NCBInr	77/75	26%
11.	5195	25.3 kDa heat shock protein	HS25P_ARATH	EC	SwissProt	61/58	49%
12.	5894	26S protease regulatory subunit 8 homolog	PRS8B_ARATH	EC	SwissProt	60/54	38%

19



Spot No.	Name of the Protein	Accession No.	EC/ NEC	Database	Score	Query coverge
13. 10288	Probable 5-epi-aristolochene synthase 4	5EAS4_NICAT	EC	SwissProt	71/58	19%
14. 3430	adenylate isopentenyltrans ferase	gi 384081618	EC	NCBInr	78/75	21%
15. 3598	serine/threonine protein kinase Stpk-v2	gi 402170021	EC	NCBInr	79/75	36%
16. 10283	glyceraldehyde-3-phosphate dehydrogenase 3	gi 573922461	EC	NCBInr	80/75	35%
17. 10284	hypothetical protein VITISV_009566	gi   147860071	EC	NCBInr	80/75	32%
18. 2121	Probable indole-3-pyruvate monooxygenase YUCCA3	YUC3_ARATH	EC	SwissProt	63/58	24%
19. 2106	Eukaryotic translation initiation factor 3 subunit Fisoform	gi 508714799	EC	NCBInr	80/75	39%
20. 6273	26S proteasome non-ATPase regulatory subunit 8 homolog A	PSD8A_ ARATH	NEC	SwissProt	60/58	36%
21. 2408	3-ketoacyl-CoA thiolase 5, peroxisomal	THIK5_ ARATH	NEC	SwissProt	64/58	31%
22. 6342	Calcium-binding protein KIC	KIC_ARATH	NEC	SwissProt	60/58	60%
23. 10302	Probable pectinesterase/ pectinesterase inhibitor	PME59_ ARATH	NEC	SwissProt	64/58	11%
24. 3587	glyceraldehyde-3-phosphate dehydrogenase 3	gi 573922461	EC	NCBInr	98/75	37%
25. 3517	Hydroxyphenylpyruvate reductase	HPPR_PLESU	EC	SwissProt	64/58	38%

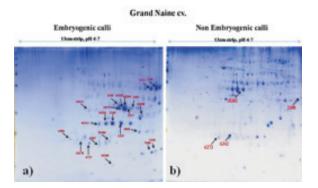


Fig. 9: a) & b) Protein gel indicating the protein spots identified by MALDI-TOF-Mass spectroscopy

# 4.1.4 Improvement of bananas through conventional breeding

A total of 419 bunches were crossed during last year and the total number of hybrid seeds were obtained in 130 bunches involving different cross combinations of AA X AA, AAB x AA, ABB X AA and ABBB (Open Pollinated) were 14,292 (Sunken-10445). All the hybrid seeds were initiated under in-vitro condition. Highest germination was recorded in ABB x AA cross combination. The total



Table. 9. Seed set and germination details of seeds developed at NRCB and Agali

			Total seeds		% of Embryo Present		Seed nated	% of Embryo germinated	
S.No	Female parent	NRCB	Agali	NRCB	Agali	NRCB	Agali	NRCB	Agali
1	Kothia	419	2976	27.39	33.8	1.7	23.28	6.25	68.8
2	Bankela	*	4433	*	30.99	*	14.25	*	45.99
3	Karpuravalli	*	973	*	36.6	*	22.19	*	60.5
4	Udhayam	448	36	43.9	30.55	1.6	0	3.9	0
5	Bangrier	456	*	19.95	*	1.9	*	9.8	*
6	Saba	703	*	21.08	*	2.1	*	10.41	*
	Tota1	2026	8416	28.08	32.98	1.82	19.90	14.23	58.43

seeds obtained both from NRCB, Trichy and SBI, Agali along with their germination details are provided in Table 9.

Successfully germinated and regenerated 270 hybrid plantlets of different cross combinations of ABB x AA, AA x AA and ABBB (open pollinated) were planted in the field. Rest is in the process of different stages such as subculture, primary and secondary hardening, etc.

## Supply of germplasm

- ◆ 120 of banana suckers from 12 accessions including NRCB Seletion-8 (Bangrier) were supplied to other AICRP centers, Horticulture College for women, TNAU, Trichy.
- ◆ 45 of banana suckers from 10 accessions including NRCB Selection-9 were supplied to TAFE, Chennai for MLT.
- ◆ 20 suckers of Saba, Rasthali, Udhayam, Namarai and Thellachakkarakeli were supplied to a progressive farmer Mr. Vinoth, Trivandrum.
- ◆ Two suckers each of Attikol and Athiakol were supplied to NBPGR, New Delhi.

# Improvement of Pisang Awak group of bananas (3X x 2X)

## **Udhayam**

A total of 29 bunches were crossed, out of which 23 bunches set seeds (79.3%). Total 484 seeds were collected, of which 206 seeds had embryos (42.4%). Percent germination with respect to total embryos was just 3.6%, while with respect to total number of seeds was very low with 1.5%.

## Karpuravalli

A total of 10 bunches were crossed, out of which 7 bunches set seeds (70.0%). Total 973 seeds were collected 362 seeds had embryos (36.6%). Percent germination with respect to total embryos was 60.5%, while with respect to total number of seeds it was low with 22.1%.

## Improvement of Cooking Bananas (ABB)

## Kothia

A total of 111 bunches were crossed, out of which 35 bunches set seeds (31.5%). Total 3395 seeds were obtained of which only 968 seeds had embryos (28.5%). Percent germination with respect to total embryos was 55.3% while with respect to total number of seeds was only 15.8%.



## **Bangrier**

A total of 52 bunches were crossed, out of which 20 bunches set seeds (38.4%). Total 463 seeds were collected, 93 seeds had embryos (19.9%). Percent germination with respect to total embryos was 9.8%, while with respect to total number of seeds was very low with 1.9%.

#### Bankela

A total of 26 bunches were crossed, out of which 15 bunches set seeds (57.6%). Total 4445 seeds were collected, 1374 seeds had embryos (30.9%). Percent germination with respect to total embryos was 45.9%, while with respect to total number of seeds was low with 14.2%.

#### Saba

A total of 66 bunches were crossed, out of which 27 bunches set seeds (40.9%). Total 703 seeds were collected, 148 seeds had embryos (21.0%). Percent germination with respect to total embryos was 10.4%, while with respect to total number of seeds was low with 2.1%.

## Development of improved diploids

During 2012-13, 271 progenies (AAB X AA, ABB x AA and AA x AA) were field planted at Agali, out of which 260 survived. This includes 7(2x), 206 (3X), 55(4X) and 1 (*E. superbum*) progenies which were confirmed through flow cytometry.

## Progeny No 115

Developed as an improved parthenocarpic diploid (AA) from the cross of ABB x AA (Karpuravalli x Pisang Jajee (Seeded)). Plant is slender with 1.7 m height with erect leaves and is free from leaf spot diseases throughout the year. Bunch is small with 5-6 hands of fruits with 2.5 kg weight, small fruits, tasty pulp with good aroma (Fig. 10). Exhibits its suitability for use as male parent with fertile pollen (>80). Random screening of roots



Fig. 10: Progeny No. 115 - a) Habitat of plant; b) Bunch; c). Male Bud; d). Fruit hand; e). L.S of fruit indicated that they are free from nematodes. This has potential for further advancement after pot screening for nematodes and Fusarium

## Progeny No 134

wilt.

Hybrid progeny of Anai Komban x Matti, was found to be free from all leaf spot diseases during the last three years. This is classified as diploid (AA) through morphotaxonomic score and confirmed through flow



Fig. 11: Habitat of plant & bunch of Progeny No. 134



cytometry. Plant is 2.3- 2.5 m height with erect leaves. Bunch is small with 4-5 hands and fruits are long (13- 15 cm) and parthenocarpic in nature (Fig. 11). Male flowers exhibit pollen fertility with >70% pollen germination up to 20<sup>th</sup> node. This exhibits potential for use as male parent in banana breeding programme.

Seed set tetraploid Bhat Manohar (ABBB) - Open pollinated bunch of tetraploid Bhat Manohar exhibited seed set to an extent of 668 seeds, out of which 400 seeds were fully developed and 268 were floating seeds. First set of 200 good seeds have been initiated under *in-vitro*, of which only 138 seeds had well developed embryos and rest had no embryo but with rich endosperm.

Establishment of E.superbum seedlings - First time, a protocol was standardized for seed priming using growth hormones and embryo culture for Ensete superbum and 12 seedlings were generated which were established at Agali.

For the first time, seed priming protocol has been standardized for germination and regeneration of *Ensete superbum* seeds through embryo culture. Seed priming in GA3 for 10 days on a shaking platform enhanced embryo regeneration by more than 50%. Presently, 38 plantlets have been regenerated through embryo culture and are being maintained *invitro* for shifting to primary hardening.

# Collection and compilation of basic information on varietal compatibility, factors affecting hybrid seed production and regeneration

Elucidation of causes for non seed setting in banana was tried using two contrast female parents (Sterile- Kozhikodu and fertile – Saba and Alpon) through histological and histochemical studies. Study indicated possible structural and hormonal reasons for poor or no seed setting. This study gave an insight into pollen ontology, time course pollen tube

movement and fertilization processes. Pollen entry through micropyle and subsequent fertilization is recorded 28 hrs after pollination.

Pollen stigma interaction studies – Apart from structural difference and ploidy incompatibility, other factors (external and internal) also play a major role in the success of seed setting. Hormonal imbalance, specific genes and their pathways, triggering initial pollen tube germination and development are some of the factors which have proven their role in many vegetatively propagated monocots. Hence preliminary studies were under taken on histology, enzyme analysis, proteomics and gene expression during compatible and non compatible crosses.

#### Hormonal basis

Studied the hormonal status (IAA, GA3, Zeatin and ABA) during pollen-pistil interaction. The pollinated samples were collected in different time intervals *viz.*1 hour, 6 hrs, 12 hrs, 24 hrs and control (without pollinations) and analyzed by HPLC method. Level of zeatin (Cytokinin) increased in Chengdawt and Rasthali and but it decreased in Saba. Pollen germinations were low in Chengdwat and Rasthali, which may due to high level of cytokinin. Several fold increased level of IAA was noticed in Saba but it was low in Chengdwat and Rasthali. Levels of GA<sub>3</sub> did not change and one fold increase of ABA was noticed in Saba.

# Evaluation of parents and progenies for their reaction to *Foc* pathogen

Ten progenies and six parents were evaluated for their reaction to Foc pathogen under green house condition. The observation on internal vascular discoloration in the corm tissues indicated that Namarai × Pisang lilin hybrid alone was free from the Fusarium wilt symptom (score 1) which was followed by Senna chenkadali × Lairawk hybrid which recorded the minimum wilt score of 1.3.



# Studies on relationship between stomatal density and ploidy in Musa

Studied the ploidy level based on stomatal density using different genomic combination of Musa accessions like AA, BB, AAA, AAB, ABB, AAAA, AABB and ABBB. It was observed that stomatal size was more in tetraploid genomes (except AAAB) followed by triploid and diploid genomes (Table 10). In general, it was observed that there is no correlation between ploidy level and stomatal characters like, length, breadth and size of the stomata as well as stomatal density. The relationship exhibited that stomatal density is highly influenced by the B genome contribution.

#### Nendran through **Improvement** of hybridization

Nendran (AAB) was used as female parent and crosses were made with three diploid male parents namely Pisang Lilin, cv. Rose and Calcutta-4. Based on the number of seed set, it was concluded that P. Lilin and cv. Rose are the best compatible male parents for Nendran, whereas Calcutta 4 was considered as the least compatible parent as the seed set was very low. Embryo germination was carried out in three different media and found that MS + Caesin

Table 10. Stomatal characters in various ploidy/genome of banana

Genome	Stomatal density/mm2	Length (µm)	Width (µm)	Size (µm²)
AA	71.3262	25.44	28.48	727.34
AB	73.10	25.21	27.4	689.52
BB	20.33	26.21	33.88	888.86
AAA	64.66	31.26	34.39	1075.99
AAB	64.21	31.14	33.28	1036.4
ABB	10.912	32.36	34.96	1130.14
AAAB	37.77	28.6	39.2	1121.12
AABB	33.33	33.6	43.4	1458.2
ABBB	25.48	35.1	46.5	1632.15

Table 11. Best media composition for Nendran based crosses

Medium	Media composition	% of Em	ıbryo germinated
		Nendran x Pisang Lilin	Nendran x Cultivar Rose
A	MS	15.39	15.01
В	MS+ Caesin hydrolysate + Morel Vitamin+ Biotin and Inositol	32.30*	3400*
С	B medium with half strength MS	0	0
	CD for medium composition at 5% level		5.12
	CD for cross combination at 5% level		6.27
	CD for interaction of medium composition	on	8.87



hydrolysate + Morel Vitamin + Biotin and Inositol was the best combination (32.2%) followed by normal MS medium (15.3%) and B medium with half strength MS (0%) (Table 11). Nendran based crossed seeds needs excess quantity of vitamins and nutrients for better embryo germination.

# 4.1.5 Improvement of Rasthali through induced mutagenesis

The shoot tips of cv. Rasthali (350 nos.) were treated with three different mutagens namely ethyl methane sulfonate (EMS), diethyl sulphate (DES) and sodium azide (NaN<sub>3</sub>) and were screened *in vitro* with fusaric acid and culture filtrate. The shoots survived are in various stages of micropropagation (Table 12).

# Screening of mutated Rasthali plants against Fusarium wilt

Mutated plantlets of cv. Rasthali were screened under pot conditions in three different batches, two using sand maize meal and one using conidial suspension of *FOC* (VCG 0124/5). Internal disease scoring was done after five

Table 12. Status of mutated cultures of cv. Rasthali (AAB)

Treatments	No. of plantlets under in vitro screening	Root ing	Primary harden ing	
ST - EMS - 2%	564	36	-	
PB - EMS - 0.6%	112	27	20	
ST - DES - 10mM	217	43	13	
PB- DES – 4mM	69	20		
ST - NaN3 - 0.02%	6 321	54	12	
PB - NaN3 - 0.01%	<sub>0</sub> 73	39	-	
Total	1356	219	50	

ST – Shoot tips; PB – Proliferating buds

and three months after inoculation, respectively (Table 13).

Out of 150 mutated plants of cv. Rasthali screened for Fusarium wilt resistance using conidial suspension, seven plants scored one with no symptom of Fusarium wilt incidence and one mutant scored 2. Out of 221 mutated Rasthali plants screened using sand maize

Table 13. Pot screening of mutated Rasthali against Fusarium wilt using sand maize meal and conidial suspension of *FOC* 

S.No.	Treatment details	Plants screened (Nos.)						
			1	2	3	4	5	6
	Conidial sus	pension						
1.	EMS ST	150	7	1	0	0	0	142
	Sand maize	meal						
1.	EMS ST	14	1	0	0	1	0	12
2.	EMS PB	34	0	0	0	0	11	23
3.	NaN3 ST	63	0	0	0	0	0	63
4.	NaN3 PB	13	0	0	0	1	2	10
5.	DES ST	36	0	0	0	0	2	34
6.	DES PB	45	0	0	0	0	0	45
7.	Control	16	0	0	0	0	0	16
	Total	221	1	0	0	2	15	203

ST - Shoot tips; PB - Proliferating buds



meal, only one mutant scored one with no symptom of Fusarium wilt. Therefore nine putative mutants including the one with disease score 2 have been initiated under *in vitro* for further multiplication.

# 4.1.6 Identification and characterization of nematode resistant genes in banana

# Cloning of *Musa* chitinase and testing their efficiency against nematode

Full length Musa chitinase gene was cloned into His.Tag - pET 21b (+) vector and transformed into BL21 strain for protein expression (Fig 12a). Optimum temperature conditions and time interval for protein expression in bacterial culture was standardized. The maximum expression was obtained after 6 hours of 1mM IPTG induction at 20°C (Fig 12b). Then chitinase protein was purified using Ni-NTA affinity chromatography and confirmed based on their molecular weight at 29 kDa (Fig. 12c). Purified chitinase protein was tested against the nematode egg. The efficiency of purified Musa chitinase was tested against nematode eggs which indicated none of the chitinase treated eggs hatched. It was also observed that all the treated eggs exhibited one or the other abnormalities like vacuole formation inside the eggs, change in shape of the eggs (from oval to round), matured eggs failing to hatch (Fig 12d) etc. This suggested that chitinase could be one of the factors imparting resistance against nematodes.

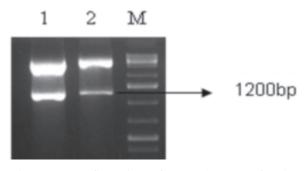


Fig. 12a: Confirmation of pET21b vector for the presence of *Musa* chitinase gene

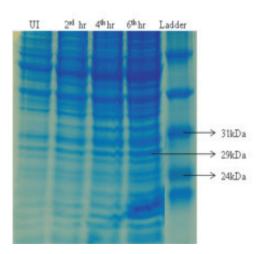


Fig. 12b: Expression of chitinase protein from BL21 crude culture filtrate

- Uninduced
- 2, 3, 4 2hrs, 4hrs, 6hrs after IPTG induction
- 5 Protein Molecular Weight Marker

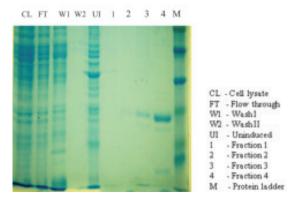


Fig. 12c: Purified chitinase protein using Ni-NTA affinity chromatography

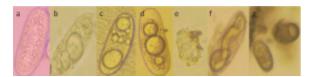


Fig. 12d: Efficiency of *Musa* chitinase against nematode eggs: a. Control egg b-g) Chitinase treated eggs

# Up-gradation of *Musa* EST-SSR database with their putative function

BLAST2GO analysis was carried out for all the SSR containing ESTs and their putative functions were linked with the respective contigs as well as singletons. Pathways were



also derived from BLAST2GO analysis. The SSR containing ESTs belongs to each metabolic pathway were separated and a link has been made to detect the SSR containing ESTs as well as their SSR primer details (Fig. 13). A total of 100 metabolic pathways were predicted and an average of 5 enzymes were involved in each pathway. It was observed that maximum (30) and minimum (1) number of SSR containing sequences were present in pentose phosphate pathway and alpha lineoleic acid pathway, respectively. This will be useful to the scientific community who are involved in development of candidate gene markers for a specific trait.



Fig. 13: *Musa* EST-SSR data base with molecular function and their metabolic pathways

# Assigning the presence of A or B genome in the progenies using genome specific markers.

The genome specific markers namely chitinase and SSR 4 were tested against the progenies of Karpuravalli (ABB) x Pisang Jajee (AA). Out of ten, five progenies showed only A specific band and the remaining had both A and B specific bands (Fig. 14). The morphotaxonomic characterization and flowcytometry analysis suggested that the progenies which is showing A specific bands are grouped under diploid with AA genome, whereas other progenies had A and B specific bands are grouped under tetraploid with ABBB genome. Thus it was again reconfirmed that these markers could be used for the prediction of

the presence of A and/or B genome in the progenies also.

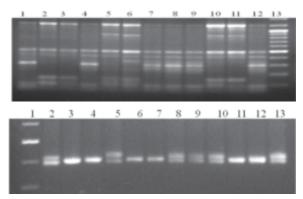


Fig. 14: Detection of A and/or B genome in Karpuravalli x Pisang Jajee progenies using genome specific markers a) chitinase marker b) SSR 4 marker

Lane 1-Karpuravalli (Femele parent ABB); Lane 2-Pisang Jajee (Male Parent AA); Lane 3-Progeny 130 (AA); Lane 4-Progeny 6 (AB); Lane 5-Progeny 111 (AA); Lane 6-Progeny 110 (AA); Lane 7-Progeny 7 (AB); Lane 8-Progeny 5 (AB); Lane 9-Progeny 3 (AB); Lane 10-Progeny 106 (AA); Lane 11-Progeny 114 (AA); Lane 12-Progeny 4 (AB) and Lane 13-100 bp ladder

# Genome and transcriptome wide analysis of chitinase

Genome wide analysis of chitinase genes of Musa acuminate ssp. malaccensis (D'hont et al. 2012) was carried out and the genes were characterized based on conserved domain. A total of 26 chitinase sequences were obtained from Musa AA genome. Of which, only 14 sequences had complete ORF whereas the remaining 12 sequences were incomplete, partial CDs. Transcriptome data obtained from nematode challenged and unchallenged resistant and susceptible cultivar were subjected to digital gene expression analysis (DGE). Based on the results, it was observed that number of over expressed chitinase isoforms (CIs) under nematode challenged condition were more in susceptible genotype (15 CIs) compared to the resistant. It was also observed that only two CIs namely GSMUA\_Achr9G02370\_001 and GSMUA \_Achr9G16770\_001 (class I and II) located in chromosome 9 were significantly over expressed with 10.495 and 6.75 fold respectively in resistant when compared with



susceptible cultivar under nematode challenged condition. Interestingly it was observed that class I CIs located in chromosome 1 and 3 (GSMUA\_Achr3G06330\_001) were significantly lower expressed with - 5.61(GSMUA\_Achr1G07320\_001) and -2.55 fold change respectively in nematode challenged resistant cultivar (Fig. 15). This result suggested that out of three class I CIs, the one located in chromosome 9 was more specific to nematode resistant mechanism.

#### Log(Fold-Change) Expression Profiles

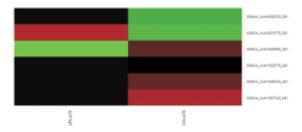


Fig. 15: Heat map representing the expression profile (> 2 fold with significant p value 0.05) of chitinase isoforms in resistant and susceptible cultivars challenged with *P. coffeae* 

UR - Unchallenged Resistant

CR - Challenged Resistant

CS - Challenged Susceptible



## 4.2 CROP PRODUCTION

# 4.2.1 Standardization of agro-techniques for banana production and productivity

# Standardization of stage wise nutrient requirement in Udhayam banana

In the second ration crop, application of 300g N and 400g K<sub>2</sub>O in the ratio of 7:2:1 N and 4:3:3 K<sub>2</sub>O in seven splits during vegetative stage (210g N & 160g K), flowering stage (60g N & 120g K) and bunch development (30g N & 120g K) stages (N1) advanced the fruit maturity (110.6 days) and also recorded the highest bunch weight (27.6 kg) and fruit TSS (31.2°B) (Table 14). Among different planting densities, planting of single sucker per pit at a spacing of 2.4X2.4m (1736 plants/ha) recorded the earliest fruit maturity in 112.3 days as against 119.6 days with planting of three suckers per pit at 3.6x3.6m spacing (2315 plants/ha). Dry matter production (DMP) at harvest was in the range of 5352 – 6065 g/ plant. Leaf NPK concentrations at harvest was ranging from 2.12% to 2.96% N, 0.29% to  $0.54\% \text{ P}_2\text{O}_5$  and 2.00% to  $2.79\% \text{ K}_2\text{O}$ . Among different nutrient levels, N1 recorded the highest nitrogen (2.57%), phosphorus (0.45%) and potassium (2.95%) contents.

The economics of different treatments showed that application of 300g N and 400g  $\rm K_2O$  in the ratio of 7:2:1 and 4:3:3 at vegetative, flowering and bunch development stages (N1) recorded the highest yield of 60.0 t/ha with the highest B:C ratio of 3.43, whereas application of RDF of N & K in the ratio of 7:3:0 N and 6:2.5:1.5  $\rm K_2O$  (N3) recorded the lowest yield (53.9 t/ha) with the lowest BC ratio of 3.04.

# Effect of organics on the BSV and BBrMV infected Poovan banana

In the second ratoon crop, application of 20 kg FYM, 0.9 kg neem cake, 2.0 kg vermicompost and 0.9 kg groundnut cake (T3) recorded the highest bunch weight of 15.9 kg with more number of hands (12.1) and fingers/ bunch (188.5) (Table 15). Better fruit quality in terms of highest TSS (26.4°B), more pulp: peel ratio (5.46) and the lowest acidity (0.35%) were also recorded under the treatment T3. Application of organics recorded the highest N, P, K, Mg and low Ca and Na contents, more DMP than 100% inorganic fertilization. The effect of organics on soil physical properties indicated, application of 20 kg FYM + 0.9 kg neem cake + 2.0 kg vermicompost + 0.9 kg groundnut cake (T3) recorded the lowest bulk density of 1.13 while the highest bulk density

Table 14. Effect of stagewise nutrition on bunch weight (kg.) in banana cv. Udhayam

Treatments	N1	N2	N3	Mean
S1 (1984 pl./ha)	29.1	25.8	24.6	26.5
S2 (1736 pl./ha)	31.2	28.7	27.4	29.1
S3 (2778 pl. /ha)	25.6	25.4	23.3	24.8
S4 (2057 pl./ha)	27.7	26.5	25.1	26.4
S5 (2315 pl./ ha)	24.5	24.1	23.6	24.1
Mean	27.6	26.1	24.8	
	S. Ed.	C.D.	Significance	<b>C.V.</b> %
Spacing (S)	0.802	2.213	**	7.15
Nutrition (N)	0.613	1.694	*	
SXN	1.422	-	NS	



Table 15. Effect of organics on bunch weight in BSV and BBrMV infected of Poovan banana (II ratoon)

Treatments	N1	N2	N3	N4	N5	N6	Mean
M1-BSV	12.8	13.9	14.4	14.1	15.3	14.8	14.2
M2-BBMV	14.3	15.1	15.5	15.2	16.3	15.3	15.5
M3-Healthy	16.6	17.1	17.8	16.6	19.1	17.1	17.6
Mean	14.5	15.3	15.9	15.3	16.9	15.7	
	S. Ed.		C.D	C.D. (5%)		ficance	C.V. %
Material (M)	1.257		2.	2.685		**	6.14
Nutrition (N)	2.011		4.	4.056		**	
MXN	3.140		6.	6.374		*	

of 1.30 and 1.36 g cc<sup>-1</sup> was recorded with application of 100% and 125% RDF inorganic fertilizers respectively.

Application of 20 kg FYM + 0.9 kg neem cake + 2.0 kg vermicompost + 0.9 kg groundnut cake (N3) as well as other organic treatments significantly improved the porosity (45.5%) as well as particle density (44.3%) as against a porosity of 40.2% with 100% inorganic (T6) that was on par with 125% inorganic fertilization (T5).

# 4.2.2 Fertilizer tailoring for targeted banana yield and sustainable soil health

# Validation of fertilizer tailoring equations for Grand Naine banana

The following fertilizer tailoring equations/ready reckoner for Grand Naine,

developed by NRC for Banana, were validated in two different locations *viz.*, Pazhur and Sirugambur villages in Tiruchirapalli district (Table 16).

$$FN = (8.80*T) - (0.73*SN) - (0.32*ON)$$

$$FP = (0.84*T) - (0.77*SP) - (0.37*OP)$$

$$FK = (11.21*T) - (0.44*SK) - (0.39*OK)$$

where, FN, FP and FK are NPK requirement through fertilizer (kg/ha), SN, SP & SK are NPK available in the soil (kg/ha), ON, OP & OK are NPK requirement through organic manure (kg/ha), and T – yield target (t/ha).

At Pazhur, the initial soil NPK contents were 260 kg, 19 kg and 311 kg per hectare respectively and at Sirugambur, they were 158 kg, 8 kg and 226 kg, respectively. Based on

Table 16. Ready-reckoner: Soil test based NPK dose (g/plant) for targeted yield of Grand Naine banana

Targe yield (			Soil N (kg/ha)										
	100	125	150	175	200	225	250	275	300	325	350	375	400
60	152	146	140	133	127	121	115	109	103	97	91	85	79
72	187	181	175	169	163	156	150	144	138	132	126	120	114
84	222	216	210	204	198	192	186	179	173	167	161	155	149
96	257	251	245	239	233	227	221	215	209	203	196	190	184
108	155	286	280	274	268	262	256	250	244	238	232	226	219
120	328	322	316	309	303	297	291	285	279	273	267	261	255



132	363	357	351	345	339	332	326	320	314	308	302	296	290
144	398	392	386	380	374	368	362	355	349	343	337	331	325
156	433	427	421	415	409	403	397	391	385	379	372	366	360
168	468	462	456	450	444	438	432	426	420	414	408	402	395
180	504	498	492	485	479	473	467	461	455	449	443	437	431
Targe					Soi	1 P (kg	g/ha)						
yıcıu(ı	6	8	10	12	14	16	18	20	22	24	26	28	30
60	15	15	14	14	13	13	12	12	11	11	10	10	9
72	19	18	18	17	17	16	16	15	15	14	13	13	12
84	22	21	21	20	20	19	19	18	18	17	17	16	16
96	25	25	24	24	23	23	22	22	21	21	20	20	19
108	29	28	28	27	27	26	26	25	25	24	24	23	23
120	32	32	31	31	30	29	29	28	28	27	27	26	26
132	35	35	34	34	33	33	32	32	31	31	30	30	29
144	39	38	38	37	37	36	36	35	35	34	34	33	33
156	42	42	41	41	40	40	39	39	38	38	37	36	36
168	46	45	44	44	43	43	42	42	41	41	40	40	39
180	49	48	48	47	47	46	46	45	45	44	44	43	43
Targe					Soi	1 K (k	g/ha)						
yield (													
	100	125	150	175	200	225	250	275	300	325	350	375	400
60	210	206	202	199	195	191	188	184	180	177	173	169	166
72	254	251	247	243	240	236	232	229	225	221	218	214	210
84	299	296	292	288	285	281	277	274	270	266	263	259	255
96	344	340	337	333	329	326	322	318	315	311	307		300
108	389	385	382	378	374	371	367	363	360	356	352	349	345
120	434	430	426	423	419	415	412	408	404	401	397	393	390
132	479	475 520	471	468	464	460	457	453	449	446	442	438	435
144 156	523 568	520 565	516 561	<ul><li>512</li><li>557</li></ul>	509 554	505 550	501 546	498 543	494 539	490	487 532	483 528	479 524
168	613	609	606	602	598	595	546	543	584	535 580	576	528	569
180	658	654	651	647	643	640		632	629	625	621		614
100	030	034	031	047	043	040	030	032	029	023	021	010	014



the initial soil test values and using equations/ ready reckoner, the NPK requirements (in g per plant) were worked out for the yield targets of 75, 90 105, 120, 135 and 150 tons per hectare and are given in the (Table 17). Simultaneously, the blanket recommendation of fertilizer dose (BRFD) of 200:30:350 g NPK/plant was also maintained for comparison.

At Pazhur village, the fertilizer dose targeting 150t/ha recorded 27.3% more plant height, 35.3% more pseudostem girth, 18.2% more numbers of leaves and 22.4% more leaf emergence rate than that of the BRFD, while at Sirugambur village, 150t/ha target fertilizer dose recorded 30.0%, 25.0%, 4.6% and 23.6% more in respective parameters than that of BRFD at 7 MAP. At Pazhur, the leaf NPK concentrations of 2.35%, 0.74% and 3.1% were recorded with fertilizer dose targeting 150t/ha, while they were 2.18%, 0.47% and 2.53% at BRFD at 7MAP. At Sirugambur, the leaf NPK concentrations of 2.47%, 0.78% and 3.22% were recorded with fertilizer dose targeting 150t/ha, while they were 2.0%, 0.34% and 2.24% at BRFD at 7MAP.

Under Pazhur condition, when the blanket recommendation of NPK (200:30:350 g NPK/plant) was applied, the actual yield recorded was 92.4t/ha with the Benefit to Cost (B:C) ratio of 2.77. The highest B:C ratio of 3.65 was observed with actual yield of 128.4t/ ha, when the adjustment equations were applied

for yield target of 135 t/ha. With the increasing targets from 75 to 90t/ha, the actual yields were slightly above the targets, with B:C ratio ranging from 2.51 to 2.80. The nutrient dose, targeting 105t/ha exactly produced the same (actual) yield of 105t/ha with B:C ratio of 3.14, which is more than that with blanket recommendation. At this target (105t/ha), 13.6% more yield was obtained than that with the blanket recommendation, with additional investment of just Rs. 954.89/ha due to fertilizer. Beyond this target level, with increasing targets, the actual yield increased with diminishing rate up to the target of 150t/ ha i.e., the actual yields were less than the targets but more than that with blanket recommendation. When the B:C ratios were observed between the targets 105t/ha and 150t/ha, the highest B:C ratio of 3.65 with actual yield of 128.4t/ha was observed at the target, 135t/ha. Beyond this target (135t/ha), the B:C ratio started declining. Thus, under Pazhur condition, the fertilizer tailoring equations for Grand Naine, holds good up to the target 105t/ha, if actual yield is the concern and suitable up to the target 135t/ha, if the return per unit investment due to fertilizer is the concern (Table 18 and Fig. 16).

Under Sirugambur condition, the NPK blanket recommendation produced yield of 85.5t/ha with B:C ratio of 2.57. The highest B:C ratio of 3.64 was worked out with the application of NPK in relevance to these fertilizer tailoring equations aiming the yield

Table 17. Tailored NPK doses for different yield targets for Grand Naine banana

Treatments		Pazhur			Sirugambur	
(Targets)	N (g/plant)	P (g/plant)	K (g/plant)	N (g/plant)	P (g/plant)	K (g/plant)
75 t/ha	156.73	16.12	234.64	181.55	18.95	247.10
90 t/ha	200.73	20.32	290.69	225.55	23.15	303.15
105 t/ha	244.73	24.52	346.74	269.55	27.35	359.20
120 t/ha	288.73	28.72	402.79	313.55	31.55	415.25
135 t/ha	332.73	32.92	458.84	357.55	35.75	471.30
150 t/ha	376.73	37.12	514.89	401.55	39.95	527.35



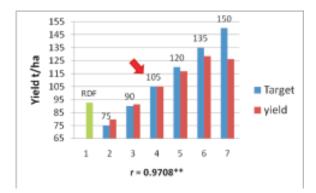


Fig. 16: Target vs Actual Yield in location I

target of 135t/ha, which actually recorded an yield of 129.3t/ha. The actual yields increased with increasing rate, as the target increased from 75t/ha to 120t/ha and were more than or on a par with the target. Beyond this target, 120t/ ha, the actual yield increased with diminishing rate. The nutrient application in relevance to these equations, aiming the target of 120t/ha produced an actual yield of 119.7t/ha (with B:C ratio of 3.46), which is on par with the target and 39.8% more yield was obtained than that with the blanket recommendation. A gradual increase in B:C ratio from 2.59 to 3.64 from the target of 75t/ha to 135t/ha and beyond that it declined. Thus, under Sirugambur condition, this set of fertilizer tailoring equations for Grand Naine, holds good up to the target 120t/ha, if actual yield is the concern and holds good up to the target 135t/ha, if the return per unit investment due to fertilizer is the concern (Table 19 and Fig. 17a & 17b). (Note: Costs of NPK are Rs. 16.5,

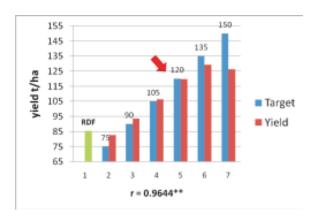


Fig. 17a: Target vs Actual Yield in location II



Fig. 17b: Grand Naine banana bunches produced by NPK tailoring

Table 18. Effect of tailored NPK for different yield targets on Grand Naine banana at Pazhur Village

Treatments	Nutrien	t dose (g	plant-1)	Actual		Profit	Cost of	Benefit
	N	$P_2O_5$	K <sub>2</sub> O	yield (t ha <sup>-1</sup> )	cent deviation	(Rs. ha <sup>-1</sup> )	fertilizers (Rs. ha <sup>-1</sup> )	Cost ratio
Blanket recommendation	200 1	30	350	92.4	0	831600	46687.5	2.77
75 t/ha	156.73	16.12	234.64	79.5	-13.96	715500	31716.89	2.51
90 t/ha	200.73	20.32	290.69	91.2	-1.30	820800	39679.64	2.80
105 t/ha	244.73	24.52	346.74	105	13.64	945000	47642.39	3.14
120 t/ha	288.73	28.72	402.79	116.7	26.30	1050300	55605.14	3.40
135 t/ha	332.73	32.92	458.84	128.4	38.96	1155600	63567.89	3.65
150 t/ha	376.73	37.12	514.89	126	36.36	1134000	71530.64	3.49
CD (p=0.05)				27.15				



Table 19. Effect of tailored NPK for different yield targets on Grand Naine banana at Sirugambur Village

Treatments	Nutrien	t dose (g	plant-1)	Actual		Profit	Cost of	Benefit
	N	$P_2O_5$	K <sub>2</sub> O	yield (t ha <sup>-1</sup> )	cent deviation	(Rs. ha <sup>-1</sup> )	fertilizers (Rs. ha <sup>-1</sup> )	Cost ratio
Blanket recommendation	200 1	30	350	85.5	0	769500	46687.5	2.57
75 t/ha	181.55	18.95	247.1	82.8	-3.16	745200	34565.66	2.59
90 t/ha	225.55	23.15	303.15	93.6	9.47	842400	42528.41	2.85
105 t/ha	269.55	27.35	359.2	106.5	24.56	958500	50491.16	3.15
120 t/ha	313.55	31.55	415.25	119.7	40.00	1077300	58453.91	3.46
135 t/ha	357.55	35.75	471.3	129.3	51.23	1163700	66416.66	3.64
150 t/ha	401.55	39.95	527.35	126.3	47.72	1136700	74379.41	3.47
CD (p=0.05)				31.51				

Rs. 58.75 & Rs. 30 per kg and market price of fruit is Rs. 9/kg – for calculation of B:C ratio).

#### Studies on nutrient dynamics in banana

Under this study, Ney Poovan (AB) and Rasthali (ABB) banana were planted in a randomized block design with of treatments viz., 50%, 75%, 100%, 125% and 150% of recommended dose of fertilizer (RDF) with an absolute control. The initial soil NPK contents were 198.4, 8.9 and 217.1 kg N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O per ha. At sucker stage (9 to 12 leaf),

in Ney Poovan, the stem NPK contents were: 1.21%, 0.28% and 5.2%, respectively whereas in Rasthali, 1.37%, 0.24% and 5.5%, respectively. At this stage, the Ney Poovan corm NPK contents were 0.72%, 0.13% and 3.2%, respectively while in Rasthali, 0.81%, 0.11% and 4.1%, respectively. During early stage of vegetative growth stage (4 months after planting), the vegetative growth parameters were recorded. The plant height ranged from 68 to 80.2 cm in Ney Poovan while it ranged from 80.4 to 89.1 cm in Rasthali.

Table 20. Effect of NPK levels on plant growth parameters in Ney Poovan and Rasthali

NPK levels		Ney Poova	n		Rasthali	
(% RDF)	Plant height (cm)	Pseudostem girth (cm)	Number of leaves	Plant height (cm)	Pseudostem girth (cm)	Number of leaves
Control	68.0	22.1	10.8	81.5	25.7	8.6
50	74.3	23.8	11.1	86.2	27.0	9.0
75	80.2	24.1	11.1	89.1	27.6	9.0
100	70.0	21.8	10.9	80.4	27.5	9.7
125	72.7	21.8	10.7	79.5	25.5	8.9
150	68.6	21.8	10.7	80.6	25.8	8.9
Mean	72.3	22.6	10.9	82.9	26.5	9.0
CD(p=0.05)	2.68	1.84	NS	5.32	1.59	NS



Table 21. NPK concentrations in different segments of Ney Poovan plant at different treatments

		N	Nitrogen (%)	(9)			Phos	<b>Phosphorus</b>	(%)			Potas	Potassium (%	(9	
Treat.	Leaf	Petiole Stem	Stem	Corm	Root	Leaf	Petiole	Stem	Corm		Leaf ]	Petiole	Stem	Corm	Root
Control	3.45	0.72	2.11		0.79	0.53	0.31 0.52 (	0.52	3.36	0.2	2.31	5.6	6.98 2.87 4.07	2.87	4.07
20%	3.43		1.15	0.85		0.54	0.32	0.58	0.3		2.18	6.04	6.83	3.16	3.49
75%	3.67		1.09			0.51	0.34	9.0	0.32		2.27	6.17	69.9	3.31	4.35
100%	3.48		1.68		0.81	0.46	0.3	0.59	0.25		2.26	5.51	6.25	2.49	4.49
125%	3.43		1.00			0.51	0.31	0.48	0.3		2.17	6.01	9.9	2.58	3.7
150%	3.26	0.72	2.07	0.77	8.0	0.49	0.29	0.42	0.25		2.1	5.85	5.75	2.47	4.4
	3.45	0.74	1.52			0.51	0.31	0.53	02.30		2.22	5.86	6.52	2.81	4.08
CD(5%)	0.151		0.927	0.120		0.072	NS	0.1111	0.095	NS	0.193	0.451	0.940	0.940 0.622 0.572	0.572

Table 22. NPK concentrations in different segments of Rasthali plant at different treatments

		Nin	Nitrogen (%	(%			Phos	Phosphorus (%)	(%)			Potas	Potassium (%)	(9	
Treat.	Leaf	Petiole	Stem	Corn	1 Root	Leaf	Leaf Petiole Stem		Corm	Root	Leaf ]	Petiole	Stem	Corm	Root
Control	2.52	2.52 0.67 1.00	1.00	0.72	0.94	0.36	0.38		0.38	0.73	3.15	0.73 3.15 6.13	6.93 3.10 5.73	3.10	5.73
20%	2.24	99.0	1.09	0.74	0.88	0.29	0.38	0.50	0.36	0.76	3.14	5.95	99.9	3.10	5.73
75%	2.31	0.73	0.98		06.0	0.36	0.39	0.48	0.39	0.76	3.08	5.31	6.33	3.43	5.68
100%	2.41	0.62	0.97			0.36	0.40	0.50	0.37	0.76	3.02	5.89	7.02	2.83	5.99
125%	2.17	0.64	0.98			0.34	0.39	0.48	0.37	0.73	3.14	5.85	6.95	3.51	5.87
150%	2.43	0.62	0.94	69.0		0.34	0.39 0.50	0.50	0.35	0.78	2.99	80.9	6.31	3.05	6.24
Mean	2.35	99.0	0.99		0.91	0.34	0.39	0.49	0.37	0.75	3.08	5.87	0.70	3.17	5.87
CD(5%)	0.227	0.094	0.051		0.107	0.068	NS		0.015	0.019	9 0.137 0	0.553	0.604	0.411	0.283





The pseudostem girth ranged from 21.8 to 24.1 cm and from 25.5 to 27.6 cm in Ney Poovan and Rasthali, respectively and the number of leaves varied from 10.7 to 11.1 and from 8.6 to 9.7, respectively (Table 20).

During early stage of vegetative growth, after the appearance of about ten broad leaves, whole plant sampling was done. The total biomass was fragmented into leaf, petiole, pseudostem, corm and root. The mean dry weights were determined as shown in the graphs. In Ney Poovan banana, the mean nitrogen contents in leaf, petiole, pseudostem,

corm and root were 3.45, 0.74, 1.52, 0.79 and 0.75% respectively. The respective phosphorus contents were 0.51, 0.31, 0.53, 0.30 and 0.20%. The respective potassium contents were 2.22, 5.86, 6.52, 2.81 and 4.08% (Table 21).

In Rasthali banana, the mean nitrogen contents in leaf, petiole, pseudostem, corm and root were 2.35, 0.66, 0.99, 0.73 and 0.91%, respectively. The respective phosphorus contents were 0.34, 0.39, 0.49, 0.37 and 0.75%. The respective potassium contents were 3.08, 5.87, 6.70, 3.17 and 5.87% (Table 22).



## 4.3 PHYSIOLOGY, BIOCHEMISTRY AND POSTHARVEST TECHNOLOGY

#### 4.3.1 Drought stress tolerance in banana

Studied the effects of drought stress during different phenological stages of cv. Grand Naine under field condition. The soil moisture deficit stress (as progressive stress) was imposed by withholding irrigation to reach -0.6 to -0.7 MPa through drip irrigation at 3<sup>rd</sup> and 5th month and at flowering stage. Soil moisture stress imposed at 3rd month resulted in 27-45 days delay in flowering. The stress imposed during 5th month after planting at 19-20% soil moisture level resulted in bunch malformation and reduction in number of fruits and bunch weight (Fig. 18). The plants primed with 0.1mM salicylic acid as foliar spray before imposing soil water deficit stress, produced normal bunch.



Fig. 18: Malformed Grand Naine bunch

The soil moisture stressed (at 75% available soil moisture) Grand Naine plants at 18-20 leaf stage recorded 20% reduction in photosynthesis (10.45 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) compared to irrigated control (13.01 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). The stomatal conductance decreased to 0.29 (mol m<sup>-2</sup> s<sup>-1</sup>) from the irrigated control of 0.31 mol m<sup>-2</sup> s<sup>-1</sup> and the transpiration increased by 12.25% over control (Fig. 19). The leaf temperature increased to 39.53°C in

stressed plants compared to irrigated control (35.27°C). Therefore, even moderate soil moisture stress in cv. Grand Naine banana plants significantly reduced the photosynthesis, stomatal conductance and increased the transpiration and leaf temperature.

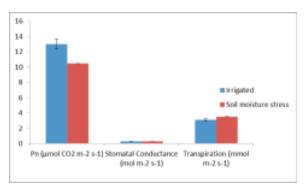


Fig. 19: Gas exchange parameters of irrigated and moisture-stressed plants

The soil moisture stress combined with wind and higher atmospheric temperature drastically reduced the photosynthetic activity and the terminal part of torn leaves (5-6 cm) dried-up. The top 6-7 fully expanded leaves had an average of 80-90 tearing and the breadth of the each torn leaf ranged from 3.0 to 6.0 cm (Fig. 20). The tearing of leaves into smaller leaf flaps leads to drying of leaves thereby it reduces photosynthetic area by 12-15%.



Fig. 20: Wind-affected banana plants



Under controlled pot studies, cv. Grand Naine plants showed increased production of ABA with 6.68 ppm / g of leaves (fr. wt.), which is 8.9% more than irrigated control, when reduced to 75-80% available soil moisture. In another pot studies, when the soil water content was reduced to 75% available soil moisture, stress tolerant banana cultivars Saba, Karpuravalli, Ney Poovan and Jwaribale recorded higher antioxidative enzymes (APX, CAT, SOD) than irrigated control plants.

Subjecting three months old cv. Grand Naine plants to 85% soil moisture deficit stress for seven days after priming with beta amino butyric acid, glycine betaine and kaolin and measuring the gas exchange parameters indicated plants primed with 20 mM glycine betaine sustained photosynthesis, stomatal conductance and transpiration by recording 5-13 fold more over unprimed drought imposed plants (Fig. 21). Besides, the primed plant maintained leaf tissue density (0.2 g/kg) and leaf succulence (0.4 mg H<sub>2</sub>O/mm<sup>2</sup>), which was on par with irrigated control after two weeks of drought stress.

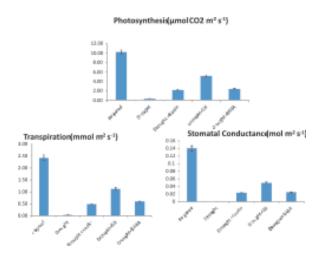


Fig. 21: Effect of soil moisture deficit stress relieving chemicals on gas exchange parameters (Photosynthesis, Transpiration and Stomatal conductance)

The drought tolerant genes, viz., DREB2, WRKY, ASR, SK<sub>3</sub>DHN were successfully amplified from genomic DNA of Grand Naine and sequenced and were all in expected amplicon sizes.

#### Salt stress tolerance in banana

Banana cultivars were phenotyped for salt tolerance stress. The salt stress was imposed at 50, 100 and 150 mM of NaCl in six banana genotypes. After three weeks stress, gaseous exchange parameters were measured. In cv. Nendran, photosynthesis decreased by 87.45% (1.52 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) in 50 mM NaCl stress plants as compared to control (12.11 imol CO, m<sup>-2</sup> s<sup>-1</sup>). Among all, cv. Saba and Karpuravalli recorded less reduction in photosynthesis (< 34%), stomatal conductance (25%) and transpiration (45.68%). At 100 and 150 mM NaCl stress, there was drastic reduction of photosynthesis (0.19 imol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (0.02 mol m<sup>-2</sup> s<sup>-1</sup>) and transpiration (0.55 mmol m<sup>-2</sup> s<sup>-1</sup>) in tested banana cultivars and there was no significant difference for these parameters among tested genotypes at higher salt stress concentration. The gas exchange parameters could differentiate salt sensitive banana plants from tolerant genotypes at 50 mM NaCl stress (Table 23). In salt stressed plants (50 to 150 mM NaCl), tolerant banana genotypes viz., Saba Karpuravalli and Ney Poovan recorded less number of leaf senescence, whereas Rasthali, Nendran recoded higher leaf senescence. Similarly the plant height, girth and new leaf production also affected in the susceptible cultivars.

The Grand Naine plants primed with 200 μM beta amino butyric acid and subsequent imposition of salt stress with 50 mM NaCl recorded two fold more photosynthesis than salicylic acid (100 µM) primed plants. But the stomatal conductance and transpiration did not vary among the primed plants. However, the gas exchange parameters were recorded 10-12



fold less in primed plants than control plants.

Farmers Trial: A trial was conducted at farmer's field at Thottiam, using soil moisture stress relieving chemicals. The moistures deficit stress relieving chemicals was given as foliar spray in cv. Rasthali one month prior to flowering. The results revealed that plants applied with ASA (0.1 mM)+ BHT (100 ppm) + KNO<sub>3</sub> (0.75%) recorded no leaf crinkling, reduced leaf drying, better bunch throw with



Fig. 22: Effect of soil moisture relieving chemicals on flowering

more space between hands (Fig. 22), whereas in control plants (non-application of chemicals) leaf crinkling, extensive leaf drying and poor bunch throw were recorded.

### Proteomic and biological functions analysis in NaCl-stressed bananas

Eighty differentially expressed proteins identified using Melanie7 software from proteomic analysis of roots of salt-resistant banana cv. Saba and salt susceptible cv. Grand Naine treated with 100 mM and 1 mM (control) NaCl for 15 days. Out of these 80 proteins, 43 major proteins differentially expressed by more than two-times were mass fingerprinted using MALDI-TOF-TOF mass spectrophotometer. Identification and annotation for biological functions of these 43 proteins from Saba and Grand Naine using MASCOT analysis revealed that proline dehydrogenase, glutamine synthetase, methionine adenocyltransferse, osmotin like protein, beta-glucosidases, cyclin-D3-2, map kinase 4, defensin-like protein 72, mitogen activated protein kinase 12 and protein dehydrogenase are important enzymes/

Table 23. Gas exchange parameters in salt stressed banana cultivars

		•			Stoma (					-	ration m <sup>-2</sup> s <sup>-1</sup> )	
	Control		100 mM NaCl		Control						100 mM NaCl	
KV	13.13	7.89	1.23	0.23	0.19	0.10	0.10	0.00	2.34	1.70	0.92	0.67
RAS	12.40	5.35	0.78	0.04	0.16	0.12	0.10	0.04	6.02	3.20	1.70	0.99
SABA	7.96	5.23	1.94	0.22	0.12	0.09	0.0a1	0.01	3.7	2.01	1.34	0.48
NEN	12.11	1.52	0.92	0.18	0.25	0.10	0.07	0.02	7.87	0.62	0.22	0.3
NEYPO	10.25	5.22	0.52	0.15	0.16	0.09	0.05	0.01	6.26	2.9	0.9	0.53
POOV	7.24	4.13	1.02	0.32	0.11	0.07	0.02	0.01	5.52	2.16	1.20	0.30
Mean	10.52	4.89	1.07	0.19	0.17	0.10	0.06	0.02	5.29	2.10	1.05	0.55
SEM	1.004	0.844	0.199	0.038	0.021	0.007	0.016	0.006	0.804	0.375	0.205	0.106



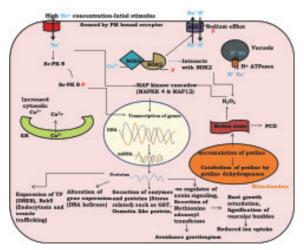


Fig.23: A generic pathway for transduction of salt stress in banana root cells based on the proteins identifiedin salt resistant and susceptible banana cvs. in response to NaCl stress

proteins regulated in response to high NaCl concentration and these proteins involve in cell cycle and development, signal transduction and proline catabolism (Table 24).

Based on the annotated proteins, a generic model for transduction of signals in the cells in response to NaCl stress in bananas showed their involvement of salt overlay sensitive (SOS), mitogen activated protein (MAP) kinase cascades, H2O2 signalling, Na ions extrusion, lignification of vascular bundles and root growth retardation pathways of plant. The transduction of signals due to salt stress is constructed as a generic model as following pathways that are interlinked (Fig. 23).

Table 24. Differentially expressed proteins identified in response to salt stree

Spot No.	Swiss prot Accession No.		Sequence ( coverage (%)		No. peptic match	des ExMr	ThpI/ ExpI	Taxonomy
73	Q5YLB4	DNA gyrase subunit B,	9	36	16	98.2/99	7.2/7.5	Nicotiana
		Cell cycle and Developme	nt					
192	Q0J3H7	Cyclin-D3-2	27	39	5	38.3/40.2	4.8/5.7	Oryza sativa Japonica Group
173	Q8LW53	Light-independent protochlorophyllide reductase subunit N	23	37	4	51.3/52	5.5/6.7	Chaetos phaeridum globosum
178	Q6F6B5	Protein accumulation and replication of chloroplasts	12 3	40	3	82.5/83.0	5.6/5.5	Arabidopsis thaliana
148	Q940D0	Two-component response regulator ARR1	14	38	30	75.1/74.6	5.9/6	Arabidopsis thaliana
		Stress						
150	Q2V2S9	Putative defensin-like protein 72	47	40	10	68.3/65.2	8.4/7.6	Arabidopsis thaliana
175	Q9LRR4	Glyceraldehyde-3-phosphadehydrogenaseC subunit	te 13	38	3	44.4/43.0	8.2/7.8	Arabidopsis thaliana
231	Q41350	Osmotin like protein	35	42	32	24.7/23.5	7.8/6.4	Solanum lycopersicum



Spot No.	Swiss prot Accession No.	Protein Name	Sequence S coverage (%)	Score	No. peptic match	les ExMr	Thp <i>I/</i> Exp <i>I</i>	Taxonomy
325	Q3L634	Mannose-binding lectin	18	38	7	16.1/15.5	5.1/4.9	Zingiber officinale
256	Q570I7	α-Glucosidases	16	36	11	10.8/9.9	9.5/8.8	Arabidopsis thaliana
314	P12653	Glutathione S transferase <b>Transcription</b>	52	32	9	23.8/22.6	5.4/5.5	Zea mays
183	A9LYH7	DNA-directed RNA polymerase subunit beta	16	40	5	78.4/77	9.1/9.5	Acorus americanus
202	Q53N90	Putative B3 domain- containing protein	13	32	6	33.1/34.0	9.6/8	Oryza sativa Japonica Group
234	Q9FT73	DNA helicase	21	32	10	79.3/78.5	7.2/7.0	Arabidopsis thaliana
		Detoxification						
181	Q96266	Glutathione S-transferase F8	27	40	9	29.2/28.1	8.5/9.2	Arabidopsis thaliana
280	Q06GU2	NAD(P)H-quinone oxidoreductase subunit	56	39	12	11.2/12.8	9.7/11.0	Drimys granadensis
		Signal Transduction						
326	O04160	Shaggy-related protein kinase theta	16	36	4	52.5/55	6.9/7.0	Brassica napus
354	Q8GYQ5	Mitogen-activated proteir kinase 12	n 17	39	5	42.4/43.2	8.0/8.2	Arabidopsis thaliana
360	Q84MA2	Type I inositol 1,4,5- trisphosphate 5-phosphatase 2	20	43	8	73.5/74.3	6.6/6.4	Arabidopsis thaliana
152	Q39024	Map kinase kinase 4	25	36	6	42.8/41	5.7/5.6	Arabidopsis thaliana
267	P29687	Rab5	35	40	9	21.9/20	8.4/8.3	Nicotiana tabacum
181	Q9M0L0	CRT/DREB	25	36	5	42.2/40.0	5.0/4.9	Arabidopsis thaliana
183	M5BF43	SOS3	28	38	10	25.6/24.9	5.1/5.5	Arabidopsis thaliana



Spot No.	Swiss prot Accession No.		Sequence s coverage (%)		No. peptid	les ExMr	Thp <i>I/</i> Exp <i>I</i>	Taxonomy
		Carbohydrate Metabolisn	n					
260	Q8L7W8	Alpha-L-fucosidase 1	11	39	6	57.1/55.6	5.2/5.0	Arabidopsis thaliana
319	Q9FFR3	6-phosphogluconate dehydrogenase, decarboxylating 2	21	51	4	53.2/54	5.6/5.5	Arabidopsis thaliana
272	Q0J8A4	Glyceraldehyde-3-phosphadehydrogenaseC subunit	ite 32	48	6	36.4/37.2	4.3/4.5	Oryza sativa Japonica Group
305	P39207	Nucleoside diphosphate kinase 1	30	37	26	16.4/17.0	6.3/7.0	Arabidopsis thaliana
		Secondary Metabolism						
222	Q8W593	Lactoylglutathione lyase	17	33	32	20.8/19.0	5.1/4.9	Arabidopsis thaliana
357	Q9FKK7	Xylose isomerase	28	36	12	53.6/55.1	5.5/4.7	Arabidopsis thaliana
347	Q6ZD89	Caffeic acid 3-O-methyltransferase	36	46	6	39.5/38.7	5.4/6.1	<i>Oryza sativa</i> subsp. <i>japonica</i>
		Lipid Metabolism						
310	Q6DUV9 desaturase	Omega-6 fatty acid	9	32	25	50.7/49.5	8.9/9.0	Brassica napus
290	~	Long-chain-fatty-acid-CoAligase	25	36	7	30.4/29.0	4.35/4.4	Arabidopsis thaliana
		Amino acid Metabolism						
254	Q8LCE1	Glutamine synthetase	22	39	5	42.4/45.0	8.0/7.8	Arabidopsis thaliana
303	P92983	Proline dehydrogenase	26	46	12	52.9/53.0	5.9/6.2	Arabidopsis thaliana
312	P19252	Glutamine synthetase	12	32	4	66.5/65.0	5.7/5.5	Pisum sativum
150	P23686	Methionine Adenosyl transferase	2	32	2	43.1/42.5	5.5/4.9	Arabidopsis thaliana
238	Q9LD55	Eukaryotic translation initiation factor 3A	26	39	13	114.2/114.0	9.2/9.0	Arabidopsis thaliana



Spot No.	Swiss prot Accession No.		Sequence S coverage (%)	Score	No. o peptid match		Thp <i>I/</i> Exp <i>I</i>	Taxonomy
		Transport Function						
227	Q9MB78	H+-ATPases	32	46	14	10.7/9.8	5.0/5.1	Nepenthes alata
175	Q84ZC0	V-ATPase	30	38	11	51.3/52	7.1/7.0	Oryza sativa subsp. japonica
193	P30298	Sucrose synthase	16	44	2	92.1/93.5	5.9/6.2	Oryza sativa subsp. japonica
211	O48946	Cellulose synthase	18	36	7	122.2/122.1	6.5/6.7	Arabidopsis thaliana
		Phytohormone metabolis	m					
304	Q9XFL9	Llipoxygenase	35	40	25	77.5/77.4	6.2/6.1	Zea mays
327	C6GMF5	9-cis-epoxy-carotenoid dioxygenase	34	42	12	23.0/23.0	4.9/5.0	Nicotiana plum baginifolia

## 4.3.2 Biochemical mechanism of resistance of bananas to root lesion nematode

#### Proteomic changes in Nendran by Pratylenchus coffeae infection

Proteomic analysis of Nendran banana cultivar susceptible to nematode was studied for confirmation of differentially expressed proteins due to infection of root lesion nematode from the total proteins isolated by phenol-ammonium acetate method from root tissues sampled at seven days after inoculation with infective juveniles of P. coffeae and control (uninoculated) using 18 cm IPG strips of pH 3-10 for first dimension and 12% SDS-PAGE for second dimension. A total of 564 and 573 proteins were detected from nematodeinoculated and control Nendran roots (Fig. 24a & b) respectively. On comparison using Melanie7 software, 45 differentially expressed (up-regulated, down-regulated, newly appeared

and disappeared) proteins were identified and 20 proteins, which recorded more than two-fold changes due to the *P. coffeae* infection were fingerprinted by MALDI-TOF mass spectrophotometer.





Fig. 24: 2-DE maps of Nendran banana root proteins: (a) control (uninoculated) and (b) inoculated with *P. coffeae* 

### Differentially expressed proteins identified in response to *Pratylenchus coffeae* infection

Eighty differentially expressed proteins were identified from root protein mapping of nematode resistant banana cv. Anaikomban



and susceptible cv. Nendran in response to infection by root lesion nematode. From these, 40 proteins with more than two-fold changes were mass fingerprinted by MALDI-TOF-TOF mass spectrophotometer and 30 proteins were annotated by database searching using MASCOT MS/MS ion search software for protein identity (Table 25). The proteins were

involved in energy metabolism, transcription of PR genes, cell wall remodelling, stress response, signal transduction, protein metabolism, metabolism, secondary replication and transcription and protein metabolism during biotic stress including nematode infection.

Table 25. Differentially expressed proteins identified in response to Pratylenchus coffeae infection

Spot No.	Swiss prot Accession No.	Protein Name	Sequence S coverage (%)	core	No. peptic match	les ExMr	Thp <i>I/</i> Exp <i>I</i>	Taxonomy
		Carbohydrate metabolism						
51	P08735	Glyceraldehyde-3- phosphate dehydrogenase	31	40	16	36.5/34.8	6.4/6.6	Zea mays
162	Q9LFA6	Beta-Galactosidase	27	32	9	82.0/81.9	8.6/8.4	Arabidopsis thaliana
12	P00840	ATP synthase subunit beta	. 19	40	9	21.1/22	9.6/9.8	Zea mays
14	D7LN95	O-glycosyl hydrolase	30	36	15	65.8/66	5.5/5.7	Arabidopsis lyrata subsp. lyrata
		Stress response						
2	Q9SBJ1	Pyruvate dehydrogenase	32	38	12	41.4/40.2	6.6/6.5	Arabidopsis thaliana
59	Q9FRL8	Glutathione reductase	22	36	6	23.4/22.6	5.7/5.5	Arabidopsis thaliana
150	Q43729	Peroxidase	52	40	15	34.0/33.5	10.0/9.8	Arabidopsis thaliana
70	Q8L727	Glutathione- S-transferase	45	36	23	20.0/20.7	8.8/8.6	Arabidopsis thaliana
4	Q9LSB8	Putative pentatricopeptide repeat-containing protein	8	38	22	76.6/77	7.9/8.0	Arabidopsis thaliana
56	Q42599	NADH-quinone oxidoreductase	28	40	4	25.5/24.2	5.3/5.5	Arabidopsis thaliana
		Signal Transduction						
8	P49103	Ras-related protein Rab-2	43	42	12	23.0/22.6	6.9/7.2	Zea mays
204	A2YMM3	F-box protein	7	44	21	18.1/17.5	9.8/9.5	Pseudend oclonium akinetum



Spo No.		Protein Name	Sequence coverage (%)	Score	No. peptid	les $ExMr$	Thp <i>I/</i> Exp <i>I</i>	Taxonomy
219	Q9C6C3	ADP-ribosylation factor GTPase-activating protein	8 1	36	12	87.8/86.5	6.2/6.5	Arabidopsis thaliana
15	Q9M310	F-box/kelch-repeat protein	13	39	4	53.7/54	5.2/5.5	Arabidopsis thaliana
130	Q9SJ32	Putative F-box/FBD/ LRR-repeat protein	23	39	14	41.2/42	5.8/6.0	Arabidopsis thaliana
		Protein Metabolism						
1	Q9FHM3	50S ribosomal protein L11	21	32	24	19.3/18.9	10.2/9.8	Arabidopsis thaliana
11	Q9LUQ6	60S ribosomal protein L19-2	29	32	25	24.3/22.2	11.2/11.0	Arabidopsis thaliana
138	Q8LGH4	Cullin-4	14	36	6	91.4/92.6	8.1/8.5	Arabidopsis thaliana
6	Q9SL42	Peptidyl-prolyl cis-trans isomerase	26	32	10	29.8/30	5.3/5.7	Arabidopsis thaliana
58	P93648	Lon protease homolog 2 <b>Transport</b>	35	40	21	18.9/19.2	7.6/7.8	Zea mays
57	Q9SZH0	V-type proton ATPase subunit G3	33	36	13	12.1/11.5	5.1/4.8	Arabidopsis thaliana
134	Q9SAF0	Oxysterol-binding protein-related protein	18	40	12	92.2/93	6.1/6.6	Arabidopsis thaliana
		Transcription						
18	P50546	DNA-directed RNA polymerase subunit α	14	38	11	121.0/121.5	8.6/8.5	Arabidopsis thaliana
9	Q9FL33	DNA replication licensing factor MCM3 homolog 1	15	40	15	42.0/42.7	8.4/8.6	Arabidopsis thaliana
17	Q9LT47	Polycomb group protein FERTILIZATION- INDEPENDENT ENDOSPERM	16	35	26	60.0/61	9.5/10	Arabidopsis thaliana
73	Q5YLB4	DNA gyrase subunit B, chloroplastic/mitochondrial	9	36	16	98.2/99	7.2/7.5	Nicotiana benthamiana
53	P17158	Maturase K	33	36	17	61.3/62	9.4/9.6	Hordeum vulgare



Spot No.	Swiss prot Accession No.	Protein Name	Sequence S coverage (%)	Score	No. peptid		Thp <i>I/</i> Exp <i>I</i>	Taxonomy
7	Q8GXA1	Methylesterase 3	14	33	6	75.1/76	7.0/7.2	Arabidopsis thaliana
9	Q9FL33	DNA replication licensing factor MCM3 homolog 1	15	40	15	42.0/42.7	8.4/8.6	Arabidopsis thaliana
		Secondary metabolism						
88	O49499	Cinnamyl alcohol dehydorogenase	8	38	22	39.1/39.4	5.1/4.9	Pseuden doclonium akinetum

### Enhancement of shelf-life of bananas by biochemicals and characterisation

To characterise and understand the mechanisms of 'green ripening' of Cavendish bananas and shelf-life enhancement of preclimacteric bananas, with biochemicals, measuring of the level of ripening-related enzyme activities, chlorophyll pigments and its catabolites is important to know the ripening status. Hence, standardization of ripeningrelated enzymes activity and also 2-DE proteomic analysis in peel and pulp tissues of Grand Naine and Poovan banana fruits were done. Polygalacturonase activity in peel and pulp of climacteric (ripening stage 6) Grand Naine and Poovan bananas was 10 and seventimes higher compared to preclimacteric green bananas. Pectate lyase and pectin methylesterase activities were around fivetimes and more than 40-times higher in climacteric Grand Naine and in Poovan pee land pulp respectively compared to preclimacteric fruit tissues. Cellulase activity in peel and pulp of climacteric (ripening stage 6) Grand Naine and Poovan bananas was 3.5 and five-times higher compared to preclimacteric green bananas (Fig. 25a & b). Chlorophyllase activity was 41 and 64-times higher in peel of climacteric Grand Naine and Poovan respectively as compared to preclimacteric peel tissues. Mg-dechelatase activity was 16 and 9.6-times more in peel of climacteric Grand Naine and Poovan respectively (Fig. 25c).

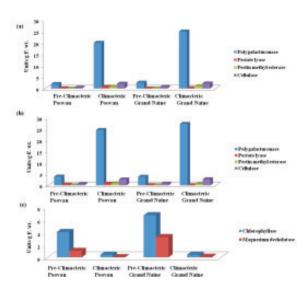


Fig. 25: Changes in activity of enzymes in pre climacteric and climacteric (stage 6) Poovan and Grand Naine bananas: (a) cell wall degrading enzymes in peel; (b) cell wall degrading enzymes in pulp and (c) chlorophyll catabolizing enzymes in peel

Identification by HPLC of pigments in green ripe and yellow ripe Cavendish banana fruit peel was standardised to study the mechanism of green ripening in Cavendish fruit. Identification of chlorophyll pigments and its catabolites, chlorophillides in comparison with retention time of standards with R were as follows: Chlorophyll a – 21.1;



Chlorophyll b - 12.8; Chlorophyllide - 6.2 (Fig. 26a & b).

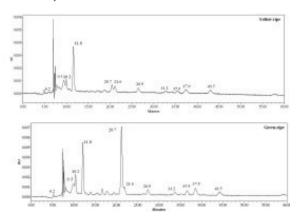


Fig. 26: HPLC chromatograms of chlorophylls and catabolites in peel of Cavendish banana: (a) preclimacteric and (b) climacteric (stage 6)

2-DE proteomic analysis for Grand Naine and Poovan peel tissues was standardised. Total proteins extraction by phenol-ammonium acetate protocol and proteome mapping with 18 cm non-linear pH 4-7 IPG strips with 150 to 800  $\mu$ g proteins and developed with silver nitrate staining. The yields of proteins from peel were 1.3 mg/g of tissue for Poovan and 1.5 mg/g for Grand Naine. The 2-DE maps showed around 600 spots in Poovan and 660 protein spots in Grand Naine with 450  $\mu$ g of protein optimal for 2-DE analysis for detection of even smaller proteins prominently (Fig. 27a & b).

Full mature Grand Naine and Poovan preclimacteric bananas were treated with 1-methylcyclopropne (1-MCP) at one μl/L concentration for 12 and 24 hrs and stored at 13.5, 21 and 29 °C. Preclimacteric bananas as control were stored without 1-MCP treatment. Prior to 1-MCP treatment, bananas were washed in running tap water, 10 ppm chlorine solution for 10 min, 500 ppm of benomyl fungicide solution for 5 min and air-dried.

Grand Naine and Poovan fingers stored at 13.5 °C had higher green life of 64 and 46 days respectively irrespective of exposure time and the control fingers had 35 and 20 days

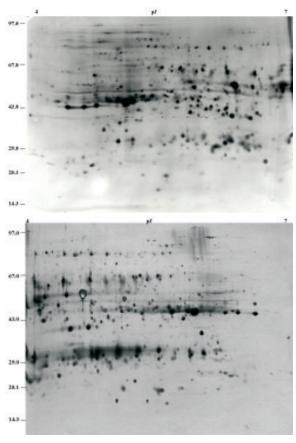


Fig. 27: 2-DE maps of whole proteome of peel tissue: (a) Poovan and (b) Grand Naine

green life. Grand Naine fingers at 21 and 29 °C storage had 14 days green life and control fingers had 5 and 4 days green life respectively. Poovan fingers at 21 and 29 °C storage had 30 and 15 days green life and control fingers had 7 and 5 days green life respectively (Table 26). The physiological parameters showed that the 1-MCP treated Poovan and Grand Naine bananas significantly delayed the onset of ethylene production irrespective of storage temperature (Fig. 28a-c and Fig. 29a-c). Contrary to the blocking of ethylene production, 1-MCP treatment increased the respiration as compared to control, which was evidenced by more O, consumption and CO, release. The enzymes activities preclimacteric and peel colour breaking stage of 1-MCP treated Poovan and Grand Naine showed that the polygalacturonase, cellulase and chlorophyllase activities were subdued in



Table 26. Duration of green life of full mature Grand Naine and Poovan bananas treated with 1-MCP

Temp.	Sample	Grand Naine	Poovan
13.5	Control	35	20
	Treatment	64	46
21	Control	5	7
	Treatment	14	30
29	Control	4	5
	Treatment	14	15

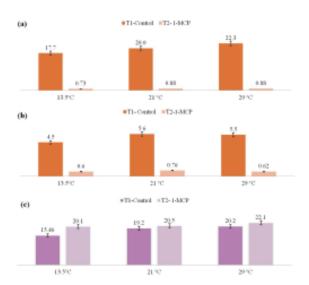


Fig. 28: (a) Ethylene; (b) O<sub>2</sub> and (c) CO<sub>2</sub> evolution in 1-MCP treated cv. Poovan at breaking stage (stage 2)

1-MCP treated bananas than in control bananas compared to preclimacteric stage of bananas at all storage temperatures (Fig. 30a-c). These biochemical characteristics and the physiological parameters observed clearly indicated the effectiveness of 1-MCP in prolonging the shelf-life of green life of Cavendish and Indian bananas at 13.5 °C.

#### 4.3.3 Postharvest Technology

### Comparative evaluation of shelf-life of banana leaves

Shelf-life of banana leaves of 10 commercial varieties and five wild species at

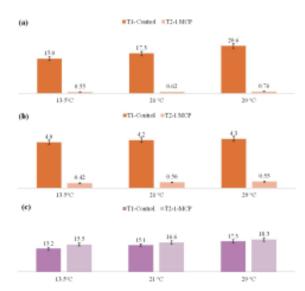


Fig. 29: (a) Ethylene; (b) O<sub>2</sub> and (c) CO<sub>2</sub> evolution in 1-MCP treated cv. Grand Naine at breaking stage



Fig. 30a-c: Enzymes activity in 1-MCP treated cv. Poovan banana at pre climacteric and breaking stage: (a) polygalacturonase; (b) cellulase and (c) chlorophyllase

room temperature varied from three to six days. Among the varieties, Poovan, Karpuravalli and Udhayam had six days shelf-life, which is comparable with wild species like Elavazhai and Phirima. Rasthali, Saba, Monthan and Kungsa wild registered five days shelf-life each, while Pachanadan recorded the minimum shelf-life of three days. Nendran, Mortaman and NeyPoovan had four days shelf-life.



#### Survey on leaf production of banana

Survey results indicated that Poovan banana is the main variety cultivated for leaf purpose in Thirukattupalli area of Thanjavur. A population of 1000 to 1200 plants/acre is maintained and the plant crop was allowed for bunch and the first (ratoon) crop for leaf purpose. Harvesting of around 550 leaves/acre is done at 3/4<sup>th</sup> stage from the sixth month onwards was done daily. The 3/4<sup>th</sup> stage leaf was tied with coarse fibre to prevent opening of the leaf and to protect it from wind damage. Whole leaf measuring 6-8' length and 1.8 - 2.3'width is bundled into 100 nos. (Table 27).

### Standardization of pre-treatments and storage in banana leaves

For extending the shelf-life of banana leaves, treatment of Poovan and Udhayam leaves with cold water at 20°C for 30 min measured eight days of shelf life against three days at room temperature without any pretreatment. Treatment at 25°C for 30 min registered a shelf-life of seven days when compared to five days at room temperature alone (control-without any pre-treatment).

### Improved postharvest management practices in banana

Under improved postharvest handling, packing and storage of banana, Udhayam banana stored up to 131 days at 13.5°C when

compared to control (14 days). Storage of 80% mature Robusta using 3% O<sub>2</sub>, 10% CO<sub>2</sub> and 87% N<sub>2</sub> gases at 13.5°C as active modified atmosphere packaging (MAP) extended the shelf-life of fruits up to 77 days when compared to control (8 days). Mortman can be stored up to 105 days under MAP at 13.5°C against 35 days under passive MAP. Poovan banana ripened with 100 ppm ethylene gas for five hours in the ripening chamber was highly accepted (with a hedonic scale of 7.41) as compared to smoking with dried leaves of banana (with a hedonic scale of 6.00).

### Development of banana coarse fiber based product

Flower baskets were prepared using coarse fiber extracted from outer-sheath of banana pseudostem (Fig. 31).



Fig. 31. Flower baskets made from coarse fibre of banana

Table 27. Cost of production of banana plant for leaf purpose

Leaf cultivation and sales details	Rs.		
Full length leaf * sold (whole sale)	5.00/full length leaf		
Tiffin leaf (retail market)	0.50 to 0.60/leaf		
Meals leaf	0.80 to 0.90/leaf		
Cost of cultivation	50-60/plant		
Net profit (through contractors)	0.9 lakhs/acre		
Net profit (farmer as producer)	1.50 to 1.75 lakhs/acre		

<sup>\* (</sup>One full length leaf can be cut into two meals leaves and four tiffin leaves)



#### Refinement of banana fig

Banana fig made into different shapes after drying for 48 hr retained its shapes perfectly as compared to other drying regimes of freshly cut shapes after drying (Fig. 32).



Fig. 32. Banana fig made into different shapes



#### 4.4. CROP PROTECTION

#### 4.4.1 Management of Banana weevils

## Isolation and Identification of weevil active volatile components of leaf sheath of susceptible cultivars

Banana leaf sheath volatile was collected from the susceptible cvs. Nendran, Poovan and Musa balbisiana cv. Attikol by solvent extraction method. The extract was subjected to GC-EAD and GC-MS for identifying the stem weevil active volatile and other volatile components. GC-MS analysis indicated 23 -27 volatile components, belong to Alkene, Acetate, Aldehyde, Ketone alcohol and fatty acid. Weevil active volatile components from the leaf sheath extracts were identified using GC-EAD facility for cvs. Poovan (18.2 and 19.8 RT) and Nendran (17.9 and 19.6 RT).where as the wild balbisiana accession did not show any response indicating that weevil active volatiles are not present in the extract (Fig. 33).

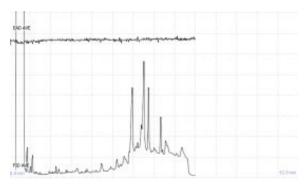
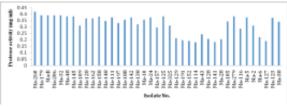


Fig. 33: GC-EAD profile of wild balbisiana (Accn.No. 2028)

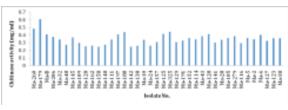
#### Consortium development of endophytic fungi for the management of corm weevil

Cuticle degrading enzymes (Protease, Chitinase and Lipase) from 25 isolates of *Beauveria bassiana* and 39 isolates of *Metarhizium anisopliae* were analyzed by spectro photometric method to develop a fungal consortium for corm weevil management. In

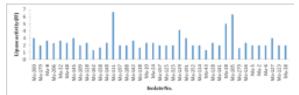
Beauveria bassiana, the maximum protease activity was 0.431 mg/ml in the isolate Ba-0508 and minimum 0.196 mg/ml in Ba-023. Maximum chitinase activity 0.573 mg/ml was recorded in NRCB Beauveria bassiana and 0.294 mg/ml in Ba-280. Maximum lipase activity of 3.0 U was recorded in Ba-0275 minimum and 1.33 U in Ba-1812 and NRCB -Bb. In Metarhizium anisopliae, the maximum protease activity was recorded as 0.417mg/ml in Ma-260 and minimum 0.180 mg/ml in Ma-114. Maximum chitinase activity of 0.608 mg/ml was recorded in Ma-179 and minimum 0.247 mg/ml in Ma-158 and Ma-142. Maximum lipase activity of 6.66 U was recorded in Ma-111 and 1.33 U in Ma-43 and Ma-162 respectively (Fig. 34a-c).



(a) Protease activity



(b) Chitinase activity



(c) Lipase activity

Fig. 34a-c: Cuticle degrading enzymes levels in different endophytic fungi (*Metarhizium anisopliae*)

### Evaluation of improved semiochemical lure for banana weevils

Evaluation of eleven volatile blends against stem weevil by Wind tunnel studies indicated the maximum weevil activity (70.0



to 73.3 per cent) in the treatment No. H and D (Fig. 35a). The time taken to reach the lure by the stem weevil inside the wind tunnel was 2.27 and 2.21 minutes. Among the six treatments tested against corm weevil by Pit fall trap method indicated the maximum weevil activity 55.0 per cent was recorded in the treatment No.H. (Fig. 35b).

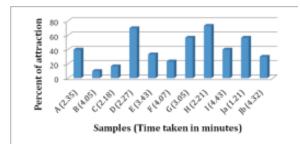


Fig. 35a: Stem weevil attraction to volatile blend

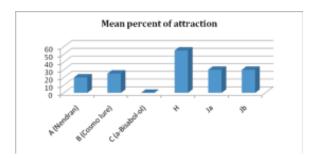


Fig. 35b: Corm weevil attraction to volatile blend , host and semiochemical.

### 4.4.2 Fungal and bacterial diseases of banana and their management

Evaluation of biocontrol agents and botanical for the suppression of Fusarium wilt disease in pre-infected banana plants cv. Rasthali (Mortman)-AAB

Field evaluation of different effective biocontrol agents individually and in different combinations for the suppression of Fusarium wilt disease in the banana plants cv. Rasthali already infected with Fusarium wilt showed that among the bio-agents, the maximum reduction of Fusarium wilt disease (disease score 2 in the 1-6 disease scale) was recorded in the banana plants treated with endophytic

T. harzianum, Endo P. pinophilum, wild P. pinophilum + rhizo T. asperellum, endo. mut. P. pinophilum + rhizo. Mut. T. asperellum, endo P. putida+ rhizo Bacillus spp., endo. mut. Penicillium spp. + rhizo. Mut. T. asperellum, Trichoderma sp. SrT3, Trichoderma sp. Kr2, Endo T. harzianum + rhizo. Mut T. longibrachiatum + Difenoconazole (0.1%) (Fig. 36). The percentage of plants sustained up to harvest was 100% in the above said treatments and only 35.0 percent in the control. Application of bio-agents also increased the plant height (6.8 to 28.4%), girth (2.6 to 27.3%), total number of leaves (5 to 38.9%), leaf area (7.3 to 43.4%), total number of hands (16.6%), number of fingers (30.8%) and bunch weight (from 41 to 101%). Significant difference was noticed in the treated plants as compared to untreated control plants.

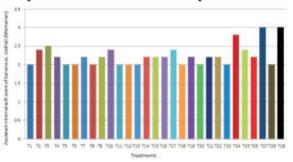


Fig. 36: Field evaluation of bio-control agents on the internal wilt disease score in banana cv. Rasthali (Mortman) pre-infected with *Foc* 

T1-Wild endo. T. asperellum (prr2), T2-Wild endo. P. pinophilum (Bc2), T3-Wild endo. Penicillium sp. (Dsr1), T4-Mut. endo T. asperellum (prr2), T5- Mut. endo. P. pino (Bc2), T6 -Mut. endo. Peni. sp (Dsr1), T7-Wild en. T. asperellum. (prr2) + Wild rhizo T. longibr., T8.-Wild. P. pino. (Bc2) + Wild rhizo. T. aspe. T9-Wild Penicillium sp. (Dsr1) + Wild rhizo T. asperellum, T10-Mut.endo. T. asperellum (prr2)+ Mut. rhizo. T. longibrachiatum, T11-.Mut.endo. P. pinophilum (Bc2) + Mut. rhizo. T. asperellum, T12-Mut.endo. Penicillium sp (Dsr1) + Mut. rhizo. T. asperellum, T13-Endo. P. putida (C4r4) + rhizo. Bacillus cereus (Jrb1), T14-Endo. Achromobacter sp. (Gcr1) + rhizo. B. cereus (Jrb5), T15-. Endo. rhizobium sp.(Lpr2)+rhizo. Bacillus cereus, T16-Endo. Bacillus flexus (Tvpr1) + rhizo. Bacillus cereus (Jrb1), T17-Endo. Bacillus flexus (Tvpr1) + rhizo. Pseudomonas putida (Jrb2), T18-. Trichoderma sp. (SRT3), T19- Trichoderma sp. (SRT3), T20-Trichoderma sp. (KR2), T21-Trichoderma sp.(KR4), T22-Trichoderma sp. (KR8), T23- Mut.endo. T. asperellum (prr2) + Mut. rhizo. T. longibra. + Difena., T24- Mut. endo. P. pinophilum (Bc2)+ Mut. rhizo. T.aspere + Difena., T25- Mut.endo. Penicillium sp. (Dsr1) + Mut. rhizo. T. aspere. + Difena., T26-Difenaconazole (0.1) % alone, T27- Carbendazium (0.1)% alone, T28- Drenching of Zimmu leaf extract, T29-Control (infected suckers).



#### Evaluation of endo and epiphytic fungal and bacterial antagonists for the management of eumusae leaf spot disease

The evaluation of 19 talc based formulations of bacterial isolates (7 epiphytes and 12 endophytes), 8 endophytic fungal isolates and one plant extract effective under in-vitro condition were tested against Mycosphaerella eumusae pathogen with standard control Propiconazole (0.1%) + mineral oil (1%) was studied in cv. Nendran in farmer's field in Vayalur area of Tiruchirapalli district. The analysis of the results on the leaf spot disease severity and youngest leaf spotted-0 (YLS-0) indicated that, five bacterial isolates viz., 9nb, 10bb, 56bb, 21acy and 6M2 recorded 51.2 to 58.3% reduction in disease severity and 8.5 to 34.1% increase in YLS-0 values as compared to untreated control plants during vegetative phase. The results of effective biocontrol agents was comparable with the effect of standard control i. e., Propiconazole (0.1%) + mineral oil (1%) (Fig. 37).

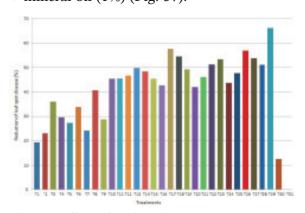


Fig. 37: Effect of native biocontrol agents on the percentage reduction of leaf spot disease over control

T1-1DF, T2- 2DF, T3- 3DF, T4- 6DF, T5- 7DF, T6 - 9DF, T7-11DF, T8-12DF, T9- 3NB, T10- 4NB, T11- 5NB, T12- 7NB, T13- 9NB, T14- 14NB, T15- 24BB, T16- 7BB, T17- 10BB, T18- 17BB, T19- 19BB, T20-56BB, T21- 60BB, T22- 10acy, T23- 21 acy, T24- 6BB, T25- 6M2, T26- 1E2, T27- 6E, T28- Zimmu leaf extract, T29- Tilt + oil, 30- Talc alone, 31- Control

### Isolation and identification of *Erwinia* spp. causing rhizome rot disease

A total of six isolates of *Erwinia* spp. causing rhizome rot were isolated, purified and

pathogenicity test conducted have resulted in identification of three isolates as *Erwinia* which caused corm rot in cv. Grand Naine banana.

### Screening of botanicals for the effective inhibition of *Erwinia* spp. of banana

A total of 11 botanicals and 11 bacterial isolates obtained from the banana rhizosphere were screened against *Erwinia* spp. under *in vitro* condition by poison food technique and agar well diffusion methods. The results showed that among the botanicals and bio-control agents, only Zimmu leaf extract at 50% conc. resulted in complete inhibition of *Erwinia* spp.

### Evaluation of potash solubilizing ability of fungal and bacterial solubilizers

Bacterial (26) and five fungal isolates were applied separately in the pots planted with cv. Grand Naine for their potash solubilizing activity. The source of K<sub>2</sub>O is Mica which was applied @ 100g/pot. The results indicated that the bacterial isolates *viz.*, NRCB KSB 16, 17, 20 and 26 were found more effective than others in sustaining the exchangeable potassium in soil with increasing time and the order of efficacy among the isolates was 16>17>20>26.

### Evaluation of *Foc* effective fungal bio-control agents on the root lesion nematode of banana

The endophytic fungal bio-control agents (BCA) such as *Trichoderma* spp. and *Penicillium* spp. were further evaluated under green house condition for the suppression of root lesion nematode *Pratylenchus coffeae* in cv. Grand Naine. The BCA were applied in two different methods *viz.*, 1. BCA application 10 days after the nematode inoculation and 2. Nematode inoculation after 10 days of BCA application. The observations on the root lesion index, population of nematodes/g of soil and root weight was taken 3 months after application. The results indicated that among different fungal BCA, soil application of *Trichoderma asperellum* and *T. viride* recorded 90-93%



reduction in root nematode population in cv. Grand Naine with a root lesion index of only 1.2 as against 2.8 in the control plants (on a root lesion index scale of 1-5) (Fig. 38). Generally soil application of BCA was effective before nematode inoculation.

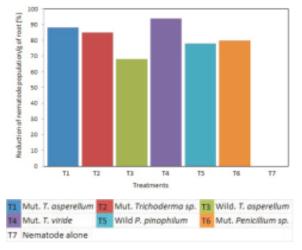


Fig. 38: Effect of Fusarium wilt suppressive biocontrol agents on the population of root lesion nematodes in banana plants cv. Grand Naine

#### 4.4.3 Studies on viral diseases and their management

#### Survey for viral diseases

Survey was undertaken in Karur, Tiruchirapalli, Thanjavur and Theni districts of Tamil Nadu for banana viral diseases. The percent incidence of BBTD varied from 30 to 100% in cv. Poovan in Thirukattupalli. Severe incidence of Banana Streak Virus (BSV) and Banana Bract Mosaic Virus (BBrMV) was recorded in surveyed orchards.

#### Molecular Characterization of banana viruses

Multiple alignments of CP gene of 49 BBrMV isolates including 22 isolates generated this year showed nucleotide (nt) and amino acid (aa) identity of 79-100 and 80-100 %, respectively. Phylogenetic analysis revealed that two isolates viz., TN14 and TN16 were distinct and the rest clustered together (Fig. 39). Eleven recombination events were detected

using Recombination Detection Program. Codon-based maximum-likelihood methods revealed that most of the codons in the CP gene were under negative or neutral selection except for codons 28, 43, and 92 which were under positive selection. Gene flow between BBrMV populations of banana and cardamom was relatively frequent but not between two different populations of banana infecting isolates.

Sequence analysis of partial fragments of RNA-1, RNA-2 and cp gene in RNA-3 of CMV infecting banana sample showed that 90-99% nucleotide identity with isolates of CMV sub group IB. Complete genome of a new BSV species spontaneously expressed from Virupakshi (syn: Hill Banana) has been amplified using RCA method and cloned. Approximately 7000 bp sequence was obtained by primer walking from the whole genome clone. The sequence analysis revealed that 99% sequence similarity with the Banana Streak Gold Finger Virus (BSGFV).

A full length genome of BSV species infecting cvs. Rasthali and Poovan were cloned into pUC18 vector. Seventeen partial RT-RNaseH gene sequences of BSMYV from cv. Poovan collected from Trichy and Karur districts of Tamil Nadu have been characterized. The sequences of 17 isolates were analyzed using Bio-edit programme. The result revealed that the partial RT-RNaseH gene of BSV isolates shared an identity of 91.0-100% at nucleotide level (nt) and 88.0-100% at amino acid (aa) level respectively. There were eight isolates representing Musiri, Kulithalai, Kattuputhur and Lalgudi which had 100 per cent sequence homology at nucleotide level and also amino acid level. Isolate LAL 3 exhibited lowest homology of 91.3% at nucleotide and 88.3 % at amino acid level when compared to other isolates.

HC-Pro gene was amplified, cloned and sequenced for 25 DAC-ELISA positive BBrMV isolates collected in a survey in and



around Trichy. The genetic diversity analysis revealed that HC-Pro gene of Trichy isolates shared identity of 85.3-98.9% at nucleotide level (nt) and 76-99.5% at amino acid (aa) level with the published sequences.

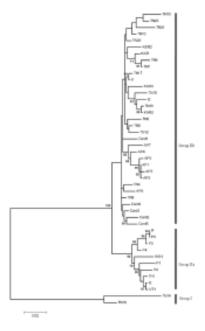


Fig.39: Phylogenetic analysis of coat protein (CP) gene of amino acid sequences of banana bract mosaic virus (BBrMV) isolates from different parts of the world. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Bootstrap scores above 50 % (1000 replicates) are placed at the tree nodes. The scale bar represents the number of nucleotide substitutions per site. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. TN- Tamil Nadu: AP-Andhra Pradesh: KER- Kerala: KAR-Karnataka. TN4, TN5, TN11, TN15, TN16, TN17, TN18, TN19, TN22, TN23 and I3-Tiruchirapalli; TN6- Pudukottai; TN8 and TN9-Theni; TN10-Karur; TN12-Cuddalore; TN13 and TN14-Thanjavur; TN21,I1 and I2-Coimbatore; AP7-Kovur; KAR2-Bangalore; KAR3-Arabhavi; KER2-Kasargod; KER3-Kayankulam. TN, AP, KER, KAR, I are belongs to Indian origins: P- Philippines: WS -Western Samoa: VT- Vietnam: THI- Thailand. Card 1, 2, 3, 5 and 6 are coat protein gene of BBrMV infecting cardamom.

#### Diagnostic techniques for banana viruses

- ◆ DAC-ELISA was standardized for detection of episomal BSMYV using recombinant antiserum of viral associated protein. The titre of the antiserum was 1:8000 dilution and 1:20 dilution of antigen.
- ◆ IC-PCR for the detection of episomal BSMYV was standardized.
- ◆ RT-PCR was more sensitive for detection of BBrMV followed by IC-RT-PCR and DIBA than DAC-ELISA
- ◆ Different parameters of Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP) technique (Fig. 40) such as temperature and concentration of betaine and MgSO₄ were optimized for the detection of BBrMV.
- Multicomponent multiplex PCR based detection of BBTV was validated using 30 samples collected in the survey. Except one sample, all six components were detected in the remaining samples.

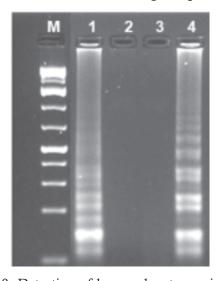


Fig. 40: Detection of banana bract mosaic virus (BBrMV) by Reverse Transcriptase Loop- mediated isothermal amplification (RT-LAMP). Agarose gel electrophoresisis analysis of RT-LAMP products: M-Marker; Lane 1, 4: BBrMV infected banana plant sample; Lane 2, 3: Healthy banana plant sample



#### Screening germplasm against banana viruses

Sixteen banana accessions from *Musa* germplasm belonging to sub-group Mysore (AAB) were screened against BSV species using RCA approach and also by IC-PCR. Twelve accessions were positive in RCA and 16 were positive in IC-PCR.

Out of 17 samples received from Arabhavi, two were positive for CMV. Among nine samples received from Fruit Research Station, NAU, Gandevi, one sample (cv. Saba) was positive for BBTV and the rest were negative for all viruses. Twenty five germplasm samples received from OUAT-Buvaneshwar were tested for four banana viruses indicated two samples was positive for BBrMV and the rest were negative for all viruses. Mother plants of cv.Udhayam were tested against BBTV, CMV, BBrMV and BaMMV for establishing mother block for mass propagation at NRCB farm.

Host-virus interaction in Banana: Molecular mechanism of resistance and susceptibility, latency, integration and episomal expression of EPRV's

## Analysis of yield, expression of BSV symptoms, symptom severity in the permanent field trail for cultivar Poovan

Non-symptomatic Poovan plants planted during the year 2005-06 were continued for 8th ratoon crop in 2013-14. This year the expression of BSV symptom was observed newly in 31 plants. Out of 560 plants, so far 127 plants have expressed the symptoms of streak disease during the last 8 years. Analysis of disease severity index, yield, girth and plant height over the years revealed that higher variation between the years and the weather factors prevailed in the corresponding year might have influenced for the wide variation.

### Comparison of BSV severity in TC and sucker grown plants of cv. Poovan

The ratoon crop data of field grown BSMYV infected Poovan plants obtained through tissue culture was compared to plants raised through conventional suckers. The streak virus severity index and yield loss were significantly higher in TC plants than healthy as well as sucker grown infected plants.

#### Develop constructs of partial dimers for different genomic components of BBTV for testing infectivity

Eighteen primers were designed to construct partial dimer of all six genomic components of BBTV. PCR amplification was standardized using newly designed primers. Initially partial dimer for BBTV-DNA 4 was cloned in pUC-18 vector and sub cloned in pBin19 binary vector and transformed to Agrobacterium strain LBA 4404 and AGL1 for infectious clone development.

### Differentially expressed transcripts/proteins in BSMYV infected/ healthy cv. Poovan.

Proteomics study to identify differentially expressed proteins in streak virus infected and healthy plants of cv. Poovan was undertaken. App.1000 reproducible spots were identified in each of the healthy and BSV infected Poovan banana plants (Fig. 41). Thirty spots which showed two- fold differences in intensity were selected for peptide mass fingerprinting. Of those, thirty proteins identified by PMF, 16 were up-regulated and 14 got down-regulated in the BSV infected samples. These proteins were found to be involved in defense, signal transduction, cell structure and function, photosynthesis and energy, plant growth, protein designation/ storage and transcription/ translation.

#### Latency studies in BBTV

When BBTV was inoculated on to TC bananas through aphids, latency was observed





Fig. 41: Leaf proteome profiling of differentially expressed proteins in BSMYV infected / healthy cv. Poovan by 2-D PAGE. Proteins were fractioned using IPG gel strips (18 cm) in first dimension and 12% SDS-PAGE in second dimension

on inoculated plants. The latent period varied between 24.6 days to more than 159 days. Semi-quantitative PCR has revealed that during latency the viral load was substantially lesser than infected (symptomatic) plants. Latency was also recorded in vectors.

## Isolation and characterization of endophytes associated with BBTV infected and healthy Grand Naine banana plants

Endophytic bacterial profile varied between BBTV infected and healthy bananas as well as between viruliferous and non-viruliferous aphids. Totally 16 endophytic bacteria were identified and associated in banana when infected with BBTV. Staphylococcus saprophyticus, Bacillus subtilis, S. scuiri, Bacillus megaterium, B. firmus, B. megaterium, B. aryabhattai strain XB168, B. licheniformis strain YC1-A, Pseudomonas sp. S3 (2013b), B. amyloliquefaciens strain AR-2, B. subtilis strain M6-7 and B. subtilis strain B068150 were some of the endophytic bacteria isolated from banana and aphids.

### Influence of endophytic bacterial treatments on BBTV infection in banana

Two endophytic bacteria namely *Bacillus* subtilis and *B. pumilus* were isolated from banana and a rhizosphere bacteria *Pseudomonas* flourescens were inoculated to tissue culture plants of cv. Grande Naine a day after BBTV

inoculation and grown in a pot culture. Plant inoculated with bio-agents expressed bunchy top symptoms earlier than control plants. The percent disease incidence ranged from 33.3 to 58.3 in bio-agents inoculated plants as compared to 16.6% in the un-inoculated control plants. Non-symptomatic, bio-agents inoculated plants significantly increased the plant growth parameters than control.

### **4.4.4** Proteomic analysis of host–BBTV interaction in banana

The healthy Hill banana cultivar grown under glass house conditions was taken as reference for the banana leaf proteome map. Protein was isolated from healthy and infected Hill banana shoot; 200 mg of protein was used for 2D analysis. 2-DE analysis was carried out from three biological replicates using the pH 4-7, 13 cm IPG strips with silver staining method. For each sample, the gels were repeated at least in triplicate and showed a high level of reproducibility. Typically, more than 1000 protein spots were reproducibly detected by Melanie software on each silver-stained gel. Out of 40 spots, 39 were annotated, spots that did not result in protein identification were probably due to low abundance of protein in the spot or occurrence of post-translational modifications or sequences absent in the database (Fig. 42). The identified proteins presented MASCOT score values ranged from 32 to 96, ensuring homology to proteins from the database used in the analysis. The reliability of the results was reinforced by the observation that all the proteins were identified as the same protein using either the banana EST database or banana genome sequence information (http://banana-genome.cirad.fr). To analyze the proteomic profile of the Hill banana leaf, all proteins identified from the reference proteomic map were functionally clustered. Most of the proteins were involved in defence (28%), followed by signal transduction (18%), metabolism (9%), plant growth (10%), cell division and structure (16%), photosynthesis/



energy (18%), protein synthesis (5%) and protein designation (5%).

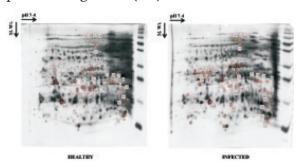


Fig. 42: Silver-stained 2-DE gel of proteins from healthy and infected hill banana shoot

The Hill banana 2-DE reference map was subsequently used for landmarking and identifying proteins that have altered levels upon BBTV infection. Upon Melanie software aided differential display, 40 of all reproducible protein spots detected by 2-DE showed at least 1.5 fold change (p<0.05). In total, 25 upregulated and 14 down-regulated protein spots were observed in the infected samples. The proteins observed with the highest induction levels were: Mitogen-Activated Protein Kinase, Calmodulin motif containing protein, ABC transporter, Cinnamyl alcohol dehydrogenase. Defence related and signal transduction proteins were among the highest induced proteins in BBTV infected Hill banana leaves. Plant metabolism and growth related proteins were up-regulated whereas protein synthesis, photosynthesis and cell division related proteins were down-regulated which can be corroborated with the symptom development due to BBTV infection.

#### Validation of proteomics results through Semiquantitative-PCR and biochemical analysis Semiquantitative-PCR

Semi-Quantitative PCR analysis was carried out for 25 genes from each functional category which showed significant differential expression in 2-DE analysis. Cinnamoyl-CoA reductase 1 (CCR), Feruloyl CoA Ortho-Hydroxylase (FCOH), Exopolygalacturonase (EXO), GDSL esterase/lipase, Farnesyl Pyro

Phosphate Synthase 1(FPPS), Peptide deformylase 1B (PD), MAPK, Calmodulin 8, Cinnamoyl Alcohol Dehydrogenase (CAD), PhotoSystem I, Oxygen Evolving Enhancer Complex (OEEC), Argonaute 16, Cytochrome 450, 40S Ribosome, RUBISCO, SUMO-activating enzyme subunit 1B-1, Vacuolar-processing enzyme, Threonine Protein Kinase, 26S proteosome regulatory complex component, Peroxidase and ATP Synthase Beta Subunit genes gave single band and corroborated with the protein result.

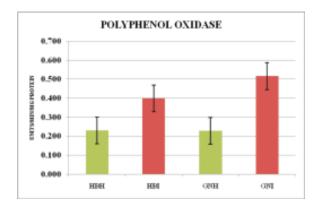
#### Estimation of antioxidant enzymes and hormones from healthy and BBTV infected hill banana plants

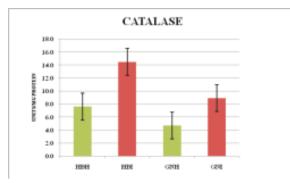
Oxidative stress due to viral infection is essential in pathogenesis. Increased ROS catalyzes the enzymes of cell membrane and DNA damage, enzymatic antioxidant system are activated which detoxifies the ROS effect. Oxidative stress in compatible virus-host plant interactions was studied in BBTV-infected Hill banana and Grand Naine plants. BBTV infected plants showed induction of catalases, peroxidases, PPO, APX, GPX, total phenol and total protein. Specific activities of these enzymes were also estimated (Fig. 43). Free radicals produced in response to hypersensitive signals the plant defense system to prevent the further penetration and proliferation of virus.

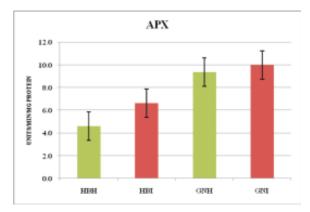
#### **Root Proteomics**

Reproducible spots (>850) were identified and 60 spots which showed two fold difference were sent for PMF by MS. Of those, 58 proteins identified by PMF, 23 were upregulated and 35 got down-regulated in the BBTV-infected samples. These proteins were found to be involved in defence, signal transduction, cell structure and function, photosynthesis and energy, plant growth, protein designation/storage and transcription/ translation. ATP synthase beta-subunit, Antiviral protein S, ETF-Beta and putative









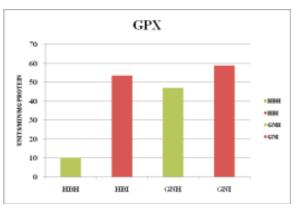
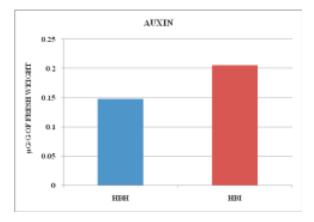


Fig. 43: Specific activity of enzymes (units/min/mg of protein)

Hormones was also quantified in BBTV-infected and healthy Hill banana plants. GA was found to be higher in healthy plants than BBTV infected Hill banana plants whereas auxin showed reverse trend (Fig. 44).



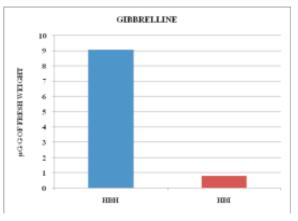


Fig. 44: Hormonal levels in BBTV-infected and healthy hill banana plants

serine/threonine protein kinase was upregulated upon infection.

#### **Phloem Proteomics**

Acetone-HCl protein extraction method was standardized for phloem protein isolation for 2-DE. This method produced good resolution on SDS-PAGE. More number of bands was observed than the other four methods. A total of >50 reproducible spots were identified and 30 spots which were found to show two fold difference were sent for PMF by MS.



#### 4.5 EXTERNALLY FUNDED PROJECT

4.5.1 Improved livelihoods through conservation and cultivation of near extinct landraces of banana of Kolli Hills (PI-S.Uma, Co-PIs-M.S.Saraswathi and S.Backiyarani)

Three batches of 300 plantlets of Karuvazhai were multiplied and distributed to the farmers of Semmedu, Mekhanikkadu and Melvazhavu villages of Kolli Hills during the first training programme. Handouts on cultivation practices were distributed to the farmers and they were trained on the method of application of manures and fertilizers and also to identify the nutrient deficiencies. They were also trained to differentiate the symptoms produced by major pest and diseases affecting banana. Periodical visits were made for recording the growth observations and monitoring the plants established at Kolli Hills.

A second training programme on macropropagation was conducted in targeted areas, in which farmers' were trained on the identification of disease free plants and criteria to be considered for the selection of suckers i.e., age, size and type of suckers. During the second training programme, kit containing growth regulators and fungicides were provided to the farmers.

Fourth set of hardened tissue cultured Manoranjitham plants (380 plants) are ready for the establishment of field demonstration





Fig. 45: a) and b) Women beneficiaries conserving the tissue cultured Manoranjitham distributed by NRCB, Trichy at their backyards

block at different locations in Kolli Hills. Manoranjitham plants supplied during Feb. 2013 have started yielding and yield data are being recorded (Fig. 45).

4.5.2 DBT-QUT Project on Biofortification and development of disease resistance in banana

Component - I Transfer and evaluation of Indian banana with PVA constructs and providing authentic virus free IMFB to Indian partners (PI - S.Backiyarani, Co-PI-S.Uma and M.S. Saraswathi)

Embryogenic cell suspension of cvs. Grand Naine and Rasthali were received from NRCB Component 3 and utilized for transformation of Gen I constructs. A total of eleven co cultivations have been carried out with Gen I PVA constructs of pBMGF-DCpBMGF-DC-34, pBMGF-DC-32, pBMGF-DC-35. The transformed cells were sub-cultured in different concentration of antibiotic medium. The survived cells were sub-cultured into regeneration medium. After two weeks the survived embryos were sub cultured into germination medium. It was observed that the response of co-cultivated cells of Rasthali and Grand Naine was found to be normal in the selection medium having Kanamycin. But the rate of proliferation is high in case on Grand Naine is high when compared to Rasthali.

Component - II Transfer and evaluation of Indian bananas with iron gene constructs (PI - M. Mayil Vaganan Co-PI - C. Anuradha)

Four Gen I iron gene constructs *viz.*, pBMGF-DC-52, pBMGF-DC-53, pBMGF-DC-57 and pBMGF-DC-58 received from QUT, Australia in a lyophilized form, was diluted with sterile water and an aliquot was visualized on 0.8% agarose gel electrophoresis. The constructs were transformed into *E. coli* cells (DH5á) by heat shock method using the



competent cells prepared with 100 mM calcium chloride. After the transformation, a single colony isolated from every plate was short streaked on a LB medium plate with 100 mg/l kanamycin, grown for 12 hrs at 37 °C and the presence of transgenes in *E. coli* cells was confirmed through colony PCR. Plasmid DNA isolated from transformed *E. coli* bacterial colonies was analyzed and confirmed with different primersets designed based on the genes sequences and also specific primer sets provided by the QUT (Fig. 46) and *E. coli* cultures were stocked in glycerol.

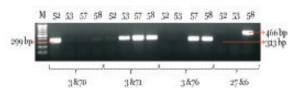


Fig. 46: PCR confirmation of iron constructs using primer sets given by QUT. Primer set 3&70 shows positive amplification for *CrFea1* gene in iron construct pBMGF-DC-52. Primer set 3&71 shows positive amplification for *OsNas1* gene in iron construct pBMGF-DC-53, 57 & 58. Primer set 3&76 shows positive amplification for *OsYSL2* gene in iron construct pBMGF-DC-57 & 58. Primer set 27&6 shows positive amplification for *SoyFerritin* gene in iron construct pBMGF-DC-52

*E.coli* cells carrying the iron constructs were individually transformed into *Agrobacterium* strain, AGL1 using the technique of Triparental mating with the help of helper strain PRK2013. The transformed cells were selected in the LB agar media with the antibiotics kanamycin (100 mg/l) and rifampicin (25mg/l). Selected *Agrobacterium* colonies were confirmed by the PCR using the construct-specific primers of QUT.

Initially, cocultivation with iron constructs was performed as trial with one ml SCV of Rasthali and Grand Naine ECS received from Component-III of the network project. In the initial stage, the Grand Naine ECS cocultivated with constructs failed to produce the embryogenic response in embryogenic medium and as a result, few of the unresponded Grand

Naine cells were discarded documentation. The embryogenic response of Rasthali ECS was higher when compared the Grand Naine ECS. Rasthali and Grand Naine ECS were cocultivated with all the four iron constructs in a round basis and eighteen cocultivations were performed till now. Twice each of Rasthali and Grand Naine cells were cocultivated with construct pBMGF-DC-52; thrice of Rasthali and twice of Grand Naine cells with construct pBMGF-DC-53; twice of Rasthali and five times of Grand Naine cells with pBMGF-DC-57 and once of Rasthali and Grand Naine cells with pBMGF-DC-58. The cocultivated cells responded well in the embryogenesis in the presence of kanamycin (100mg/1) (Fig. 47). Standardization of embryogenesis and its germination are in progress.

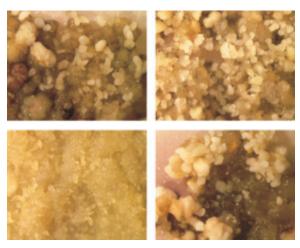


Fig. 47: Embryogenesis of cocultivated ECS

Component –III: Development of efficient ECS for Rasthali and providing to Indian partners (PI-S.Uma, Co-PI-S.Backiyarani)

### I: Supply of immature male flower buds to partner institutes

Immature male flower buds of cv. Rasthali and Grand Naine and ECS were supplied to partner institutes NABI, BARC (Table 28 & 29).



Table 28. Immature male flower buds for transfer to partner institutes

Name of the partner institute	su	te of pply	Cultivar	Number of buds supplied
NABI	Aug.	8/2013	Rasthali	52
			Grand Naine	100
BARC	Oct.	19/2013	Rasthali	-
			Grand Naine	50
NABI	Apr.	4/2014	Rasthali	50
			Grand Naine	100

Table 29. List of ECS supplied to partner institutes

Name of the partner institute	supply	Cultivar	SCV
BARC	Oct. 19/2013	Grand Naine	1 m1
IIHR	Apr. 4/2014	Rasthali	1 ml
TNAU	Mar. 20/2014	4 Rasthali	1 m1
NABI	Sep. 15/2013	Rasthali	6 ml

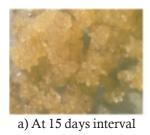
### Suspension establishment and maintenance

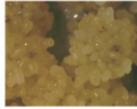
### Medium standardization to improve the proliferation of suspension cultures

Few embryogenic clumps were transferred to 3-5ml medium with gradual scaling up of the medium. On comparision, cv. Rasthali responded well in MA2 (without zeatin) medium as well as in M2 (supplemented with zeatin), whereas cv. Grand Naine responded only in M2 medium. Proliferation capacity was maximum during first 3-4 months after initiation of suspension culture and attained a stable proliferation rate after 5 months (Fig. 48a-d).

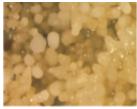
#### Establishment and regeneration of ECS

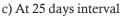
ECS once developed has the capacity to multiply and regenerate beyond 18-24 months.

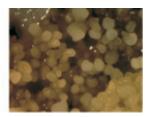




b) At 20 days interval







d) At 45 days interval

Fig. 48a-d: Stages in development of somatic embryos

But regeneration with better germination and development into plantlets was found to deteriorate over time. In cv. Rasthali best regeneration was observed during 5-6 months after initiation of ECS.

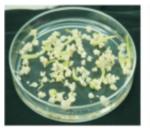


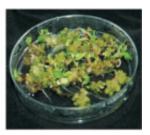
QUT team visit to NRCB

### Germination of somatic embryos into complete plantlets

After 45-50 days, well developed mature somatic embryos were transferred to regeneration medium. Compared to M4 medium, MA4 medium was found to be the best for germination of plantlets. Healthy plants with well developed shoots and roots was obtained in MA4 whereas shoots were slender and pale in M4 medium (Fig. 49a-c).

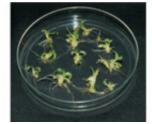






a) After 10 days

b) After 30 days



c) After 45 days

Fig. 49a-c: Different stages of development of somatic embryos to complete plantlets

Functional genomics for Sigatoka (PI-S.Uma Co-PI-R. Thangavelu, S. Backiyarani and M.S. Saraswathi)

#### 'B' Genome whole transcriptome

Next generation sequencing was performed for Musa balbisiana cv. Attikol using Ion torrent platform. Musa transcriptome reads were assembled using a MIRA tool and generated 82413 contigs. BLAST2GO pipeline program against SWISSPROT, TrEMBL, COG and PlantCYC databases was used to functionally annotated. 35783 protein coding genes were identified from the BLAST results and 193826 Gene ontology terms were assigned as a function for these proteins. 3301 unigenes were found to be homologous to known defense-related genes and pathways like Perception of PAMPs by Pattern Recognition Effector-triggered Receptors (PRRs), immunity (ETI), Ion fluxes, Transcription factors (TFs), Oxidative burst, Pathogenesisrelated proteins (PRs), Programmed cell death (PCD), Plant hormones, Cell wall modification, etc. (Fig. 50 a-c).

4780 SSRs and 7818 SNPs were identified using MISA tool and Ion torrent software suite.

523 primers were designed. 30 SSR primers which are related to plant defence mechanism were synthesized and out of which 17 EST-SSR primers were validated for about 37 samples in which 20 were from B genome cultivars. Results indicated that these defense related SSR could clearly delineate *M. balbisiana* and *acuminata* accessions. Four primers, SSR17, 21, 23 and 26 (UDP-arabinopyranose mutase, Phosphoenolpyruvate carboxykinase, xyloglucan endotransglucosylase and Uridine cytidine kinase respectively) exhibited unique bands in *M. balbisiana*.

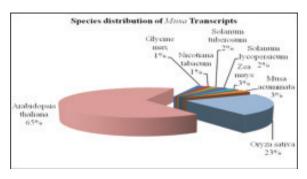


Fig. 50a: Species distribution of *Musa* unigenes from the annotation results

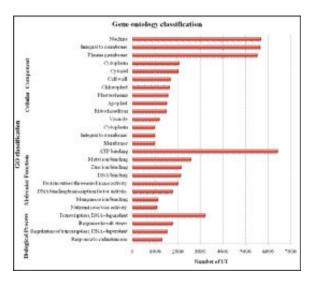


Fig. 50b. Histogram representation of Gene Ontology Classification of the *Musa* unigenes distribution in Cellular component, Molecular function and Biological process



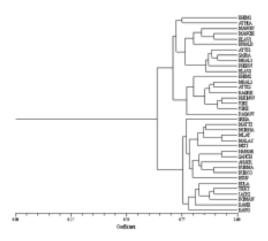


Fig. 50c: Dendrogram for phylogenetic relationship between B and other genome in banana based defense related EST-SSR markers resulted from B genome whole transcriptome.

### Host pathogen (Musa – M. eumusae) Gene Expression Studies

Musa - Sigatoka gene expression was performed for 12 defense related genes which were from Sigatoka challenged Musa cDNA-SSH library which resulted in cytochrome oxidase, lipoxygenase, metallothionein, falvin containing monoxygenase were found to be upregulated only in resistant cultivar (Fig. 51a&b; 52a&b and 53a&b); whereas auxin responsive factor, serine glyoxylate, retrotransposon ty1copia were found to be highly up-regulated in resistant cultivar, when compared to susceptible cultivar. Brownhopper induced resistant protein were up-regulated only in susceptible cultivar; even though ethylene responsive factor, senescence-associated protein, aleurain-like protease and zinc finger were found to be highly up-regulated in susceptible cultivar, compared to resistant cultivar.

Next generation sequencing was performed using Illumina Hiseq platform to study resistance mechanism involved in banana against Eumusae leaf spot disease; which is one of the most devastating diseases. Samples were collected from *Mycosphaerella eumusae* challenged, four leaves stage Tissue cultured

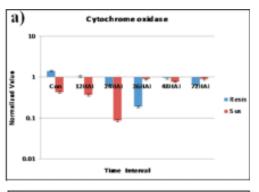




Fig. 51a&b: Real Time PCR profiles of flavin containing monoxygenase and cytochrome oxidase

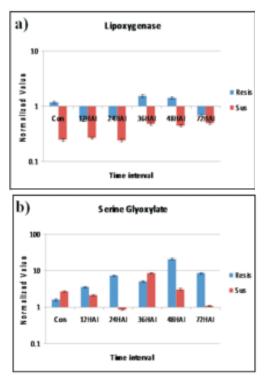
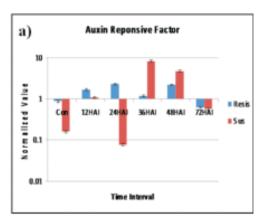


Fig. 52a&b: Real Time PCR profiles of flavin containing lipoxygenase and serine glyoxylate





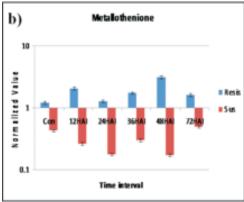


Fig. 53a&b: Real Time PCR profiles of Auxin Responsive factor and Metallothenione.

plants of contrasting cultivars (resistant cv. Manoranjitham and susceptible cv. Grand Naine) with similar genetic background, under controlled conditions. Pair end sequencing was performed with mean read length of 100 bp. Approximately 36 million reads were generated with 100% high quality reads. Reads were assembled using TOPHAT2 and Cufflinks using *Musa acuminata* genome sequence as reference, and generated contigs of about 45609 in each sample. Contigs were annotated against *Musa* and *Viridae Plantae* databases.

#### Musa Sigatoka Transcriptome Studies

Cuffdiff V.2.0.1 program then calculates the differential gene expression. Differential gene expression (DGE) was performed various combinations like challenged resistant and susceptible; unchallenged resistant and susceptible. Results were categorized into up, down and neutral. In challenged resistant and susceptible totally 16761 and 18068 were neutrally regulated; 4315 and 4658 were up regulated; 3247 and 3261 were downregulated in *Musa* and *Viridae Plantae* database respectively. With respect to unchallenged resistant and susceptible about 9895 and 10564 neutrally regulated; 5660 and 6135 were found to be up-regulated; 6782 and 6625 were down regulated in *Musa* and *Viridae plantae* database respectively.

SSR were predicted using MISA perl program and resulted in about 27,214, out of which about 8470 from challenged resistant and 9239 from challenged susceptible; others from unchallenged samples and primers were also designed. SNP discovery was done using Illumina software suite, totally 355 SNPs were resulted in challenged resistant and in susceptible about 271.

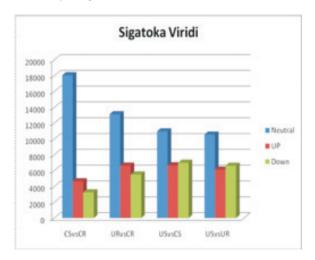
KEGG pathway analysis revealed defense related pathway like Phenylpropanoid, Flavonoid, Flavone, Carotenoid, Steroid and flavonol biosynthesis; Linoleic acid, Arachidonic acid, Riboflavin, Cyanoamino acid, Amino sugar and nucleotide sugar metabolism, etc.

Sigatoka resistant transcripts were subjected to identify the transcription factors by using PlantTFcat: An Online Plant Transcription Factor and Transcriptional Regulator Categorization and Analysis Tool. Totally 93 transcription factors were identified and C<sub>2</sub>H<sub>2</sub> Zinc finger protein was found to be most abundant. 1054 SNPs and 211 Indels were identified from Musa Transcriptomics which includes Sigatoka, Drought and Nematode. SNAP tool analysis indicated that 288 were synonymous and 750 were non synonymous among the SNPs identified in resistant and susceptible cultivars of Sigatoka, nematode and drought transcriptome with reference to banana genome. 1054 SNPs were annotated by using Blast2GO tool. 16, 48



Novel miRNA and its targets were also predicted for the unique genes of Sigatoka, Nematode transcriptome using Plant MicroRNA database (PMRD) respectively.

Molecular mechanism involved was derived from unigenes resulted from CS (challenged susceptible) vs CR (challenged resistant) Digital Gene Expression (DGE) of banana *Eumusae* transcriptomics. About 20 biological processes were involved in resistance namely Cell wall related (46), Antifungal (31), JAS (19), SA (16), Plastid (16), Innate & Basal immunity (13), Plasma membrane (11), Phenyl propanoid pathway (11), Transcription factors (11), Hydrogen Peroxide (10), Cell death (10),



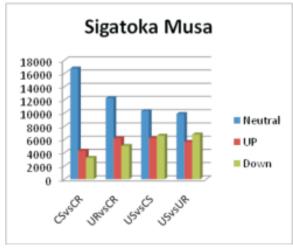


Fig. 54: DGE results of Musa Eumusae Transcriptome

Ethylene pathway (10), PR (10), Hypersensitive reaction (10), PAMPS (9), Lignin pathway (8), Signal transduction (8), Photosynthesis related (7), ROS (7), Auxin (3).

Musa Sigatoka resistance mechanism was derived using 256 up-regulated unigenes of CR vs UR resulted from DGE (Fig. 54). Unigenes were categorized into cell wall related, PAMPS (Pathogen Associated Molecular Patterns), (Reactive Oxygen Species), hypersensitive reaction, Salicyclic acid pathway, jasmonic acid, Phenylalanine ammonia-lyase, antifungal Phenylpropanoid pathway, NPR1, Shikimate pathway, Transcription factors. Among which about 83 unigenes were found to be involved in cell wall related and PAMPS; whereas for ROS is 44 and with regards to transcription factors is around 26.

## 4.5.3 Developing DUS guidelines for banana (PI-S.Uma Co-PIs S.Backiyarani and M.S.Saraswathi)

Draft framework of DUS guidelines has been prepared and submitted to the Authority based on the discussions held during two task force meetings held for finalizing the DUS guidelines for Banana.

# 4.5.4 Accreditation Test Laboratory for Certification of Tissue culture raised plant material under NCS-TCP (PI-S.Uma Co-PI-M.S.Saraswathi)

Three hundred and nineteen batches of tissue cultured bananas at various stages of production inclusive of three varieties namely Grand Naine, Robusta and Monthan have been tested for their genetic fidelity using SSR and ISSR markers under DBT – ATL and issued test reports. This alone has generated an income of Rs. 5.37 lakhs to the Institute.



# 4.5.5 Development of banana central core stem slicer and juice extractor (PI-K.N.Shiva)

As a collaborative work, banana central core stem slicer and juice extractor was developed at Central Institute of Agricultural Engineering-Regional Centre, Coimbatore.

# 4.5.6 DST Project: Identification of molecular strategies for the control of Cosmopolites sordidus—a major pest of bananas (PI-B.Padmanaban)

#### In-vitro screening of selected genotypes

The corm weevils collected from different banana growing areas were screened against selected genotypes namely Nendran (AAB), Poovan (AAB), Attikol (BB) and Karpuravalli (ABB). After 35 days of weevil introduction, the number of adult weevils, eggs, larvae and pupae were recorded.

Among the four cultivars screened against corm weevil collected from various locations, Nendran was found as a highly susceptible cultivar and a minimum fecundity of 14.5 and maximum 21.25 was recorded. In cv. Karpuravalli maximum weevil mortality was recorded indicating that the cultivar is resistant to corm weevil.

### Bio-assay trials using the wheat inhibitor against banana corm weevil grub, C. sordidus

Amylase inhibitor bioassay was studied for monitoring of banana weevil larval growth in amylase inhibitor infiltrated banana stem discs. One grub per stem disc was released and observed daily for grub mortality. Amylase inhibitor assay was conducted against 2<sup>nd</sup> instar corm weevil grub. Among the four treatments (15 mg/20 ml, 37.5 mg/20 ml, 75 mg/20 ml, Control), 100% mortality was recorded in the maximum concentration (75 mg/20 ml) on the 8<sup>th</sup> day.

# 4.5.7 DBT Project: Molecular approaches for the control of *Odoiporus longicollis* Olivier) (Coleoptera: Curculionidae) a major pest of bananas (PI-B.Padmanaban Co-PI R.Thangavelu)

### In-vitro screening of selected genotypes against stem weevil

The collected weevils from different banana growing areas (TN, KL, KNK.MH and Assam) were screened under *in vitro* conditions. The selected genotypes *viz.*, Poovan (AAB), Attikol (BB), Karpuravalli (ABB) and Nendran (AAB) stem pieces were cut in to 30 cm length and kept in a 40L container for feeding and fecundity development. Four weevils were released in each container and observation was taken after 35 days. Among these four cultivars, Poovan was founded as highly susceptible and Attikol as resistant to stem weevil infestation based on their fecundity and mortality.

### Bio-assay trials using the wheat inhibitor against banana stem weevil grub, O. longicollis

Amylase inhibitor bioassay was conducted for the monitoring of banana weevil larval growth in amylase inhibitor infiltrated banana stem discs. One grub per stem disc was released and observed daily for grub mortality. Amylase inhibitor assay was conducted against stem weevil grub of second instar larvae. Among the three treatments tested

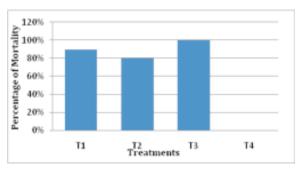


Fig. 55: *In vitro* screening of amylase inhibitor to stem weevil grub.

T1-7.5mg/20ml, T2-18.75mg/20ml, T3-37.5mg/20ml, T4- Control



(7.5mg/20ml, 18.75mg/20ml and 37.5mg/20ml), 100% mortality of banana stem weevil was recorded in the treatment 37.5mg/20ml (Fig. 55). Minimum mortality (33.3 per cent ) was recorded on the 3<sup>rd</sup> day and 100 percent mortality was recorded on 7<sup>th</sup> day.

# Isolation and purification of DNA from the entomopathogens for molecular characterization

Genomic DNA was isolated from 36 *Metarhizium anisopiae* and four *Beauveria bassiana* isolates and standardized the methods to arrest degradation of DNA during storage at low temperature condition.

#### Isolation and identification of native entomopathogens for the management of stem weevil

Isolates of endophytic *Metarhizium* spp., (18), 3 isolates of endophytic *Beauveria bassiana* and 10 isolates of rhizospheric *Metarhizium* spp. were isolated from rhizosphere and various tissues of banana, which belong to different genomic groups. These isolates were identified based on cultural morphology and spore characters microscopically.

# 4.5.8 Coffee Board Project: "Eco-friendly approaches for the management Coffee white Stem borer, *Xylotrechus quadripes* Chev. (Coleoptera: Cerambycidae) (PI-B. Padmanaban)

Eighteen isolates of *Metarhizium anisopliae* were screened against CWSB adult beetles under *in vitro*. Four isolates indicated 75.0 per cent mortality and two isolates (Ma- 125, and Ma-157) indicated mortality on the third day itself. The effective isolates (NRCB, CCRI, RCRS, Ma- 125, and Ma-157) were evaluated under field condition indicated that NRCB isolate of *B.bassiana* followed by CCRI isolate was effective in preventing CWSB attack.

Talc formulation of NRCB isolate *Beauveria bassiana* was incorporated in the PVC pipe trap to study the beetle infection. The liquid formulation of NRCB isolate *Beauveria bassiana* was smeared on the vane trap to study the beetle infection. In order to prevent the mass trapping of non-target insects in the cross vane trap, a PVC pipe trap was designed, which minimized the trapping of non-target insects.

As an integrated approach to CWSB management, the beetles emerging from the up-rooted coffee stems were fumigated with bio and non-bio fumigants and the results indicated that aluminum phosphide tablet was very effective followed by Nuvan and Menma.

#### 4.5.9 Contract Research Project

M/s Novozymes South Asia Pvt. Ltd. Evaluation of MET 52 EC and granules on banana corm weevil, *Cosmopolites sordidus*.

Treatments were imposed on banana plants in the farmers' field in Muthalapuram and Erasai (Theni District). Observations to be recorded.

4.5.10 ICAR project: Outreach project on phytophthora, fusarium and ralstonia diseases of horticultural and field crops

Evaluation of biocontrol agents and botanicals for the management of Fusarium wilt disease of banana cv. Grand Naine - AAA

A field trial conducted at Muthalapuram in Theni district with endophytic and rhizospheric fungal, bacterial isolates and botanical in cv. Grand Naine indicated that combined application of fungal endophytic *Penicillium pinophilum* Bc 2 + rhizospheric *T. asperellum* and liquid formulation containing endophytic *Trichoderma harzianum* Prr 2 + *Bacillus flexus* Tvpr 1 and single endophytic *Penicillium pinophilum* Bc 2 and Zimmu leaf extract (50% Conc) in the soil recorded the



maximum reduction of internal wilt disease score (1.1, 1.2 and 1.4 and 1.5 respectively) as against 5.8 in the control plants (Fig. 56&57). (disease scale 1-6 where 1 is healthy and 6 is 100% infected or dead) These treatments also



Fig. 56: Effect of soil drenching of liquid formulation of *Trichoderma* sp. +*Bacillus flexus* on the suppression of Fusarium wilt disease in cv. Grand Naine under field condition

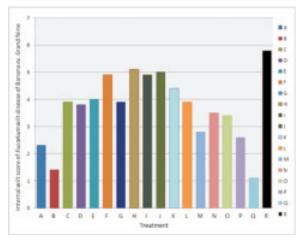


Fig. 57: Evaluation of endophytic and rhizospheric fungal and bacterial antagonistic microbes against Fusarium wilt disease of banana cv. Grand Nine

A	Mut.Endol[asperelluml(Prr2)		Endo. Bacillus sp. (Enbr1) + Endo.
^		1	Burkholderia sp. (GcTcr1)
11	EndoP.pinophilum (Bc2)	к	Endo.Bacillus sp. (Enbr1) + Endo.Bacillus
-		ъ.	sp. (Enbr1)
С	Mut.EndoPenicillism sp (Dsr1)	L	Endo.Bacillus sp. (Enbr1) + EndoBacillus
			sp. (GcTc2)
D	Mut.RhiTrichoderma sp.	M	Zimmu alone
Е	Mut.RhiT. asperellum	N	Endo. Pseudomonas putida (C4r4) +
E.		24	Zimmu leaf extract
F	Mut.RhiT.viride (140c)	0	Endo. Achromobacter sp. (Gcr1) + Zimmu
		o	leaf extract
G	EndoLysinibacillus (Dsr1)+	p	Endo. Bacillus flexus (Tvpr1)+Zimmu leaf
۳	Endo.Bacillus sp. (Enbr1)	r	extract
н	EndoLysiniBucillus (Dsr1) +		Drenching of liquid formulation containing
11	EndoOchrobactrum sp. (pjr1)	Q	T. harzimum (Pvr2) +B. flexus (Tvpr1)
	Endo. Burkholderia sp.		
1	(GcTcr1) + EndoOchrobactrum	R.	Control
	sp. (pjr1)		

increased the plant growth and yield attributing characters such as plant height (47.7%) girth (49.2%), total number of leaves (80.3%), leaf area (95.4%) number of hands (87%), number of fingers (up to 75.4%) and bunch weight (170.5%) significantly as compared to control plants. Interestingly, 11 different treatments recorded 100% harvest of good bunches as compared to only 35% harvested in the control plants (Fig. 58).



Fig. 58: Effect of soil application of Fusariom wilt suppressive endophytic *Penicillium pinophilum* (Bc 2) + rhizospheric *Trichoderma asperellum* on bunch weight of cv. Grand Naine (The application of these biocontrol agents has recorded almost complete control of the disease and 100% harvest of the crop in the Fusarium wilt sick plot).

# Transcriptomic analysis of gene expression due to the interaction of *Foc* pathogen and effective bio-control agents in banana

Suppressive Subtractive Hybridization (SSH) was carried out in cv. Grand Naine to identify the differentially expressed genes due to the interaction of *Foc* pathogen and effective biocontrol agent in banana. Total RNA was isolated from the roots of *Foc* alone-inoculated control (Driver) and *Foc* + *T. harzianum* 



inoculated (Tester) banana plants. The total RNA concentration of Tester and Driver RNA was 103.7µg/µl and 106.8µg/µl respectively. mRNA was isolated and the concentration of Tester and Driver mRNA was 0.5µg/µl and 0.9µg/µl respectively. The mRNA isolated was immediately converted into their respective cDNAs and subjected further to SSH analysis. Finally the resultant SSH products were run in 1.8% agarose gel and the PCR products of subtracted cDNA was ranged from 250 bp to 1000bp whereas the unsubtracted cDNA was ranged from 400 bp to 1000 bp.

The subtracted cDNA was subjected to cloning using INSTA cloning kit (Fermentas) and transformed using One shot Omnimax 2T1-phage resistant cells. A total of about 808 clones were obtained and Plasmid DNA was isolated from all these clones and double digested with ECORI and Pst1 enzymes for checking the presence of inserts.

# Characterization of *Foc* pathogen affecting cv.Grand Naine in Theni district of Tamil Nadu by VCG analysis

Incidence of Fusarium wilt disease in Cavendish group of banana was recorded recently in cv. Grand Nine which is normally infected by VCGs 01213/01216 of tropical race 4 Foc pathogen and also by VCGs 0120, 0126, etc. of subtropical race 4. The samples from different banana growing villages of Theni district were collected and the pure culture of the Foc pathogen was isolated and characterized by VCG analysis using the nit-M testers obtained from Australia. The results indicated that the Foc infecting Cavendish belongs to VCG 0124 and 0125 of race 1 in Theni district of Tamil Nadu (Fig. 59). Earlier the Foc pathogen infecting Cavendish was identified as VCG 0124 and now VCG 0125 is also identified besides some unknown VCGs, which require further analysis by molecular method.

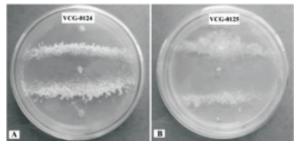


Fig. 59: VCG analysis of *Foc* infecting banana cv. Grand Naine in Theni district of Tamil Nadu

4.5.11 DBT project; Development of biopesticide formulation for reducing post harvest losses and for achieving export quality and increased shelf life of banana fruits (PI-R.Thangavelu)

Isolation, purification and characterization of post harvest pathogens collected from post-harvest disease infected samples from different markets

Samples of post harvest disease infected banana fruits (95) were collected from different parts of Tamil Nadu, Andhra Pradesh, Karnataka and Kerala (Fig. 60). From these samples, 54 isolates of *Colletotrichum musae*, 28 isolates of *Lasiodiploidia theobromae* and 13 isolates of *Fusarium* sp were isolated and purified.

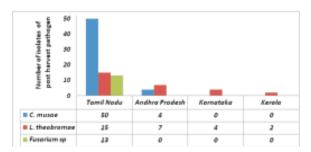


Fig. 60: Number of post harvest pathogens isolated from different markets in South India

Identification of effective, native bio-agents having multiple actions against different isolates of post-harvest pathogen

Bacterial (61) and nine fungal isolates of epiphytic and endophytic nature were collected



from culture collection of Plant Pathology, NRCB, Trichy were screened for their antagonistic activity against post-harvest pathogens (C. musae and L. theobromae). The results indicated that among the bacterial isolates, five bacterial isolates showed inhibitory activity (over 50%) against C. musae and none of the isolates inhibited L. theobromae pathogen. Among the fungal isolates, T. asperellum and Trichoderma sp. completely inhibited of C.musae whereas Trichoderma sp. inhibited 60% of mycelial growth and T. asperellum completely inhibited L. theobromae. These antagonistic microbes were evaluated for the inhibition by volatile compounds, two fungal isolates viz. T. asperellum and Trichoderma sp. showed inhibitory activity (over 50%) against C. musae and no fungal/bacterial isolates inhibited L. theobromae pathogen. However, non-volatile compounds produced by fungal isolate viz. T. asperellum and Trichoderma sp. showed inhibitory activity (100%) against C. musae and T. asperellum completely inhibited L. theobromae pathogen (Fig. 61).

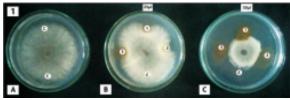


Fig. 61: Effect of non-volatile metabolites of *Trichoderma* sp on *L. theobromae* 

1) Effect of metabolites extracted from *T. asperellum on L. theobromae.* 1) Metabolite extracted using ethyl acetate, 2) Metabolite extracted using *n*-butanol, 3) Combined effect of metabolites extracted from ethyl acetate and *n*-butanol, C) Control (MeOH only)

#### Screening and identification of effective botanicals for the management of postharvest disease of banana

Among the 55 botanicals screened against *C. musae* and *L. theobromae*, only 50% concentration of Zimmu leaf extract exhibited 100 % inhibition of mycelial growth of both *C. musae* and *L. theobromae* pathogen under *in vitro* condition.

## Evaluation of ACC deaminase production by native epiphytic and endophytic bacterial isolates

Isolates of bacteria numbering 39 were screened for ACC deaminase production (increase shelf life of fruit) and two isolates were found to produce ACC deaminase under *in vitro* condition.

## Evaluation of mutated Rasthali banana plants for their resistance against Fusarium wilt disease

A total of 371 mutants of Rasthali plants were screened by two-inoculation techniques viz., inoculation with conidial suspension and sand maize meal inoculum against Fusarium wilt disease. The observation on the internal vascular discoloration by Fusarium wilt disease indicated that among 150 conidial suspension treated plants, seven plants with EMS treatment and derived from shoot tip culture showed no symptom of Fusarium wilt disease and out of 221 plants, which were inoculated with maize meal inoculum of *Foc*, only one plant, which was treated with EMS, showed no symptom of wilt disease.

### Evaluation of parents and progenies for their reaction to *Foc* pathogen

A total of 10 progenies and six parents were evaluated for their reaction to *Foc* pathogen under green house condition. The observation on internal vascular discoloration in the corm tissues indicated that Namarai × Pisang lilin hybrid was free from the Fusarium wilt symptom (score 1) which was followed by Sannachenkadali × Lairawak hybrid which recorded the minimum wilt score of 1.3.

# 4.5.12 DBT project: Evaluation of transgenic banana for resistance to *Banana Bunchy Top Virus* (Rep mediated) (PI-R. Selvarajan, Co-PI- C. Anuradha)

Fifty three plants were multiplied in tissue culture from five southern positive transgenic



lines through shoot tip culture. These plants were inoculated with viruliferous banana aphids and kept in the greenhouse to monitor the symptom expression. All the untransformed control plants showed typical BBTD symptoms after 11-15 days of inoculation whereas all tested transgenic lines did not show any symptoms for 3 months of postinoculation. Molecular analysis was performed for virus challenged plants using PCR with CP, rep and â-actin gene (internal control). Three plants in R2 transgenic line and five plants in R3 line were PCR positive for CP gene but these plants did not express BBTV symptom probably due to less titer of virus. Eight lines of embryogenic cell suspension (ECS) for Hill banana has been established (Fig. 62) and being maintained. Eighty six batches of cocultivation was carried out with eight ECS-lines using BBTV-rep gene construct to generate transgenic plants. Twelve plants got regenerated which are in the hardening stage.

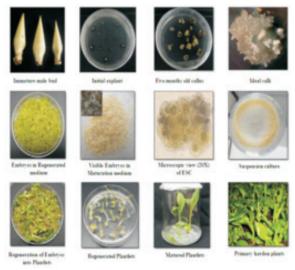


Fig. 62: Establishment of embryogenic cell suspension (ECS) for Hill banana for developing transgenic resistant to BBTV

# 4.5.13 Network Project on Transgenic in Crops –Transgenic Component (PI-R.Selvarajan)

Twelve fresh lines of ECS have been established for cv. Virupakshi. Co-cultivation

with 16 batches was done with pBINAR::BBTVCP construct. Twenty batches of co-cultivation was carried out with eight ECS-lines using RNAi construct for multivirus to generate transgenic plants. The co-cultivated batches are under selection and some are in maturation and regeneration stage. Southern positive (BBTV-CP) plants were maintained in transgenic glass house. These plants were mass propagated through shoot tip culture. These plants were inoculated with BBTV using viruliferous aphids which were kept in a contained area for monitoring symptom expression or resistance in banana.

### 4.5.14 DBT-ATL scheme for virus indexing (PI- R. Selvarajan)

Mother cultures of Tissue culture banana plant received from forty seven TC industries were tested for banana viruses under contract service as well as DBT –ATL scheme. Totally 35464 samples (8887 is for BBTV, 8790 for BSMYV, 8770 for BBrMV and 9017 for CMV) were tested for the presence of virus. The number of positives for BBTV, BSMYV, BBrMV and CMV were 24, 21, 8 and one, respectively. This year certificate of quality was issued for 30.04 million TC plants.

# 4.5.15 DST project: Proteomic studies of host-pathogen interactions in Banana-Banana Bract Mosaic Virus (BBrMV) system (PI-C.Anuadha)

Protein was isolated from healthy and infected Nendran shoot. 200 mg of protein was used for 2-DE analysis. 2-DE analysis was carried out from three biological replicates using the pH 3-10, 18 cm IPG strips with silver staining method. For each sample, the gels were repeated at least in triplicate and showed a high level of reproducibility. Typically, more than 1000 protein spots were reproducibly detected by melanie software on each CCB stained gel and 40 spots which were found to show two fold difference were sent for PMF by MS.



#### 5 TECHNOLOGY ASSESSED AND TRANSFERRED

#### 5.1. Training

- Post harvest handling, packing and storage techniques in banana for export to Mr. M. Buvanendran, Madurai and Mr. P. Gunasekaran, Sengunthapuram, Karur Dist., Tamil Nadu.
- ◆ About 4800 banana farmers/ entrepreneurs/ Horticultural/ Agricultural Officers/ College students visited NRCB and were briefed about improved production technology, postharvest management and value addition of banana.



Banana farmers from Theni visited NRCB for training on 27.06.2013

#### 5.2. Radio talks through All India Radio, Tiruchirapalli

Name of the Scientist	Topic	Date of broadcast
I. Ravi	Use of plant hormones in banana production (Tamil)	17.10.2013
V. Kumar	Season based banana cultivation techniques (Tamil)	19.09.2013
K.J. Jeyabaskaran	Nutrient management in banana cultivation— Question and Answer (Tamil)	19.07.2013
	Fertilizer management in banana (Tamil)	24.07.2013
M.S. Saraswathi	Importance of tissue culture banana (Tamil)	28.05.2013

#### 5.3 Exhibitions conducted/ participated

Name of the Events	Organiser/ venue	Date(s)
International Conference on Tropical Roots and Tubers Crops for sustainable Liveli hood under Changing Agro Climate	Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala	9-12 Jul., 2013
Kissan Mela - 2013	NRCB, Tiruchirappalli, Tamil Nadu	21 Aug., 2013
8 <sup>th</sup> National Conference on KVK-2013	ICAR & UAS, GKVK campus, Bangalore, Karnataka	23-25 Oct., 2013
Banana Festival - 2013	CII and Tamil Nadu Banana Grower Federation, CODISSIA, Coimbatore, Tamil Nadu	14-15 Dec., 2013



Name of the Events	Organiser/ venue	Date(s)
Agri Expo - 2013	Dinamalar (Tamil Daily), Thanjavur,	19-22 Dec.,
	Tamil Nadu	2013
26th Kerala National Science	Kerala State Council for Science,	28-31 Jan.,
Congress- National Science	Technology & Environment, Kalpetta,	2014
Expo	Wayandu, Kerala	
National Agriculture Fair cum	CII, Govt. of Maharashtra and Govt. of	9-13 Feb., 2014
Exhibition	India, Central Cotton Research Institute,	
(Krishi Vasanth - 2014)	Nagpur, Maharashtra	
Agri Expo - 2014	Puthiyathalaimurai Media, National	21-23 Feb.,
	College, Tiruchirappalli, Tamil Nadu	2014



Shri Sharad Chandra Pawar, Hon'ble Union Minister for Agriculture & Food, Govt. of India and Dr. S. Ayyappan, Secretary-DARE & Director General-ICAR, New Delhi visit to NRCB stall during 8<sup>th</sup> National Conference on KVK- 2013, Bangalore, Karnataka on 23.10.2013



Farmers visit to NRCB stall during Agri Expo – 2013, Thanjavur, Tamil Nadu on 19.12.2013



#### 6 EDUCATION AND TRAINING

#### 6.1 Students guided

Guide	Degree	Project title	Name
B. Padmanaban	M. Sc. (Microbiology)	Isolation of endophytic  Lecanicillium lecanii (Zimm.)  from Musa sp. and evaluation of the same fungi against banana aphid, Pentalonia nigronervosa (Coq.) (Homoptera: Aphididae)	K. Ragavi
	M. Sc. (Microbiology)	Isolation of endophytic  Metarhizium anisopliae (Metsch.) from Musa sp. and evaluation of the same fungi against banana aphid, Pentalonia nigronervosa (Coq.) (Homoptera: Aphididae)	R. Sudha
R. Selvarajan	M. Sc. (Applied Microbiology)	Screening of banana cultivars for resistance against Banana Bunchy Top Virus (BBTV)	S.A. Karthick
	M. Sc. (Biotechnology)	Cloning, sequencing and diversity analysis of HC-Pro gene of Banana Bract Mosaic Virus (BBrMV) isolates	R.S. Sukanya
	M. Sc. (Biotechnology)	Sequencing and genetic diversity analysis of Banana Streak Virus (BSV) isolates infecting cv. Poovan	R. Thilagavathi
	B. Tech (Biotechnology)	Serological and molecular based detection of banana viruses	S. Vinusri
I. Ravi	M. Sc. (Biotechnology)	Quantification of ABA and amplification of drought tolerant genes in banana cv. Grand Naine	A.R. Sam Paul
	M. Sc. (Biotechnology)	Drought tolerance: Biochemical changes and amplification of drought tolerant genes in banana cultivars	P. Sujatha
M. Mayil Vaganan	M. Sc. (Biochemistry)	Comparative enzymatic and proteomic analysis of two banana cultivars - Rasthali and Poovan - in relationship with finger drop	L. Sridevi
	M. Sc. (Biotechnology)	Cloning of soybean ferritin gene and its transient expression analysis in embryogenic cell suspension of banana cv. Rasthali	S. Saranya



Guide	Degree	Project title	Name
S. Bakiyarani	M. Sc. (Bioinformatics)	Mining of SNPs from banana transcriptome data and their validation	M. Leevitha
M. S. Saraswathi	B. Tech. (Biotechnology)	Standardization of tissue culture protocol for mass multiplication of banana ( <i>Musa</i> spp.) cv. Ney Poovan	S. Mehnath
	B. Tech. (Biotechnology	Genetic fidelity testing of tissue culture raised banana using ISSR markers	S. Akila
C. Anuradha	M. Sc. (Biotechnology)	Fluorescence <i>In situ</i> Hybridization (FISH) for finding viral genome in banana	C. Priyadharshini
	M. Sc. (Biotechnology)	Evaluation of BSVMys derived promotors	B. Elakiya

#### 6.2 List of trainings offered

Title	Date (s)	Nos.	Course Coordinator
Post harvest handling, packing, storage and ripening in banana for domestic and export markets	9-13 Dec., 2013	7	K. N. Shiva
Crop protection scientists of AICRP banana Centers-Training cum workshop	4-5 Mar., 2014	16	B. Padmanaban, P. Sundararaju, R. Thangavelu and R. Selvarajan
Awareness cum training programme on protection of plant varieties and farmers rights	24 Mar., 2014	100	M. M. Mustaffa S. Uma



Participants of the training cum workshop on Crop Protection Scientists of AICRP (Fruits) Centers



Participants of the training on Post-harvest handling, packing, storage and ripening in banana for domestic and export markets



#### 6.3 Off-Campus Training

		No. of	
Title of the training/ Venue	Date	Partici pant(s)	Coordinator & Resource persons
Exposure training on Genetic fidelity testing of tissue cultured banana to a Senior Scientist of RPRC, Bhubaneshwar, Odisha	16-18.4.2013	1	M.S. Saraswathi
Demonstration on High Density Planting Techniques in Banana organized by SKVK, Karur and NABARD, Karur under the NABARD FTTF Scheme, Kulithalai, Karur	15.5.13	50	V. Kumar
Field Demonstration of Use of Banana Farm Implements' and Scientists- Farmers Interactive Meeting organized by CIAE, Coimbatore-NRCB, Trichy- Farm Fresh Banana, Chinnamanur	16.5.13	80	M.M. Mustaffa and V. Kumar
DBT sponsored Training programme on Macropropagation at Semmedu village, Kolli Hills, Namakkal dt., Tamil Nadu	5.9.2013	150	S. Uma, M.S. Saraswathi, S. Backiyarani, B. Padmanaban and R. Thangavelu
USAIDConsultancy Programme for Transforming Eastern India's Economies Through Innovative Rural Business Hubs (RBH) in Collaboration with CII-FACE, New Delhi in South 24 Parganas and Nadia dt., West Bengal	16-20.12.201	3 500	M.M. Mustaffa, V. Kumar and K.J. Jeyabaskaran
ICAR sponsored Integrated Crop Management Programme on High Density Planting Techniques for Banana organized by Saraswathy KVK, Maruthur in Kulithalai, Karur.	4.2.2014	40	V. Kumar
Improved Cultivation Practices for Banana to the Tribal Banana Growers, Agali, Palghat, Kerala under the TSP Programme	15.2.2014	100	M. M. Mustaffa, V. Kumar R. Thangavelu and K.N. Shiva
Packaging of fruits and vegetables with special reference to banana, Breeze Residency, Tiruchirappalli, Tamil Nadu	3.4.2013	150	K.N. Shiva
Value added products in minor millets for marketing through SHGs, organized by Saraswathi-KVK, Pulutheri, Karur Dt., Tamil Nadu	8.1.2014	20	
Launching programme of solar drier for drying banana, Thottiyam Trichy Dt., Tamil Nadu	30.1.2014	30	



#### 7 AWARDS AND RECOGNITIONS

#### 7.1 Awards

Name	Award	Awarded by / Organizer/ Date
S. Uma, V. Kumar and S. Backiyarani	Fellow of CHAI	Confederation of Horticultural Associations of India, New Delhi
R. Selvarajan	Best Poster	Asia Pacific Congress of Virology (Virocon-2013), Amity University Uttar Pradesh, Noida, 17-20 <sup>th</sup> Dec., 2013
V. Kumar	Best Lead Paper Presentation	National Seminar-cum Workshop on Canopy Management and HDP in Sub-tropical Fruit Crops, CISH, Lucknow during 22-24 Oct., 2013
K.N. Shiva	Best Oral Presentation	National Seminar on Plastics in Agriculture, Indian Plastics Institute, Chennai, 21st Sept., 2013

#### 7.2 Recognitions

Name	Particulars
B. Padmanaban	Special invitee for XIX and XX IMC Meetings of NRCB Member Secretary for 15 <sup>th</sup> Research Advisory Committee Meeting of NRCB
S. Uma	Review committee member for the DBT project International Examiner for the M.Tech student, Vaal University, Andries Potgieter Boulevard, South Africa
	Member, Editorial Boards of Indian Journal of Horticulture and Current Horticulture
	Review member of international Journal on Biotechnological Advances
	External Examiner for the evaluation of Ph.D. (Horticulture) thesis of UHS-Bangalore, UAS - Dharwad, GKVK-Bangalore and TNAU-Coimbatore
	Group leader to guide the discussions on Needs of the germpalsm collection managers – diversity and knowledge during <i>Musa</i> Net Diversity Working Group, Center for International Forestry Research (CIFOR), Bogor, Indonesia
R Selvarajan	External member, Institute Biosafety Committee, IIHR, Bangalore
	Editor of Virus disease (formerly Indian Journal of Virology), Indian Society of Virology, New Delhi



Name	Particulars
	Doctoral committee member for Ph.D theesis of Bharathidasan University-Tiruchirapalli, Madurai Kamaraj University- Madurai and TNAU, Coimbatore
M. Mayil Vaganan	Member of Doctoral Committee of two Ph.D. students, Bharathidasan University, Tiruchirapalli
K.N. Shiva	Member of ITMC to Sugarcane Breeding Institute, Coimbatore, Tamil Nadu
	Member of the Executive Council of Indian Society for Spices, IISR, Calicut for year 2013-'15
	Member, Editorial Board of Kadali Samachar – AIPUB, Newsletter
	Editor, <i>E-Newsletter of ITMU</i> , NRC Banana, Tiruchirapalli, for year 2013-2014
C. Anuradha	External member for setting question papers for M.Sc., Bharathidasan University-Tiruchirapalli
M. S. Saraswathi	Doctoral committee member for Ph.D. students, Bharathidasan University, Tiruchirapalli
	Co-Nodal officer and Member Secretary of the RFD cell and member of the Institute RFD committee



#### 8 LINKAGES AND COLLABORATIONS IN INDIA AND ABROAD

- ♦ A collaborative project was initiated with CIAE, Regional Station, Coimbatore for Developing banana central core stem slicer and juice extractor and Developing postharvest mechanization package for banana central core.
- ◆ A MoU was signed with St. Joseph College, Tiruchirapalli, Tamil Nadu for training woman in banana post harvest and value addition.

#### 9 PUBLICATIONS

## 9.1 Research Papers Published in journals

#### 9.1.2 National

- Backiyarani, S., Sundararaju, P., Mayilvaganan, M., Uma, S., Saraswathi, M.S. and Arunkumar, G. 2013. Time Course expression studies during *Musa Pratylenchus coffeae* interaction. *Ind. J. Hort.* **70**(2): 217-222.
- Mary Sheeba, M., Selvarajan, R. and Mustaffa, M.M. 2013. Prediction and identification of microRNA from banana infected with Banana Streak Mysore Virus (BSMYV). Madras Ag. J. 100(4-6): 513-518.
- Mayil Vaganan, M., Ravi, I., Sarumathi, S., Nandakumar, A., Sundararaju, P. and Mustaffa, M.M. 2014. Phenylpropanoid enzymes and phenolic polymers and metabolites as chemical defenses to infection of *Pratylenchus coffeae* in roots of resistant and susceptible bananas (*Musa* spp.). *Ind. J. Expt. Biol.* 52(3): 252-260.

#### 9.2.2 International

- Arun, K., Uma, S., Saraswathi, M.S., Backiyarani, S. and Durai, P. 2013. Seed priming studies on *in-vitro* germination and regeneration of hybrid banana embryos (*Musa spp*). *Seed Sci. Res.* 41: 439-451.
- Backiyarani, S., Uma, S., Arunkumar, G., Saraswathi, M.S. and Sundararaju, P. 2013. Cloning and characterization of NBS-LRR resistance gene analogues of *Musa*

- spp. and their expression profiling studies against *Pratylenchus coffeae. African J. of Biotech.* **12**(27): 4256-4268.
- Backiyarani, S., Uma, S., Arunkumar, G., Saraswathi, M.S. and Sundararaju, P. 2014. Differentially expressed genes in incompatible interactions of *Pratylenchus coffeae* with *Musa* using suppression subtractive hybridization *Physiol. and Mol. Pl. Pathol.* **86**: 11-18.
- Balasubramanian, V. and Selvarajan, R. 2013. Genetic diversity and recombination analysis in the coat protein gene of banana bract mosaic virus. *Virus genes.* **48**(3): 509.17.

#### 9.3 Popular articles

- Jeyabaskaran, K.J. and Mustaffa, M.M. 2014. How to manage problem soils in banana cultivation?, 'Gyan Manthan'-Knowledge Sharing workshop on Tropical Fruits Banana, Mango and Pomegranate for value chain management and farm profitability enhancement', organized by CHAI, ASSOCHAM, TNAU, JISL and AIPUB, Coimbatore, 1-2, March.
- Mustaffa, M.M. and Kumar, V. 2014. High density planting and canopy management in banana'. In Souvenir: *National Seminar-cum Workshop on 'Canopy Management and HDP in Sub-tropical Fruit Crops'*, CISH, Lucknow during 22-24 Oct., 2013.
- Mustaffa, M.M. and Shiva, K.N. 2013. Advances in production and processing



- of banana. In: Souvenir *SYMSAC-VII Post-harvest processing of spices and fruit crops* (Eds. Sasikumar, B et al.,) 27-29 November. IISR, Karnataka, pp.77-87.
- Mustaffa, M.M. and Shiva, K.N. 2013. Use of plastics in banana production and processing. In: *National Seminar on plastics in agriculture*, Indian Plastics Institute, Chennai chapter, 21st September.
- Muthamil Selvan, M., Ravindra Naik, Annamalai, S.J.K. and Kumar, V. 2014. Wet banana pseudostem chipper for compost industry. *ICAR News*, 20(1): 8-10.
- Kumar, V. and Mustaffa, M.M. 2014. Strategic management of mat and bunch for quality production of banana. In: Souvenir of Gyan Mantha- Knowledge sharing workshop on tropical fruits- banana, mango and pomegranate for Value Chain Management and Farm Profitability Enhancement, Coimbatore 1-2. March.
- Krishna Surendar, K., Durga Devi, D., Ravi, I., Krishnakumar, S., Rameshkumar, S. and Velayudham, K. 2013. Water Stress in Banana-A Review. Bull. *Env. Pharmacol. Life Sci.* 2 (6): 1-18.
- Padmanaban, B and Mustaffa, M.M. 2013. Eco-friendly steps can control stem weevil, The Hindu, dated 22, Sept. page No. 3.
- Padmanaban, B and Mustaffa, M.M. 2014. Management of banana aphids. *Vanoli Uzhavar Sanga Seithikkathir*, March. pp 8-9.
- Padmanaban, B and Mustaffa, M.M. 2013. Stem weevil management in banana cultivation. *Dinamalar – Vyivasayamalar*, Tiruchirapalli- dated Nov.30. page No.16.
- Padmanaban, B. 2014. Strategic management of insect pests of banana for improved production, In: Souvenir of *Gyan Manthan- Knoweldge sharing workshop on Tropical fruits-Banana, mango and*

- Pomegranate for value chain management and farm profitability enhancement, CHAI and ASSOCHAM, Coimbatore, 1-2 March. pp 55-64.
- Shiva, K.N. 2013. How to sort and grade the banana for export? (Tamil) *Pasumai vikadan*, 25<sup>th</sup> Dec., pp. 38-39.
- Shiva, K.N. and Mustaffa, M.M. 2014. Technological innovations in value chain management of banana. In: Souvenir of Gyan Manthan-Knowledge sharing workshop on tropical fruits Banana, mango and pomegranate for value chain management and farm profitability enhancement. [Eds. P. Rethinam et al 1-2 March, CHAI, ASSOCHAM, Coimbatore, pp. 38-44.
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- Uma, S., Backiyarani, S., Saraswathi, M.S. and Mustaffa, M.M. 2013. Banana genome to enrich fruit basket. *Ind. Hort.* March-May 2013. Pp 3-6.

#### 9.4 Chapters in books

Selvarajan, R. and Balasubramanian, V. 2014. Host-virus interactions in bananainfecting viruses (Eds-R.K. Gaur et al). Plant virus-host interaction molecular



- approaches and viral evolution. Elsevier academic press. pp.57-78. isbn: 978-0-12-411584-2.
- Selvarajan, R., Thangavelu, R., Balasubramanian, V. and Mustaffa, M.M. 2013. Banana. In. (H.P. Singh- Eds), Advances in horticulture biotechnology diagnostics for horticulture crops (Vol. VII), Westville Publishing House, New Delhi. pp. 30-57. ISBN: 9788185873961.

#### 9.5 Technical Bulletin

Shiva, K.N., Mustaffa, M.M. and Uma, S. 2014. (2<sup>nd</sup> Edn). Protection of plant varieties and Farmer's Rights (PPV & FRA) and Copy rights. National Research Center for Banana, Trichy, Tamil Nadu. pp. 26.

#### 9.6 Extension folders/Reports/Handouts

- Mustaffa, M.M., Jeyabaskaran, K.J. and Ravichamy, P. 2014. NRCB at a glanceextension folder in Hindi (Revised Edn. for Exhibition on Krish Vasanth-2014, Nagpur). P. 4
- Mustaffa, M.M., Padmanaban, B., Mayil Vaganan, M., Anuradha, C. and Ravichamy, P. 2013. NRCB Annual Report 2013-2014. National Research Centre for Banana, Tiruchirapalli, Tamil Nadu. P. 102.
- Padmanaban, B., Mayil Vaganan, M., Anuradha, C., Mustaffa, M.M. and Ravichamy, P. 2013. *NRCB Newsletter* Vol. XII/No.2. National Research Centre for Banana, Tiruchirapalli, Tamil Nadu. P. 4.
- Shiva, K.N. and Mustaffa, M.M. (Eds.). 2013. *E-Newsletter of ITMU*, Vol. II, Issue No. 1. National Research Centre for Banana, Tiruchirapalli, Tamil Nadu. P. 4.
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# 9.7 Research papers/ abstracts presented in Seminar/ workshop/ Symposium/ Conference, etc.

- Anuradha, C and Selvarajan, R. 2013. Proteomic analysis of host pathogen interaction in banana bunchy top virus infected hill banana (AAB). *In. National symposium on Symposium on pathogenomics for diagnosis and management of plant disease*. CTCRI, Thiruvananthapuram, Kerala, 24 25 Oct. pp 104.
- Anuradha, C., Balasubramanian, V. and Selvarajan, R. 2013. Sequence motif comparison and homology modelling of helper component proteinase (HC-Pro) of banana bract mosaic virus. pp 23. *Ibid*.
- Balasubramanian, V., Anuradha, C. and Selvarajan, R. 2013. Genetic variation of HC-Pro gene among the isolates of *Banana bract mosaic virus* from India. pp 21. *Ibid.*
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- Mustaffa, M.M. and Shiva, K.N. 2013. Advances in production and processing of banana, *Symposium on post-harvest* processing of spices and fruit crops. Madikeri, Karnataka, 28th Nov.
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- Saraswathi, M.S and Uma, S. 2014. Banana conservation strategies current status and future plan. In: *Brainstorming meeting and training cum demonstration on cryopreservation and in vitro conservation in horticultural crops.* IIHR, Bengaluru, 21 & 22 Feb.
- Saraswathi, M.S., Kannan, G., Selvasumathi, M., Uma, S., Mustaffa, M.M. and Backiyarani, S. 2014. Cost effective tissue culture protocols for mass multiplication of commercial banana varieties (*Musa spp.*) In: *Knowledge sharing workshop on tropical fruits with the theme value chain management and farm profitability enhancemen*, Coimbatore, 1-2, March.
- Selvarajan, R., Prasanya Selvam, K., Balasubramanian, V. and Anuradha, C. 2013. *Agrobacterium* mediated transformation of embryogenic cell suspension of Hill banana for developing transgenics to BBTV. In: *Asia Pacific Congress of Virology,* Amity University, Noida, Uttar Pradesh, India, 17-20th Dec.

- Selvarajan R. and Balasubramanian, V. 2013. Immunodiagnosis of banana bract mosaic virus (BBrMV) using the polyclonal antiserum developed against recombinant coat protein. In. National symposium on pathogenomics for diagnosis and management of plant disease. CTCRI, Thiruvananthapuram, Kerala, 24 25 Oct. pp 77.
- Selvarajan, R., Mary Sheeba, M. Balasubramanian, V. and Gayathrie, T. 2013. Production of polyclonal antiserum against recombinant viral associated protein of banana streak Mysore virus (BSMYV) and its application in diagnosis. In: *Asia Pacific Congress of Virology.* Amity University, Noida, Uttar Pradesh, India, 17-20th Dec.. pp 105.

#### 9.9 Training Manual

- Kumar, V. and Mustaffa, M.M. 2013. Improved production technologies for banana cv. Grand Naine- a promising variety for domestic and export market for fresh fruit & processing industries. Under the USAID Consultancy Programme for Transforming Eastern India's Economies Through Innovative Rural Business Hubs (RBH) in Collaboration with CII-FACE, New Delhi.
- Mustaffa, M.M. and Shiva, K.N. 2013. Banana Production: Global & National Scenario. In: Post harvest handling, packing, storage and ripening in banana for domestic and export markets [Eds. K.N. Shiva et al., NRCB, Trichy, Tamil Nadu, pp. 1-4.



## CONSULTANCY SERVICES AND COMMERCIALIZATION OF TECHNOLOGIES

- ♦ A total of 35,464 samples were tested for the presence of virus and a gross amount of Rs 65,66,092/- has been generated under virus testing contract service.
- ◆ 319 batches of tissue cultured bananas inclusive of three varieties namely Grand Naine, Robusta and Monthan have been tested for their genetic fidelity using SSR and ISSR markers under DBT − ATL and generated an income of Rs. 5.37 lakhs.
- ◆ Polyclonal antiserum produced for CMV, BBrMV and BBTV has been sold to tissue culture companies and also to the State Agricultural Universities *viz.*, KAU, APHU and Department of Horticulture, Kerala. An amount of Rs. 35,000/- has been realized from the sale of antisera.

- ◆ USAID Consultancy Programme for Transforming Eastern India's Economies through Innovative Rural Business Hubs (RBH) in Collaboration with CII-FACE, New Delhi, Sahai-e-Village Ltd., Kolkata.
- Mother culture of cv. Udhayam banana sold to HRC, Nagicherra through MTA.
- ◆ Licensing of Technical Know-How technology transferred to four entrepreneurs *viz*, Banana Fig to Mr. K. Prabhakaran, Malappuram, Kerala; Post-Harvest Handling, Packing and Storage of Central Core Stem to Mr. A. Ananth, Namakkal, Tamil Nadu; Flower (thokku) and Stem pickles to Mr. S, Sreejith, Kollam, Kerala and Mr. J. Venkatesan, Bengaluru, Karnataka.



#### 11 RAC/ IMC/ IRC MEETINGS

#### **RAC Meeting**

Research Advisory Committee meeting of the Centre was conducted on 27 and 28 December, 2013, wherein all the members of RAC including the Chairman Dr. G. L. Kaul, Former VC, AAU attended the meeting. Recommendations generated from the meeting were approved by the Council and communicated the same to all the members.



Dr. G. L. Kaul chairing the RAC meeting

Sl. No	. Name and address	Position
1.	Dr. G. L. Kaul, Former VC, AAU, Ghaziabad, U.P.	Chairman
2.	Dr. R. K. Tyagi, Head, Division of Germplasm, NBPGR, Pusa, New Delhi	Member
3.	Dr. G. Chandra Sekar, Head, Dept. of Plant Pathology, Tamil Nadu Agril. University, Coimbatore	Member
4.	Dr. M. M. Mustaffa, Director, NRC for Banana, Tiruchirapalli	Member
5.	Shri Shaker Nagarajan, President, TNHBGF, Pattiveeranpatti, Tamil Nadu	Member (IMC Rep.)
6.	Shri Bopanna Venkata Rao, Banana Growers, Kovvur, A.P	Member (IMC Rep.)
7.	Dr. B. Padmanaban, Principal Scientist, NRC for Banana, Tiruchirapalli	Member Secretary

#### Recommendations

- ◆ To safeguard the collected germplasm, a duplicate set of germplasm may be planted in the organization/institute of similar agro-climatic conditions located in respective states/area from where it has been collected to ensure survival of the collected germplasm till the same gets established in the NRC field gene bank. Reasons for non-survival may be studied to assist in avoiding the mistakes done for the collection of germplasm especially from difficult areas.
- Banana germplasm exploration should be intensified mainly in North Eastern region.
   Exact and realistic details of Collection

Plan may be prepared and followed up year-wise and area-wise.

- ◆ Embryogenic cell cultures (ECS) of cv.Virupakshi and Rasthali may be stored on a long term basis. In this context, the cryopreservation of ECS and Musa seeds may be explored to use the expertise and facility available at NBPGR for long term storage.
- ◆ Attempts may be made to collect required germplasm from ITC collections from Belgium. Since repeated supply from ITC becomes difficult it is suggested that all possible efforts should be made to safeguard the ITC germplasm received in previous years.

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- Resistant genes identified for Musa nematodes needs to be validated in the field.
- It is mandatory to evaluate the leaf production potential of germplasm accessions.
- The banana cultivation is gaining importance in non-traditional areas such as Uttar Pradesh (Lucknow), Punjab (Mohali). There is a need to gather information on frost resistant banana genotypes. In this context, Director, Research, PAU, Ludhiana may be contacted and provided with the list of banana germplasm suitable for growing under Punjab condition.
- Attempts may be made to extend the banana cultivation in non-traditional areas. In this connection Dr. P.N. Mathur, Bioversity International, New Delhi may be contacted for identification of suitable places for banana cultivation using the climate analogue software available with Bioversity International

#### **IMC Meeting**

The eighteenth meeting of the Institute Management Committee was held on 13.5.2013, 4.10.2013 and 19.3.14 under the chairmanship of Dr. M. M. Mustaffa, Director, NRC for Banana. During this meeting, various policy decisions were discussed and recommended for approval to the Council.

- In the germplasm survey, GIS tools are very important and may be utilized to find out the gaps in collections and planning the exploration and collecting missions in future.
- Trait-specific banana germplasm identified by NRCB needs to be registered. Proposal may be sent to the Director, NBPGR, New Delhi or Member Secretary, Plant Genetic Resource Committee, NBPGR, New Delhi for registration.
- The methodology used for the soil salinity studies by the Physiologist may be verified. CSSRI Karnal may be contacted for the same for modification required, if any. Validate the methodology on soil sampling for salinity etc.
- Pilot testing of all value added products of banana developed at the Centre should be attempted for commercial exploitation
- A demonstration trial on leaf spot disease management be undertaken in Andhra Pradesh.



Dr. M.M. Mustaffa, Director NRCB, chairing one of the IMC meetings

The following members participated in the meeting

Sl. No.	Name and address	Capacity
1.	Dr. M.M. Mustaffa, Director, NRCB, Tiruchirapalli	
2.	Dr. N. Kumar, Dean (Horticulture), TNAU, Coimbatore	Member
3.	Dr. R. Chithiraiselvan, Principal Scientist, IIHR, Bangalore	Member
4.	Dr. K.B. Patil, Vice-President, Jain Irrigations System, Jalgaon	Member
5.	E.I. Jonathan, Director, Crop Protection, TNAU, Coimbatore	Member



Sl. No.	Name and address	Capacity
6.	Dr. S.V. Hittalmani, Director of Horticulture Incharge, Government of Karnataka	Member
7.	Dr. S. Mariappan, Dean TNAU, Coimbatore	Member
8.	Shri Shaker Nagarajan, President, TNHBGF, Pattiveeranpatti, TN	Member
9.	Shri Bopanna Venkata Rao, Banana Grower, Kovvur, AP	Member
10.	Shri. M. Krishnan, Administrative Officer, NRC for Banana, Tiruchirapalli	Member Secretary

#### **IRC** Meeting

The seventeenth Institute Research Council meeting was held on 4, 17 and 20 April, 2013 under the Chairmanship of Dr.M.M. Mustaffa, Director, NRCB. The Member Secretary welcomed the Chairman and other members of IRC. After introductory remarks by the Chairman, the research programmes, comments of the last IRC, action taken on the comments, salient achievements for the year 2012-13 and technical programme

for the year 2013-14 were presented by the scientists were reviewed.



Dr.M.M. Mustaffa, Director NRCB, chairing the 17<sup>th</sup> IRC meeting



# TRAINING /REFRESHER COURSE/SUMMER/WINTER 12 INSTITUTES/ SEMINARS/ CONFERENCE/ SYMPOSIA/ WORK SHOP ATTENDED BY THE SCIENTISTS

#### 12.1 Training/ Refresher courses attended by the Scientists (HRD)

Name of the Scientist	Name of the training programme /Venue	Period
S. Uma, R. Thangavelu and R. Selvarajan	Management Development Programme on Leadership Development NAARM, Hyderabad	26 Nov.–7 Dec., 2013 26 Aug 6 Sept., 2013
I. Ravi	Nodal officer installation - cum - training workshop for NAIP' SSCNARS for NARS, NAARM, Hyderabad	18-19 Oct., 2013
	Adaptation and mitigation strategies for climate resilient agriculture, CRIDA, Hyderabad	22-31 Oct., 2013
K.N. Shiva	National Training on Non-thermal and Non- chemical Processing Technologies – Application of High Pressure and Pulsed Light Technology for Food Processing, CIFT, Cochin, Kerala	18-31 Oct., 2013
S. Backiyarani	International training programe on Bioinformatics training course applied to the <i>Musa</i> genome, Montpellier, France	18-22 Nov., 2013
M.S. Saraswathi	International training on standardization of protocols of tissue culture and somaclonal variant selection in <i>Musa</i> improvement, TBRI, Pingtung, Taiwan	21-26, Oct., 2013

#### 12.2 Workshop/seminars/conference/symposia/meetings, etc. attended by the Scientists

Name of the Scientist	Name of the training programme /Venue	Period
M. M. Mustaffa	Farmers Meet – TN Banana Growers Federation, Theni, Tamil Nadu	16 May, 2013
	International Conference on Water Quality and management for climate resilient in agriculture, Jalgaon, Maharashtra	27-30 May, 2013
	Scientific Advisory Committee Meeting of KVK, Needamangalam, Tamil nadu	24 June, 2013
	International Conference on Tropical Roots, Tubers for sustainable livelihood under changing agro-climate, CTCRI, Thiruvananthapuram	11-12 Jul., 2013
	ICAR Instt. Horticulture Division Meeting / ICAR Foundation Day, New Delhi	14-17 Jul., 2013



Name of the Scientist	Name of the training programme /Venue	Period
	ICAR-Bioversity International Work Plan signing Ceremony/ Workshop, ICAR, New Delhi / National Citrus Meet at NRCC, Nagpur	12-14 Aug., 2013
	Meeting on Post Harvest Losses reduction in Horticultural Crops, ICAR, New Delhi	29-30 Aug. 2013
	First review meeting of Banana Biofortification	14-17 Sept., 2013
	Project at NABI, Mohali	
	National Meet on Fruits, NBAII and National Conference on KVK-2013, UAS, Bangalore	21-23 Oct., 2013
	National Seminar on Canopy Management and High Density Planting in Subtropical Fruit Crops, CISH, Lucknow, U.P.	24-27 Oct., 2013
	SYMSAC Conference, Madikeri	27-29 Nov. 2013
	IICPT Board Meeting, Ministry of Processing, New Delhi	1-2 Dec., 2013
	Interactive Workshop on Admn. and Financial matters for ICAR Instts., NAARM, Hyderabad	8-10 Dec., 2013
	Off-campus Training Programme on Improved Banana Production Technology under Consultancy programme, Kolkatta	16-17 Dec., 2013
	TNAU, Coimbatore – to give Lead Lecture in the National Workshop on Precision Farming Technologies for Banana	10 Jan., 2014
	Bioversity Work Plan Review Meeting – NRCG, Pune	17 Jan., 2014
	ICAR Instt. Directors Conference, Baramati	19-20 Jan., 2014
	Group Discussion of AICRP on Fruits, Dapoli	22-25 Jan., 2014
M. M. Mustaffa, R. Thangavelu, K. J. Jeyabaskaran and K. N. Shiva	First Stakeholders Consultative Meet on Banana, organized by CII & AC & RI (TNAU), Killikulam, Tuticorin Dt., Tamil Nadu	21 Jun., 2013
M. M. Mustaffa, S. Uma, R. Thangavelu, K.J. Jeyabaskaran and K. N. Shiva	Second Stakeholder Consultative Meet on Banana conducted by CII-Tamil Nadu Agriculture & Food Processing Panel and IICPT, Thanjavur, Tamil Nadu	31 July, 2013
B. Padmanaban	National Symposium on Emerging trends in Eco-friendly Insect Pest Management, TNAU, Coimbatore	22-24 Jan. 2014



Name of the Scientist	Name of the training programme /Venue	Period
B. Padmanaban, R. Thangavelu, R. Selvarajan, K.J. Jeyabaskaran and V. Kumar	Knoweldge sharing workshop on Tropical fruits - Banana, Mango and Pomegranate for value chain management and farm profitability enhancement, TNAU, Coimbatore organized by CHAI and ASSOCHAM	1-2 Mar., 2014
S. Uma	Consultation meeting on 'In-situ conservation and On farm Management of Musa wild species and landraces in India' between, organized by BIOVERSITY- International, New Delhi	10-11 Mar., 2014
	Workshop on Best Practices for <i>Musa</i> Germplasm Collection and Data Management, CIRAD in Guadeloupe (French West Indies) organized by BIOVERSITY and CIRAD, France	9-14 Dec., 2013
	Banana Global Musa Expert Workshop, Kampala, Uganda organized by BIOVERSITY- East Africa	8-11April, 2013
	ICAR- BIOVERSITY work plan meeting, NASC complex, New Delhi, organized by BIOVERSITY, India	14 May, 2013
	Knowledge Sharing Workshop on Banana Mango and Pomegranate, Coimbatore, organized by CHAI in collaboration with AIPUB, Trichy and JISL, Jalgaon, Maharashtra	1-2 Mar., 2014
	2 <sup>nd</sup> DUS Task Force meeting, PPV & FRA, New Delhi	17 Jul., 2013
M. M. Mustaffa, S. Uma, R. Thangavelu, and V. Kumar	Biannual AICRP (TF) workshop, MPKV, Dapoli, Maharashtra	20-23 Feb., 2014
S. Uma and S. Backiyarani	2 <sup>nd</sup> National Conference on Horticultural Biotechnology, IIHR, Bengaluru	13 Jun., 2013
M. M. Mustaffa, S. Uma and M. Mayil Vaganan	Review meeting of the Indian partners under the DBT-BIRAC funded Indo-Australian project, BIRAC office, New Delhi	24 Aug., 2013
R. Selvarajan	Asia-Pacific Congress of Virology, Amity University, Noida, New Delhi	17-20 Dec., 2013
	National symposium on Pathogenomics for diagnostics and management of plant diseases, CTCRI, Thiruvananthapuram	24-25 Oct., 2013
	Seminar on Horticultural Biotechnology IIHR, Bengaluru	14 June, 2013
	IBSC meeting, IIHR, Bangalore	16 Dec., 2013



Name of the Scientist	Name of the training programme /Venue	Period
I. Ravi	International Conference on Climate change and implication for water resources and nutrition security, Bangalore	15-16 Nov., 2013
K.J. Jeyabaskaran	Banana Farmers Meet' organized by the Saraswathi KVK, Kumaramangalam, Tamil Nadu	21 Nov., 2013
	Banana Tissue Culture – Awareness Meeting organized by the CII /FACE, Jai Krishnapur, Krishnanagar Dt., West Bengal	16 Dec., 2013
V. Kumar	International Conference on Water Quality and Management for Climate Resilient Agriculture, Jalgaon, Maharashtra	28-31 May, 2013
	International Conference on Tropical Roots and Tubers for sustainable livelihood under Changing Agro-Climate, CTCRI Thiruvananthapuram.	11-12 July 2013
	RFD Nodal Officers' Meeting, KAB-II, New Delhi	1-2 Aug., 2014
	Seminar on Horticulture, Dinamalar AGRI-EXPO, Vellore, Tamil Nadu	4 Aug., 2013
	National Seminar- cum Workshop on Canopy Management and HDP in Sub-tropical Fruit Crops, PFDC, CISH, Lucknow	22-24 Oct., 2013
M. M. Mustaffa and V. Kumar	Panel Discussion on Varietal Release of Banana Cultivars, TNAU, Coimbatore	9 Jan., 2014
V. Kumar	National Workshop on Precision Farming Technologies for Banana, TNAU, Coimbatore	10-11 Jan.2014
	Biennial Group Discussion Meeting of the AICRP (Fruits) at Dr. BSKVV, Dapoli, Maharashtra	22-25 Jan., 2014
	ICAR Special Programme on Integrated Crop Management in Banana, Maruthur, Karur, Tamil Nadu	4 Feb., 2014
	Group Discussion cum Review Meeting of ICAR Platform Project on Seed and Planting Material, IIHR, Bangalore	22 Feb., 2014
	XIII Scientific Advisory Committee Meeting of TNAU KVK, Santhiyur, Salem Dist, Tamil Nadu	12 June, 2013
	VI Scientific Advisory Committee Meeting of TNAU KVK, Tindivanam, Villupuram Dist, Tamil Nadu	19 June, 2013
	XVIII SAC Meeting of TNAU KVK, Vriddhachalam, Cuddalore Dist, Tamil Nadu	20 June, 2013
	Fifth SAC Meeting of TNAU KVK, Needamangalam, Tiruvarur Dist, Tamil Nadu	24 June, 2013



Name of the Scientist	Name of the training programme /Venue	Period
	38th SAC Meeting of TNAU KVK, Sirugamani, Tiruchirapalli Dist, Tamil Nadu	26 June, 2013
	Farmers-Scientists Interactive Meeting, Cumbum, Theni Dist., Tamil Nadu	16 May, 2013
M. M. Mustaffa and V. Kumar	Conducted a survey in banana growing areas of Fatehpur, Koshambi, Allahabad and Barabanki districts of Uttar Pradesh	25-26 Oct., 2013.
V. Kumar	Banana Farmers Meeting organized by KRIBHCO, Thottiam, Tamil Nadu	4 Mar., 2014
•	Annual Review meeting and transformation training of DBT/BIRAC Project: Biofortification and evaluation of Indian banana with iron constructs; NABI, Mohali, Chandigarh	15 – 17 Sept., 2013
M. Mayil Vaganan	National Conference on Bioactive compounds and functional foods in health and disease management and made an oral presentation; Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonepat, Haryana.	15-16 Nov., 2013
K. N. Shiva	Packaging of Fruits and Vegetables with special reference to Banana, organized by TGI Packaging Pvt. Ltd., Breeze Residency, Tiruchirappalli, Tamil Nadu	3 April, 2013
	National Seminar on Microbes and Human Welfare, organized by National Academy of Biological Sciences, Chennai and Dept. of Microbiology, Bharathidhasan University, Tiruchirappalli, Tamil Nadu	20 July, 2013
	One-day Workshop on Repeat study on Assessment of postharvest losses in Hort. Crops, Animal & Fishery products, NASC Complex, New Delhi	29 Aug., 2013
	NAIP-Organization & Management Committee (OMC) meeting at KAB-II, ICAR, PUSA, New Delhi	30 Aug. 2013
	AGRICON 1 <sup>st</sup> Steering Committee Meeting, organized by CII, Tiruchirappalli, Tamil Nadu	13 Sept., 2013
	National Seminar on Plastics in Agriculture, organized by Indian Plastics Institute, Chennai Chapter, Tiruchirappalli, Tamil Nadu	21Sept., 2013



Name of the Scientist	Name of the training programme /Venue	Period
	NAIP-sponsored MDP Workshop on Supply Chain Management in Agriculture, NAARM, Hyderabad	8-10 Oct., 2013
	Symposium (SYMSAC-VII) on Post-harvest Processing of Spices and Fruit Crops, Madikeri, Karnataka	27-29 Nov., 2013
S. Uma and S. Backiyarani	National Seminar on Horticulture Biotechnology, IIHR, Bangalore	13 Jun., 2013
M. S. Saraswathi	Brainstorming meeting and Training cum Demonstration on Cryopreservation and <i>in vitro</i> conservation in Horticultural crops, IIHR, Bengaluru.	21&22 Feb., 2014
	Knowledge sharing workshop on Tropical Fruits with the them Value chain management and Farm Profitability Enhancement, Coimbatore	1&2 March, 2014
	NABMGR meeting, NRC for Grapes, Nagpur	29 Nov., 2013
	First Task Force meeting of the DBT funded project on Income generation through conservation and cultivation of near extinct landraces, Kolli hills, Tamil Nadu	12&13, Sep., 2013
C. Anuradha	National symposium on Pathogenomics for diagnostics and management of plant diseases, CTCRI, Trivandram	15&16 Nov., 2013
	ICAR sponsored National Workshop (Intellectual Property and Technology Management in Agriculture -AgrIP 2013), Cochin	24&25 Oct., 2013
	DBT task force meeting and presented the project report of Evaluation of transgenic banana for resistance to Banana bunchy top virus, New Delhi	4 Feb., 2014.



Field visit by Dr. M.M. Mustaffa, Director and Dr. K.J. Jeyabaskaran, Pr. Scientist at Kulithallai, Tamil Nadu



Dr. M.M. Mustaffa, NRCB delivering a key–note address at National meet on fruit crops at Bangalore



#### WORKSHOPS, SEMINARS, SUMMER INSTITUTES, FARMERS 13 DAY, TRAINING PROGRAMME, ETC. ORGANIZED AT THE **CENTRE**

#### KISAN MELA

The National Research Centre for Banana celebrated its 20th Foundation day as "Banana Kissan Mela" with the theme on "Banana fruit care" on 21st August 2013. Dr.M.M. Mustaffa, Director, NRCB presided over the function and Thiru P. Velmurugan, B.L., Principal District and Sessions Judge, Tiruchirapalli was the chief guest of the function and the delivered chief guest address. On the occasion, a compilation



Farmer receives the "Best Banana Grower" Award at Kisan Mela

on 20 years of research achievements was released by the chief guest.

An exhibition was arranged to display technologies for banana fruit care, production, quality tissue culture plants, value added products and management of pest and diseases. Scientists of NRCB delivered talks on banana fruit care. Around 500 banana growers and many agricultural and horticultural officers, entrepreneurs from various districts of Tamil Nadu participated in the field day.



Farmers from different districts of Tamil Nadu at Kisan Mela meeting

#### 14 DISTINGUISHED VISITORS

Name and Address	Date
Dr. N. Kumar, Dean (Horticulture), TNAU, Coimbatore	13.5.2013
Dr. E.I. Jonathan, Director, Crop Protection, TNAU, Coimbatore	13.5.2013
Dr. R. Chittirai Selvan, Principal Scientist, IIHR, Bangalore	13.5.2013
Dr. S.V. Hittalmani, Director of Horticulture Incharge, Govt. of Karnataka	13.5.2013
Dr. K.B. Patil, Vice-President, Jain Irrigations System, Jalgaon	13.5.2013
Prof. Harvna Yakubu, V.C, University of Development Studies, Tamale, Ghana, Africa	13.5.2013
Prof. Dr. S. Sathiamoorthy (Former Director, NRCB), Coimbatore	16.5.2013



Name and Address	Date
Shri Siraj Hussain, IAS, Secretary, Ministry Food Processing, GOI, New Delhi	8.6.2013
Dr. A.K. Singh, Head Division of Fruits, IARI, New Delhi	20.7.2013
Dr. S. Mariappan, Dean TNAU, Coimbatore	4.10.2013
Dr. S. Ayyappan, Secretary-DARE & Director General-ICAR, New Delhi	9.10.2013
Dr. K.B. Patil, Vice-President, Jain Irrigations System, Jalgaon	11.9.2013
Dr. H.P. Singh, Former DDG (Hort), ICAR, New Delhi	19.9.2013
Dr. G.L. Kaul, Former VC, AAU, Ghaziabad, U.P.	27.12.2013
Dr. R.K. Tyagi, Head, Division of Germplasm, NBPGR, Pusa, New Delhi	27.12.2013
Dr. G. Chandra Sekar, Former Head, Dept. of Plant Pathology, TNAU, Coimbatore	27.12.2013



RAC members with Directore abd Scientists of NRCB on 27.12.2013



Shri Siraj Hussain, IAS, Secretary, Ministry Food Processing, Govt. of India, New Delhi visit to NRCB on 8.6.2013



Prof. Harvna Yakubu, V. C, with his team from Ghana, visited the Centre on 11.04.2013



Thottium (Tiruchirapalli Dt.) farmers attending one day training programme on banana cultivation at NRCB on 11.11.2013

#### Farmer's visits

More than 4800 farmers, agricultural & horticultural officers, self help group members and students visited the Centre.





#### 15 EMPOWERMENT OF WOMEN

About 1550 women including students, SHG members and other women entrepreneurs from different parts of country visited NRCB



SHG women attending one day training programme on banana fibre extraction at NRCB

and learnt various technologies available at NRCB on Crop Improvement, Crop Production, Crop protection and Postharvest.



TNAU horticulture students visit to NRCB

#### 16 PERSONNEL

#### Staff news

Name	Position	w.e.f
Dr. P. Sundararaju, Principal Scientist	Superannuated from service	30.04.2013
Ms. Richa Sood, Assistant	Resigned from service	30.05.2013
Mr. R. Krishnamurthy, Assistant	Promoted to Assistant Administrative Officer	01.07.2013
Dr. I. Ravi, Senior Scientist	Promoted to Principal Scientist	01.01.2011
Dr. V. Kumar, Senior Scientist	Promoted to Principal Scientist	01.01.2011
Dr. K.J. Jeyabaskaran, Senior Scientist	Promoted to Principal Scientist	15.07.2012
Mr. R. Neela Mega Shymala Kannan, Steno Gr. III	First Financial up-gradation granted under MACPS	01.09.2013

#### **Scientific Staff**

S. No.	Name	Designation
1	Dr. M.M. Mustaffa	Director
2	Dr. P. Sundararaju	Principal Scientist (up to 30.04.2013)
3	Dr. B. Padmanaban	Principal Scientist
4	Dr. S. Uma	Principal Scientist
5	Dr. R. Thangavelu	Principal Scientist
6	Dr. R. Selvarajan	Principal Scientist



S. No.	Name	Designation
7	Dr. I. Ravi	Principal Scientist
8	Dr. V. Kumar	Principal Scientist
9	Dr. K.J. Jeyabaskaran	Principal Scientist
10	Dr. M. Mayil Vaganan	Senior Scientist
11	Dr. K.N. Shiva	Senior Scientist
12	Dr. S. Backiyarani	Senior Scientist
13	Dr. M.S. Saraswathi	Senior Scientist
14	Mr. R. Natarajan	Scientist
15	Dr. C. Anuradha	Scientist

#### **Technical Staff**

S. No.	Name	Designation
1.	Dr. S. Palanichamy	Senior Technical Officer (Field)
2.	Dr. P. Durai	Senior Technical Officer (Field)
3.	Mr. P. Ravichamy	Technical Officer (Journalism)
4.	Mrs. T. Anitha Sree	Technical Officer (Field)
5.	Mrs. C. Sagayam Jacqueline	Senior Technical Assistant (Computer Programmer)
6.	Mr. D. Ramachandramurthi	Senior Technical Assistant (Civil Overseer)
7.	Mr. V. Selvaraj	Senior Technical Assistant (Field)
8.	Mr. T. Sekar	Senior Technical Assistant (Lab)
9.	Mr. R. Pitchaimuthu	Technical Assistant (Field)
10.	Mr. N. Marimuthu	Technical Assistant (Lab)
11.	Mr. K. Kamaraju	Technical Assistant (Lab)
12.	Mr. M. Bathrinath	Technical Assistant (Field)
13.	Mr. A. Subramanian	Technical Assistant (Driver)
14.	Mr. P. Mohan	Technical Assistant (Driver)
15.	Mr. V. Manoharan	Technical Assistant (Driver)

#### Administrative, Audits & Accounts and Supporting Staff

S. No.	Name	Designation
1.	Mr. M. Krishnan	Administrative Officer
2.	Mrs. C. Gomathi	Asst. Finance & Accounts Officer
3.	Mr. R. Krishnamurthy	Assistant Administrative Officer (w.e.f. 01.07.2013)
4.	Mr. M. Krishnamoorthy	Private Secretary
5.	Mr. P. Murugan	Assistant



S. No.	Name	Designation
6.	Ms. Richa Sood	Assistant (up to 30.05.2013)
7.	Mr. R. Sridhar	Personal Assistant
8.	Mr. R. Neela Mega Shyamala Kannan	Steno Gr. III
9.	Mrs. S. Durgavathy	Upper Division Clerk
10.	Mr. M. Devarajan	Lower Division Clerk
11.	Mrs. A.U. Suja	Lower Division Clerk
12.	Mr. R. Mohanraj	Mali SSG-IV
13.	Mr. V. Pandiyan	Mali SSG-III
14.	Mr. V. Thangaraju	Messenger SSG-II
15.	Mr. P. Kamaraj	Mali SSG-II
16.	Mr. V. Ganesan	Mali SSG-I
17.	Mrs. K. Mariammal	Safaiwala SSG-I



Dr. P. Sundararaju, Principal Scientist, superannuated from service on 30.04.2013.



#### 17 OTHER INFORMATIONS

## Director General, ICAR visits NRC Banana, Tiruchirapalli

Dr. S. Ayyappan, Director General-ICAR and Secretary-DARE, Government of India, New Delhi, visited National Research Centre



Dr. S. Ayyappan, Director General, ICAR declare open the Screen House at NRCB on 9.10.2013

## Protection of Plant Varieties and Farmers' Rights Awareness Programme

An awareness programme on protection of plant varieties and farmers' rights was held on 24. 03. 2014 at National Research Centre for Banana, Tiruchirapalli to create an awareness to the farmers about their rights on the local varieties and landraces of plants and the need for protection of the indigenous plant types. In this programme, lectures were delivered by Scientists. Around 100 progressive farmers from different parts of the state participated.



for Banana on 9.10.2013 and inaugurated the newly constructed Screen House, Net House and Glass House at NRCB, Tiruchirapalli. He encouraged the scientists to go for more patenting of the technologies.



Dr. S. Ayyappan, Director General, ICAR visiting Screen House and in discussion

Dr. S. Sathiamoorthy, Former-Director of the NRC banana was the chief guest and gave away awards to the farmers for conservation of seeds and plant materials. Dr. M. M. Mustaffa, Director in his presidential speech dealt upon the role and importance of PPV and FR and emphasised the need of the farmers for protecting the traditional varieties of rice, banana and other crops.

Mr. Siddhar from Thanjavur and Mr. Thirumalai of Kolli hills, Namakkal district were felicitated and given awards for their conservation efforts of different traditional rice varieties and banana varieties like karuvazhai,







Namarai and sambal vazhai. In the technical session, Dr. Sriram of TNAU spoke about the provisions of the PPV&FR Act, 2001 and the ways to register a new or important plant variety with the PPV&FR Authority, New Delhi. Dr. Geetha Rani, Gene Bank Manager, MSSRF, Chennai spoke on her experience of conserving the traditional rice varieties and other crops with the help of local tribal states of Odisha and Kerala.

### National Science Day Celebration at NRCB



The National Research Centre for Banana (ICAR), Trichy celebrated 'National Science Day' on 28th February, 2014 remembering 86th year of Dr. C. V. Raman's Nobel Prize winning discovery of "Raman Effect" with a theme



on 'Fostering Scientific Temper' among school and college students. Director of the Centre, Dr. M. M. Mustaffa inaugurated the seminar and exhibition and delivered the presidential address. The primary objective was to expose the students to recent research developments and banana also to create an awareness and motivation among the students. The day was open to students to visit the exhibition and laboratories to interact with the scientists. Over 650 students from different schools and colleges visited the Centre on this occasion.

Scientists of the Centre explained research activities on banana varieties, tissue culture techniques, drip irrigation, fertigation method, eco-friendly bio-control agents available for management of pests, diseases, nematodes and banana value added products from banana fruits and plant.



#### **ANNEXURE - I**

#### **ON-GOING PROJECTS DETAILS**

#### I. List of On-going Institute Projects

1.	Cron 1	<b>Improvement</b>	

2	2000711002	Crop improvement of banana through conventional breeding (M.M. Mustaffa)
2	2000711004	Improvement and management of banana genetic resources in Indian subcontinent (S. Uma)
2	2000711005	Identification and characterisation of nematode resistance genes in banana (S. Backiyarani)
4	2000711006	Improvement of Rasthali through induced mutagenesis (M.S. Saraswathi)

#### 2. Crop production & Postharvest Technology

- I I	
2000713001	Standardization of agro-techniques for banana production and productivity (V. Kumar)
2000713006	Fertilizer tailoring for targeted banana yield and sustainable soil health (K.J. Jeyabaskaran)
2000713007	Studies on nutrient dynamics in banana (K.J. Jeyabaskaran)
2000716002	Drought stress tolerance in banana: Understanding the physiological, biochemical and molecular mechanism of drought tolerance (I. Ravi)
2000716003	Salt stress tolerance in banana: Understanding the physiological, biochemical and molecular mechanism of salt tolerance (I. Ravi)
2000716004	Physiological and biochemical mechanism of nematodes and pseudostem weevil resistance and identification of 'biomarker metabolites' in banana (M. Mayil Vaganan)
2000716005	Biochemical and molecular basis of ripening of banana fruit and its manipulation with biochemicals (M. Mayil Vaganan)
2000717003	Development of pre and post harvest techniques for leaf production in banana (K.N. Shiva)
2000717004	Development of modified atmosphere packaging techniques in banana and plantain for domestic and export markets (K.N. Shiva)
2000717005	Development and refinement of value added products in banana and plantain (K.N. Shiva)



#### 3. Crop Protection

2000715006	Management of Banana weevils (B. Padmanaban)
2000715003	Investigation on fungal and bacterial diseases of banana and their management (R. Thangavelu)
2000715005	Studies on viral diseases of banana and their management (R. Selvarajan)
2000715007	Host-virus interactions in Banana: Molecular mechanisms of resistance and susceptibility, latency, integration and episomal expression of EPRV's (R. Selvarajan)
2000715008	Proteomic analysis of host-BBTV interaction in banana (C. Anuradha)

#### **II. ICAR Funded Projects**

S. No.	Project Title
1	Network Project on Transgenic in crops – Banana functional genomicsSigatoka component Drought component (S. Uma)
2	Outreach Project on Phytophthora, Fusarium & Ralstonia diseases of horticultural and field crops (R. Thangavelu)
3	Network Project on Harnessing arbuscular mycorrhizae in horticultural crops (R. Thangavelu)
4	Network Project on Transgenic in Crops - Transgenic Component: Development of transgenic banana resistant to Banana Streak Virus (BSV) and Banana Bunchy Top Virus (BBTV) (R. Selvarajan)
5	Intellectual Property Management and Transfer/ Commercialization of Agricultural Technology – IPR (C. Anuradha)
6	Development of banana central core stem slicer and juice extractor (K.N. Shiva)

#### III. Projects Funded by other Agencies

S. No.	Project Title	Funded by
1	Improved livelihoods through conservation and rejuvenation of near extinct banana landraces of Kolli hills of Tamil nadu (S. Uma)	DBT
2	Framing Crop Specific DUS Guidelines for Banana (S. Uma)	PPV&FRA
3	Accredited Test Laboratory and National Certification System for Tissue Culture raised Plants (NCS-TCP) Genetic Fidelity Testingcomponent Virus Indexing component (R. Selvarajan and M.S. Saraswathi)	DBT



S. No.	Project Title	Funded by
4	Identification of molecular strategies for the control of Cosmopolites sordidus Ger.) ( <i>Coleoptera: Curculionidae</i> ) (a major pest of bananas (B. Padmanaban)	DST
5	Molecular approaches for the control of <i>Odoiporus</i> longicollis (Oliver) (Coleoptera: Curculionidae) (a major pest of banana) (B. Padmanaban)	DBT
6	Eco-friendly approaches for the management of coffee white stem borer, <i>Xylotrechus quadripes</i> Chev. (Coleoptera: Cerambycidae) (B. Padmanaban)	Coffee Board
7	Biofortification and development of disease resistance in banana	DBT
	Component-I: Transfer and evaluation of Indian banana with pro Vitamin-A (PVA) constructs (S. Backiyarani)	
	Component-II: Transfer and evaluation of Indian banana with Iron constructs (M. Mayil Vaganan)	
	Component-III: Development of efficient ECS for Rasthali and providing authentic virus free IMFC to Indian Partners (S. Uma)	
8	Evaluation of transgenic banana for resistance to Banana Bunchy top virus (Replicase mediated) (R. Selvarajan)	DBT
9	Development of Bio-Pesticide formulation for reducing post harvest losses and for achieving export quality and increased shelf life of banana fruits (R. Thangavelu)	DBT
10	Proteomic studies of host-pathogen interactions in Banana - Banana Bract Mosaic Virus (BBrMV) system (C. Anuradha)	DST

#### IV. Contract Research Projects

Evalution of MET 52 EC and granules on banana against corm weevil (Cosmopolites sordidus). (B. Padmanaban)



#### **ANNEXURE - II**

#### **METROLOGICAL DATA**

Month	Min. Temp. (°C)	Max. Temp. (°C)	Relative Humidity (%)	Rain Fall (mm)
April 2013	27.43	39.80	78.36	_
May 2013	27.80	40.54	76.22	10.0
June 2013	27.20	36.30	78.66	5.0
July 2013	26.77	36.77	68.96	_
August 2013	25.96	36.29	73.18	134.8
September 2013	24.65	34.88	78.45	143.5
October 2013	24.41	34.75	92.00	106.5
November 2013	22.86	31.06	90.50	112.0
December 2013	22.60	30.50	88.14	46.2
January 2014	21.75	32.92	88.90	_
February 2014	22.68	33.56	89.90	_
March 2014	23.79	36.85	88.40	_
Total				558.0





## राष्ट्रीय केला अनुसंधान केंद्र

भारतीय कृषि उनुसंधान परिषद तायनूर पोस्ठ तोगमलै रोड तिरूच्चिरापिक ६२० १०२, तमिल नाडु

#### **National Research Centre for Banana**

(Indian Council of Agricultural Research)

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