



# ICAR - NRCB NEWSLETTER

ICAR - National Research Centre for Banana

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## From Director's Desk

At ICAR-NRCB, one of the main areas of research of Crop Production Section is biochemistry and research carried out is summarized and presented under IN FOCUS of this Newsletter. The suppression of pheophosphide *a* oxygenase activity at higher temperature was found to be the biochemical basis of green ripening in Cavendish bananas. The biochemical mechanism of resistance to root lesion nematode has indicated the elevation of key enzyme, cinnamyl alcohol dehydrogenase activity, leading to higher synthesis of phenolic metabolites. By metabolomic analysis during ripening, isomaltulose with low glycemic index has been found in the banana fruit. Pre- and post-harvest green life of bananas using ripening retarding biochemicals have been developed for long time storage and long distance transportation and biochemical mechanism of delay in ripening has also been worked out. An efficient protocol for proteomic analysis in banana roots, a recalcitrant tissue, is standardised. Further, putative proteins/ enzymes *vis-à-vis* finger drop, a major physiological disorder associated with ripening of bananas have been suggested by proteome analysis.



Fifty nine exotic accessions including 19 from ITC, Belgium and 40 hybrids of IITA have been introduced for evaluation. One of the mutants of cv Rasthali has been promising with resistance to Fusarium race 1. In crop production, nutrient composition of vermicomposted banana residues varied with varieties. Vacuum oven drier was found better to dry banana slices with good quality attributes. Nendran flour exhibited higher biomolecules compared to other varieties.

Record of *Fusarium* wilt TR4 in Uttar Pradesh is of main concern and effective sensitization programmes have been conducted by ICAR-NRCB in five states in collaboration with respective state departments, Quarantine personnel, SAU's and Bioversity International. Lateral Flow Immune strip test kit has

been developed for quick, easier and onsite detection of BBrMV and validated across strains and the same was released by the Honourable Agriculture Minister, Shri Radha Mohan Singh during 89<sup>th</sup> foundation Day on 16.7.2017.

ICAR-NRCB was successful in sea shipment of banana consignment, at one seventh the cost of air freight. We signed MoU with prestigious NIT, Trichy, for developing technologies and student exchange for PG research, MoC with AP state government as their knowledge partners. Mega National Banana Festival was conducted in collaboration with the TN State government and TNAU where more 100 banana varieties were showcased. ICAR-Bioversity-IITA collaborative project supported by Melinda Gates Foundation was also launched. The Centre conducted 2 training programmes on 'Hi tech Horticulture' for Bayer officials and Technology exposure to more than 500 farmers and 30 entrepreneurs and woman SHG's. Two products have been commercialized. One DST Postdoc award and one Young Scientist project to work at ICAR-NRCB have been awarded by DST. Kisan Mela was celebrated with three foreign delegates from IITA, NARO and Bioversity. This being the beginning of our Silver Jubilee Year (1993-2018), yearlong celebrations with diverse programs are envisaged.

*S. Uma*  
(S. UMA)

## INFOCUS

**Biochemical mechanism of resistance of bananas to root-lesion nematode, *Pratylenchus coffeae***

### Phenylpropanoid enzyme activities

The biochemical mechanism of resistance of banana cultivars to the root lesion nematode, *Pratylenchus coffeae*, revealed the temporal activities of first and last enzymes of phenylpropanoid pathway, phenylalanine ammonia lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD), in response to nematode infection in the roots of both resistant cvs. Yangambi Km5 and Anaikomban and susceptible cvs. Nendran and Robusta

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increased from 4<sup>th</sup> day and reached the maximum activity at 7<sup>th</sup> day post-inoculation. However, the activity induction of PAL and CAD at 7<sup>th</sup> day in resistant cultivars was 6.2-times (163 pico katal/mg protein) and 18-times (360 pico katal), respectively higher than the constitutive levels and 5.2-times and 7.3-times greater than in susceptible cultivars.

#### Phenolic acids induction levels and lignin accumulation

The infection of *P. coffeae* caused a significant increase in total soluble phenolic acids from the 4<sup>th</sup> day reaching maximum at 7<sup>th</sup> day post-inoculation with 4.2-times (82 µg per g fresh wt.) higher than the uninfected control (19.4 µg per g fresh wt.) and 3-times (27.5 µg per g fresh wt.) greater than in the susceptible cultivars. The increase in lignin deposition in the roots of resistant cultivars was rapid and reaching the maximum at 7<sup>th</sup> day with 188 µg per mg root cell wall dry wt., which was 4.3-times higher than the uninfected control (44 µg per mg root cell wall dry wt.) and 3.3-times (57 µg per mg root cell wall dry wt.) greater than susceptible cultivars.

#### Phenolic metabolites vis-à-vis *P. coffeae* infection

Six phenolic metabolites viz., protocatechuic acid ( $R_t = 6.47$ ), vanillic acid ( $R_t = 10.13$ ), caffeic acid ( $R_t = 11.54$ ), *p*-coumaric acid ( $R_t = 12.69$ ), ferulic acid ( $R_t = 21.76$ ) and sinapic acid ( $R_t = 25.60$ ) were found in root tissues of resistant cv. Yangambi Km5 and susceptible cv. Nendran and no qualitative change existed in the number of metabolites between resistant and susceptible cultivars. Upon nematode infection, accumulation of vanillic, *p*-coumaric, ferulic and sinapic acids were 3.70-, 2.86-, 2.97- and 3.19-times higher in resistant cultivar compared to the uninfected control. The accumulation of phenolic acids was 2-times lesser in susceptible cultivar.

#### Protein extraction method for banana rooteomics analysis

Development of an efficient protocol for protein extraction from banana root, a recalcitrant tissue, for proteomic analysis is the first essential step. Out of four methods viz., the Trichloroacetic acid-acetone (TCA), Phenol-ammonium acetate (PAA), Phenol/SDS-ammonium acetate (PSA) and Tris base-acetone (TBA) evaluated with modifications from Grand Nain roots, the PAA yielded highest protein (0.89 mg/g) and produced highest number of protein spots (584) in 2-DE gels.

#### Banana root proteomics response to *P. coffeae* infection

Forty differentially expressed proteins (DEPs) with more than 2 fold changes were mapped between resistant banana cv. Anaikomban and susceptible cv. Nendran upon root lesion nematode infection. Biological annotations of 30 out of 40 DEPs showed that these proteins/enzymes were involved in energy metabolism, transcription of PR genes, cell wall remodeling and stress. Validation by semi-quantitative RT-PCR of three highly over-expressed enzymes viz., glutamine reductase, β-galactosidase and cinnamyl alcohol dehydrogenase showed that the transcript levels of these enzymes/proteins were higher in the roots of Anaikomban and Nendran infected with root-lesion nematode compared to uninfected control roots.

#### Metabolomic analysis and detection of palatinose

Metabolomic analysis is a novel tool to understand the biochemistry of biological processes. Methanol: chloroform: acetonitrile (2:1:1 v/v) solvent system and 10 mg peel and pulp tissues in one ml of solvent were found best for banana with detection of more than 1500 metabolites including 250 known metabolites from NIST library. Analysis of metabolites in Grand Nain fruit during ripening revealed presence of palatinose (isomaltulose), a structural isomer of sucrose with noncariogenic and low glycemic index characteristics.

#### Pre- and post-harvest enhancement of banana green life

Enhancement of green life of bananas is essential to minimize the post-harvest losses and to transport to long distance markets. Treatment of 1-methylcyclopropene (1-MCP) at 1 µl/L concentration for 12 hrs enhanced the green life of full mature (95%) Grand Nain and Poovan fruits by 10 days over the control at ambient temperature and 19 and 26 days more at 13.5°C. Similarly, treatment of full three quarter mature (85%) Grand Nain and Poovan fruits with 1-MCP increased the green life by 20 days at ambient temperature and by 44 and 26 days at 13.5°C over control fruits. Enhancement of green life of 95% and 85% mature bananas of both genotypes at 21°C was similar to storage at ambient temperatures. The physiological, biochemical and quality parameters of 1-MCP treated and ripe bananas were similar to untreated control bananas.

Pre-harvest treatment of mature (100%) Poovan bunches with 1-MCP at a concentration of 1 µl/L for 12 hr and covering bunches with polythene sleeves airtight enhanced *in planta* green life for 10 days after which the bunches showed maturity browning.

#### Mechanism of 1-MCP action on delayed ripening

Assay of S-adenosylmethionine synthetase, 1-aminocyclopropane 1-carboxylase (ACC) synthase and ACC oxidase and quantification of precursors of ethylene biosynthesis such as methionine, S-adenosylmethionine and 1-aminocyclopropane 1-carboxylic acid during ripening for 7 days in 1-MCP treated and untreated control Poovan bananas showed lower ACC synthase activity (2.32 nmol/g) coupled with higher levels of S-adenosylmethionine in 1-MCP treated bananas against control (5.26 nmol/g) on 5<sup>th</sup> day of treatment indicating the 1-MCP tends to block the ACC synthase activity and consequently causing accumulation of S-adenosylmethionine, the substrate for ACC synthase.

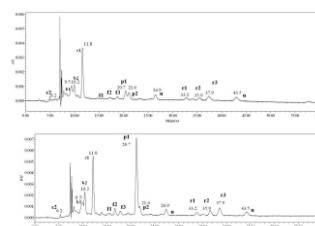
#### Biochemistry of finger drop in bananas

Finger drop is a major physiological disorder associated with postharvest ripening of bananas. A comparative analysis between Rasthali (a sensitive cultivar) and Poovan (a resistant cultivar) showed complete (100%) dropping on 5<sup>th</sup> day of ripening in Rasthali whereas no dropping occurred in Poovan even on 6<sup>th</sup> day of ripening stage 7. Assaying of ACC synthase and polygalacturonase in tissues at fruit pedicel rupture area showed significantly higher activity levels on 4<sup>th</sup> and 5<sup>th</sup> days in susceptible cv. Rasthali coinciding with finger drops than in resistant cv. Poovan.

By proteomic analysis, five finger drop-responsive proteins viz., ACC oxidase, polygalacturonase, pectate lyase, xyloglucan endotransglycosylase/hydrolase and expansin are identified as putative candidates of finger drop phenomenon.

#### Green ripening of Cavendish (Grand Nain) bananas

Investigation of biochemical mechanism of stay green trait (failure in chlorophyll breakdown) during ripening of Cavendish bananas at higher temperatures revealed that between bananas ripened at 21 and 31°C, fruits stored at 31°C remained green and retained higher levels of chlorophylls, exhibited very low



**Fig. 1.** Profiles of chlorophyll and catabolites in peel tissues of Grand Nain at 21°C (top) and 31°C (bottom); pheophorbide a (peak p1 with Rt 20.7)



phosphorhydrolase activity coupled with higher accumulation of PaO's substrate, implying partial impairment of PaO activity contributing to the 'stay green' trait in Cavendish bananas at higher temperatures.

#### Proteomic analysis of NaCl stress in banana

Proline dehydrogenase, glutamine synthetase, methionine adenocyltransferase, osmotin like protein, beta-glucosidases, cyclin-D3-2, map kinase 4, defensin-like protein 72, mitogen activated protein kinase 12 and protein dehydrogenase involved in cell cycle and development, signal transduction and proline catabolism were important enzymes/ proteins highly regulated in roots of cv. Saba (salt resistant) and cv. Grand Nain (salt sensitive) in response to high NaCl (100 mM) concentration.

Based on the expressed proteins, a generic model for transduction of signals in the cells in response to NaCl stress in bananas is constructed and showed their involvement of salt overlay sensitive (SOS), mitogen activated protein (MAP) kinase cascades, H<sub>2</sub>O<sub>2</sub> signaling, Na ions extrusion, lignification of vascular bundles and root growth retardation pathways of plant.

### RESEARCH HIGHLIGHTS

#### Crop Improvement

Seven accessions were collected from BRS, Kannara and nineteen accessions were introduced from ITC, Belgium through NBPGR, New Delhi. Morpho-taxonomic characterization was completed for two hybrid progenies of Pisang Jajee x Matti and Saba x Pisang Lilin. Ornamental banana hybrids have been developed by crossing among *Musa laterita*, *M. ornata*, *M. siamensis*, *M. rubra*, and *M. velutina*. Field evaluation of banana cv. Udhayam derived from three different explants recorded higher yield in flower bud derived plants (26 kgs in 373 days) than sucker derived plants (20.3 kgs in 391 days). Evaluation of elite clones of banana cvs. Grand Nain and Ney Poovan collected from different parts of Tamil Nadu indicated that clones of cvs. Grand Nain and Ney Poovan which were collected for specific traits like dwarfness, short duration and higher yield maintained their traits in the first crop. Multiple shoots have been obtained through embryo culture of Pro-207 self pollinated seeds. LD50 have been determined for EMS and DES using both shoot tip (1% for 5 hrs and 15mM for 5 hrs) and ECS (0.2% for 1.5 hrs and 2 mM for 1.5 hrs) explants of cv. Grand Nain. Rasthali mutant, NRCB RM 217 was found resistant to fusarium wilt under both pot as well as under wilt sick plot. Genotyping of 153 germplasm accessions was completed for 15 EST-SSR primers. Amino-methyltransferase, 3-dehydroquinate dehydratase, Phospho-methylpyrimidine-synthase and Tubby-like F-box protein 1 genes showed polymorphism between drought tolerant and susceptible cultivars.

#### Crop Production

Amount of nutrients (N, P, K, Cu, Zn and Fe) removed through bunch harvest by cv. Rasthali was 20% more than cv. Ney Poovan. Nutrient content of vermicompost made from residues of cv. Ney Poovan was > 20% higher than those from residues of cv. Rasthali. The Quantum Yield (YII) of PS II under steady state in field grown banana plants subjected to drought stress alleviation chemicals (Acetyl Salicylic Acid + Butylated Hydroxy Toluene) recorded higher (0.47 to 0.53) than untreated drought plants (0.37 to 0.41). The photosynthesis decreased in all drought treatments (0.89 to 2.03  $\mu\text{mol of CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) compared to irrigated control (18.07  $\mu\text{mol of CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) after the soil water potential reached -0.8 to -0.9 MPa. However, the plants primed with ASA+BHT recorded 65% more photosynthesis than non-primed drought plants. Banana flour based pasta products were standardised with banana flour and maida in the ratio of 70:30. Banana flour based

extruded products such as pasta enriched with carrot and beet root juices were standardized and developed. Successful sea shipment of Nendran banana to Dubai was accomplished at 1/7<sup>th</sup> the cost of air freight without affecting fruit quality. The removal of water from the banana slices was faster with vacuum oven drier (300 min) compared to solar drier (600 hrs). Color, texture, flavor and taste was superior with vacuum drying conditions than solar drier which tends to make the slices a bit darker. With the increase in temperature, the swelling power of flour of some banana cultivars was increased. The order of increase in swelling was Monthan (13.27 %) > Saba (12.93 %) > Nendran (11.33 %) > and Grand Naine (10.85 %) with Nendran flour having higher accumulation of bioactive compounds.

#### Crop Protection

Out of 47 *Musa* germplasm accessions screened, 20 were found to be moderately susceptible to banana stem weevil. Volatiles from banana pseudostem weevil were identified using NIST library. Botanical consortium having non-edible oils and aqueous plant extracts screened against stem weevil under *in vitro* and field condition indicated 100 and 91% weevil mortality, respectively. Heavy incidence of red palm mite, *Raoeilla indica* Hirst (Acari: Tenuipalpidae) was observed on banana during June-August. Two predators, *Stethorus pauperculus* Weise (Coleoptera: Coccinellidae) and *Scolothrips rhagebianus* Priesner (Thysanoptera: Thripidae), were found to be very effective against all stages of the mite. A recent survey along with ICAR-CSSRI, Lucknow conducted in the month of September 2017, in the villages Katrauli, Mangalsi and Muksoonganj of Sohawal block of Faizabad district, in Uttar Pradesh indicated the confirmed presence of *Fusarium* wilt disease in cv. Grand Nain and the incidence was ranged from 30 to 45 percent and about 95% of the area under banana was affected. The farmers were sensitized about the measures to contain the further spread of the disease to uninfected areas and also the management of the disease in the infected fields.



Field of banana (cv. Grand Nain) affected by the *Fusarium* wilt (TR-4) in Uttar Pradesh

Recombinase polymerase amplification assay has been standardized for banana streak mysore virus (BSMYV). Lateral flow immune-strip test kit was developed for quick, easy and onsite detection of banana bract mosaic virus (BBBrMV) and this dipstick has been validated by testing more than 30 isolates. A LAMP based kit developed for banana bunchy top virus (BBTV) has been validated by testing 70 hill banana samples from lower Pulney hills. About 6911 tissue culture banana samples were tested and 31.66 million tissue culture plants were certified. Genes from banana bract mosaic virus and cv. Nendran were cloned in yeast vectors. Quantitative  $\beta$ -galactosidase assay was performed. Six eIF4E genes from banana were amplified with specific primers, cloned and sequenced. Further they were sub-cloned in yeast vectors for studying its interaction with VPg. Nano formulations of zimmu and quercetin were found effective in controlling root-lesion nematode with 98.84 and 92.74% mortality under *in vitro* conditions.

Events	Date	Details
Inauguration of Indo-African research project titled 'Improvement of banana for smallholder farmers in the Great Lakes Region of Africa'	1 April, 2017	Bioversity International- IITA-ICAR-NRCB collaborative mega project with research component titled 'Breeding for improved banana with <i>Fusarium</i> wilt resistance' was launched at ICAR-NRCB, Tiruchirappalli.
Signing of MoU for Nendran export	20 April, 2017	ICAR-NRCB signed MoU with APEDA & M/s. Fair Exports India Ltd., Kochi for export of banana cv. Nendran by seaways.
IMC meet	23 May, 2017	ICAR-NRCB, Tiruchirappalli.
International yoga day	20 June, 2017	Staff of ICAR-NRCB were taught yoga by Jai Ranga Nature Cure Hospital and Yoga Research Centre, Tiruchirappalli.
Training on "Hi-tech banana cultivation for enhancing the production and productivity of quality bananas".	21-23 June, 2017	Three day training on various aspects of banana cultivation was provided to officials of Bayer Crop Sciences Ltd., Mumbai at ICAR-NRCB, Tiruchirappalli.
Release of dip-stick test kit at the ICAR foundation day by the Union Minister of Agriculture and Farmer's Welfare, Government of India.	16 July, 2017	The dip-stick was developed by ICAR-NRCB for the on-site detection banana bract mosaic virus (BBrMV).
National banana festival at AC&RI, Madurai, Tamil Nadu	21-23 July, 2017	ICAR-NRCB scientists participated, delivered lectures and displayed around 75 bunches of different banana cultivars and value added products of banana.
Signing of MoA with NIT, Tiruchirappalli	26 July, 2017	ICAR-NRCB signed MoA with NIT, Tiruchirappalli for developing technologies and employing students for internship and Post Graduate research programmes.
Sensitization program on <i>Fusarium</i> wilt TR-4 in collaboration with Bioversity International, India	5 August, 2017	Stakeholders from AICRP-Fruits, UHS, Bagalkot, UAS, Bangalore under the chairmanship of Dr. B.N.S. Moorthy, Horticulture Commissioner, GOI, New Delhi.
24 <sup>th</sup> ICAR-NRCB foundation day & Kisan Mela	21 August, 2017	Dr. C. Anandharamakrishnan, Director, IIFPT, Thanjavur was chief guest of the event. Dr. Rony Swennen, IITA & KUL, Belgium; Dr. Allan Brown, IITA, Tanzania & Dr. Robooni, NARO, Uganda were guests of honour. Special lectures were delivered by Drs. Rony Swennen, R. Thangavelu and K. N. Shiva.
Sadbhavna divas	18 August, 2017	Staff of ICAR-NRCB took pledge.
Sewa divas	17 September, 2017	Cleaning activities by staff at staff quarters of ICAR-NRCB.
Swachhta Hi Seva	20-25 September, 2017	Cleaning activities by staff at and near ICAR-NRCB.
Hindi Pakhwara	14 -28 September, 2017	Conduction of various Hindi competitions and distribution of prizes.



Signing of MoC by Director, ICAR-NRCB with Chief Minister of Andhra Pradesh



Release of publications during ICAR-NRCB Foundation day & Kisan Mela, 2017



Signing of MoU by ICAR-NRCB with APEDA and Fair Exports for export of Nendran to Dubai via seaport

### STAFF NEWS

Name	Event	Date
Mr. B. Sathish, Senior Administrative Officer	Transferred to ICAR-IIRR, Hyderabad	21 April, 2017
Mr. V. Selvaraj, Senior Technical Assistant	Promoted to Technical Officer	w.e.f. 5 March, 2017
Mr. T. Sekar, Senior Technical Assistant	Promoted to Technical Officer	w.e.f. 10 March, 2017
Mr. K. Kamaraju, Senior Technical Assistant	Promoted to Technical Officer	w.e.f. 10 March, 2017
Mr. R. Mohanraj, Skilled Supporting Staff	Promoted to Lower Division Clerk	w.e.f. 21.04.2017
Mr. P. Mohan, Senior Technical Assistant	Superannuation	31 July, 2017

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