

# ICAR - NRCB

## Newsletter



ICAR - National Research Centre for Banana

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### DIRECTOR'S DESK

I am happy to share that ICAR-NRCB successfully hosted and conducted the International Conference on Banana (ICB-2020) during 23-25 February, 2020. The conference was attended by more than 500 participants including international delegates from Australia, Belgium, France, Czech Republic, Turkey, South Africa, Zambia, Kenya, Uganda, Nigeria, Philippines and Indonesia, besides scientists and other stakeholders from different parts of India, students, entrepreneurs and farmers. Scientific deliberations and discussions were held in 11 technical sessions and a satellite workshop on "Fusarium wilt, Tropical Race 4 (*Foc-TR4*)" was also conducted. In this newsletter, 'Fusarium wilt - Tropical Race 4 – A destructive disease in Banana' is discussed 'In Focus'. Occurrence of *Foc-TR4* was reported from Bihar and Uttar Pradesh during 2015. Since then, surveys on its occurrence, distribution and damage and numerous sensitization and awareness programs were conducted by the centre. The centre has identified resistant sources by screening at *Foc-TR4* hotspot areas and also identified effective biocontrol agents for its management.



This year, three elite selections identified and developed by the centre, Kaveri Saba, Kaveri Haritha and Kaveri Kanya, have been recommended for release as central varieties. The centre collected 13 species of wild banana; developed NCR 17, a promising Nendran based hybrid; identified promising hybrids resistant to Fusarium wilt, race 1 and root-lesion nematode and also developed TBM-9, a promising dwarf mutant line which was recommended for MLT under ICAR-AICRP (Fruits). Significant research achievements by the Crop Production section include working out of nutrient dynamics for cvs. Grand Naine and Nendran; development of clump management technology for cvs. Ney Poovan and Poovan; identification of drought tolerant cultivars suitable for different water stress regimes; development of technologies for reducing finger drop; and profiling of flavonoids and anthocyanins in banana peel and flower. Fruits of banana cultivars with better glycemic index were identified. In Post-Harvest management, extension of shelf-life of banana leaf and fruits was standardized. Banana flour and peel powder based products were prepared and characterized. Research on banana starch, peel flour, stem powder and fibre has led to an array of products and technologies. In Crop Protection section,

species complex of banana leaf and fruit scarring beetle was documented from different states of India. Isolates of microbial biocontrol agents effective against stem weevil and volatile compounds for use as attractants / deterrents against weevils from different banana cultivars were identified. Promising microbial isolates and their effective consortia were identified for management of Fusarium wilt race 1 and tropical race 4. Molecular characterization of various banana viruses was carried out. The centre also developed and validated a lateral flow immunoassay device for on-site detection of Cucumber Mosaic virus.

During 2019-20, the centre successfully conducted one international conference, two workshops and 17 trainings. A total of 27 research articles were published in various journals of national and international repute and 25 presentations in various national and international conferences / seminars were made by the researchers of the centre. The Centre has signed MoU's with VFPC, Kerala and provided consultancy services towards a sea shipment protocol for exporting Nendran banana to the European Union. Various post-harvest value added products were commercialized through training cum sale of technology. The centre has research linkages with international institutes like Bioversity International, France, QUT, Australia, IITA-Nigeria, NARO-Uganda and more than 30 national institutes.

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### IN FOCUS

#### Fusarium wilt –Tropical race 4 –A devastating disease of banana

Although India is bestowed with more than 20 commercial varieties, the Indian banana trade mainly depends on Cavendish banana (cv. Grand Naine). Cv. Grand Naine occupies 52% and 64% of the area and production respectively. Among the production constraints, the tropical race 4 of Fusarium wilt disease (*Foc TR4*) has become most important as it is considered as the most devastating disease on Cavendish bananas around the world. *Foc TR4* has been reported from countries such as China, Taiwan, Philippines, Indonesia, Malaysia, Australia, Pakistan, Laos, Vietnam, Oman, Jordan, Mozambique, Israel and Colombia. Financial losses due to *Foc TR4* were estimated

to be 121 million USD in Indonesia, 253 million USD in Taiwan and more than 7.5 million USD in northern Mozambique. In Australasia more than 100,000 ha of commercial banana plantations have been devastated by *Foc* TR4.

### Survey, identification and characterization of *Foc* TR4 in Bihar and Uttar Pradesh

In December 2015, wilt disease symptoms were observed in the cvs. Grand Naine and Robusta grown in Barari village of Katihar district, Bihar. Analysis of the samples at ICAR-NRCB confirmed that the *Fusarium* wilt observed belonged to Tropical race 4 (VCG 01213/16). Subsequent surveys conducted in Uttar Pradesh and Bihar confirmed the presence of *Foc* TR4 in Uttar Pradesh (Ayodhya and Maharajganj districts) and Bihar (Katihar and Purnia districts).



*Fusarium* wilt TR4 devastated field in Uttar Pradesh

### Genome sequence analysis

To understand the genome organization of *Foc* strain, TR4 (VCG01213/16), genomic fungal libraries of ~300bp were sequenced using the Illumina NextSeq® 500 system for 150×2 cycles. The sequence analysis indicated that the genome of *Foc* TR4 of VCG 01213/16 is 47.38 mb with 51.1% GC content. A total of 15,508 (96.15%) proteins were annotated from 16,129 using UniProt database with a cutoff E-value of 10<sup>-5</sup> and remaining 621 (3.85%) were unannotated or uncharacterized proteins. Plant Host Interaction (PHI) search showed that there were 365 putative virulence-associated genes that have been identified against reference *Foc* TR4 of which 19 are unique. The genes that are found to change the phenotype of the organisms specifically the pathogenicity are ABC1, kdpB, acrB, oqxA & B and pstB which belong to cellular transporter protein-encoding unigenes essential for import of nutrients and export of secondary metabolites. Among the 14 secreted in xylem (SIX) protein gene clusters (SIX1-SIX14), SIX1, SIX2, SIX6, SIX8 and SIX9 have been found to be present in the *Foc* TR4 genome. Moreover, the presence of homologues SIX8, both SIX8a and SIX8b in the genome of *Foc* TR4 strains is a major difference noticed.



Syntony map of *Foc* TR4 in comparison with the reference genome, Rfoc 4287 and Rfoc TR4

### Possible impact of *Foc* TR4 in India

*Foc* TR4 is a soil borne pathogen that persists in the soil for many years even in the absence of the host. As *Foc* TR4 spreads through planting material, bunches, soil, irrigation water and bunch stalk, chance for further spread of this strain to other banana growing states will be a serious threat to the livelihood and sustainability of banana farmers.

### ICAR-NRCB initiatives for the management of *Foc* TR4

ICAR-NRCB has carried out several sensitization programs and research activities for preventing the further spread of *Foc* TR4 and its management.

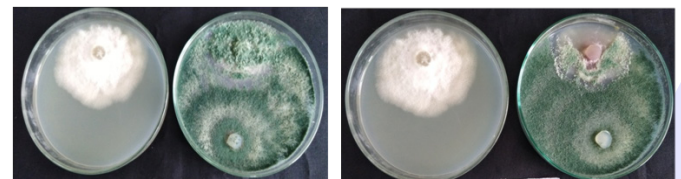
### Sensitization programs

A total of 29 sensitization and awareness meetings have been conducted to officials belonging to plant quarantine, tissue culture industries, SAUs, KVKs, ICAR, NHB, ATMA, Department of Agriculture, students, research scholars of various SAUs and progressive farmers. The ICAR-NRCB has released fact sheets, posters, extension folders and technical folders, in English, Hindi and Tamil and distributed to the stakeholders for creating awareness. Extensive surveys have been conducted to detect the presence of *Foc* TR4 in major banana growing states like Gujarat, Maharashtra, Madhya Pradesh, West Bengal and Tamil Nadu.

### Management of *Foc* TR4

#### Glass house evaluation

Native endophytic and rhizospheric bioagent combinations such as *Trichoderma* sp. NRCB3 + *Penicillium pinophilum* and *Trichoderma asperellum* prr2 + *Bacillus flexus* were evaluated against *Foc* TR4 under glass house conditions in cv. Grand Naine with promising results. Maximum disease suppression was observed in the treatment *Trichoderma* sp. NRCB3 + *Penicillium pinophilum* followed by *Trichoderma* sp. NRCB3 + *Penicillium pinophilum*. Besides, application of biocontrol agents also significantly increased the plant growth parameters.



Mycelial growth inhibition of *Foc* TR4 by endophytic *Trichoderma asperellum*



Suppression of *Foc* TR4 by biocontrol consortia in cv. Grand Naine

#### Field evaluation

Three field experiments using four different consortia of biocontrol agents have been initiated for the management of *Foc* TR4 in Uttar Pradesh, Bihar and Gujarat. The preliminary results showed application of consortia of biocontrol agents resulted in 55-60% reduction in disease incidence besides increasing the plant growth.

#### Mass production and delivery of biocontrol agents

A method of mass production of biocontrol agents using easily available, cost-effective organic materials through

solid-state fermentation was developed and validated. Among the organic substrates tested, farmyard manure (FYM) was found suitable for colonizing *T. asperellum* (Prr2). Moreover, the other ingredients added to FYM further enhanced the population of *T. asperellum* Prr2. The shelf-life of the formulation was excellent for up to six months of storage at room temperature.



Mass multiplication of *Trichoderma asperellum* (Prr2) in bamboo basket using FYM formulation

### Host plant resistance

A total of 279 banana genotypes belonging to different genomic groups (diploids, triploids and tetraploids including wild types) were evaluated in the *Foc* TR4 hot spot area at Katihar district, Bihar. The results indicated that out of 279 genotypes, 26 were immune, 14 were highly resistant and 20 were resistant to *Foc* TR4.

## RESEARCH HIGHLIGHTS

### Crop Improvement

- Three ICAR–NRCB selections, viz. Kaveri Saba, Kaveri Haritha and Kaveri Kanya were recommended for release as central varieties.
- Field screening of hybrids led to the identification of one immune (progeny no. 778) and two resistant (progeny no. number 754 and 756) progenies to Fusarium wilt (race 1).
- Four Nendran based hybrids (NPL 30, NPL34, NPL 36 and NCR 8) were found to be resistant to root-lesion nematode, *Pratylenchus coffeae* under glasshouse conditions.
- Multi-location trials on hybrid NCR 17 confirmed it to be a stable yielder across diverse agro-climatic conditions.
- TBM-9, a promising dwarf mutant line was recommended for MLT under ICAR-AICRP (Fruits).
- One hundred diverse Indian *Musa* accessions were evaluated for profiling of nine mineral elements, viz. Ca, K, Mg, Na, P, B, Fe, Mn, and Zn.
- Five putative mutants of Grand Naine derived from EMS and DES treated ECS showed resistance to Fusarium wilt Tropical Race 4 (*Foc* TR4).
- Putative androgenic haploids of cv. Ney Poovan (20 nos.) have been developed. The germination of embryogenic calli for *in vitro* production of androgenic haploids followed by *in vitro* shooting and rooting of putative androgenic haploids were also achieved on the modified MS media.
- The protocol for the mass multiplication, regeneration and germination of somatic embryos using SERV has been

fine-tuned and found to be highly cost efficient.

- The effect of monochromatic LEDs of different wavelengths on micropropagation of cv. Red Banana indicated that the highest percentage conversion of somatic embryos and plantlet development were obtained under blue and red light spectrum.
- RGA2 gene expression was studied in *Foc* TR4 resistant and susceptible Indian landraces. Ten SSR primers were designed in R genes present in chromosome 3 and tested in resistant and susceptible cultivars for developing gene specific markers of which a single primer 29400 showed polymorphism between resistant and susceptible cultivars.



Kaveri Saba

Kaveri Haritha

Kaveri Kanya

### Crop Production and Post-Harvest Technology

- Nutrient dynamics was worked out for cvs. Grand Naine and Nendran.
  - Two *Bacillus* strains with PGPR activity were tested for their efficacy on tissue culture derived plants of cvs. Red Banana and Grand Naine during hardening and they not only promoted growth but also significantly increased the activity of defense related enzymes.
  - Under clump management studies, allowing 4 side suckers per clump significantly reduced the bunch weight and delayed flowering in cvs. Ney Poovan and Poovan, respectively, compared to single sucker plants.
  - Banana cultivars with ABB genome, viz. Bluggoe, Peyan, Kotha Bontha and Saba were found drought tolerant.
  - Banana genotypes from North Eastern states, viz. Athiakol, Karthobiumtham, Bhimkol, and Kechulepa recorded lesser reduction of chlorophyll content under drought conditions compared to irrigation and the drought stress prolonged the flowering by 21 days in Agni Malbhog and 10 days in Kach Kela.
- Treatment of bunches of cv. Grand Naine with 6% CaCl<sub>2</sub> reduced the finger drop by 60% and higher concentrations of CaCl<sub>2</sub> caused blackening at the pedicel zone and injury to the fruits.
- The threshold temperature at which the ‘green ripening’ of Cavendish bananas takes place is 26°C. Very low activity of pheophorphydase *a* oxygenase (PaO) and accumulation of pheophorphydases were observed in the peel of green ripe Grand Naine bananas.

- Cultivars Monthan, Rasthali, Poovan, Karpooravalli and Udhayam possessed about 350 mg of flavonoids in peel.
- Among cultivars from the North Eastern states, Beeji Kela, *Musa balbisiana*, Kach Kela, Chinali, Nepali Chinia and Batheesa Chiriya contained high quantity of flavonoids. Central core stem of cvs. Manjahaji and Kanai Bansi contained higher flavonoids.
- Peel flavonoids of Monthan, Pachanandan, Nendran and Karpooravalli showed highest anti-oxidant (DPPH scavenging) activity.
- Transgenic banana was developed with 5.5 times higher iron in cv. Grand Naine and their large-scale multiplication using immature male flower buds and suckers was initiated.
- Pulp of banana cultivars with B genome (Attikol, Bhimkol, Beejikela and *M. balbisiana*) contained higher Fructans than the A genome cultivars.
- Under leaf production studies, cv. Karpuravalli produced maximum number of leaves, whereas cv. Poovan recorded the highest leaf area. Maximum shelf-life (11 days) was recorded with leaves of cv. Sakkai at 13.5°C against 4 days at room temperature.
- The post-harvest losses ranged between 11.03 and 38.77% in various parts of South India.
- Treating banana fruits with carbendazim and dipping in wax at 13.5°C enhanced the green life of cv. Ney Poovan by 55 days.
- A combination of 5% banana flour, 0.6% modified starch, 0.6% peel flour along with 93% refined wheat flour was found to be good for preparation of *pizza* base.
- Value added products developed from banana include low sugar, fibre-rich cookies (from center core stem powder of cv. Nendran); basil seed suspended ready-to-drink juice; personal hygienic products from fibre; disposable plates form leaf; bio-plates from leaf sheath and biodegradable bio-plastic from fruit peel.
- Enzymes, viz. chitinase, lipase and protease extracted from promising isolates of fungal bioagent *Beauveria bassiana* were found promising *in vitro* against stem weevil, *Odoiporus longicollis*.
- Three isolates of *Akanthomyces lecanii* caused total mortality of *Pentalonia nigronervosa in vitro* on the third day.
- From 10 cultivars belonging to various genomic groups, 90 volatile compounds including 57 insect attractants and 33 deterrents / anti-microbial compounds were identified using GC-MS.
- Surveys in farmers' fields in Tamil Nadu showed root-knot nematode (*Meloidogyne* sp.) was the predominant plant nematode associated with cvs. Red Banana and Nendran, whereas spiral nematode (*Helicotylenchus* sp.) was abundant in the root samples of cv. Poovan.
- Foliar application of salicylic acid at 100 and 200µM concentration under pot conditions reduced the reproduction of root-knot nematode, *Meloidogyne incognita* by 60-80% over inoculated control.
- Rhizome rot incidence on tissue cultured cv. Grand Naine was found to be 2–15% in Madhya Pradesh, Maharashtra, Gujarat, Uttar Pradesh and Bihar.
- Totally 60 isolates of rhizome rot pathogen were collected and characterized as *Pectobacterium* sp., *Achromobacter* sp., and *Klebsiella* sp. based on cultural characteristics on CVP and 16s rDNA sequencing.
- A simple, rapid bioassay methodology was developed for rhizome rot disease of banana which can be completed within 10-20 days.
- Among 25 microbial isolates evaluated for growth promotion in cv. Grand Naine under glass house conditions, H4 BC1, H6 BC3, H7 BC2 and H8BC1 significantly enhanced the plant height and girth.
- Two promising isolates, BCB 2-4 and BCNA5-3, were characterized by 16s rDNA sequencing.
- Foc isolates from cv. Grand Naine (Surat, Gujarat) were confirmed using molecular tools as belonging to VCG 01220 and 0125 of *Foc* race 1 while *Foc* infected samples collected from Kerala (5 nos.) and Tamil Nadu (5 nos.) were identified as *Foc* race 1 belonging to VCG 0124.
- Fifty-five potential phosphate solubilizing bacteria (PSB) were isolated from rhizospheric soil of 12 germplasm accessions and studied *in vitro*, of which PSB27 (6.21), PSB39 (5.36), PSB45 (5.36), PSB52 (5.27) and PSB54 (5.37) recorded highest phosphate solubilization index. PSB52 and PSB54 were identified by 16SrRNA analysis as *Enterobacter hormaecheis* sp. *xiangfangensis* and *Leclercia adecarboxylata*, respectively.
- Banana bract mosaic virus (BBrMV) was recorded for the first time in Hill banana at Lower Pulney Hills, Tamil Nadu and in Pisang Madu from ITC collections.



Hands of cv. Grand Naine treated with 6% (left) and 8% (right) CaCl<sub>2</sub> for management of finger drop

### Crop Protection

- *Basilepta subcostata*, *Bhamoina varipes* and *Sphaeroderma cruenta* were recorded as leaf and fruit scarring beetle species of banana in north and northeastern India.
- Sequencing of populations of *B. subcostata* from Bihar, West Bengal, Assam, Meghalaya, Uttar Pradesh, Odisha and Manipur showed that all the populations belonged to a single morpho-species.

- Cucumber mosaic virus (CMV) incidence was recorded in more than one million NCS-TCP certified TC banana plants and it was up to 16% in Burhanpur (Madhya Pradesh).
- Totally 43 diploid germplasm accessions (AA and BB) were screened for banana bunchy top virus (BBTV) resistance of which 13 AA diploids expressed typical symptoms but BB diploids were asymptomatic even after three consecutive inoculations. *Musa flaviflora* and *M. burmannicoides* Type AP also were infected by BBTV.
- Nanopore sequencer (MinION device) was used to detect and characterize BBTV from cv. Poovan (AAB) including asymptomatic samples and the copy number could be enriched by RCA method. The whole genome sequences obtained were more accurate with >99 % homology when compared to reference sequences.

## OTHER INFORMATION

### International Conference on Banana (ICB – 2020)

International Conference on Banana 2020 - “Innovations in Sustainable Production and Value Chain Management in Banana” jointly organized by the ICAR-National Research Centre for Banana, Tiruchirappalli; Society for Promotion of Horticulture, Bangalore; and Alliance Bioversity International-CIAT, was held at Tiruchirappalli, Tamil Nadu, during 22-25 February, 2020. Dr. A.K. Singh, DDG (Hort. Sci.), ICAR, New Delhi was the Chief Guest of the inaugural ceremony. Other dignitaries were Dr. Alagusundaram, DDG (Agrl. Engi.), ICAR, New Delhi; Dr. W.S. Dhillon, ADG (Hort. Sci.), ICAR, New Delhi; Dr. S. Uma, Director, ICAR-NRCB, Tiruchirappalli; Dr. N.K. Krishnakumar, Regional Representative of Bioversity International for South and Central Asia; Dr. N. Kumar, Vice Chancellor, TNAU, Coimbatore; Mr. Sivasasu, Collector, Tiruchirappalli District and Ms. Sumathi Ramachandran, Post Master General, Tiruchirappalli. During the inauguration, a ‘Banana Expo’, showcasing the vast diversity of bananas in India and other banana related enterprises was inaugurated by Mr. Sivasasu, Collector, Tiruchirappalli District. A special postage cover marking the Silver Jubilee Year of establishment of ICAR-NRCB was released by Ms. Sumathi Ramachandran. Awards were distributed and publications were released on the occasion. Mobile apps on banana information, banana cultivation, export and value addition, and pest and disease management, an ELISA kit for virus detection and a liquid formulation of banana sakthi (a micronutrients mix) were also released for the benefit of banana farmers.

The conference was conducted in 11 Technical Sessions on identified theme areas and a Satellite Workshop on Tropical Race 4 of Fusarium wilt, a dreaded disease of banana in many countries including India, was held as part of the ICB 2020. Many international collaborators such as IITA, Alliance Bioversity International-CIAT, FAO and QUT participated. Over 500 delegates including international delegates from Australia, Belgium, France, Czech Republic, Turkey, South Africa, Zambia, Kenya, Uganda, Nigeria, Philippines and Indonesia took part in ICB-2020. A ‘Banana Expo’ displaying fruits of 180 banana cultivars and 60 stalls related to banana industry was visited by more than 15,000 farmers, students and the public. A training on Foc TR4 was conducted by ICAR-NRCB in collaboration with the Alliance Bioversity-CIAT and it was attended by 45 people including scientists, plant quarantine officials and students.



Release of publications by delegates during inaugural ceremony of ICB-2020

### 26<sup>th</sup> ICAR-NRCB Foundation Day and Kisan Mela

The ICAR-NRCB celebrated its 26<sup>th</sup> foundation day as farmers’ day on 21 August, 2019 with the theme “Recent Interventions for Doubling the Banana Farmers’ Income”. Dr. S. Uma, Director, ICAR-NRCB, motivated the farmers’ to double their income through the use of technologies developed by the centre and also informed about the release of ICAR-NRCB selections, viz. Kaveri Kalki (a high yielding cyclone tolerant selection), Kaveri Sugantham (a selection with unique aroma) and Kaveri Saba (a drought tolerant selection). Mr. S. Sivasasu, IAS, Collector, Tiruchirappalli was the Chief Guest of the event and Mr. T.V. Manjunatha, Additional Principal Chief Conservator of Forests, Chennai was the Guest of Honor. During the event, awards for Best Farmer, Best Entrepreneur and Technology Disseminator were given by the Chief Guest. About 900 participants including banana farmers, entrepreneurs, KVK scientists, state horticultural officers and exporters attended the event. An exhibition was also arranged showcasing various agriculture inputs and banana based products.



Participants of ICB-2020 during the inaugural ceremony



Awardees with Director, ICAR-NRCB and Chief Guest at ICAR-NRCB foundation day

### Training on Banana fiber extraction and its utilization

The ICAR-NRCB organized a five-day training program (8-11 July, 2019) on banana fiber extraction and its utilization for three women entrepreneurs from Manipur. The entrepreneurs had hands on training on the technology of extraction, preservation and utilization of banana fiber for making handicrafts, bio-plates, sanitary napkin, etc. The trainees were also exposed to more than 30 different value added products produced from flour, ripe banana and wastes like central stem, flower and pickle. The training was supported by the ICAR-Manipur centre.



Women trainees from Manipur with Director, Course Co-ordinators, ICAR-NRCB and Mr. Adithya Senthil Kumar, IAS, Sub-Collector, Srirangam, Tamil Nadu

### Hands on training on Macropropagation

A one-day training program on 'Macropropagation Technology' was organized at the centre for the benefit of the Gaja Cyclone affected banana farmers of Thanjavur and Namakkal districts of Tamil Nadu. The training program was jointly funded by ICICI foundation, Trichy Zone and SEED Division-DST, New Delhi. Macropropagation is a low cost farmers' friendly technology for the mass multiplication of banana planting material at the farm level by the farmers. More than 100 farmers participated and benefitted by the

program. Dr. S. Uma, Director, ICAR-NRCB, in her inaugural address explained the significance of this low cost technology and the need for its spread in various parts of the country. Mr. Asif Iqbal, Project Manager (Trichy) and Mr. P. Satyanathan, Project Manager (Madurai), ICICI foundation attended the training and addressed the farmers.

### Signing of MoU with VFPC, Kerala

A memorandum of Understanding was signed by ICAR-NRCB with Vegetable and Fruit Promotion Council Keralam (VFPC), Kerala on 27 November, 2019 for export of cv. Nendran to Europe via sea.



Signing of MoU between ICAR-NRCB and VFPC, Kerala

### Opening of ICAR-NRCB sales counter

A sales counter was opened on 11 July, 2019 for the benefit of the public and promotion of value added products of banana. The Chief Guest, Mr. Adithya Senthil Kumar, IAS, Sub-Collector, Srirangam inaugurated the sales counter and the first product was sold to the Guest of Honor, Mr. K. Natarajan, IBS, Station Director, AIR, Tiruchirappalli. Dr. S. Uma, Director, ICAR-NRCB congratulated the team of scientists and other staff for their concerted efforts in opening the sales counter.



Director, ICAR-NRCB with guests during the opening ceremony of ICAR-NRCB sales counter

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