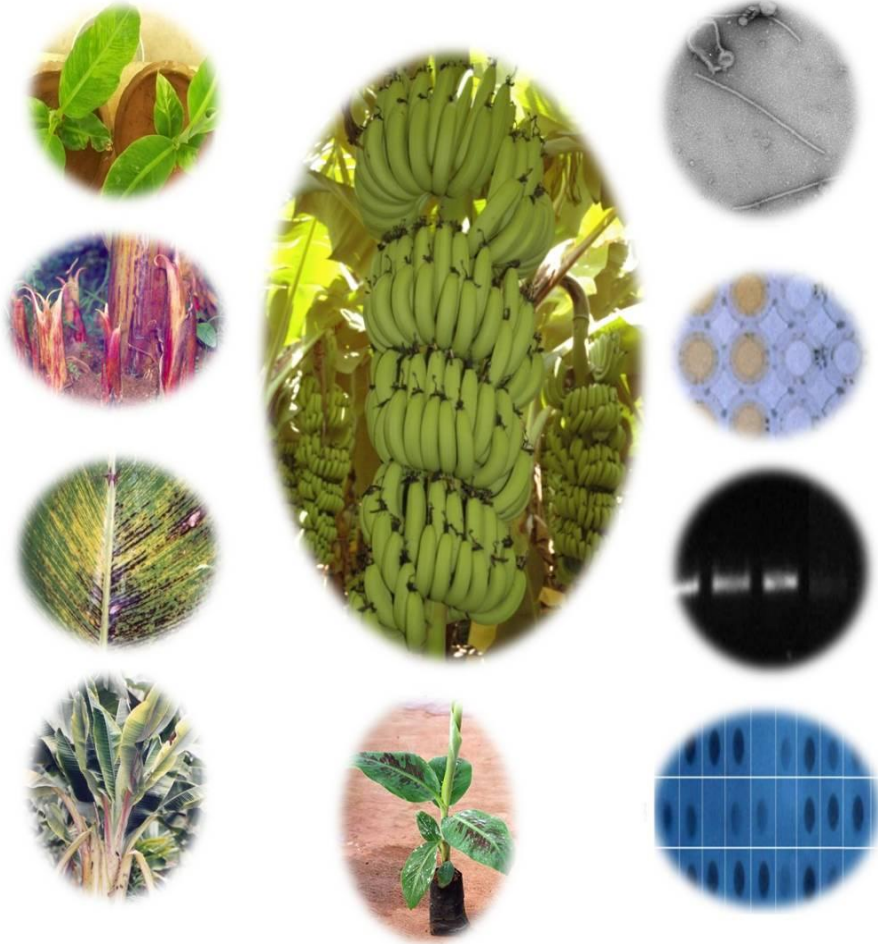


VIRUS INDEXING - A TECHNOLOGY FOR THE PRODUCTION OF QUALITY PLANTING MATERIAL IN BANANA



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(R. Selvarajan and V. Balasubramanian)

Preamble

India is the world largest producer of banana, contributing 22.5% of global banana production with 30.20 million tonnes per annum (NHB, 2016). There is a need for more than 900 million plants per annum to substitute the suckers with the tissue culture (TC) plants. But, at present approximately 150 million tissue culture plants are produced annually. Tissue culture technology in banana has revolutionized the banana cultivation and increased the production and productivity tremendously in India. The demand for the TC plants is enormous because the TC banana plants performance is much superior and insect pests like weevils and nematodes, pathogens like fungal and bacterial origin are also eliminated through shoot tip culture but viral pathogens are not removed because of its obligate nature and lives inside the cells of shoot tissues. Moreover, the planting materials, which act as reservoir for the viral pathogens also aggravate the incidences and spread.

Viruses are the major problem in TC bananas causing serious loss in production. Bananas are affected by four major viruses such as banana bunchy top virus (BBTV), banana streak Mysore virus (BSMYV), cucumber mosaic virus (CMV) and banana bract mosaic virus (BBrMV). BBTV causes annual loss of production worth US \$ 50 million in India alone. Timely and early detection of viruses can save the crop. For detection, efficient oligonucleotide primers for polymerase chain reaction (PCR) based detection and immuno-based kits using the polyclonal antisera produced against bacterially expressed recombinant viral proteins for viruses infecting banana were detected. Highly efficient primers were designed from the highly conserved part of the genome for PCR based detection of banana bunchy top virus and banana bract mosaic virus. ELISA based kits developed are sensitive and cheaper. Cost effective simple protocols for extraction of viral nucleic acid from banana tissues for the detection was also

developed. The total cost for sample preparation to detection for four viruses is just Rs 500/- per sample.



Bunchy Top Virus affected banana plants



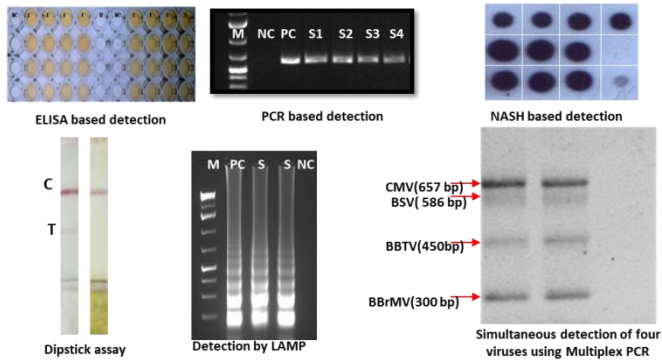
Bract Mosaic Virus affected banana flower



Banana Streak Virus symptom on banana leaf



Banana Mosaic Virus affected banana plant



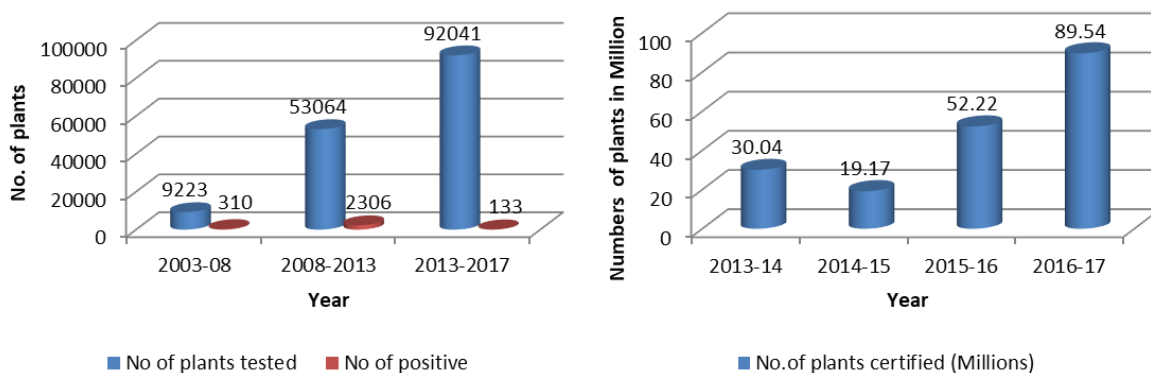
Virus indexing techniques developed:
M- DNA ladder; NC-Negative control; PC- Positive control;
S1-S4: Survey samples tested; C- Control line; T- Test line



PCR based virus detection technology was transferred on Turn-Key basis and established virus testing facility for producing virus free banana plants for Government of Andhra Pradesh

Virus indexing and certification

The mother plants are indexed and assured to be free of viruses before micropropagation and the resulting TC plants undoubtedly superior to suckers. The banana tissue culture industry in India could sustain its growth since the molecular diagnostic technologies helped to produce virus free planting materials. The molecular virology lab has been accredited by DBT, Govt. of India in 2007. The kits are being used in indexing system under National Certification System for Tissue Culture raised Plants (NCS-TCP) implemented by DBT, Government of India. Till date, 200 million TC plants were certified from our facility. Based on our virus testing record the number of positives to virus in mother culture in TC banana has reduced significantly over the years (Figures).



Total number of plants tested and certified by the Centre

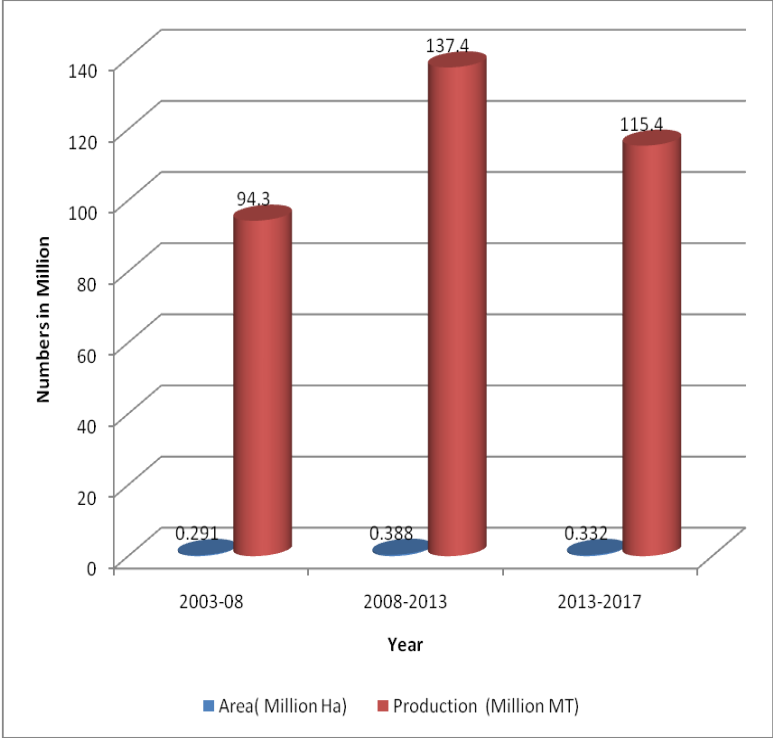
Ensuring banana germplasm free of viruses

India is one of the areas of origin for bananas and plantains and the largest producer of banana in the world. Around 310 banana germplasm are available at NRCB field gene bank. Banana germplasm are also conserved at different centres of All India Coordinated Research Programme-Tropical Fruits (AICRP-TF) for breeding purpose, international exchange and testing. Timely diagnosis and exclusion of virus from the germplasm is utmost important to safeguard our vast germplasm wealth of *Musa* in India. Germplasm conserved in the field gene bank at different locations (AICRP centres, NRCB) and *in vitro* gene bank of NBPGR numbering 1596 were tested for presence of banana viruses. Totally, 414 putative positive

germplasms were found really positive against one or the other of four viruses. The technology was validated by testing enormous number of banana samples. The recombinant antiserum developed in this technology development was supplied TCPUs and research stations working on banana.

Impact of the virus indexing in banana

- Virus diagnostics developed at NRCB is used for certification programme for the supply of quality tissue culture banana plants. The incidence has come down drastically over the years of implementation of certification programme for tissue culture banana in India.
- The banana production in India was 18.9 Million tons in 2005, which increased to 30.20 Million tons in 2016. This production increase is mainly attributed to the adoption of virus free quality planting material supplied by TC industries.
- TC banana industries supplying virus free plants have increased their production of quality tissue culture plants over the years compared to the industries, which don't follow the virus indexing.
- Virus indexed Hill banana (syn: Virupakshi, GI-0124) has helped to rejuvenate in the lower Pulney Hills of Tamil Nadu.
- Ensuring the virus free genetic resources helped the banana improvement programme and international exchange of germplasm.



Area and production of certified tissue culture banana plants over the years