PP(S5)/03: Specific detection of *Fusarium fujikuroi* causing bakanae of rice through recombinase polymerase amplification

Prashantha S.T., **Bashyal B.M.*** Sangeeta Gupta and Rashmi Aggarwal
Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi – 110012, India
**Email: bishnumayabashyal@gmail.com*

Bakanae of rice is a major devastating disease caused by *Fusarium fujikuroi.* The disease causes a significant yield loss in major rice growing parts of the country. Symptoms of the disease include yellowish leaves, elongation, rotting and adventitious roots formation. These visible symptoms are helpful in identification of the pathogen associated with the disease. Several workers attempted to identify the pathogen based on PCR, real time PCR, LAMP techniques. But a rapid point of care diagnostics to identify the pathogen in rice seed/infected plants is not available. The isothermal amplification techniques like RPA, LAMP are useful in development of field based detection of the pathogen. These techniques can be integrated with lateral flow devices for rapid identification in field samples. The unique secondary metabolite gene cluster present in *Fusarium fujikuroi* has been utilized in this study to amplify the NRPS31 in *Fusarium fujikuroi* isolates. The gene sequence was downloaded from NCBI database and identified a unique region through in silico analysis. Five set of RPA primers were designed to amplify the NRPS31 gene. Among them, only one manually designed primer amplified the target gene. The target gene was amplified only in *Fusarium fujikuroi* and did not amplify in other sps of *Fusarium* such as *Fusarium proliferatum, Fusarium verticillioides, Fusarium thapsinum, Fusarium andiyazi, Fusarium mangiferae* and *Fusarium oxysporum.* The amplification produced a 280bp product in *Fusarium fujikuroi* isolates. The technique described here is specific, sensitive and useful in developing rapid diagnostic tools.

PP(S5)/04: Genetic variability of *Sugarcane bacilliform virus* causing leaf fleck of sugarcane in India

Janiga Gopinath, K. Nithya and **R. Viswanathan***
ICAR-Sugarcane Breeding Institute, Coimbatore – 641007, Tamil Nadu, India
**Email: rasaviswanathan@yahoo.co.in*

Sugarcane bacilliform virus (SCBV) (Genus: *Badnavirus*, Family: *Caulimoviridae*), a plant pararetrovirus which causes leaf fleck in sugarcane considered as economically important pathogen, limiting the exchange of its germplasm worldwide. To explore the prevalence and genealogies of SCBV from germplasm and cultivated varieties, 358 leaf fleck affected samples from cultivars and germplasm were collected and subjected to PCR to amplify 726bp reverse transcriptase/ribonuclease H region of ORF3. 104 PCR amplicons were sequenced and the contigs derived from each fragment was assembled using CAP3 contig assembly and variability analysis was performed in MEGA X using the maximum likelihood method with tamura-Nei model. Phylogenetic analyses of the sequences revealed formation of three major groups, in which CBJ 46 alone formed a separate group with 40% similarity to the remaining isolates. The sequences had shown 85-99% similarity with other SCBV partial sequences (ORF 3 region) from NCBI genbank. Overall, SCBV-BT and SCBV-BRU genotypes are found to widely present in India and existence of newer genotypes viz. CHN 1, CHN 2 from China, SCBV-IM and SCBV MO from Australia were identified from India for the first time. Even though the isolate CBJ 46 showed >80% similarity to ORF 3 of SCBV YN –YZ 20602 from China, in phylogenetic analysis it has formed a clear distinct separate group which indicates the possible emergence of new SCBV variant. Huge phylogenetic diversity existing among the SCBV isolates throws light on the variation within the SCBV species in India and their variation from the other genera of Badna viruses. Thus the present study helps to understand the SCBV population structure in India and will address the spread of new genetic variants from other countries through germplasm exchange.