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REPORT

A NOVEL TECHNIQUE FOR MASS INDEXING OF TISSUE CULTURED BANANA PLANTS FOR BANANA BUNCHY TOP VIRUS (BBTV)

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Among the four major viral diseases infecting banana (*Musa* spp.), banana bunchy top virus (BBTV) is the most devastating, which can cause heavy yield losses if planting materials have been pre-infected. One of the disadvantages of raising tissue-cultured plants is the possibility of viral infection when sensitive, rapid and reliable indexing procedures are not followed to get rid of the infected plants at any stage of the tissue culture protocol. Thus, indexing mother plants and planting materials for being free-of-viruses becomes more important in order to assure the availability of healthy planting materials to the growers. It is theoretically ideal to test and index all or a large proportion of the mother plants and plantlets before releasing to farmers. However, on practical point of view, it is rather difficult and costly for indexing large number of samples with currently available techniques and facilities. Testing each sample through Polymerase Chain Reaction (PCR) method is laborious and costly, and warrants quick and sensible mass detection techniques. Therefore, a novel, low cost technique was standardized to address this problem and provide results within a shorter time period.

Large number of samples was assayed with a relatively small number of PCR reactions using combinatorial screening in which 144 samples are arranged as in 12 x 12 spatial format. Equal amount of banana tissue (50 mg from each sample) from 12 samples arranged as a row were pooled (composite sample) for DNA extraction using modified CTAB method and subjected to row PCR with BBTV specific primers in order to identify the rows that accommodated BBTV infected sample(s). Similarly, equal amount of banana tissue (50 mg from each sample) from 12 separate samples were arranged as a column, pooled (composite sample) for DNA extraction, and subsequently used for PCR amplification in which columns accommodating infected samples could be easily traced. Considering the results of both row and column PCR, possible samples suspected to be infected with BBTV were recognized. These samples were individually subjected to PCR for exact identification of the BBTV-infected samples. The technique developed for mass indexing of banana samples for BBTV is the first such technique reported, and has many advantages over the conventional PCR techniques currently used.