行政院國家科學委員會專題研究計畫　成果報告

香蕉委縮病病毒病嚴重型品系基因體的分析與比較

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REPORT

Many viruses were known to infect banana and plantain and cause seriously damage. Among banana viral disease *Banana bunchy top virus* (BBTV) disease has been the most common and destructive viral disease in the Asia and Pacific regions, and has been considered to be one of the most important plant viral diseases around the world. BBTV genome consists of at least six single stranded circular DNA (ssDNA) components (BBTV DAN 1-6). BBTV DNA 1 encodes replication associated proteins (Reps), and in vitro protoplasts inoculation assay reveled BBTV DNA 1 is replication competent and it also could support the replication of other non-Rep encoded components. No major open reading frame (ORF) has been identified for DNA 2 and the function of DNA 2 is currently unknown. DNA 3 encodes the viral coat protein. DNA 4 and DNA 6 encodes proteins are very similar to BC1 and BV1 protein encoded by *Squash leaf curl* begomovirus. While DNA 6 encodes possible nuclear shuttle proteins and DNA 4 encodes proteins can redirect the DNA 6 gene products to cell periphery, and both have been suggested to be involved in virus movement. DNA 5 was shown to contain an LXCXE motif and the binding between DNA 5 gene products and retinoblastoma (Rb) had been demonstrated by yeast two-hybrid analysis, and may involved in host-cell cycle manipulation. BBTV DNA 1-6 could be detected in all isolates collected around the world and were suggested to be the integral components of BBTV. Besides the six components, several additional components have also been reported associated with some BBTV isolates. These components were BBTV DNA component 2 (designed here as BBTV DNA Y), BBTV DNA 1-4 (designed here as BBTV W1-4), BBTV DNA S1-2, and BBTV DNA S3. Based on sequence comparisons it has been suggested that BBTV DNA Y and W1, and BBTV S2 and W2, were variants of one another, and that

BBTV S1, S3 were separate molecules. Except BBTV Y, all the additional components encode Rep. The published BBTV Y encodes two small ORF with unknown function. However manipulation sequences of BBTV Y at two sites created an ORF which also potentially encodes Reps. It has been demonstrated that BBTV S1 are replication competent; however, they could not support the replication of BBTV non-Rep encoded components.

All BBTV and its associated components have a stem loop structure with a conserved sequences TA(G/T)TATTAC in the loop region which is common to all nanovirus. Interestingly among BBTV components, the conserved sequence in the loop of all BBTV integral component is TATTATTAC , however, the conserved sequence in the loop of other Rep encoded components is TAGTATATTAC. A stretch of 69 nucleotides (nt) sequences surrounding and include the stem loop region share at least 62% homology between BBTV DNA 1-6, and has been defined as stem-loop common region (CR-SL). Another stretch of 66-92 nt located at 5'of CR-SL share at least 76% sequence homology between BBTV DNA 1-6 have also been identified and defined as major common region (CR-M).

In Taiwan, BBTV strains have been collected and could be differentiated by symptoms and by PCR-genome types. All BBTV severe strains induce dwarf and typical bunchy top symptoms. However they induce different extent of vein clearing. Type I induce moderate vein clearing, and type II cause mild vein clearing. The severe strains could be differentiated by the pattern of PCR amplification products using three different primer pairs C1, CR-SL and TS. C1 primer pairs were designed from conserved region of all BBTV DNA 1 identified at present. CR-SL primer pairs were designed from the CR-SL region of BBTV Taiwan severe strain components DNA 1,3. TS primer pairs were designed from the conserved region of BBTV Y /BBTV W1, BBTV W2/S2. All primer pairs were designed in an

immediately adjacent outward extending direction and can amplify the whole genome of related BBTV components. All three primer pairs could be used in PCR reaction to amplify a ca. 1.1 kb DNA fragments from nucleic acids purified from BBTV type I infected banana. Two primer pairs C1, CR-SL but not SR-CR primer pairs could be used in PCR reaction to amplify a ca. 1.1 kb DNA fragments from nucleic acids purified from BBTV type II infected banana. This suggests BBTV type I and II may differed in with or without the association of BBTV Y / W1 and /or BBTV W2/S2. Because BBTV type I and type II cause different symptom, will the association of additional components is the sole difference remain to be identified.

A symptom attenuate BBTV strain cause moderate stunting, mild leaf trophy and moderate vein clearing have also been reported and designed as BBTV strain IV. Only C1 and TS but not CR-CL primer pairs could be used in PCR reaction to amplify a ca. 1.1 kb DNA fragments from nucleic acids purified from BBTV type IV infected banana. This is intriguing since CR-CL were designed from conserve region of the CR-CL of BBTV severe strain DNA 1 and 3, and this primer pairs could potentially amplify BBTV severe strain DNA 1,3,4 and 5. The sequence of BBTV type VI DNA 1 had been determined. It revealed that 9 nucleotides deletion occurs at the CR-SL. It partially explain why C1 primer pairs but not CR-CL could be used in PCR reaction to amplify a ca. 1.1 kb DNA fragments from nucleic acids purified from BBTV type IV infected banana. However it did not explain why CR-CL could not amplify other BBTV type IV components, and the genome organization of other BBTV type IV components remains to be resolved.

Here we have detail characterized the genome organization of Taiwanese BBTV severe strain type I, type II and type IV. It revealed that the genome organization of Taiwanese BBTV type I and type II essential components are similar except they are different in the association of additional components. Based on the presence or

absent of additional Reps, however, BBTV could be differentiated into 7groups. The genome organization of BBTV type IV is unexpected. We have cloned 90 BBTV type IV components 1, and 89 of them all have a deletion in the open reading frame region. Only one clone have complete open reading frame. All the identified BBTV type IV components CR-CL region are nearly identical and are similar to BBTV component 6. Interestingly we were unable to find BBTV type IV component 6 even three pairs of primers designed from conserved region of BBTV DAN 6. Unlike all BBTV essential component loop conserved sequence in the CR-CL region is TATTATTAC, the BBTV type IV component 2 conserved sequence in the CR-CL region is TAGTATTAC.