学 位 論 文 要 旨

Research on begomoviruses isolated from cucurbits in Indonesia

インドネシアのウリ科作物から分離されたベゴモウイルスに関する研究

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Begomoviruses cause many serious cucurbit viral diseases in the world. The members of begomovirus can be either bipartite (two circular, single-stranded DNA components known as DNA-A and DNA-B) or monopartite (single component homologous to the DNA-A of bipartite begomoviruses). DNA-A and DNA-B of bipartite begomoviruses have similar size, approximately 2.5-3.0 kb. The DNA-A component contains six open reading frames (ORFs), encoding the pre-coat protein (V2 in monopartite/AV2 in bipartite) and coat protein (CP, V1/AV1) in the virion-sense strand, and the replication enhancer protein (REn, C3/AC3), transcription activation protein (TrAP, C2/AC2), replication associated protein (Rep, C1/AC1) and uncharacterized C4 or AC4 protein (C4/AC4) in the complementary-sense strand. DNA-B has only two ORFs, encoding the nuclear shuttle protein (NSP, BV1) in the virion-sense strand and the movement protein (MP, BC1) in the complementary-sense strand. The nucleotide sequences of DNA-A and DNA-B are different, except for a short common region (CR) of approximately 200 nucleotides that is very similar or identical in the both DNAs. The CR contains repeated sequence motifs known as iterons, that are sequence-specific recognition sequence for Rep, and the predicted stem-loop structure containing the conserved nonanucleotide (TAATATT/AC) forming the origin of replication (ori), which is required for the cleavage and joining of the viral DNA during replication.

Melon (*Cucumis melo*) is one of the important cucurbit crops cultivated in Indonesia. During the field survey in Indonesia in 2015-2017, the severe symptoms of begomovirus infection were observed on melon fields. To our knowledge, no studies have fully characterized the begomoviruses infecting melon crops in Indonesia at the molecular level. Considering the emerging and spreading rapidly of begomoviruses infection, surveys were conducted to collect and analyze molecularly several begomoviruses from symptomatic melon plants in Indonesia. A begomovirus, *Tomato leaf curl New Delhi virus* (ToLCNDV), was identified from melon plants in Yogyakarta,

Indonesia [Indonesia:Yogya:Melon:2015] (ToLCNDV-[ID:YG:Mel:15]) and the complete nucleotide (nt) sequence of the isolate was determined. ToLCNDV-[ID:YG:Mel:15] has a bipartite genome (DNA-A and DNA-B), and both DNAs showed highest identity to ToLCNDV-[Indonesia:Java:Cucumber:2008] (ToLCNDV-[ID:JV:Cuc:08]). However, there was some variability in the ORFs in the viral sense of ToLCNDV-[ID:YG:Mel:15]. In a phylogenetic analysis based on AV1 nucleotide sequences, ToLCNDV-[ID:YG:Mel:15] clustered with *Squash leaf curl China virus* (CN-SLCCNV) isolates. Recombination analyses revealed that ToLCNDV-[ID:YG:Mel:15] was a recombinant between ToLCNDV and SLCCNV. Thus, the virus is a new recombinant of ToLCNDV derived from a begomovirus naturally infecting melon in Indonesia. The infectivity of the recombinant ToLCNDV-[ID:YG:Mel:15] isolate was confirmed by biolistic inoculation with partial tandem repeats of DNA-A and DNA-B clones. The inoculation induced mosaic and leaf curl symptoms on melon, but not on tomato, which is the primary natural host of ToLCNDV. To our knowledge, this is the first report of a recombination event between ToLCNDV and SLCCNV, an event that may have increased the severity of this disease in melon.

The recombination of begomoviruses with other viruses including intraspecies and interspecies has commonly occured. The assembled DNAs of begomovirus genomes were identified from symptomatic melon plants by NGS analysis. Each of DNA-A and DNA-B from two melon samples showed high identity with ToLCNDVs. Interestingly, two different DNA-As and one DNA-B were detected from one melon plant, that one DNA-A had high identity with ToLCNDVs, while another with SLCCNV, and for DNA-B had high identity with ToLCNDVs. From forty-two melon samples collected from one melon field, bipartite ToLCNDV genomes were detected from 35 samples, while ToLCNDV with DNA-A of SLCCNV were detected from 7 samples. However, there was no plant infected with DNA-B of SLCCNV. The infectious clones of SLCCNV DNA-A (SA) and ToLCNDV DNA-A (TA) / DNA-B (TB) were constructed from the sample. Inoculation of TA alone induced mild symptoms, while TA+TB inoculation induced severe symptoms and high infectivity to all tested plant species. Combination of TA+TB+SA inoculation induced very severe symptoms and high infectivity on all inoculated plants. Inoculation of SA alone showed no infectivity on all inoculated plants. Inoculations of TA+SA and SA+TB also did not induce symptoms with low infectivity. This study showed the novel occurrence of tripartite-like begomovirus with bipartite ToLCNDV and SLCCNV DNA-A-like molecule on melon plants.

Infection by begomoviruses has been detected and spread rapidly on *Cucurbitaceae* and *Solanaceae* plants in Indonesia. A rapid and simple detection assay for begomoviruses under field conditions for routine sampling of plants is needed. Primers for a loop-mediated isothermal amplification (LAMP) assay were designed based on the sequences of three Indonesian begomoviruses, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV), infecting *Cucurbitaceae* and *Solanaceae* plants. LAMP assays using a Genelyzer^{TM} FIII portable fluorometer with a toothpick method successfully detected these begomoviruses in infected melon, pepper, and eggplant samples. LAMP assays conducted during a field survey for detection of the three begomoviruses on 104 fresh leaves indicated that most of the samples were positive; the results were confirmed by PCR using universal primers of begomoviruses as a common detection method. These results demonstrate that this simple and rapid LAMP assay using a fluorometer portable device can be used to achieve real-time detection of begomoviruses under field conditions.

This research may indicate the evolution of begomovirus to adapt melon plants by recombination and tripartite-like genome strategies.