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GENOME ANALYSIS OF GEMINIVIRUS IN PEPPER CULTIVATION FROM WEST SUMATERA

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Abstract

Analysis of DNA components that composed genome of geminivirus is a prerequisite in order for effectively combating this pathogen. For that reason primer walking strategy was applied. Forty-six primers in total have been synthesized and tested for the identification of DNA components in the geminivirus genome. All the designed primers have been tested in six different isolates. The result successfully identified of DNA-A, and satellite DNA but no DNA-B could be amplified from geminivirus from West Sumatera Sequencing method was performed bidirectional in order to elucidate the genome structure. After editing the sequence data, currently 1464 bases was collected and analyzed. The length is approximately 54% from the total of gemini virus genome. Analysis of β-component (satellite DNA) was successfully amplified from isolate TD-21. The sequence composed of 1347 bases. In conclusion some facet could be mentioned that, genome structure of geminivirus from West Sumatera is monopartite as proved by the absence of DNA component and the presence of β-component. About 54% of the DNA-A component was successfully sequenced and analyzed and the other 46% is still in progress.

Keywords

Gemini Virus, DNA-A, DNA-B, satellite DNA, Contig.

INTRODUCTION

Geminivirus is one of the main constraints in pepper cultivation. It was identified in the first time at 1999 in West Java [4], and known as a causing agent of Pepper Yellow Leaf Curl Disease. Since 2000 the disease has spread in all Java Island and was regarded as epidemic in all pepper cultivation [9]. Field identification of the disease symptom caused by geminivirus is compounded by the symptom caused by other viruses which are mosaic, stunt, yellow leaf, curly and malformation. The symptom mainly could be observed during flowering stage and very rare during transplantation period.

Currently no effective strategy has been reported. Resistant cultivar should be an effective and economic strategy [1]. However, no resistant cultivar fur this purpose is currently available so far. With the advent of DNA engineering technology it is possible to create transgenic crop that could be resistant against geminivirus infection. One of the strategies that have been used is pathogen derived resistance (PDR) [8]. In strategy the availability of pathogen derived gene prerequisite. Fulfilling this prerequisite genome of the pathogen must be elucidated.

This research was aimed to map the genome structure of geminivirus a causing-agent of Pepper Leaf Curl Disease in pepper cultivation. Furthermore number of genes and their detail structure was

Material and Method

Isolates Collection and DNA Isolation

We collected 50 infected plants from six regions in West Sumatera. Every region represents geographical conditions (lowland, medium land and land). Virus DNA isolation was done as described many authors [2, 3, 6]. Briefly leaf of infected plant prepared in 1,5 ml microcentrifuge tube. One hundred microlitre of buffer containing 0,1 M Tris (pH 8.0), M EDTA and 1% SDS was added inside. Leaf tissue grinded well with plastic pistil before incubated for minutes at 65°C. Three point five microliter of 8M tassium acetate was added and the tube stored for 30 minutes on ice. After that, the tube was trifuged for 15 minutes in 10.000 rpm. nol:Chloroform (1: 1) extraction was done two times finally saturated in TE. The supernatant was transferred into the new sterile tube and precipitated with 2,5x nol absolute prior incubation in -20°C for 15 minutes. Supernatant was discarded and the pellet was washed with 500 µl of 70% ethanol. Pellet was dried and resuspended in 25 µl of sterile H_2O.

PCR Reaction and Sequence Analysis.

A universal primer pair as described by Rojas was used as starting point of sequence analysis. The primers were PAL1v1978 (5'-GCATCTGCAGGCCC CATYGTCTTYCCNGT-3') and PAR1c715 (5' GATTTC-TGCAGTTDATRTTYTCRTCCATCCA-3'). A fragment of 1600 bp in length should be produced using this primer pair. Amplification was done in 25 µl total volume reaction, containing 1 µl of DNA template, µl of combined primer pair (5 pmol/µl), and 1 bead RTG PCR bead (Ge-Healthcare-UK). PCR reaction was done in a Biometra Thermalcycler by applying one denaturation (94°C in 4 minutes), 30 core cycles

for denaturation, 55°C;2 minute for extension. ... performed for 10 minutes prior 4°C ... PCR was checked by means of ... and documented in JPEG ... in PT Charoen Phokphand ... Polymerase Chain ...

... Contig Analysis.

... the sequence data, primer walk- ... applied. Additional primers were de- ... region of the previous sequence ... and reverse orientations. In some ... happened doubly in the vicinity to ... Primers were synthe- ... with ... Invitrogene-Sweden). Sequence ... and contig assembling was done with ... (Madison-USA).

... primer pairs) were synthe- ... the experiment. Thirteen primer pairs ... region of DNA-A genome, six ... to amplify DNA-B genome and 1 ... to amplify satellite DNA (β- ... primers, 18 primers were designed ... isolates. These primers ... on two specific isolates, namely ... were selected since they ... from three different geo- ... represented low-land region ... to represent isolates from high- ... primers designed from PSS-14 ... PSS13L-1, PSS14-PYLCV- ... -353R, while group of primers ... were TD-24L, D-24 R, TD- ... TD-21 PAR, TD-21-PAR Compl, ... TD-21-PYLCV-455 F, TD-21- ... Universal primer containing degenerated ... were also designed to amplify ... which was designated as: WS- ... RAWGTTHGGATGGAA WS-PYLCV- ... MAGYTCCGA-YGTCA. In ... were tested until October 2009. This ... PCR reactions.

... analysis was focused on isolate ... primers exhibited 1-to 5 fragments. ... single fragment were used in the ...

... annealing temperature was optimized ... PCR (Esco-Malaysia).

... one from 6 used DNA-B primer ... PCR product from both isolates. ... and PSS-14 did not contain DNA-B ... they were monopartite Gemini virus ...

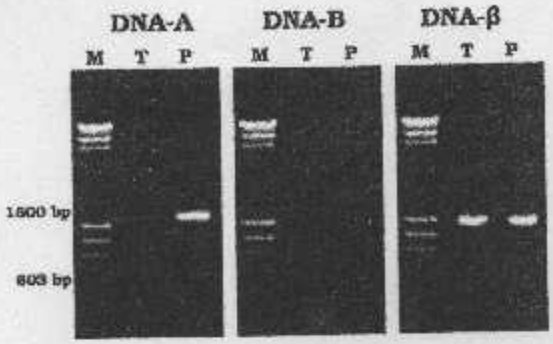

Figure 1. Electrophoresis performance of some primer pairs used to amplify DNA-A, DNA-B and satellite DNA (DNA-β) in TD-21 (T) and PSS-14 (P).

Mapping of whole genome structure of Gemini virus from isolate TD-21 was performed with primer walking strategy. The additional primers were designed in the internal region of existing previous sequence data. Nucleotide sequence data indicated that 54% of TD-21 genome was successfully elucidated. This was achieved by applying both primers PAR1c1975 and PAL1c1978 in sequencing of 1600 bp fragment.

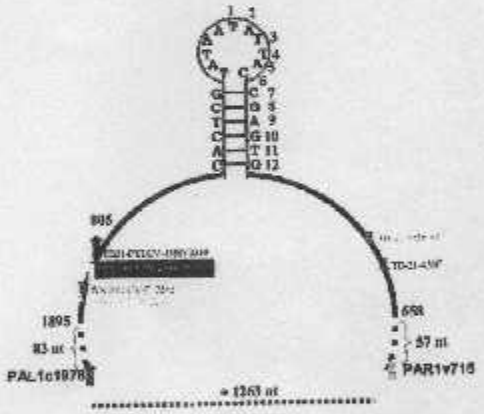

Figure 2. Schematic diagram of DNA-A genome of TD-21 isolate. Predicted gap might be 1,3 kb (1263 bases). Markers developed for primer walking are designated with arrowhead.

CTCTCCCTTCTATGAATGGCTGATGGGCCTCCTG
GGCACGCCAAGCGGCTTCCATCACATTTTCAGCA
GCCCATTGTCGTAGTTCCTCTGGAACTTGGTCAA
ACGACGACGACGGATATGGTGAAACAAATGTAT
CTACAGGTTTAGCAAATATTTTATCGAAATTAAC
ACTTAAATTGTGGTACTGTAGAACATAGTCTTTC
GGAGCAAGCTCTTTAATCAATCGAAGAGCATCG
GACTTACTTCCACAATTTAGGGCCTGCGCGTAAA
CATCGTTTACTGCGTGCGGACCACCTCGTGCAGA
TCGGCCATCTATCTGAAATTCTCCCCATTCGGTG
GTATCACCGTCTTTGTCCATGTACGCCTTGACAT

CGGAACTTGATTTAGCTCCCTGAATGTTCGGATG
GAAATGTGCTGACCTGGTTGGGGATACCAGGTC
GAAGAATCTGTTGTTCGTGCAGACGTATTTCCCT
TCGAATTGTATGAGCACATGGAGATGAGGGCTC
CCATCTTCGTGAAGTTCTCTACAGATCTTGACAA
ACAGCTTGTTGACAGGAGTGTTGATGGATTTTAA
TTGTTCGAGTGCTTCTTCTTTGGTTAGAGAACAG
TGTGGATATGTTAAGAAATAGTTTTTACTCTGTA
ACTTAAAACGACGTGGTGGAGGCATTTGGAGTG
TCGTTTTGTATT[redacted]TCCTTCTATCCCCAT
ATATT[redacted]TATATACTTAAGTTC
TATAAGGGCTTCTAAGGTAATTTTGTAACCCCCT
TGAATGATTAAAGCGGC[redacted]
[redacted]CCGCGAAAATTTTTGAGAAGGGGTCCC
CAGAAAGCCCAACCTTTTGATGACCAATCACGTG
GCATTCTGCAAAGCTTAATTAGTGGGGCGCCCAC
TATAAAGATAGGAACCACCCTCCACTATCCAGGG
ATGGGGGAACCGCTTATTCCTCCTTTTCCTGAAA
CCCTACACGGGTTTCGATGCATGTTGGCTATCCA
AGTATCTGCAAAGCTTAGAGGCTACGTATTCTCC
TGATACGGTTGGAGGTGAATTCGTGAGGGATTT
AATTTGTCTGTTGCGGTGTAAAAATTATGCCGAA
GCGTTCCATCGATACAGTGTCGTCGTTGCCAATG
TCTATAACACGCCGGAGACTAAACTACGCGAGTC
AGTACAGTCTCCCTTGCTGCTGCCCCCACTGCCC
CAGGCATGTCGTACAAACGAAGAGCATGGGTAA
ATCGGCCTATGAATCGGAAACCCAGATTCTACAG
GGGTCGAAGGAGCAGTGATGTTCCGCGGGGTTG
TGAAGGACCTTGTAAGGTCCAATCTTTTGAACAG
CGACATGACATAACACATACTGGGAAGGCCCTTT
GCGTTTCCGATGTCACAAGAGGTAATGGTTTTGA
ACTGTTGTGCATCTGCAGGCCCACATTTGTCTTC
CGGCAATAATT

[redacted] = Iteron 1 (688-694)

[redacted] = Iteron 2 (715-728)

TATATA = TATA Box (729-734)

[redacted] = nanonucleotide (800-812)

[redacted] = inverted region (IR), where T predicted as first base in the genome map.

Figure 3. Nucleotida sequence of DNA-A genome from TD-21 isolate spanned 1464 bases in length. Iteron, nanonucleotide and IR region was showed in legend.

Primer PAR1c1975 and PAL1c1978 produced high quality of sequence data in average 862 bases for each primer. Contig assembling of nucleotide sequence from both primers showed an overlapping region in length of 259 bases. The contig spanned along 1465 bases. Considering total genome of TD-21 genome was 2, 7 kb, it meant 1,3 kb should be obtained using primer walking (Figure 2). Partial detail nucleotide sequences of DNA-A genome can be seen in Figure 3.

[redacted]TTAGCTACG
CCGGAGCTTAGCTCGCCACCGTTATAATATTACC
GGTGGCGAGCGGTTCCTCTGGTGTCCAGATGGTG
GGACCCACTCCCTGGGGAAGATAAAAGA
TGGGCCTGGCATAGGCCCATCAATATTCC
TTGGTGTAATAATTGAGTCGGCCTTTAAT
ACTCTTTATTTACTTGACTTGAAACGT
ATCAAGTTTTGGATACAATAGAAATAC
TACAATGACCCCATTAAATACATGGTA
CCTAAATCTATAGCCATCACTGGAGCC
TCAGGATTATATCGATCATCTCTATCA
CTGCTTGAAATCTCCAATGGTAGATC
ATGTCTTTGAGCATATGGCATATGCT
GACTGTTGAAGTCGAATGGTGGTATG
GTGGCCGTATGGGATGAGGAAACTC
AGTGCTGCTGACTTGGTGGAGAACA
GCACAAGAATTGAATCATCAGCCCT
GTTGATGATGAACTGCATCCCCTTC
TACGTGATCGTCATTGTTGTTGTTT
TGCTCCCATGTTGTTTGTTTAAATA
CAGGATGATATGTGGACATGAATCT
TCAATTGCTGTCCTAGTATTTCTATG
GTTACTTATGTCTGATAGTTACATC
CATGTAATTAAGAGAAAGAAAAGT
AATCATCCACCATGATTATGAAGA
TAAGAAGCGAAAACCACATAAAACC
AGAAGAACTAGATGGAGAGATATA
AGAAATGGGAGCGCAGCGACAAAA
TCGGCGTGAAAAAATTAACGTTGG
TCTAAATCACAAAAAACAAAACAA
CCTTTAAAAATCTGCAAGTCCGTA
TATTTACTGAGTTACCACACGGTA
TGCTATTTACCCCTGGTAAATAGG
ATGAGTCCCCAATAGGTAAATTGT
TCGGATACCAATTGGAGACAGGAA
AATAAGAAAGACAAAAATACCCTT
TCTATTAGGCGCGTGGGAGTGCTC
AGACCTTCTCTCTCCAAAACTCAC
GATACAGGCTGATCCCGGCATCAA
GCGCGGCGGT[redacted]
[redacted]

Figure 4. Complete nucleotide sequence of [redacted] from TD-21 spanned 1347 bp. [redacted] leotide was primer sequence [redacted] reaction.

Further analysis was focused on the [redacted] (β-component). Amplification of gemini [redacted] β-component primer pair (Beta-01 and Beta [redacted] fully produced about 1300 bp fragment. The [redacted] acceptable compared to satellite DNA described [redacted] authors for instance Cotton Leaf curl virus [redacted] Mesta yellow vein mosaic virus (MYVMV) [redacted] 1359 bases and 1354 bases respectively. [redacted] satellite DNA is longer than satellite DNA [redacted] from tomato leaf curl virus (ToLCV) which [redacted] 632 bases (Figure 4.)

of Gemini virus genome indicated that ... genome containing only DNA-... to DNA-B.

... of satellite DNA showed 1347 ... in length.

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