KARAKTERISASI MOLEKULER KANDIDAT GEN KETAHANAN NBS-LRR DAN PROFIL METABOLIT TERPAUT KETAHANAN MELON (*Cucumis melo* L.) TERHADAP *BEGOMOVIRUS*

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INTISARI

Begomovirus merupakan salah satu ancaman serius dalam produksi melon. Namun data mengenai jenis *Begomovirus* yang menginfeksi tanaman melon di Indonesia masih minim dan belum diperbaharui secara periodik. Pengembangan kultivar melon tahan *Begomovirus* merupakan strategi pengendalian *Begomovirus* yang dinilai efektif dan ramah lingkungan. Guna menunjang keberhasilan pengembangan kultivar tersebut diperlukan studi tentang ketahanan terhadap *Begomovirus* secara genomik, transkriptomik, maupun metabolomik. Tujuan penelitian ini adalah untuk mengetahui mekanisme respon ketahanan tanaman melon terhadap *Begomovirus* pada aras genomik dan metabolomik. Penelitian ini diawali dengan studi keragaman *Begomovirus* di Yogyakarta dan Jawa Tengah berdasarkan sekuen *coat protein*. Selanjutnya, dilakukan karakterisasi genom *Begomovirus* yang akan dijadikan sumber inokulan dengan metode kloning sekuensing. Terdapat 9 kultivar melon yang diinokulasi *Begomovirus* melalui perantara *Bemisia tabaci*. Pengamatan dan skoring keparahan infeksi dilakukan 14 hari setelah infeksi. Sampel daun yang dikoleksi digunakan untuk deteksi molekular infeksi *Begomovirus,* identifikasi kandidat gen resisten NBS-LRR, dan analisis profil metabolit dengan *Nuclear Magnetic Resonance.* Deteksi infeksi *Begomovirus* dilakukan dengan PCR menggunakan primer Krusty Homer. Kandidat gen ketahanan NBS-LRR terhadap *Begomovirus* diidentifikasi dengan 4 kombinasi *degenerative* primer NBS-LRR. Hasil amplifikasi kemudian disekuensing dan dilakukan analisis *amino acid identity, conserved motif*, *alignment* sekuen asam amino, dan filogenetik dengan menggunakan aplikasi bioinformatik. Selanjutnya, dilakukan desain kandidat primer RGA terpaut ketahanan terhadap *Begomovirus.* Selain itu, dilakukan juga analisis profil metabolit terhadap kultivar paling tahan dan paling rentan berdasarkan hasil uji ketahanan. Sampel daun dikeringkan dengan vakum dan dipreparasi dengan pelarut CD_3OD kemudian dianalisis dengan ^{1}H-NMR. Spektra sinyal hasil NMR dianalisis menggunakan *Principal Component Analysis* (PCA) untuk mengetahui perbedaan respon metabolit antara melon terinfeksi dan tidak terinfeksi serta kultivar tahan dan rentan *Begomovirus.* Hasil penelitian menunjukkan bahwa infeksi *Begomovirus* pada tanaman melon di Yogyakarta dan Jawa Tengah disebabkan oleh SLCCV dan ToLCNDV. Hasil karakterisasi genom juga menunjukkan hasil yang serupa bahwa isolat sumber inokulan merupakan ToLCNDV yang menunjukkan similaritas DNA-A sebesar 98,25% dengan ToLCNDV-[IN:JV:Luf;17] dan similaritas DNA-B sebesar 97,7% dengan ToLCNDV [IN:Cu:11]. Setelah melon uji diinokulasi virus diketahui bahwa melon ‘Tacapa GB’ dan ‘Tacapa Silver’ menunjukkan respon *moderate resistant* terhadap *Begomovirus* dengan nilai 10 < VI < 30 % sedangkan ‘Melona’ dan

'Tacapa Gold' menunjukkan ketahanan paling rendah terhadap *Begomovirus* dengan nilai VI berturut-turut 37,5% dan 47,5%. Kultivar 'Tacapa GB' dan 'Tacapa Silver' digunakan sebagai sampel untuk identifikasi gen ketahanan NBS-LRR. Hasil analisis menunjukkan terdapat 6 motif yang teridentifikasi, yaitu P-Loop, RNBS-A, Kinase-2, Kinase-3a, RNBS-C, dan GLPL. Motif P-Loop dan GLPL merupakan motif yang paling lestari. Gen ketahanan yang teridentifikasi merupakan golongan TIR-NBS-LRR dan menunjukkan hubungan kekerabatan dengan gen *N* dari tembakau dan *L6* dari *A.thaliana* yang juga merupakan gen TIR-NBS-LRR. Berdasarkan sekuen gen ketahanan tersebut, berhasil didesain sebuah marka RGA terpaut ketahanan terhadap *Begomovirus.* Disisi lain, secara metabolomik diketahui bahwa terdapat perbedaan antara kultivar terinfeksi dan tidak terinfeksi *Begomovirus*. Senyawa kolin dan alanin menunjukkan penurunan konsentrasi yang signifikan ($P < 0.001$) antar kedua perlakuan. Kultivar kontrol tahan dan kultivar tahan menunjukkan perbedaan respon metabolit baik saat terinfeksi maupun tidak terinfeksi *Begomovirus,* meskipun keduanya menunjukkan fenotip *moderate resistant*.

Kata Kunci : Gen NBS-LRR, *Begomovirus,* karakterisasi gen, profil metabolit, ^{1}H-NMR

MOLECULAR CHARACTERIZATION OF *NBS-LRR* RESISTANCE GENE AND METABOLITE PROFILE LINKED TO RESISTANCE AGAINST BEGOMOVIRUS IN MELON (*Cucumis melo* L.)

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ABSTRACT

Begomovirus is one of the serious threats in melon production. However, scientific reports regarding *Begomovirus* species that infect melons in Indonesia are still limited and have not been updated periodically. The development of *Begomovirus*-resistant melon cultivars is a *Begomovirus* control strategy that is considered effective and environmentally friendly. In order to support the success of developing cultivars, a study of resistance to *Begomovirus* genomic, transcriptomic, and metabolomic level is needed. The purpose of this study was to determine the resistance mechanism of melon to *Begomovirus* in genomic and metabolomic levels. This study was begun with a study of *Begomovirus* diversity in Yogyakarta and Central Java based on a protein coat sequence. Furthermore, the characterization of the *Begomovirus* (as a source of inoculants) genome was carried out by sequencing cloning method. There were 9 melon cultivars inoculated with *Begomovirus* through the vector-inoculated methods. Observation and scoring of infection severity were carried out 14 days after infection. Then, leaf samples were collected to be used for molecular detection of *Begomovirus* infections, identification of candidate NBS-LRR-resistant genes, and metabolite profile analysis with Nuclear Magnetic Resonance. Detection of *Begomovirus* infection was conducted using PCR with a Krusty Homer primer while NBS-LRR resistance gene candidates were identified using 4 combinations of NBS-LRR degenerative primer. The amplification results were then sequenced and analyzed which were included analysis of amino acid identity, conserved motif analysis, alignment of amino acid sequences, and phylogenetic tree construction using bioinformatics applications. Furthermore, the sequence were used as the basis for the construction of RGA markers linked to the resistance of melons to *Begomovirus*. Finally, the profiling of melon metabolites was carried out by the ^{1}H-NMR method. The samples used were the most resistant cultivars ('Tacapa GB' and 'Tacapa Silver') and the most susceptible ('Tacapa Gold' and 'Melona') (based on the results of the resistance test) and cultivar control 'Gracia'. Leaf samples were vacuum dried and prepared with CD_3OD solvents. Signal spectra of NMR results were analyzed using PCA to determine the difference in metabolite response between infected and non-infected melons and between *Begomovirus*-resistant and susceptible cultivars. The results showed that *Begomovirus* infection in melon plants in Yogyakarta and Central Java was caused by SLCCV and ToLCNDV. Genomic characterization results also showed similar results that inoculant source isolates were ToLCNDV which showed DNA-A similarity of 98.25% with ToLCNDV- [IN: JV: Luf; 17] and DNA-B similarity of 97.7% with ToLCNDV [IN: Cu: 11]. After the inoculated test melon, it was found that 'Tacapa GB' and 'Tacapa Silver' melons showed a *moderate resistant* response to

Begomovirus with a value of 15% and 27.5% respectively, while 'Melona' and 'Tacapa Gold' showed the lowest resistance to *Begomovirus* with VI values 37.5% and 47.5% respectively. Hence, 'Tacapa GB' and 'Tacapa Silver' cultivars were used as samples for identification of NBS-LRR resistance genes. The results showed that there were 6 motif identified, namely P-Loop, RNBS-A, Kinase-2, Kinase-3a, RNBS-C, and GLPL. The P-Loop and GLPL motives are the most conserved motifs among them. The identified resistance genes were the TIR-NBS-LRR group and showed a genetic relationship with the *N* gene from tobacco and *L6* from *A.thaliana* which is also the TIR-NBS-LRR gene. Based on the sequence of resistance genes, an RGA marker linked to resistance to *Begomovirus* was constructed successfully. On the metabolomic level, it is known that there was a difference response between infected and not infected with *Begomovirus*. choline and alanine had been shown a significant decrease in concentration after *Begomovirus* infection. Moreover, sucrose and β-glucose compounds were also not identified in *Begomovirus*-infected samples. The metabolite response between control resistant with resistant cultivars also showed a different pattern even though both showed *moderate resistant* phenotype, either when infected with *Begomovirus* or not.

Keywords: NBS-LRR genes, *Begomovirus,* gene characterization, metabolite profile, ^{1}H-NMR