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Identification of DNA Markers Linked to CMV Resistance Gene(s) in Hot Pepper

Identifikasi Marka DNA yang Terkait dengan Gen Pengendali Ketahanan terhadap CMV pada Cabai Merah

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ABSTRACT

CMV has caused severe damages in hot pepper and its infection caused major yield reduction in Indonesia. Inheritance study on resistance to CMV showed that resistance to CMV was controlled by at least three recessive genes. The objective of this study was to identify DNA markers linked to CMV resistance gene(s) in hot pepper using bulk segregant analysis (BSA) strategy. Molecular markers were developed by RAPD analysis on the F2 generation generated from a cross between C 1024 and C *frutesscent* as the mapping population. DNA genomes were isolated from seventy two randomly sampled plants of a segregated F2 population and were amplified with six groups of random primers from Operon Technologies of OPA, OPC, OPE, OPF, OPH and OPM each of which consisted of 20 primers. Result of the experiment obtained 20 CMV resistance specific RAPD markers. These RAPD markers are grouped into two linked groups and one of the markers ($OPH5_{500}$) was shown to be associated with one of the three CMV resistance genes with the log-likelihood (LOD) value of 3.64. The $OPH5_{500}$ marker linked to a CMV resistance gene with a distance of 8.1 cM, and may be used to assist hot pepper breeding programs for CMV resistance.

Key words: DNA markers, RAPD, hot pepper, CMV resistance

INTRODUCTION

One of the objectives of hot pepper breeding programe in Indonesia is developing high yielding-virus resistance cultivars, especially cucumber mosaic virus (CMV) resistance. Among 45 identified viruses infecting hot peppers in Indonesia, CMV infection has caused severe damage and resulted in up to 75%-100% loss of hot pepper fruit production (DEPTAN, 1999; Duriat 1996; Duriat *et al.*, 1993; Eliyanti, 1998; Sari *et al.*, 1997; Sulyo *et al.* 1996; Rustikawati, 2000).

Result of the inheritance study of CMV resistance character in hot pepper has been inconsistence among different published reports (Singh and Thakur, 1997; Rusko and Csillery, 1980; Pochard *et al.*, 1983; Lapidot *et al.*, 1997; Hobbs *et al.*, 1996). Inheritance study on resistance to CMV showed that resistance to CMV was controlled by at least three recessive genes (Rustikawati, 2000; Herison *et al.*, 2004)

The transfer of CMV resistance characters from resistance donor parent to recurrent recipient one can be done by backcross breeding. This conventional hybridization approach usually requires 10-15 backcross generations and takes years to complete the whole cycles. To overcome this constraint, alternative approaches such as molecular aided backcross breeding technique has been suggested.

Random Amplified Polymorphic DNA (RAPD) is one of the various molecular techniques commonly applied in plant breeding. The RAPD has been used to identity DNA markers link to various diseases resistance genes in cucumber,

hot pepper, muskmelon and sweet pepper (Wechter *et al.*, 1995; Baoxi *et al.*, 2000; Horejsi *et al.*, 2000; Sanjaya *et al.*, 2002).

The objective of this study was to identity DNA markers linked to CMV resistance gene(s) in hot pepper using bulk segregant analysis (BSA) strategy and RAPD technique.

MATERIALS AND METHODS

The establishment of DNA Pool using Bulk Segregant Analysis (BSA) Method

Seventy two individuals of F2 mapping population generated from a cross of a resistance genotype (C 1024) with a susceptible one (C *frutescent*) were inoculated and grouped into highly susceptible (score 5) and resistance (score 0). DNA from the identified highly resistance plants were isolated and combine into resistance DNA pool (R pool). Similarly, DNA from the identified highly susceptible plants were isolated and combine into susceptible DNA pool (S pool). The quality and purity of DNA were determined by calculating the ratio of absorbance value of the prepared DNA at A_{260} to A_{280}. The value of 1.8 – 20 indicated good quality DNA (Sambrook *et al.*, 1989). The R pool and S pool DNA were used as template to generate RAPD markers using a number of random DNA primers. The RAPD markers were generated through polymerase chain reaction (PCR) using PE 2400 gene Amp-DNA thermal cycler.

RAPD Analysis by BSA Strategy

Subsequence steps were conducted to generate the desired RAPD markers:

1. 120 of random primers from 6 groups of Operon Primers (OPA, OPC, OPE, OPF, OPH and OPM) were used to generate RAPD markers. These random primers were used to amplify template DNA of R pool ad S pool in PCR and generated RAPD markers were separated in 0,8% agarose gel electrophoresis
2. The presence or absence of amplified products (RAPD markers) generated by the tested primers were scored for R pools ad S pools template DNA. The numbers and sizes of the generated RAPD markers were recorded and compared with 1 kb ladder marker
3. Primer producing R pool specific polymorphic marker were selected, and the R pool specific RAPD markers were identified.
4. Only the R pool specific RAPD markers were used to genotype individual plant of the F2 segregating-population. The presence or absence of R pool specific RAPD and S pool specific RAPD markers were scored for each F2 plant.

Linkage Analysis among RAPD Markers with genes Controlling CMV Resistance

5. The presence specific RAPD markers were combined with scoring data for symptoms of CMV infection. Morphological responses to CMV infection were grouped into resistance, mild, and susceptible.
6. Linkage map of RAPD markers and resistance score were constructed by MAPMAKER/ Exp application software. Linkage analysis was conducted by MAPMAKER/Qtl version 3.0. The program calculated the genetic distance of each RAPD marker to the resistance trait through the calculation of the proportion of recombinants. Calculation of the proportion of recombinants is a method to identify linkage among genes used by Morgan (Crowder, 1993).

RESULTS AND DISCUSSION

Bulk segregant analysis (BSA) was used to accelerate identification of markers associated with the CMV resistance phenotype. BSA analysis is useful to identify linkage among markers with simple genes controlling desirable characters (Paterson 1996).

PCR amplification resulted in only 28 primers out of 120 random primers tested produced polymorphic markers. Out of 28 primers producing polymorphic markers, only 12 primers produced RAPD markers specific for CMV resistance pool (R pool). From these selected primers, 23 RAPD markers were identified.

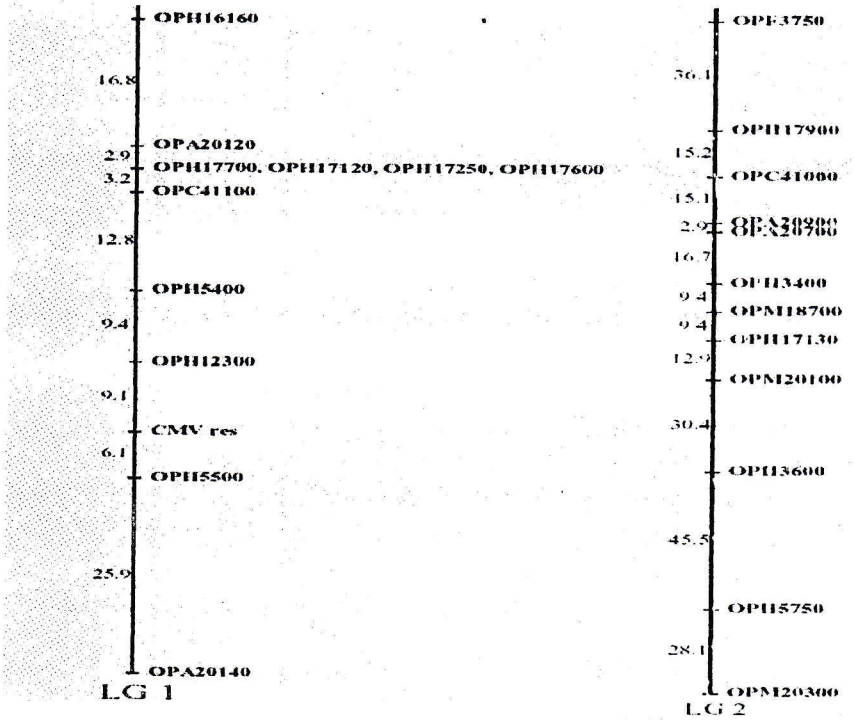

Figure 1. Linkage groups among CMV resistance specific RAPD markers identified by bulk segregant analysis method in hot pepper

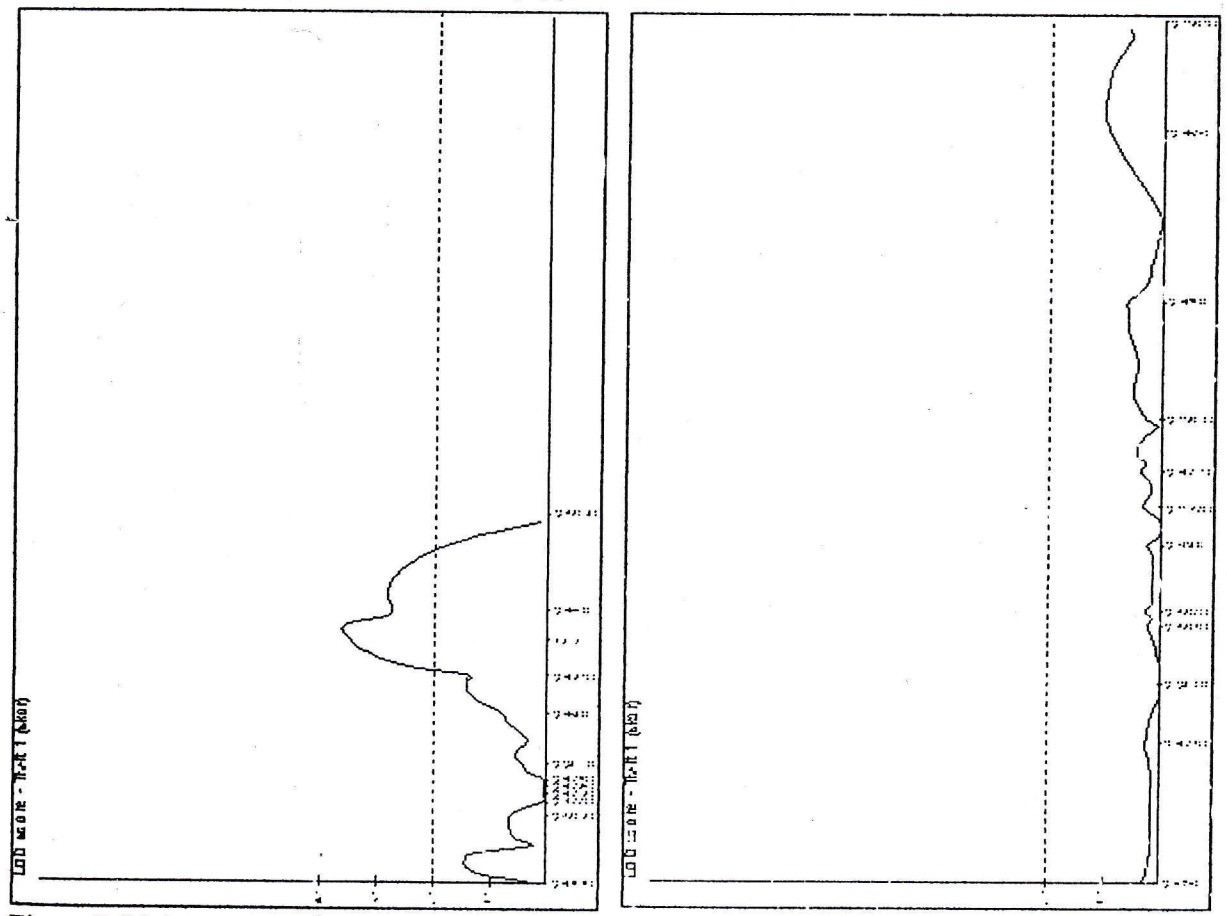

Figure 2. Linkage patern among markers within linkage group LG1 and LG2 with symptom scores

Linkage analysis among those RAPD markers showed they belonged into two different linkage groups with the log-likelihood (LOD) value 3,64. (Figure 1). Linkage group (LG) No.1 consisted of 11 RAPD markers and covering for 86,2 cM of the total genome. The linkage group (LG) No.2 consisted of 12 RAPD markers and covering for 221,7 cM of the total genome. The genetic distance among markers were commonly in a range of 0 – 50 cM, and opened to be saturated with other markers in the future.

When the disease response scores were combined into linkage analysis, one out of three genes controlling he CMV resistance characters identified in the previous genetic study was linked to one of the CMV resistance specific RAPD marker identified using BSA. The OPH5500 and $OPH12_{300}$ markers was linked and flanked to one of the CMV resistance gene with the distance of 6,1 and 9,1 cM respectively. Two genes were considered linkage when the distance between them was less than 50 cM (Crowder, 1993).

MAPMAKER/QTl analysis on symptom category data resulted almost similarly to that on qualitative data of CMV resistance as a marker. The closest genetic distance to CMV resistance controlling gene was $OPH5_{500}$ and $OPH12_{300}$ with the distance of 6,1 cM and 9,1 cM respectively, with the log-likelihood (LOD) value 3,64 (Figure 2). The existence of $OPH5_{500}$ and $OPH12_{300}$ markers will appear coincidentally with the CMV resistance gene at the probability of 93,9 and 90,9%, respectively. Therefore, those markers can be used to assist selection on CMV resistance.

CONCLUSION

With bulk segregant analysis method, 20 CMV resistance specific RAPD markers were identified. These RAPD markers are grouped into two linkage groups and two of them ($OPH5_{500}$ and $OPH12_{300}$) were shown to be associated with one of the three resistance genes. The $OPH5_{500}$ marker linked to CMV resistance gene with a distance of 6.1 cM, and may be used to assist breeding programs of hot pepper for CMV resistance.

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