LINGKAGE ANALYSIS OF RAPD (RANDOM AMPLIFIED POLYMORPHIC DNA) MARKER WITH FUSARIUM WILT RESISTANCE OF WATERMELON (Citrullus lanatus (Tumberg) Maksum and Nakai)

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) analysis was aplied to analyze watermelon (Citrullus lanatus (Tunberg) Maksun and Nakai) using two pool extreem fusarium wilt resisten (Charleston-Gray, Long Dragon AG-13, New Long ET-03, and Sugar Baby-2 and pool extreem susceptible (Dragon Giant 145, Kaisar, dan Lokal kupang). 3 primer used for amplification contains P01, 109, and G10. The research shown that P01 could be linked and produced a different fragment polymorphic DNA between resisten and non-resinten population of watermelon in 700bp. P01 was reported previosly as linked (1,6 cM) to race 1 fusarium wilt resistance in watermelon. The result by RAPD analysis showed that genotype of Charleston-Gray, Long Dragon AG-13, New Long ET-03, dan Sugar Baby-2 susceptible fusarium wilt resistance gene, and genotype Dragon Giant 145, Kaisar, and Lokal kupang were resistante to fusarium wilt.

Keywords: ***Citrullus lanatus*** **(Tunberg) Maksum and Nakai), RAPD, fusarium wilt resistance, primer**

INTRODUCTION

Watermelon (*Citrullu lanatus* (Thunberg) Matsum & Nakai) is come from dry tropic an subtropic of Africa, and than spread out to another place to China, Japan, until Indonesia. In Indonesia, watermelon is included one of populer fruit by Indonesian society because of it's nice taste, cheap, and it is easy to plant for our farmers. The other benefit from watermelon is in it's leaf, for several Indonesian society, it's leaf can be consumted fresh or cooked (Prajnanta, 1996). Center of cultivation in Indonesia is in Center Java (D.I. Yogyakarta, Magelang Kulonprogo); West Java (Indramayu, and Karawang); North Java (Banyuwangi, and Malang); and also Lampung. Capacity of cultivation areal for waterlmelon is about 28.725 Ha with nastional production about 14.28 ton/ha and productivity 410.195 ton/year (Deptan, 2004).

Breeding of watermelon in Indonesia still not maximal, so stok seed from the best variety of watermelon still suspended from impor. Impor of watermelon seed in the 2002 was 22 ton (Sumarno, 2003). There are several importance factories that need to be attanted to release best variety of watermelon with hight productivity. One of it is tolerance trait to pathogen agression. Agression of fungus, bacterial, and virus could be reducing the productivity and quality of watermelon fruit (Bruton, 1998; Nagel *et al.*, 1992). The example of several pathogen are fusarium wilt that caused by *Fusarium oxysporum*, dumping of by *Pythium ultimum*, antracnose, bacterial wilt, putrid fruit *Phytophtora*, *downy mildew, powdery mildew*, viruses, and fisiologis wilt (Kalie, 1993).

Intensive research about wilt resistances in watermelon has been done especially to fuasrium wilt. This desease can attact to each stage of growth in watermelon (Tiserat, 2006), because this desease were classified to seed born desease, so its couldn't

be controlled by chemical sprayer, rather with planting water that have fusarium wilt resistance or rotation cultivation.

Moleculer technique were useful for step up breeding program efficiency. This technique is possible for doing rapid selection to get traits that we want by using moleculer markers lingkage to some trait. One of technique moleculer that can be used is RAPD analysis. This technique is using one oligonucletide-primer with short number of bases that will adhere with randomly on PCR (*Polymerase Chain Reaction*) reaction and resulting some product to be analyzed with gel electroforesis.

RAPD analysis were used in various study, there are differensiation analysis, lingkage of *phylogenetic.* Pure line identification and verifycation, medical study and epidemology, and food technology. RAPD technique was begun by William *et al.* (1990) after being succed in amplification of DNA that has polimorphisme by using randomly primer with suported by taq-polymerase DNA. This analysis used in bacterial, fungus, insect, plant, algae, and also human researches. RAPD analysis have several benefits than others moleculers analysis, that are not influenced by environment, rapid analysis, need little DNA, and also can detecting the expression of DNA in all growth stadia and all tissues of plant so some characters of plant can be detected early.

This research purpose were to get RAPD marker that lingkage with fusarium wilt resistance trait.

MATERIALS AND METHODS

The research were done in Research Group Crop Improvement (RGCI) laboratory of Bogor Agriculture University. Two pool extreem fusarium wilt resistance (Charleston-gray, Long dragon AG-13, New long ET-03, and Sugar baby-2) and pool extreem susceptible (Dragon giant 145, Kaisar, and Lokal kupang). There Genotypes have been analyzed by morphological marker in others research before (Hawini, 2006).

Three kind of primers, P01 (5'GTA GCA CTCC'3), G10 (5'AGG GCC GTCT'3), and I09 (5'TGG AGA GCAG'3) were used for this analysis. RAPD procedures consist of four stage. Firstly, DNA extraction stage by using Invitrogen™ (USA) methode with using Kit Invitrogen DNAzol liquid, Et-OH (75% and 100%), buffer TE, and CIA (Cloroform Isoamil Alkohol). 0.2 gram sample leaf from each genotype were used to get total DNA. The second stage was quality and quantity analysis by using sambrook methode (1989). Analysis of DNA quality by runing electroforesis in 1% gel agarose. While, quantitative analysis of DNA was using spectrofotometer. Third stage was DNA amplyfication by using PCR. 25 µl PCR mix consist of 1 unit enzime of *Taq*-DNA *Polymerase* green mix, 2 µM randomly primer, 50 ng/µl DNA, dan *nuclease free water.* PCR methodes was use optimation methode of PCR from RGCI laboratorium. And the final stage was electroforetion. 10 µl PCR mix was using for runing electroforetion with 1.5% consentration of agarose gel. Electroforetion result was visualized by using UV illuminator and documented by using digital camera.

Analysis of DNA polymorfisme from PCR amplification from each genotype and each primer were identified based on present and absence of DNA band.

DISCUSSION

Amplifikasi DNA by RAPD (Random Amplified Polymorphic DNA) analaysis

There are polymorfisme from DNA amplification result between resistance and susceptible genotype by using RAPD analysis with using 3 randomly primer (Figure 1).

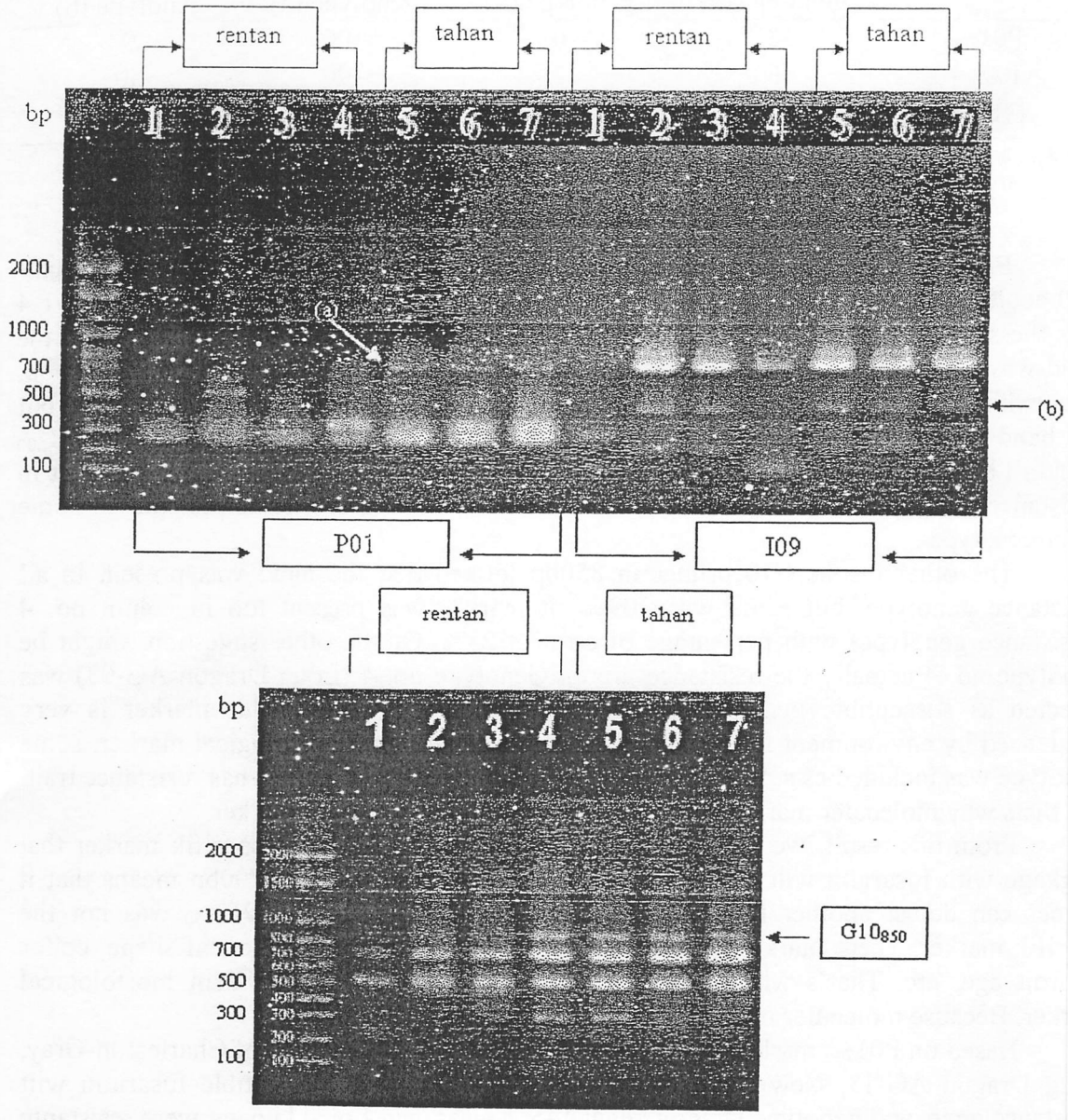

Figure 1. DNA polymorfisme by P01, I09, and G10 primer

Notes: susceptible genotype (1 : Charleston Gray; 2 : Sugar baby ; 3: New Long et-03; 4 : Long Dragon AG-13), resistance genotype (5 : Dragon Giant 145; 6 : Lokal Kupang; 7 : Kaisar)

Table 1. Persentage of present DNA band in each marker

Marker	Presentation of DNA band/ group population		% presentation/group population	
	Resistance genotype (a)	Susceptible genotype (b)	Resistance genotype (a)	Susceptible genotype (b)
$P01_{700}$	3	0	100	0
$I09_{500}$	3	2	100	50
$G10_{850}$	3	1	100	25

Notes : number of resistance genotype = 3 ; number of resistance genotype = 4

$c = \frac{a}{3} \times 100\%$; $d = \frac{b}{4} \times 100\%$

Based on figure 1 and table 1, the research was result that P01 primer in base pair 700 could differentiate between susceptible genotype and resistance genotype. Colum 1-4 was the susceptible genotype and colum 5-7 was the resistance genotype. At $P01_{700}$, the band was 100% present in all genotype in resistance group and 0% in susceptible group. Meanwhile, I09 primer result in 500bp the band was present in all resistance genotype, but the band was present too in genotype no. 1 and 4 (included susceptible group). $P01_{500}$ couldn't be categoric as spesifik marker like $P01_{700}$, because 50% the band was present in resistance genotipe. It is the number of error marker to sugest resistances trait in some plant genotype.

The other result, G10 primer in 850bp looked that the band was present in all resistance genotype, but same with $I09_{500}$, it marker was present too in colum no. 4 (resistance genotype) with persentage of error at 25%. Or the other sugestion, might be genotype no. 4 actually the resistance group. Genotype no. 4 (Long Dragon AG-03) was selected as susceptible genotype by using morfological marker. This marker is very influenced by environment for it phenotype. So, it could be in morfological marker, some genotype was included as suscaptible genotype but the real of it genetic has resistance trait. So, thats why moleculer marker is more effective than morfological marker.

From this result, we take some conclussion that $P01_{700}$ is the spesifik marker that lingkage with fusarium wilt resistance. Fragmens that formed above 700bp means that it primer can detect another trait in watermelon. $I09_{500}$ marker and $G10_{850}$ was not the specifik marker. There marker might be lingkage with another trait like leaf shape, collor of fruit age, etc. That's why moleculer marker was more effective than morfological marker. Because moleculer marker wasn't influence by environment.

Based on $P01_{700}$ marker, RAPD analysis showed that genotype of Charleston-Gray, Long Dragon AG-13, New Long ET-03, dan Sugar Baby-2 susceptible fusarium wilt resistance gene, and genotype Dragon Giant 145, Kaisar, and Lokal kupang were resistante to fusarium wilt.

This result has support to Xu *et al.* (1997) research. They reported that $P01_{700}$ was lingkage 1.6 cM with gene resistance for fusarium wilt race 1 in watermelon. This marker has been sequenced by them as SCAR primer with sequnces (5'GTA GCA CTC CAA CAT TTA TTC TAA TTTC, dan 5'GTA GCA CTC CCA ACT – CAT ACA AAT). Levi *et al.* (2001) was observe and got a result that $P01_{700}$ was ligkage to fusrium wilt for the other population of watermelon - BC_1[(P1 296341-tahan layu fusarium x New Hampshire Midget-rentan layu fusarium)] x 'New Hampshire Midget' that was resulted mapping design consist of 155 RAPD markers. So, we sugest that this marker can be used to another population of watermelon in Indonesia to select the resistance watermelon for fusarium wilt.

CONLUSSION

$P01_{700}$ is the spesifik marker that lingkage with fusarium wilt resistance. $I09_{500}$ marker and $G10_{850}$ was not the specifik marker. RAPD analysis showed that genotype of Charleston-Gray, Long Dragon AG-13, New Long ET-03, dan Sugar Baby-2 susceptible fusarium wilt resistance gene, and genotype Dragon Giant 145, Kaisar, and Lokal kupang were resistante to fusarium wilt based on marker $P01_{700}$. $P01_{700}$ marker maybe can used to another population of watermelon in Indonesia

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