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INHERITANCE OF RESISTANCE TO KYURI GREEN MOTTLE MOSAIC VIRUS IN MELON

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SUMMARY

Kyuri green mottle mosaic virus (KGMMV) is one of the virus which can reduce the quality and the amount of melon production in Indonesia. Resistance breeding is one of the solution to overcome the disease by crossing of PI 371795 and Andes to study the inheritance of resistance gene to KGMMV in melon. The aims of this research were to study the inheritance of resistance gene to KGMMV and to study various phenotypes of melon fruit produced by crossing between PI 371795 and Andes. Reciprocal breeding experiments were conducted between PI 371795 and Andes to obtain F_1 population. Furthermore, F_1 individuals were selfed to produce F_2 population. On the other hand, another F_1 individual was crossed to Andes to produce test cross population. F_1, F_2 and test cross individuals were then inoculated manually by KGMMV melon isolate (KGMM-YM). Resistance to KGMMV-YM was analyzed by symptom observation and serological detection using double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). The DAS-ELISA results of F_2 and test cross population were analyzed by chi-square test. Results of this research indicate that melon PI 371795 and F_1 are resistance to KGMMV-YM, while Andes is susceptible. F_2 and test cross population showed segregation and it follows Mendelian law with ratio F_2 segregation is 3:1 (34 resistant, 17 susceptible) while the ratio in test cross is 1:1 (6 resistant, 7 susceptible). The results of this study revealed that the inheritance of resistance gene to KGMMV-YM is controlled by a single dominant gene and it is proposed to be called *Krey* for the allele symbol (KGMMV-YM resistance gene). Results of these breeding experiments also show four variations of melon fruits which have the potential to become a commercial melons.

Key words: *Cucumis melo* L., DAS-ELISA, KGMMV-YM, *Krey*.

Key findings: This is the first report of the inheritance of resistance to KGMMV in melon. This result confirmed the utility of symptom observation and serological analysis to screen KGMMV resistant melon that may readily be used in melon breeding programs.

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INTRODUCTION

Melon (*Cucumis melo* L.) is in the *Cucurbitaceae* family and appears to have originated in Africa with a high phenotypic variation probably due to a large diversification center from the Mediterranean Sea to Eastern Asia and several independent domestication events. In the 14th century, melon was introduced to America by Columbus, then

planted in Colorado, California and Texas. Commercial melon production in Indonesia has been significantly increasing over the last decade (Delahaut, 1998; Avivi and Dewanti, 2005).

Based on report from the Department of Information, Yogyakarta province stated that melon is a commodity that ranks as 5th income in fruits and in export aspect 6th level in 2002. Export volume of melon in 2002 reached up to 334.11 ton equal to US $ 173,852. Some countries of target for export of melon were Singapore, Malaysia, Japan, Korea and Hongkong. Recently, requirement of melon in Indonesia is 1.34-1.50 kg/capita/year, and this condition support development of melon cultivation in Indonesia.

However, one of the devastating diseases of the melon crop in Indonesia is caused by a virus (Somowiyarjo, 1992). *Kyuri green mottle mosaic virus* (KGMMV) is a member of the genus *Tobamovirus*, a serious disease agent of cucurbits including melon and causes significant economic losses in several countries (Tan *et al.*, 2000). This virus was first detected in Tokushima, Japan where greenhouse-grown cucumber was an important crop (Inouye *et al*., 1967) and also reported in South Korea[3] (Lee *et al*., 2000). Furthermore, an isolate of KGMMV (KGMMV-YM) from melon fields in Klaten, Central Java, Indonesia has been isolated and characterized symptomatologically and serologically, as well as the genome structures (Daryono *et al*., 2005). To face the virus, Daryono et al. (2005) reported that melon cultivars such as; *Mawatauri, Kohimeri, PI 161375* and *PI 371795* were resistance to KGMMV-YM while other melon cultivars such as; *Yamatouri, Vakharman, Andes, Sinjong* were susceptible.

KGMMV-YM virus attacks melon and causes considerable losses in KGMMV-YM-infected melon and other cucurbit plants. This virus frequently causes substantial loss in yield and inferior fruit quality, especially in the early melon seeding. Resistance breeding is one of the solutions to overcome the disease by crossing of PI 371795 and Andes melon. Therefore, the objective of this research was to study the inheritance of resistance gene to KGMMV in melon.

MATERIALS AND METHODS

Plant material

The F_1 population was derived from a cross between PI 371795 which resistance to KGMMV-YM and Andes which susceptible to KGMMV-YM (Daryono et al., 2005). The F_1 individuals were then self-pollinated to produce F_2 population, whereas test cross individuals were derived from a cross between F_1 individual and Andes.

Virus maintenance and inoculation procedures

The KGMMV-YM inoculum was preserved from Laboratory of Genetics, Facullty of Biology, Gadjah Mada University and propagated on Zucchini (*Cucurbita pepo* L.) at Laboratory of Plant Virology, Department of Pest and Plant Diseases, Faculty of Agriculture, Gadjah Mada University. Inoculation of KGMMV-YM were used to detect resistance gene in F_1, F_2 and test cross individuals. Inoculation was done on surface leave of melon 10-days old, completely-expanded seedlings were lightly dusted with carborundum (400-600 mesh) and rub-inoculated with virus-infected sap (approximately 1:10 dilution leaf material: buffer) using sponge plugs, and grown for 30 days.

Serological detection

Serological detection was used to determine concentration of virus in melon which were inoculated KGMMV-YM then tested using *double antibody sandwich enzyme-linke immunosorbent assay* (DAS ELISA) (modified by Daryono et al., 2005). The assay was carried out in polystyrene microtiter plates. Microtiter plate covered KGMMW antibodies (dilution with buffer phosphate 1:200) and incubated for 12-16 hours at 4^{o}C, then washed with PBST four times. About 0.2 g ground samples were mixed with 2 ml buffer carbonate until powder then was vortexed and filtered with sterile cotton. The supernatant poured well micro titer plate with micropipette each 100 µl. The empty well was filled sterile water and incubated for 12-16 hours

at 4ºC. The sap was removed and micro titer plate was washed with PBST four times then incubated with conjugate (ratio buffer phosphate 1:200) each well 100 µl for 18 hours at 4ºC. Conjugate removed and micro titer plate washed with PBST 4 times, then filled substrate 100 µl at each wells. After 30-60 minutes, micro titer entered to ELISA reader (405 nm), obtained absorbance values ELISA test. A sample was considered positive for KGMMV-YM infection if the ELISA absorbance value was three times greater than the average absorbance value of healthy control tissues and infected to F_1, F_2 and test cross individuals after inoculated KGMMV-YM for four observations.

RESULTS

Symptom development of melon cultivars by KGMMV-YM

All of the inoculated progenies between Andes and PI 371795 showed or developed characteristic symptoms after manual inoculation with KGMMV-YM inoculation, whereas all PI 371795 did not show any KGMMV symptoms.

The symptom observation on melon infected KGMMV-YM was conducted to study the inheritance of resistance pattern to KGMMV-YM based on symptom development and to determine melon fruits type from a cross between Andes and PI 371795 which potential to produce commercially. The symptoms in the inoculated F_1, F_2 and test cross individuals showed systemic symptoms that included mosaic symptoms, chlorotic mottling and leaf deformation (Figure 1).

Healthy | Chlorotic | Mottle | Leave deformation

Figure 1. The symptoms of KGMMV-YM segregation on F_2 and test cross population.

Serological detection

The objective of serological detection was to detect the existence of KGMMV-YM on F_1, F_2 and test cross individuals derived from crossing between Andes and PI 371795. The serological detection based on the reaction between antigens to antibody. This reaction happened if antigenic binding site on antibody protein match antigenic determinant site from protein-containing antigen. A virus reaction with antibody which matches and is not be caused with antibody from the other virus (Clark and Adams, 1977). Serological detection used on this research using *double antibody sandwich enzyme-linke immunosorbent assay* (DAS ELISA). The advantages were since it is robust, such that it is able to identify large samples, cheaper and faster. This method is also more specific than *indirect enzyme-linked immunosorbent assay* (I-ELISA) (Somowiyarjo, 1992). The results of DAS-ELISA are shown in Tables 1 and 2.

Table 1. DAS-ELISA test on F_2 population was derived from a cross between PI 371795 and Andes.

Generation	Symptoms[a)]	ELISA[b)]			Reactions[c]
1	2	3			4
F2.1	0	0.3790	±	0.1131	R
F2.2	0	0.1950	±	0.0354	R
F2.3	0	0.1980	±	0.0212	R
F2.4	1	0.6040	±	0.2560	S
F2.5	0	0.2140	±	0.0127	R
F2.6	0	0.3690	±	0.0594	R
F2.7	0	0.4200	±	0.1061	R
F2.8	2	0.8075	±	0.0940	S
F2.9	0	2.0460	±	0.3422	S
F2.10	0	1.3120	±	0.5091	S
F2.11	2	0.7865	±	0.5339	S
F2.12	0	0.3135	±	0.0332	R
F2.13	0	0.5345	±	0.4052	R
F2.14	0	0.4170	±	0.3026	R
F2.15	0	0.5885	±	0.3953	R
F2.16	2	1.0425	±	0.7941	S
F2.17	0	1.0500	±	0.5812	S
F2.18	0	0.9640	±	0.0552	S
F2.19	0	0.6135	±	0.4660	S
F2.20	0	0.4385	±	0.2355	R
F2.21	0	0.4175	±	0.3175	R
F2.22	0	0.2690	±	0.1089	R
F2.23	0	0.2970	±	0.1089	R
F2.24	0	0.6230	±	0.2150	S
F2.25	2	2.2395	±	0.0544	S
F2.26	0	1.6880	±	0.2178	S
F2.27	2	1.3815	±	0.5650	S
F2.28	1	0.705	±	0.2227	S
F2.29	0	0.366	±	0.0870	R
F2.30	0	0.264	±	0.0198	R
F2.31	0	0.441	±	0.2595	R
F2.32	0	0.239	±	0.0325	R
F2.33	0	0.527	±	0.1831	R
F2.34	0	0.587	±	0.3465	R
F2.35	0	0.545	±	0.1513	R
F2.36	2	0.893	±	0.0233	S
F2.37	1	0.646	±	0.0530	S
F2.38	0	0.375	±	0.0113	R
F2.39	0	0.334	±	0.0113	R
F2.28	1	0.705	±	0.2227	S
F2.29	0	0.366	±	0.0870	R
F2.30	0	0.264	±	0.0198	R
F2.31	0	0.441	±	0.2595	R
F2.32	0	0.239	±	0.0325	R
F2.33	0	0.527	±	0.1831	R
F2.34	0	0.587	±	0.3465	R
F2.35	0	0.545	±	0.1513	R
F2.36	2	0.893	±	0.0233	S
F2.37	1	0.646	±	0.0530	S
F2.38	0	0.375	±	0.0113	R
F2.39	0	0.334	±	0.0113	R
F2.40	0	0.333	±	0.0092	R

Continued...

Generation	Symptoms[a)]	ELISA[b)]			Reactions[c]
1	2	3			4
F2.41	0	0.367	±	0.0445	R
F2.42	0	0.374	±	0.0219	R
F2.43	0	0.342	±	0.0431	R
F2.44	0	0.383	±	0.0573	R
F2.45	0	0.419	±	0.0573	R
F2.46	0	0.563	±	0.0870	R
F2.47	2	0.854	±	0.3613	S
F2.48	0	0.471	±	0.0354	R
F2.49	0	0.397	±	0.1485	R
F2.50	0	0.371	±	0.0898	R
F2.51	0	0.379	±	0.1188	R
HK[d)]		0.195	±	0.0354	
HK x 3		0.5850			
PK[e)]		2.005	±	0.2998	

a) Symptoms score: 0 = no symptom, 1= symptom mosaic light, 2= symptom weight; b) The average of DAS-ELISA value ± standard deviation (405 nm); c) R= resistant (≤ 0.5850); S= susceptible (> 0.5850); d) HK = healthy control (negative control); e) PK = positive control

Table 2. DAS-ELISA test on F_1 population derived from a cross between PI 371795 and Andes.

Generation	Symtomps[a)]	ELISA[b)]			Reactions
F1.1	0	0.4420	±	0.0665	R
F1.2	0	0.5480	±	0.0948	R
F1.3	0	0.5130	±	0.1259	R
F1.4	0	0.4495	±	0.0841	R
TC.1	2	0.6335	±	0.5325	S
TC.2	2	1.3675	±	0.5254	S
TC.3	2	1.6635	±	0.0559	S
TC.4	2	0.7330	±	0.1230	S
TC.5	0	0.2360	±	0.0424	R
TC.6	0	0.8585	±	0.7149	R
TC.7	2	2.6155	±	0.0021	S
TC.8	2	2.2280	±	0.2489	S
TC.9	2	1.6575	±	0.2949	S
TC.10	0	0.5060	±	0.0509	R
TC.11	0	0.4850	±	0.0552	R
TC.12	0	0.3345	±	0.0714	R
TC.13	0	0.4030		0.1259	R
HK[d)]		0.1950	±	0.0354	
HK x 3		0.5850			
PK[d)]		2.0050	±	0.2998	

a) Symptoms score: 0 = no symptom, 1= symptom mosaic light, 2= symptom weight; b) The average of DAS-ELISA value ± standard deviation (405 nm); c) R= resistant (≤ 0.5850); S= susceptible (> 0.5850); d) HK = healthy control (negative control); e) PK = positive control

The chi-square test from DAS-ELISA test (Table 3) has shown that X^2_{count} F_2 and test cross = 1,469 to F_2 and 0 to test cross. The X^2_{table} values searched on signification 5% df = 1 and found X^2_{count} values = 1,469 on signification (X^2_{table}) 0,10-0,30 and 0 on signification 0,99-1 and follows Mendel's law with ratio F_2 segregation is 3:1 (34 resistant, 17 susceptible) while the ratio in test cross is 1:1 (6 resistant, 7 susceptible).

The resistance mechanisms observed in these cultivars could also be related to the existence of mechanisms that inhibit movement of the virus from inoculated leaves to upper leaves. Resistance could also involve cellular membrane changes that impede the diffusion or transport of infective virus particles from cell to cell, or an inhibition of virus particle replication in the leaf tissue of resistant host plants, as has been described for virus diseases in some cucurbit species (Gray et al., 1988), or even a combination of these two mechanisms (Daryono, 2003).

Furthermore, results of this breeding experiments also show four variations of melon fruits which may have the potential to become a commercial melons (Figures 2 and 3). The results of DAS-ELISA test from a cross PI 371795 (resistance to KGMMV-YM) and Andes (susceptible to KGMMV-YM) exhibited segregation resistance that follows Mendelian law and it is controlled by a single dominant gene. Therefore it is proposed that *Krey* for the allele symbol for KGMMV-YM resistance gene.

Enclosure 1: Test cross population derived from a cross between F_1B with Andes

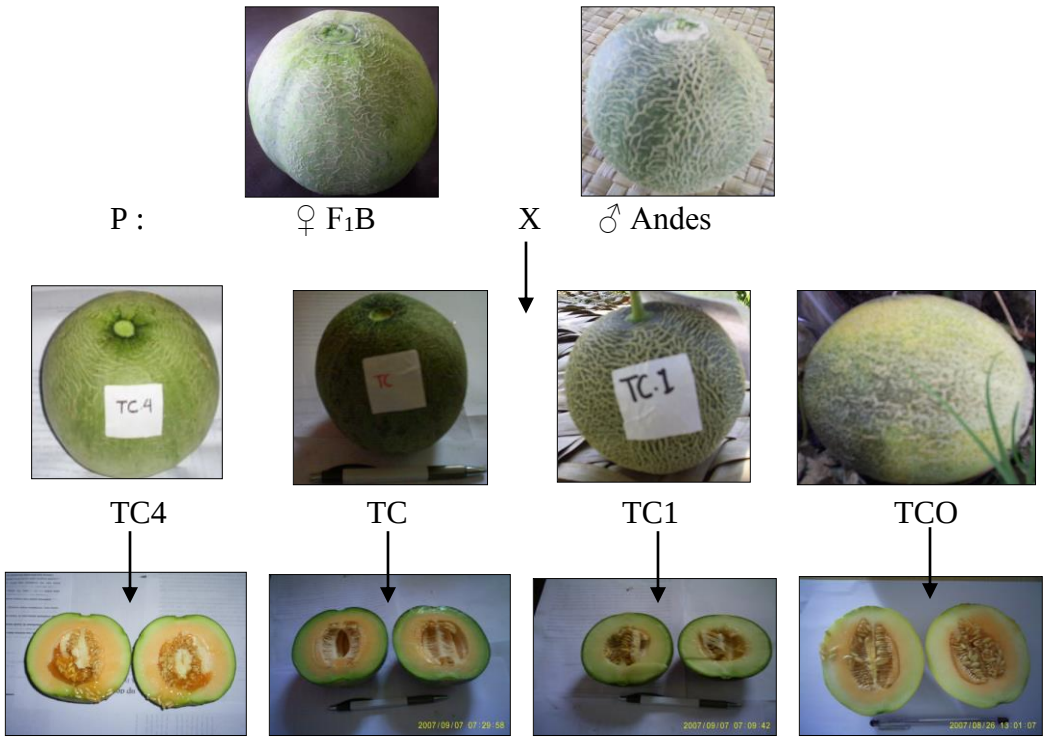

Figure 2. Test cross population between ♀ F_1B with ♂ Andes and fruit variation.

Table 3. Chi-square test on resistance segregation from DAS-ELISA test in progenies from crosses between Andes and PI 371795 after inoculation with KGMMV-YM.

Generation	Number of plant			$X^2_{(0.05)}$ c)		
	Total	R a)	S b)	Ratio	Value	Probability
PI 371795	5	5	0			
Andes	5	0	5			
F_1	4	4	0			
F_2	51	34	17	3:1	1.469	0.10-0.30
Test cross	13	6	7	1:1	0	0.99-1.00

a) R = resistant; b) S = susceptible; c) Signification 0.05 (5%)

Enclosure 2: F_2 population derived from a cross between F_1B with F_1B

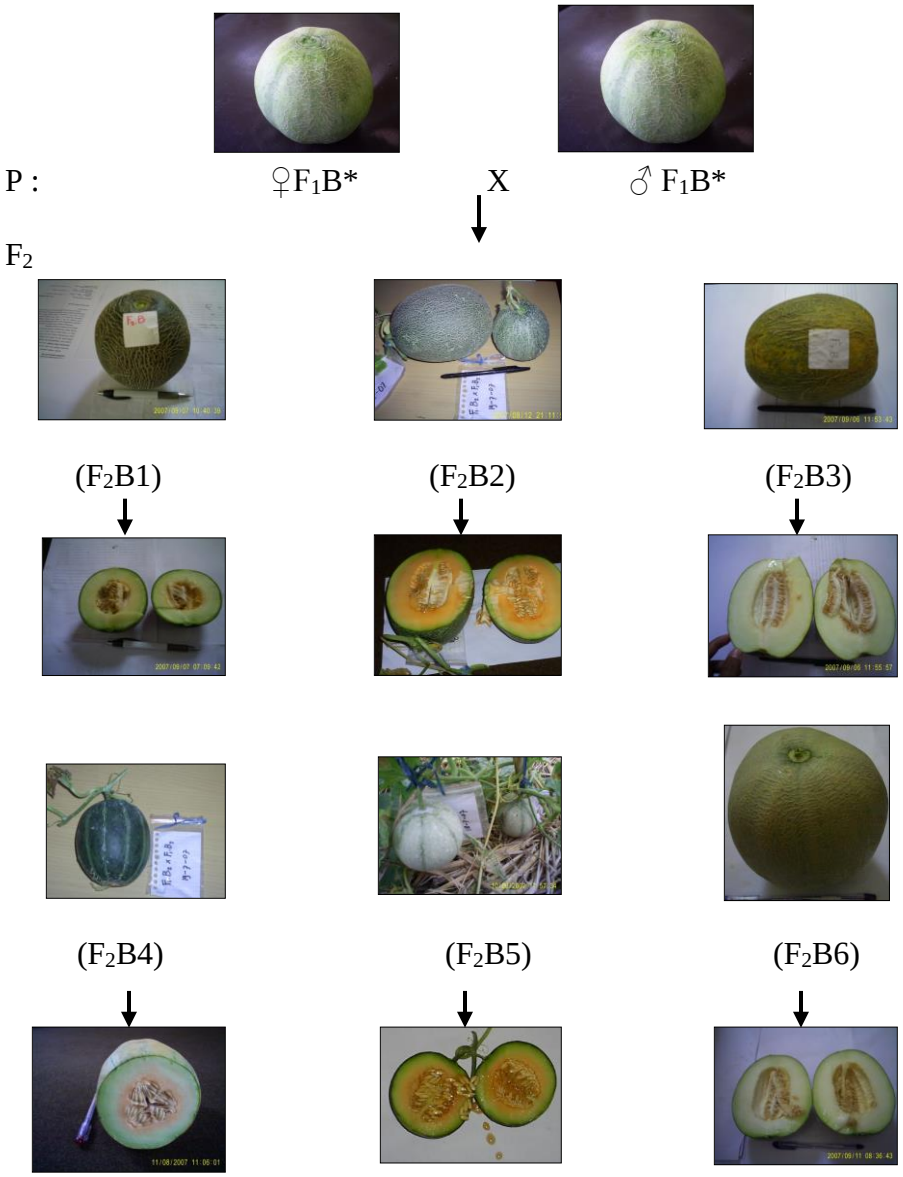

Figure 3. F_2 population was derived from a cross between ♀ F_1B with ♂ F_1B and fruit variation
*F_1B : melon derived from a cross between ♀ Andes with ♂ PI 371795.

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