Jurnal Perlindungan Tanaman Indonesia, Vol. 12, No. 1, 2006 : 53-61.

INHERITANCE OF RESISTANCE TO *PAPAYA RINGSPOT VIRUS*-PAPAYA STRAIN IN MELON (*Cucumis melo* L.)

PEWARISAN GEN KETAHANAN TERHADAP PAPAYA RINGSPOT VIRUS STRAIN PAPAYA PADA MELON (Cucumis melo L.)

Budi Setiadi Daryono
Laboratory of Genetics, Faculty of Biology, Gadjah Mada University, Yogyakarta 55281, Indonesia.
E-mail: bs_daryono@mail.ugm.ac.id
Keiko T. Natsuaki
Laboratory of Tropical Plant Protection, Graduate School of Agriculture, Tokyo University of Agriculture, Japan.

ABSTRACT

Papaya ringspot virus *(PRSV) is one of potyviruses causing severe damage to the production of cucurbit crops including melon, however resistant melons to PRSV have not yet commercially available. To find resistant genetic source of melons against PRSV, sixty-three melons were manually inoculated with PRSV-papaya strain (PRSV-P) isolated in Thailand. Levels of resistance to PRSV-P accumulation in melon leaf tissue were evaluated using a combination of visual symptom observation and RT-PCR analysis. Among melons tested, Yamatouri, Mawatauri, PI 414723, and PI 371795 showed to be resistance to PRSV-P. To study the inheritance of resistance to PRSV-P, breeding experiments were conducted by crossing the resistant Yamatouri with susceptible Vakharman. The genetic analysis results revealed that a single dominant gene conferred resistance to PRSV-P in Yamatouri and its generation.*

Key words: *Cucumis melo* L., Resistant cultivar, ELISA, RT-PCR

INTISARI

Papaya ringspot virus (PRSV) adalah salah satu virus tanaman anggota Potyvirus yang seringkali menginfeksi dan menurunkan produktivitas tanaman labu-labuan termasuk melon. Meskipun demikian, sampai sekarang belum tersedia secara komersial benih melon yang tahan terhadap PRSV. Untuk menemukan sumber genetik melon yang tahan terhadap PRSV, 63 kultivar melon telah diuji dengan inokulasi PRSV strain papaya (PRSV-P) yang diisolasi dari Thailand, sedangkan derajat ketahananya dianalisis melalui pengamatan perkembangan gejala mosaik serta RT-PCR analisis. Hasil uji ketahanan menunjukkan bahwa melon Yamatouri, Mawatauri, PI 414723, dan PI 371795 tahan terhadap infeksi PRSV-P. Perkawinan silang antara melon Yamatouri yang tahan dengan melon Vakharman yang rentan telah dilakukan untuk mempelajari pewarisan gen ketahanan terhadap PRSV-P. Hasil analis genetik menyimpulkan bahwa sifat ketahan terhadap PRSV-P pada melon

Yamatouri dan keturunanya dikendalikan oleh gene tunggal dominan.

Kata kunci: *Cucumis melo* L., PRSV, RT-PCR

INTRODUCTION

The goal of plant breeding is to contribute to a qualitative and quantitative improvement in crop production. The phenomenal increases in food production during recent years depend on a great deal the development of disease resistant cultivars of food crops (Khertapal et al., 1998). However, in the agrarian based developing countries like Indonesia, breeding for resistance to diseases caused by cucurbit viruses has not been developed in melon. It is because melon is a new horticultural crop to Indonesia and not yet important in the term of crop value. Moreover, the virus diseases are more difficult to recognize and need advance technology for its identification. This situation leads the importance of research study on breeding and production of virus resistance in melon.

Papaya ringspot virus (PRSV) is one of potyviruses causing severe damage to the production of cucurbit crops worldwide (Purcifull et al., 1984). This virus has two strains, the papaya strain (PRSV-P) and the watermelon strain (PRSV-W), formerly known as watermelon mosaic virus-1 (WMV-1). PRSV-P infects both papaya and cucurbits, while PRSV-W infects cucurbits but not papaya (Gonsalves, 1993). To identify sources of resistance to PRSV-W in melon, Webb (1979), Pitrat and Lecoq (1983) reported that two melon accessions PI 180280 and PI 180283 were resistant to PRSV-W and showed that the resistances from these two sources are allelic. Another source of resistance to PRSV-W has also been identified in melon accession PI 414723 and its breeding line PI 414723-4S3. The resistance to PRSV-W in the line PI 414723-4S3 was further studied and it was controlled by a single dominant gene (Anagnostou et al., 2000).

However, classification of inheritance patterns and transfer of resistance from sources are complicated by isolate specificity, genotype of the susceptible parent, and may be overcome by different pathotypes (Pitrat and Lecoq, 1980; Danin-Poleg, et al., 1997; Grube et al., 2000). Therefore, in order to determine the inheritance of resistance to PRSV-P (Th 110), resistant Yamatouri was crossed with susceptible Vakharman. Levels of resistance to PRSV-P accumulation in melon leaf tissue were evaluated using a combination of visual symptom observation and RT-PCR analysis.

The objective of this study is to determine the inheritance of resistance to PRSV-P in melon cultivar Yamatouri.

MATERIAL AND METHODS

Virus maintenance and inoculation procedures. PRSV-P Th110 was used as virus sources for inoculation. PRSV-P (Th 110) was propagated in zucchini (*Cucurbita pepo* cv. *Diner*). Inoculum from PRSV-P was prepared by grinding 0.01g systemically infected tissue in a pre-chilled mortar and pestle with 1 ml of 10 mM sodium phosphate buffer, pH 7.0. Seedlings at the first true leaf stage were lightly dusted with carborundum (600

mesh) and rub-inoculated with virus infected sap using sponge plugs. Inoculated plants were kept in growth chamber (26°C) and grown for 30 days. Visual scoring was done twice a week for 30 days. Plants were considered resistant (R) if viral symptoms were absent in upper leaves and susceptible (S) when symptoms were continuously observed.

Genetic materials and breeding experiments. Breeding between the resistant Yamatouri and the susceptible Vakharman was conducted in green house at Kakegawa Breeding Experimental Station, Sakata Seed Co., in 2003 (Fig. 1). Before its use as a parent, Yamatouri plant was self-pollinated many times and each generation determined to be homozygous for resistance to CMV-B2. Yamatouri plant was then crossed with the susceptible Vakharman plant by manual pollination to obtain seeds of F_1 generations. The F_1 progeny were self-pollinated to produce the F_2 generation or crossed to parents to produce reciprocal backcross families (F_1 x Yamatouri and F_1 x Vakharman). Plants of the two parents and their F_1, F_2, and reciprocal backcross populations were inoculated mechanically with PRSV-P on the cotyledons. The inheritance of resistance to PRSV-P (Th110) was evaluated on the basis of leaf symptoms and RT-PCR detection of PRSV-P on inoculated leaf at 21 days after inoculation. As control, three non-inoculated plants from each of the parents, F_1, F_2, and backcross populations were kept under the same conditions during the inoculation. Chi-square analyses were performed to analyze segregation ratios of the F_2 and BCs populations.

RT-PCR analysis. Viral RNA was extracted from upper leaf of each melon cultivars by incubation in 0.3% sodium dodecyl sulfate (SDS) following phenol chloroform extraction (Rosner et al., 1983). The PRSV specific primers were amplified by reverse transcription-polymerase chain reactions (RT-PCR). First strand cDNAs for CP gene was synthesized with First-Strand cDNA Synthesis Kit (Amersham Biosciences, UK) and a primer complementary to the 3' of the gene on 461-481 of PRSV with accession number D50591 (5' TACCCAGGAGAGAGTGC ATG-'3). This primer and a forward primer on 891-910 of PRSV (5'-TAGCACAAAAACTGGAGA GAGAG-'3) were used to amplify the cDNA product of the first strand synthesis with Takara Ex Taq™ PCR buffer (Takara Biomedicals, Japan) and following by PCR conditions: for one cycle at 92°C for 5 min followed by 30 cycles of 92°C for 30 s, 48°C for 1 min, and 72°C for 1.5 min, and finally with an extension temperature at 72°C for 10 min. Amplified products (10 µl each) were electrophoresed in 1.5% agarose gel and stained with ethidium bromide.

RESULTS

Agricultural trait of cultivars Yamatouri and Vakharman. Cultivars Yamatouri and Vakharman were selected for further breeding experiments because they showed obvious differences in disease resistance and horticultural traits. Fruit of Yamatouri is small-medium sized (600 g) of round-elongated shape, with silver-green, smooth and hard skin, and low content of soluble solids (9° Brix). While Vakharman fruit is large (2,000 g) of elongated shape, with yellow-green, smooth and hard skin, and high content of soluble solids (15° Brix). The cultivar Yamatouri was obtained in 1984 by crossing Mawatauri with Andes, followed by several generations of self-

pollination to obtain homozygous-resistant lines (personal communication with melon breeder, H. Ogawa). Breeding experiments of resistant Yamatouri with susceptible Vakharman is presented in Figure 1.

Genetic determination of the resistance to PRSV-P (Th 110). Vakharman plants developed continuous, severe systemic and local infection, including mosaic and chlorotic symptoms after inoculated by PRSV-P. On the

Fig.1. Breeding experiments of resistant Yamatouri with susceptible Vakharman conducted in green house at Kakegawa Breeding Experimental Station, Sakata Seed Co., Shizuoka, Japan. A: Yamatouri x Vakharman; B: F_1 self-pollination; C: Vakharman x Yamatouri; D: Vakharman x F_1.

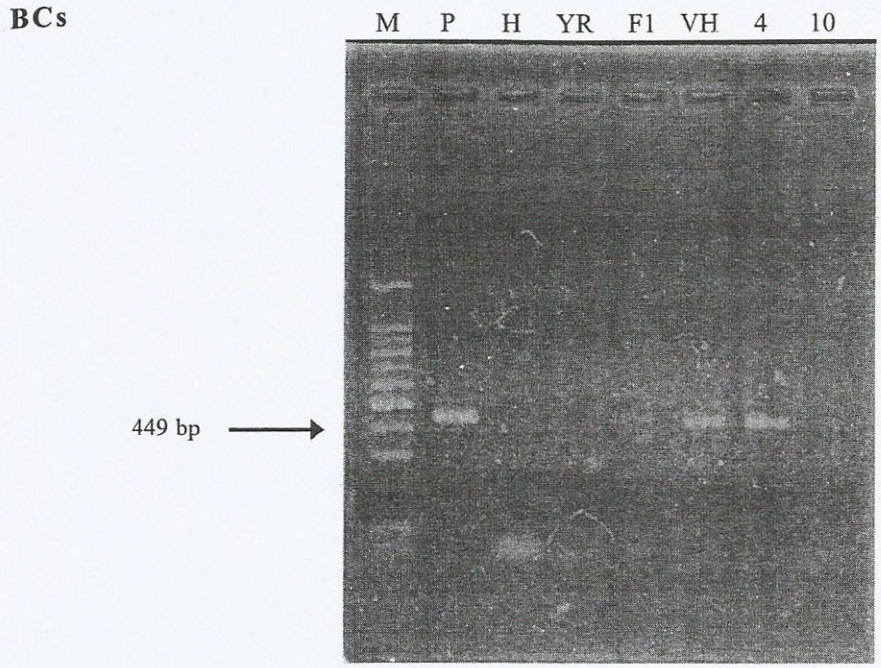

Fig. 2. Gel electrophoresis of RT-PCR products amplified from total RNA extracted from melon inoculated with PRSV-P (TH 110). M: 100 bp DNA marker (Promega); P: Positive control (Vakharman infected PRSV-P Th 110); H: Healthy melon; YR: Yamatouri; F_1: F_1 progeny; VH: Vakharman; BCs-4: backcross progeny no. 4; BCs-10: Backcross progeny no. 10. Single arrow indicates coat protein of PRSV-P (TH 110).

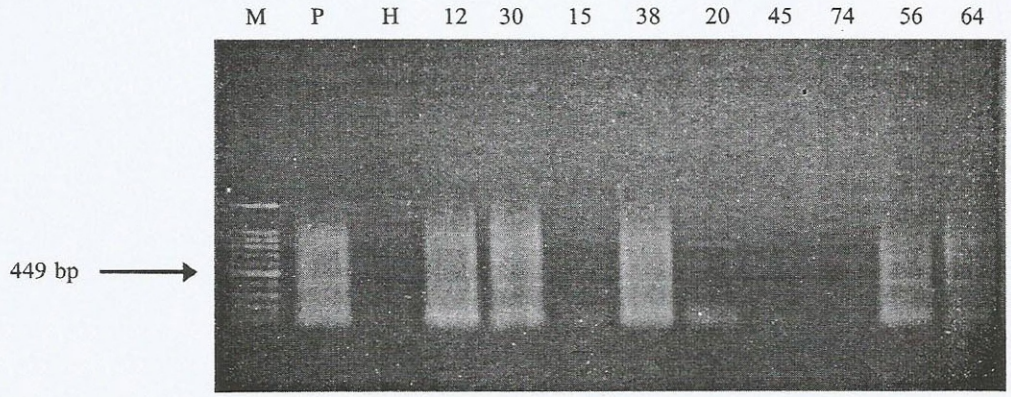

Fig. 3. Gel electrophoresis of RT-PCR products amplified from total RNA extracted from melon inoculated with PRSV-P (TH 110) in F_2 progeny. M: 100 bp DNA marker (Promega); P: Positive control (Vakharman infected PRSV-P Th 110); H: Healthy melon; F_2: susceptible F_2 progeny no. 12, 30, 38, 20, 56 and 64; F_2: resistant F_2 progeny no. 15, 45 and 74.

other hand, Yamatouri plants and F_1 progenies showed no symptoms in upper leaves after inoculated with PRSV-P (Th 110). Furthermore, the backcross of F_1 to Vakharman showed segregation with a ratio of 1 resistant: 1 susceptible after inoculated with PRSV-P (Th 110) in 25 BCs plants. Fifteen BCs plants showed no symptoms and negative by RT-PCR analysis in upper leaves, while 10 plants clearly showed both symptoms and coat protein gene (Table 1). These data suggest that a single dominant gene in Yamatouri parent confers resistance to PRSV-P (Th 110). Furthermore, the segregation result of PRSV-P (Th 110) in F_2 population showed ration of 3:1 in 75 F2 plants. It could be concluded that resistance to PRSV-P in Yamatouri is controlled by a single dominant gene in. By RT-PCR analysis, PRSV-P (Th 110) was detected in upper leaves of Vakharman, and susceptible BCs and F_2 plants, however not detected in upper leaves of-Yamatouri, F_1 progenies, resistant BCs and F_2 plants (Fig. 2 and 3).

DISCUSSION

Available hybrid melon cultivars usually show symptoms by infection of most strain of cucurbit viruses including PRSV, while resistant melon to viruses has not yet available commercially. To produce virus resistant melon against PRSV, breeding experiments and the inheritance of resistance to these viruses in melon were conducted. Resistant cultivar Yamatouri was crossed with susceptible cultivar Vakharman. Backcross between F_1 and Vakharman were also conducted. All of F_1 plants tested showed resistance to these viruses, while F_2 and BCs individuals were segregated into resistant and susceptible.

Previous studies have identified two PRSV resistance genes, *Prv1* and *Prv2* in two melon accessions PI 180280 and PI 180283 respectively (Webb, 1979; Pitrat and Lecoq, 1983). In contrast, the genetic analysis of this study supports the hypothesis that the resistance to PRSV-P in Yamatouri is controlled by a single dominant gene. Similarly Anagnostou et al. (2000) reported that the single dominant gene controls resistance to PRSV-W in a breeding line derived from PI 414723 (PI 414723-4S3). Pitrat and Lecoq (1984) identified a single dominant gene, *Zym* conferred the resistance to *Zucchini yellow mosaic virus* (ZYMV) in melon accession PI 414723, while Gilbert et al. (1994) reported that another single dominant gene in melon accession PI 414723 controls resistance to *Watermelon mosaic virus 2* (WMV). Resistance to CMV-B2 in Yamatouri was reported and it is controlled by a single dominant gene, *Creb-2* (Daryono et al., 2003). Therefore, cultivar Yamatouri showed to be resistant to both CMV and PRSV-P and its control by each single dominant gene.

Co-segregation of resistance to two or more viruses has been previously reported in several crops including pepper, pea, and bean (Grube et al., 2000; Fisher and Kyle, 1994; Kyle and Dickson, 1988; Providenti, 1991). In cucumber, linkage between a recessive gene for WMV resistance, and a gene for ZYMV resistance was reported in the TMG-1 line (Wai and Grumet, 1995). More recently, the TMG-1 line was further characterized and reported to be resistant to PRSV (Wai et al., 1997; Kabelka and Grumet, 1997). Host genes conferring resistance to different potyviruses may be tightly linked due to their evolution as gene cluster and it has also been suggested that one gene may protect against multiple viruses (Anagnostou et al., 2000). For example, a single *Cucurbita moschata* gene conferred resistant

Table 1. Resistance segregation in progenies from crosses between Yamatouri and Vakharman after inoculation with PRSV-P (Th 110)

Generation	Number of plants				χ^2	
	Total	R[a]	S[b]	Ratio	Value	Probability
P_1 = Yamatouri	26	26	0			
P_2 = Vakharman	12	0	12			
F_1 (P_1 x P_2)	24	24	0			
F_2 (P_1 x P_2)	75	62	13	3:1	1.96	0.1-0.2
BCs: P_2 (P_1 x P_2)	25	15	10	1:1	0.16	0.2-0.3

a) Resistant plants without symptoms and CP gene not detected in the upper leaves by RT-PCR analysis
b) Susceptible plants with mosaic symptoms and CP gene detected in the upper leaves by RT-PCR analysis

to both WMV and ZYMV (Gilbert-Albertini et al., 1993), and a cucumber gene protected from both ZYMV and PRSV (Kabelka and Grumet, 1997).

Resistance to PRSV showed in cultivar Yamatouri could be defined as a property of the plant that reduces or prevents virus multiplication, spread within the plants or symptom expression. In this study, PRSV were limited to initially infect on inoculated leaf and it was unable to move into upper leaf, or inability of the virus to replicate in initially inoculated leaf. According to Zaitlin and Hull (1987) interaction between a virus and a plant can be considered at four levels: (1) **tolerance**, whereby the virus produces mild symptoms on systemic infection of the plant; (2) **hypersensitive response**, whereby the virus is restricted to a few cells around the site of infection usually by necrotic response of the host; (3) **subliminal infection**, whereby the virus is limited to initially infected cell from which it is unable to move, and (4) **true immunity**, i.e., the inability of the virus to replicate in the initially infected cell. Thus, it could be concluded that interaction between PRSV-P in cultivar Yamatouri was considered at subliminal infection to true immunity. In addition, resistance gene to PRSV-P identified in this cultivar would be important to obtain a better understanding of pathogen variation relevant to breeding efforts and for understanding the organization of resistance genes in key germplasm resources.

ACKNOWLEDGMENTS

The authors thank Mr. Takuya Chiba, Kakegawa Breeding Experimental Station, Sakata Seed Co., Shizuoka-Japan for technical assistant on breeding experiment and kindly providing seeds.

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