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SYMPTOMS INDUCTION BY PSEUDORECOMBINATION OF RNA SPECIES BETWEEN CMV STRAINS

PENGIMBASAN GEJALA OLEH PSEUDOREKOMBINASI SPESIES ARN DI ANTARA STRAIN-STRAIN CMV

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INTISARI

Dua puluh tujuh strain CMV telah dikumpulkan dari beberapa wilayah di Pulau Jawa. Strain-strain tersebut memiliki empat spesies ARN dan tidak ada satu pun yang memiliki satelit ARN. Pertukaran spesies ARN dari strain-strain yang termasuk subkelompok I dan II untuk pseudorecombinasi, mengimbas gejala yang ringan sampai parah pada Nicotiana glutinosa. *Dengan penularan afid, psedorekombinan CMV dapat menghasilkan gejala yang khas satu dari strain induknya atau menghasilkan gejala yang tidak mirip dengan gejala yang ditunjukkan oleh strain-strain induknya pada* N. glutinosa. *Beberapa tanaman dengan gejala ringan dapat terdeteksi positif dengan uji ELISA tidak langsung.*

Kata kunci: CMV, pseudorekombinan, kenampakan gejala

ABSTRACT

Twenty seven strains of CMV have been collected throughout Java, they have four RNA species and none of them contain satellite RNA. The exchange of RNA species of strains belonging to subgroup I and II for pseudorecombination induced mild to severe symptoms on *Nicotiana glutinosa.* By aphid transmission, the CMV pseudorecombinant could produce a typical symptom of one of its parental strain or expressed unlikely symptoms as those of either parental strains on *N. glutinosa.* Some plants with mild symptoms were detected to be positive by Indirect ELISA.

Key words: CMV, pseudorecombinant, symptom expresssion

INTRODUCTION

Cucumber mosaic virus (CMV) is a member of Cucumovirus group, it has four species of genomic RNA. The main three RNA species (RNA-1, -2, and -3) are needed for the successfulness of infectivity (Lot *et al.,* 1974). RNA 1 is for initiating the virus replication, however, its role in the pathogenecity is less determined (Rossinck & Palukaitis, 1990; Zitter & Gonsalves, 1991). Both RNA-1 and -2 are responsible for the type of symptom appearance (Hanada, 1986; Rossinck & Palukaitis, 1990), and RNA-3 is responsible for the mechanism of coat protein synthesis, the aphid transmissibility and determines the serological specificity (Mossop & Francki, 1977; Gera *et al.,* 1979). One of the techniques for determining function of each RNA species is pseudorecombination. Pseudorecombinant

is constructed by the exchange of RNA species of two strains or two viruses, *e.g.* CMV and TAV (Habili & Francki, 1974), strains CMV-T and CMV Price-6 (Gera *et al.*, 1979). Moreover, pseudorecombinant with very mild symptoms is proposed for a challenger in cross protection (Mossop & Francki, 1977; Rao & Francki, 1981).

CMV has a lot of strains and not all of the strains can be transmitted by aphid, *e.g.* strain M. It is known that more than 60 aphid species can transmit CMV, but *Aphis gosypii* and *Myzus persicae* are found to be more frequently to transmit the virus in the field (Kaper & Waterworth, 1981). This paper present the kind of RNA species of CMV strains collected throughout Java and symptom variation induced by pseudorecombinant constructed from a mild strain with severe one.

MATERIALS AND METHODS

Virus isolates and its propagation. Nineteen strains belonging to subgroup I of CMV and eight strains belonging to subgroup II were used as parental viruses for pseudorecombination are shown in Table 1. CMV isolates from Malang were kindly provided by Mr. Mintarto M. (Faculty of Agriculture, Brawijaya University) and CMV isolates from Yogyakarta were provided by Mrs. Sri Sulandari (Faculty of Agriculture, Gadjah Mada University). These strains were classified into two subgroups on the basis of serological method and the migration rate of RNA-1 and -2 on either AGE or PAGE (Wahyuni & Sulyo, 1997; Wahyuni *et al.*, 1997, unpublished). Each of the strains were propagated on *Nicotiana glutinosa* and harvested on 12 days after inoculation.

Single stranded (ss-) RNA extraction and electrophoresis. Viral RNA was extracted by the modification methods of Rao & Francki (1981) The ss-RNA concentration was determined using the value $E_{(0.1\%,260)} = 25$ (Peden & Symons, 1973). The RNAs were electrophoresed on 1.5–1.5 % agarose in 1x TAE buffer for one hour and stained with ethidium bromide.

***Pseudorecombinant construction and its inoculation to* N. glutinosa.** The pseudorecombination was constructed according to Rao & Francki (1981) and Rao *et al.* (1982). The parental strains for pseudorecombination were chosen from the member of either subgroup I or II strains which showed different symptoms, respectively. Each of RNA species (RNA-1, -2, -3) was cut with a sterile blade and then combined the RNA's of virus belonging to the subgroup I or II strains. The RNA-4 was not used in this pseudorecombination because RNA-4 is a subgenomic RNA-3. The combined RNAs were added with 50–100 µl 1x TAE buffer and 5 μl RNase in dialysis membrane and run in the electrophoresis tank for 30-40 minutes or until ethidium bromide disappeared from the gel. The combined RNAs (designated as pseudorecombinant) released in the dialysis membrane was added with 5 % sucrose and kept at -20°C until ready to inoculate.

Inoculation of pseudorecombinant to *N. glutinosa* (in 3–5 leaves stage) was done by the modification methods of Gera *et al.* (1979) and Chen & Francki (1990). *A. gossypii* or *Myzus persicae* was starved for 2-3 h then let them to probe with the RNAs-pseudorecombinant suspension on membrane of parafilm for 7 min. at ± 25°C, ten aphids per plant.

Table 1. Plant origin of isolate and locality of CMV strains as parental strains for pseudorecombination, and symptoms induced by pseudorecombinant on *N. glutinosa*

Strains	Plant origin of isolates	Locality	Subgroup	Symptoms on *N. glutinosa*
1	Bird pepper (*Capsicum frutescens*)	Pangalengan	I	Severe chlorotic mosaic
2	Bird pepper (*Capsicum frutescens*)	Cimangkok	I	Vein chlorotic, severe chlorotic mosaic, distortion
3	Tomato (*Lycopersicon esculentum*)	Segunung	I	Severe greening mosaic, distortion
4	Tree tomato (*Cyphomandra betacea*)	Segunung	I	Severe greening mosaic, distortion
5	Cucumber (*Cucumis sativus*)	Sukabumi	I	Severe greening mosaic, enation, distortion
8	Chili (*Capsicum annuum*)	Segunung	I	Mild-severe greening mosaic
11	Chili (*Capsicum annuum*)	Grati, Pasuruan	I	Severe greening mosaic, distortion
13	Cowpea (*Vigna unguiculata*)	Subang	II	Vein and tip necrosis, severe chlorotic mosaic, distortion, shoestringing
14	Banana (*Musa sapientum* cv. Tanduk)	Lumajang	I	Severe greening mosaic, distortion
15	Banana (*Musa sapientum* cv. Ambon)	Segunung	I	Severe greening mosaic, distortion
16	Banana (*Musa sapientum* cv. Ambon)	Yogyakarta	I	Severe greening mosaic, distortion
17	Banana (*Musa sapientum* cv. Ambon)	Jakarta	II	Severe chlorotic mosaic, tip and vein necrosis, and distortion
18	Tobacco (*Nicotiana tabacum* cv. F1N)	Jember	I	Mild mosaic
20	Tobacco (*Nicotiana tabacum* cv. H-382)	Jember	I	Mild mosaic
21	Tobacco (*Nicotiana tabacum* cv. H-382)	Ajung, Jember		Severe greening mosaic, distortion, shoestringing
23	Ginger (*Zingiber officinale*)	Yogyakarta	II	Severe tip, stem and vein necrosis, chlorotic, mosaic
28	Tobacco (*Nicotiana tabacum* cv. Kasturi)	Ajung, Jember	II	Severe mosaic, interveinal, chlorotic mosaic, distortion, shoestringing
33	Chili (*Capsicum annuum*)	Tumpang, Malang	II	Vein clearing, severe greening mosaic
37	Cucumber (*Cucumis sativus*)	Poncokusumo	I	Severe greening mosaic, distortion
40	Cucumber (*Cucumis sativus*)	Poncokusumo	I	severe greening mosaic, distortion
41	Ixora (*Ixora* sp.)	Rangkasbitung	I	Mild chlorotic mosaic
42	Pumkin (*Cucurbita moschata* cv. Kabuca)	Segunung	I	Moderately chlorotic mosaic
46	Tobacco (*Nicotiana tabacum* cv. H-877)	Antirogo, Jember	II	Vein and tip necrosis, severe chlorotic mosaic, distortion
47	Tobacco (*Nicotiana tabacum* cv. H-382)	Antirogo	II	Severe chlorotic mosaic, systemic necrotic
48	Tobacco (*Nicotiana tabacum* cv. H-382)	Sukowono	II	Tip necrosis, severe chlorotic mosaic
51	Chili (*Capsicum annuum*)	Tangggul	I	Mild mosaic
52	Bean (*Phaseolus vulgaris*)	Segunung	I	Severe greening mosaic

RESULTS AND DISCUSSION

Determination of RNA species of CMV strains. Result of electrophoresis on either PAGE (Wahyuni & Sulyo, 1997) or AGE (Fig. 1) showed that all strains collected have four RNAs, RNA-1, -2, -3, and RNA-4, with the RNA 4a additionally for some strains. None of the strains contain satellite RNA (RNA-5). As the migration rate of RNA-2 belonging to subgroup I CMV strains, the migration rate of RNA-2 CMV strains 37, 3, 11 was more slowly than that of subgroup II (*i.e.* strains 17, 23) (Fig. 1).

Aphid transmission. During the experiment of inoculation pseudorecombinant by aphid (March to November 1996), the aphid was found to be very hard to rear on both *Capsicum annuum* or *Brassica campestris* ssp. *chinensis* under laboratory or glass house (Instalasi Penelitian Tanaman Hias Segunung, Cipanas, Bogor) conditions. In general, not all inoculation of the RNA pseudorecombinant suspension with aphid to either *N. glutinosa* or *N. tabacum* cv. Xanthi were successful, although *N. glutinosa* was susceptible to both parental strains. Pseudorecombinant of RNA-1 (CMV strain-18)+RNA-2 (CMV strain-18)+RNA-3 (CMV strain-41) which designated as CMV pseudorecombinant 18+18+3, failed to infect *N. glutinosa* but it induced mild symptom on *N. tabacum* cv. Xanthi nc. as with the symptoms of CMV strain-41 (Table 2). This was also found by Rao & Francki (1982) with the pseudorecombinant of CMV strains -M, -K and -U and also with the reassemble virus of RNA-CMV strain M and coat protein CMV strain U (Chen & Francki, 1990).

Symptoms induction by CMV pseudorecombinant. Pseudorecombination constructed by the exchange of RNA species between CMV strains is shown on Table 2. Among twenty pseudorecombinants, some expressed variable symptoms on *N. glutinosa* (Fig. 2), and some of them indicated derivative symptoms from one of the parental strain, particularly the strain where RNA-1 and -2 originally came. For example, CMV pseudorecombinant 8+8+5 showed similar symptoms of CMV strain-8 (Fig. 2a) and CMV pseudorecombinant 2+2+23 showed similar symptoms of CMV strain-2 (Fig. 2e). Another pseudorecombinants induced symptoms unlikely those of either parental strains of virus such as with CMV pseudorecombinant 33+42+42 (Fig. 2c) and CMV pseudorecombinant 48+48+5 (Fig. 2g). For some pseudorecombinants which induced a mild, symptomless or no symptoms, for example CMV pseudorecombinant 48+11+11 and CMV pseudorecombinant 18+18+41 (Table 2) was re-examined by indirect-ELISA according to Mowatt & Dowson (1987). Once we did to observe the concentration of total RNA pseudorecombinant in *N. tabacum* cv. Xanthi showed mild symptoms (pseudorecombinant CMV 18+18+41, Table 2) and it was 0.022 mg/ml, so this was shown to be positive by ELISA (A405 nm = 0.362). This low concentration of virus and total RNA virus in the plant may one of the caused of symptoms expression too hard to develop.

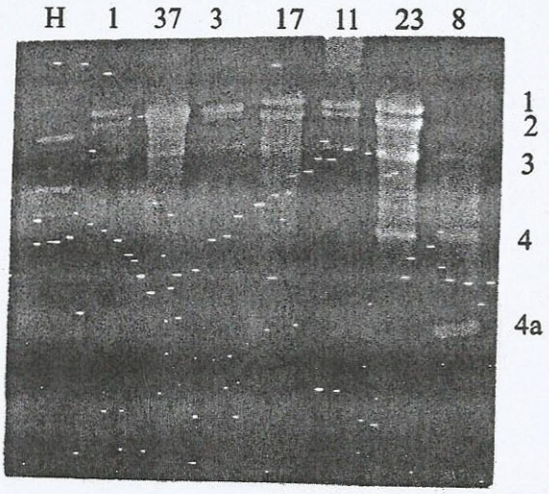

Figure 1. CMV RNAs pattern (RNA 1, 2, 3, 4 and 4a) on 1.8 % agarose with 1x TAE buffer. Strains 1, 37, 3, 11, and 8 belong to subgroup I. Strains 17 and 23 belong to subgroup II. H is RNA from healthy *N. glutinosa*.

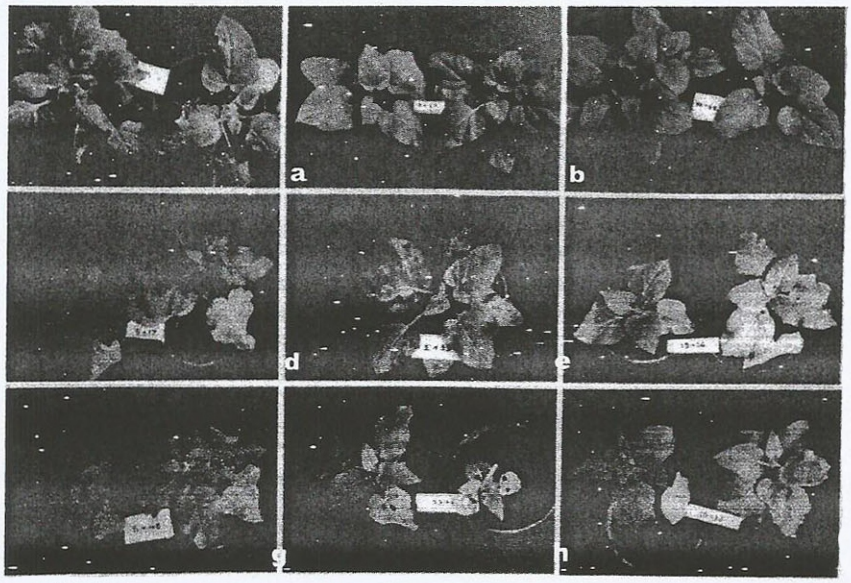

Figure 2. Symptoms variation on *N. glutinosa* induced by pseudorecombinant of CMV-RNAs, after 2527 days inoculation by aphid. a. CMV pseudorecombinant 8+ 8+5, b.CMV pseudorecombinant 8+8+23, c. CMV pseudorecombinant 33+42+42, d. CMV pseudorecombinant 17+5+5, e. CMV pseudorecombinant 2+2+23, f. CMV pseudorecombinant 13+13+14, g. CMV pseudorecombinant 48+48+5, h. CMV pseudorecombinant 23+23+1, and i. CMV pseudorecombinant 28+28+13.

Table 2. Pseudorecombination of RNA species between CMV strains which inoculated by aphid to *N. glutinosa*, and symptom appearance

CMV pseudorecombinant			Transmitted by	No. plant	Symptoms on
RNA 1	RNA 2	RNA3	species aphid	infected	*N. glutinosa*
1	1	17	*A. gosypii*	3/5	Mild mosaic and positive by ELISA
2	2	23	*A. gosypii*	2/5	Vein chlorotic, severe chlorotic mosaic, distortion (as CMV 2)
4	4	18	*A. gosypii*	1/5	Mild mosaic and negative by ELISA
5	5	52	*M. persicae*	2/3	Severe greening mosaic, enation, distortion (as CMV 5)
8	8	5	*A. gossypii*	1/5	Mild-severe greening mosaic (as CMV 8)
8	8	23	*M. persicae*	2/4	Mild-moderately greening mosaic
11	11	48	*M. persicae*	1/5, 2/3	Mild mosaic in both *N.glutinosa* and *N. tabacum* cv. Xanthi and positive by ELISA
13	13	14	*A. gossypii*	2/4	Vein and tip necrosis, severe chlorotic mosaic, distortion, shoestringing (as CMV 13)
17	5	5	*M. persicae*	2/5	Severe greening mosaic, enation, distortion (as CMV 5)
17	17	41	*A. gossypii*	1/5	Mild mosaic and negative by ELISA
18	18	41	*M. persicae*	1/3, 2/5	Symptomless on *N. glutinosa* but moderately mosaic on *N. tabacum* cv. Xanthi (as CMV-41) and positive by ELISA
48	11	11	*M. persicae*	0/5	No symptoms and negative by ELISA

It was a pity that in this experiment, we did not cross check the RNA pattern of pseudorecombinant with Northern blot hybridization to observe whether the symptoms produced on inoculated plants was caused by the RNA pseudorecombinant or just infected with one of the RNA parental strains used as with Sackey & Francki (1991) did. So, we are not able to conclude that the symptoms showed on Table 2 and Fig. 2 were really controlled by either RNA-1, 2 or RNA-3 of one or both of the parental strains.

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