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Response of Various Tomato Genotypes to Begomovirus Infection and Its Improved Diagnostic

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Begomovirus infection was identified from tomato growing areas in West Java (Bogor), Central Java (Boyolali), and D.I. Yogyakarta (Kaliurang). Efforts to reduce the infection among others are planting resistance varieties. This research was undertaken to evaluate 14 tomato genotypes for their response to the infection. Dot blot hybridization using nonradioactive (digoxigenin) DNA probe was employed to determine the presence of begomovirus in inoculated plants. Polymerase chain reaction-amplified product of DNA clone of *tobacco leaf curl virus* –Indonesia was used as a source of DNA probe. All of tomato genotypes evaluated in this study was infected separately by three strain of begomovirus (GVPSlm, GVABy, GVCBgr). Tomato genotypes *Bonanza, Jelita, Safira, Permata, Presto*, PSPT 8, PSPT 5B, *Apel-Belgia, Karibia, Mitra*, PSPT 9, *Marta,* and PSPT 2, showed susceptible or highly susceptible response to the three strains of begomovirus. Exception to those was shown by cv. Intan which resulted in moderate resistance when inoculated with GVCBgr although it resulted susceptible response with the other two strains. Dot-blot hybridization technique was proved to be a powerful tool to detect begomovirus infection in plants showing symptom as well as symptom-less plants. Accumulation of the virus in those plants was relatively high, except in cv. *Bonanza* and *Apel-Belgia*. Dot-blot hybridization technique using DIG-labeled DNA probe was able to detect begomovirus DNA in infected tissue up to 10^{-2} dilution factor.

Key words: Geminivirus, hybridization, resistance, tomato

INTRODUCTION

Whitefly-transmitted geminiviruses (WTGs) (Geminiviridae, *Begomovirus*) are known world wide as economically important pathogens causing serious diseases in food crops such as beans, peppers, cucurbits, cassavas, and tomatoes. Significant losses has been reported for tomato production in Medditteranian (Middle East, Northern Africa, and Southern Europe), Southern to Eastern Asia, Northern and Southern America, and Carribean (Pico *et al*. 1996; Czosnek & Laterrot 1997; Moriones & Navas-Castillo 2000). In Indonesia, tomato-infecting begomovirus was first reported early 2000 in West Java (Sudiono *et al*. 2004). Later on, different isolates of tomato-infecting begomoviruses were identified from different tomato growing areas at West Java, East Java, Central Java, and Yogyakarta (Kon *et al*. 2003; Sudiono *et al*. 2004; Aidawati *et al.* 2005; Sukamto *et al*. 2005; Tsai *et al.* 2006). Begomovirus infection affected 50 up to 70% tomato growing areas (Sudiono *et al.* 2004; Aidawati *et al*. 2005).

Major approach to control begomovirus infection involved a strategy to reduce the whitefly population through insecticide application and physical barriers (Polston & Anderson 1997; Palumbo *et al.* 2001). The use of insecticides has been proven effective only when the whitefly population is considered low. It was becoming a problem when population of whitefly is very high during dry season. Physical barriers such as the use of insect screen, or UV-absorbing film in the greenhouse (Antignus *et al.* 2001) have been evaluated for reducing disease spread. Unfortunately, all of those application was considered high cost crop management. Therefore, introduction of resistant tomato varieties was proposed as an alternative component for disease management.

It has been reported earlier that the source of resistance to begomoviruses in tomato was identified from wild species of *Lycopersicon*, such as *L. peruvianum*, *L. chilense*, *L. pimpinellifolium*, and *L. hirsutum* (Lapidot *et al.* 1997; Vidavsky *et al.* 1998). Effort to find resistance source for tomato in Indonesia was initially done through screening tomato germplasms. Sugiarman and Hidayat (2000) reported that tomato cv. *Donna* showed tolerant response to begomovirus infection, whereas cv. *Intan*, *Glory*, *Ratna*, *Pointed*, and *Mahkota* were susceptible. It was then suggested to involve a large number of tomato germplasm in order to recognize potential sources of begomovirus resistance. A tool to conduct such screening activities required selective methods to facilitate detection of virus infection. Molecular approach using spesific DNA probe for nucleic acid hybridisation was known as a promising method for detection of begomovirus infection in different host plants (Gilbertson *et al.* 1991; Rom *et al.* 1993; Lapidot *et al.* 1997; Pico *et al.* 1996; Rubio *et al.* 2001). We reported in this paper the application of non radioactive-labeled DNA probe for screening tomato genotypes for resistance to begomovirus.

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MATERIALS AND METHODS

Maintenance of Virus, *Bemisia tabaci*, and Plants. Three isolates of tomato-infecting begomoviruses were selected for this study, i.e. GVPSlm, GVABy, and GVCBgr, collected from Kaliurang (Yogyakarta), Boyolali (Central Java), and Bogor (West Java), respectively and has been characterized by Aidawati *et al.* 2005. The isolates were maintained on tomato plants (*L. esculentum* cv. *Arhtaloka*) by insect transmission. Tomato plants were grown separately in a whitefly-proof screenhouse. Adults *B. tabaci* for transmitting viruses were obtained from broccoli (*Brassica olereaceae* var. *Italica*) plants in Bogor (West Java) and identified using identification key of Martin (1987). The insects were reared on broccoli plants in whitefly-proof cages. Evaluation of tomato germplasms involved commercial tomato varieties, i.e. *Presto*, *Marta*, *Jelita*, *Safira*, and *Permata*, and genotypes collection of Center for Plant Breeding, Department of Agronomy and Horticulture, Bogor Agricultural University, i.e. *Intan*, PSPT 8, PSPT 5B, PSPT 9, *Apel-Belgia*, *Karibia*, *Mitra*, and *Bonanza*. Tomato plants were grown in whitefly-proof screenhouse and inoculated with begomovirus by *B. tabaci* one month after transplanting.

Evaluation of Tomato Genotypes. Inoculation of begomovirus by *B. tabaci* were conducted using cylindrical cages with mesh tops which were inverted over individual leaves. Adults *B. tabaci* were introduced into the cage through a hole which was sealed subsequently. The insects were given access to begomovirus-infected tomato plants in separate whitefly-proof cages. After a 24-h acquisition access period the whiteflies were recollected individually using an aspirator and transferred to separate caged of healthy tomato plants, 10 adults in each plant, for a 48-h inoculation access period. After the period, the whiteflies were removed, and the plants were sprayed with an insecticides and held for symptom development in an insect-proof screen house. Twenty five of four weeks-old tomato plants were used for each genotype, and five plants were treated using nonviruliferous whiteflies as control.

Classification of Plant Response. Response of different tomato genotypes was determined based on disease incidence using modified criteria previously used by Maruthi *et al.* (2003) (Table 1). Disease incidence was observed and calculated using the formula below:

$$\text{Disease incidence} = \frac{a}{a+b} \times 100\%$$

a: number of diseased plant, b: number of healthy plant

Dot Blot DNA. Plant extract preparation was carried out following procedure by Gilbertson *et al*. (1991). As much as 5 µl of plant extract from each sample was dotted to nylon membrane (Hybond-N, Amersham). Plant extract was diluted to 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} to evaluate sensitivity of hybridisation technique for detecting begomovirus in different tomato genotypes.

DNA Probe Labelling. Preparation of DNA probe was employed following a method described by manufacturer (Boehringer Manheim EMBL, Germany). DNA clone of *tobacco leaf curl Indonesia virus* (Gen bank Acc no. AB246171), which consists of conserved region of begomovirus, was selected as DNA probe. The DNA fragment was purified using absolute ethanol and sodium acetate, following PCR amplification using geminivirus specific primer, PAL1v 1978 and PAR1c 715. Purified DNA was then subjected for labeling using Random Primed Labeling with DIG-High Prime (Boehringer Manheim EMBL, Germany).

Table 1. Grouping of plant response to infection of begomovirus

Disease incidence (%)	Plant response
0	Immune
X< 10	Resistant
10 < X < 20	Moderately resistant
20 < X < 30	Moderately susceptible
30 < X < 50	Susceptible
X > 50	Highly susceptible

Hybridisation and Colorimetric Staining. Hybridisation was conducted following method developed by Dietzgen (1997). Nylon membrane containing sample DNA was submerged in prehybridisation solutions containing 10 ml *Dig Easy Hyb* solutions and subjected to prehybridisation condition, i.e. 42 °C for 60 min, followed by hybridisation condition, i.e. 42 °C for 24 h with gentle shaking. Colorimetric-based detection was employed with the use of Nitroblue Tetrazolium (NBT) and X-phosphate. The reaction was considered positive when purple color developed on the nylon membrane where the samples were dotted.

RESULTS

Symptom Development on Tomato Genotypes. Visual symptoms developed following inoculation of three isolates of begomovirus was varied among different tomato genotypes (Table 2). In general, the symptoms were leaf yellowing, upward or downward leaf curling, leaf cupping, leaf dwarfing, leaf banding, and plant stunting.

Evaluation of Tomato Genotypes. Disease incidence calculated based on the symptoms was varied among different tomato genotypes, i.e in the range of 16 up to 100%. Using Maruthi *et al*. (2003) classification, response of tomato genotypes to the virus infection was considered moderately susceptible up to highly susceptible (Table 3-5), except cv. *Intan* which was moderately resistance to GVCBgr infection (Table 5). In general, the symptoms were developed seven up to nine days after the inoculation, although some genotypes required longer incubation period including *Bonanza*, *Jelita*, *Safira*, and PSPT2.

Dot-Blot Hybridisation for Detection of Begomovirus. Digoxigenic-labelled DNA probe used in dot-blot hybridisation was able to detect the virus on plant extract from infected tomatoes up to 10^{-2} dilution factor. It is also interesting to find out that the infection was detected from symptomless tomato plants (Figure 1), although it was only tested for GVPSlm isolate. We assessed the DNA probe only for GVPSlm considered as the severe isolate of begomovirus. Based on the intensity of the color on the nylon membrane, the detection level could be differentiated from weak to strong reaction (Table 6). It might be correlated with the titer of the virus in the host plants. Therefore, we predicted that

Table 2. Symptoms of the begomovirus infection on tomato genotypes

Tomato genotype	Isolate of begomovirus*		
	GVCBgr	GVCBy	GVPSlm
Bonanza	Dk, St, Vt, Yl	B, Cp, Md, Vt	Dk, Kr, Md, St, Vt
Intan	Md, St, Vt	Dk, St, Vt, Yl	Cp, Dk, Md, Vt
Jelita	Vt, Yl	Kr, Md, St, Vt, Yl	Cp, Dk, Vt, Yl
Safira	B, Dk, Md, Vt, Yl	B, Dk, Kr, Md, Vt, Yl	Dk, Md, St, Vt, Yl
Permata	Md, Vt, Yl	Cp, Vt, Yl	Cp, Dk, Vt, Yl, St
Presto	B, Dk, Md, St, Vt, Yl	Dk, Md, Vt, Yl	B, Dk, Kr, Md, St, Vt, Yl
PSPT 8	B, Cp, Dk, Vt	B, Cp, Dk, Kr, St, Vt, Yl	Cp, Dk, Kr, St, Vt, Yl
PSPT 5B	B, Cp, Dk, Vt, Yl	B, Cp, Dk, St, Vt	Dk, Kr, Mg, Vt
Apel-Belgia	B, Dk, St, Vt, Yl	Dk, Md, St, Vt, Yl	Dk, Md, St, Vt, Yl
Karibia	B, Dk, Vt, Yl	Md, Vt, Yl	B, Dk, St, Vt, Yl
Mitra	B, Dk, Md, Vt	Dk, Md, Mg, Vt	Dk, Vt
PSPT 9	Dk, Md, St, Vt, Yl	Dk, Md, Vt, Yl	Dk, St, Vt, Yl
Marta	Dk, Md, St, Vt, Yl	Dk, St, Vt	Dk, Kr, Yl, Vt
PSPT 2	Dk, Md, St, Vt, Yl	Dk, Md, St, Vt, Yl	Dk, Ml, Vt

*Isolate of begomovirus: GVCBgr: begomovirus from Bogor, West Java; GVCBy: begomovirus from Boyolali, Central Java; GVPSlm: begomovirus from Kaliurang, Yogyakarta. Code for symptoms: B: leaf crumpling, Cp: leaf cupping, Dk: reduction of leaf size, Kr: leaf curling, Md: upward or downward leaf curling, St: stunting, Mg: leaf rolling, Vt: vein banding, Yl: yellowing

Table 3. Responses of 14 tomato genotypes to the infection of begomovirus from Kaliurang (GVPSlm)*

Genotype	Disease incidence		Incubation period (days)	Plant response
	Ti/T**	(%)		
Bonanza	7/24	29	9-14	Moderately susceptible
Intan	12/24	50	9-18	Susceptible
Jelita	25/25	100	10-12	Highly susceptible
Safira	10/25	40	8-16	Susceptible
Permata	19/25	76	7-12	Highly susceptible
Presto	25/25	100	7-12	Highly susceptible
PSPT 8	25/25	100	7-20	Highly susceptible
PSPT 5B	24/25	96	7-23	Highly susceptible
Apel-Belgia	24/25	96	12-25	Highly susceptible
Karibia	22/25	88	9-22	Highly susceptible
Mitra	12/25	48	7-25	Susceptible
PSPT 9	24/25	96	7-16	Highly susceptible
Marta	25/25	100	7-20	Highly susceptible
PSPT 2	20/25	80	11-20	Highly susceptible

*Inoculation was carried out by using whitefly with a 24-h acquisition feeding period, a 48-h inoculation feeding period, ten insects in each plant. **Number of plant showing symptom (Ti)/number of total plant tested (T)

Table 4. Responses of 14 tomato genotypes to the infection of begomovirus from Boyolali (GVCBy)*

Genotype	Disease incidence		Incubation period (days)	Plant response
	Ti/T**	(%)		
Bonanza	11/24	45.8	9-28	Susceptible
Intan	10/24	41.7	11-17	Susceptible
Jelita	16/25	64	10-14	Highly susceptible
Safira	12/25	48	8-14	Susceptible
Permata	9/25	36	10	Susceptible
Presto	6/25	24	10-12	Moderately susceptible
PSPT 8	25/25	100	7-17	Highly susceptible
PSPT 5B	25/25	100	9-23	Highly susceptible
Apel-Belgia	25/25	100	12-25	Highly susceptible
Karibia	18/24	75	12-16	Highly susceptible
Mitra	16/25	64	12-25	Highly susceptible
PSPT 9	17/25	68	9-22	Highly susceptible
Marta	23/25	92	7-20	Highly susceptible
PSPT 2	22/25	88	13-22	Highly susceptible

*Inoculation was the same as in Table 3. **Number of plant showing symptom (Ti)/total number of plant tested (T)

virus titer in cv. *Bonanza* might be low (Table 6). It was also observed that the number of plants showing symptom in this cultivar was relatively small compared to other cultivars (Table 3). We might expect cv. *Bonanza* was tolerant to GVPSlm infection.

DISCUSSION

Visible symptoms on tomato plants following inoculation with three isolates of begomoviruses were varied among 14 genotypes. However, the infection produced general

Table 5. Response of 14 tomato genotypes to the infection of begomovirus from Bogor (GVCBgr)*

Genotype	Disease incidence		Incubation period (days)	Plant response
	Ti/T**	(%)		
Bonanza	16/25	64	11-15	Highly susceptible
Intan	4/25	16	10-20	Moderately resistant
Jelita	12/25	48	9-19	Highly susceptible
Safira	21/25	84	7-19	Highly susceptible
Permata	8/25	32	11-14	Susceptible
Presto	20/25	80	9-14	Highly susceptible
PSPT 8	25/25	100	7-12	Highly susceptible
PSPT 5B	25/25	100	7-27	Highly susceptible
Apel-Belgia	25/25	100	7-25	Highly susceptible
Karibia	23/25	92	12-25	Highly susceptible
Mitra	22/25	88	9-22	Highly susceptible
PSPT 9	24/25	96	9-22	Highly susceptible
Marta	25/25	100	9-22	Highly susceptible
PSPT 2	16/25	64	12-21	Highly susceptible

*Inoculation was the same as in Table 3. **Number of plant showing symptom (Ti)/total number of plant tested (T)

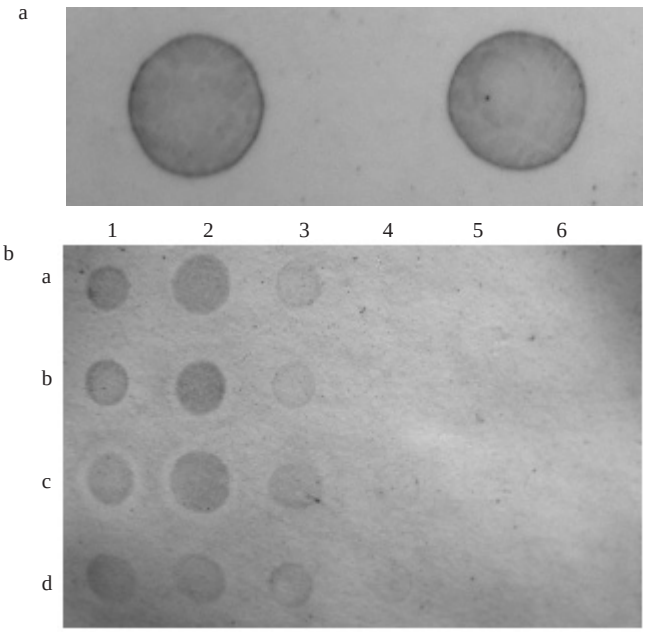

Figure 1. Detection of begomovirus from Kaliurang (GVPSlm) using digoxigenic-labelled DNA probe in dot-blot hybridisation method: a. Positive control (DNA-clone of TLCV), b. Tomato cv. *Safira* following inoculation with GVPSlm: (1a-1d) plants showing symptom; (2a-2d) plants showing no symptom; (3a-3d, 4a-4d, 5a-5d, 6a-6d) plant extract from plant showing symptom after dilution factor of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, respectively.

symptoms such as leaf yellowing and upward or inward leaf curling. It was stated by Polston and Anderson (1997) that these symptom variations were due to virus strain, time of infection, plant genotype, and environment factors. We further noticed that the interaction between the virus strain and the plant genotype may contribute to the severity of the disease. In this study it determined the response of the plants. Germplasm evaluation conducted in this study demonstrated different plant response to different isolates of begomovirus. This fenomena was also observed by Roossinck (1997) and Rubio *et al*. (2001) that demonstrated different response of one tomato genotype when inoculated with different virus strain. Therefore it is very important to consider the virus strain that will be selected in germplasm evaluation for disease resistance.

Table 6. Detection of begomovirus infection on tomato genotypes using Dot-Blot hybridisation method

Tomato genotype	Plant showing symptom		Plant with no symptom	
	Number	Hybridisation reaction	Number	Hybridisation reaction
Bonanza	7	+++	17	+
Intan	12	+++	12	+++
Jelita	25	+++	0	TD
Safira	10	+++	15	+++
Permata	19	++	6	++
Presto	25	+++	0	TD
PSPT 8	25	++	0	TD
PSPT 5B	24	+++	1	+++
Apel-Belgia	24	+++	1	+
Karibia	22	+++	3	+++
Mitra	12	+++	13	++
PSPT 9	24	++	1	++
Marta	25	+++	0	TD
PSPT 2	20	+++	5	+++

TD: Not tested because all plants showing symptom. Reaction of hybridisation: +++: strong, ++: moderate, +: weak

Based on the number of plants showing symptoms, cv. *Intan* was found moderately resistance to the virus from Bogor, whereas other genotypes were found susceptible to highly susceptible. Although resistant genotypes were not observed in this study, some genotypes demonstrated tolerant-like responses to the virus infection. It was shown by longer incubation period and fewer (≤ 50%) infected plants, for instances on cv. *Bonanza*, *Intan*, *Safira*, and *Mitra* when inoculated with the virus from Kaliurang, or cv. *Bonanza*, *Intan*, *Safira*, *Permata*, and *Presto* when inoculated with the virus from Boyolali, or cv. *Jelita*, and *Permata* when inoculated with the virus from Bogor. These results would suggest that appropriate tomato genotypes, i.e. carrying resistance or tolerance to the virus infection, might be difference for different localities with the infection. For instance, we might recommend cv. *Intan* for Bogor, West Java since it was found moderately resistance to the virus from Bogor.

Further investigation on tomato genotypes response should be carried out, for example through analysis of the disease impact on yield components and the measurement of virus titer on infected plant (Lapidot *et al*. 1997). In this study we tried to estimate the virus titer on the infected plants using DNA probe in dot-blot hybridisation method. Using similar

approach Rom *et al*. (1993) and Pico *et al.* (1996) demostrated that DNA accumulation in the plant tissues was correlated with symptom intensity and the level of DNA accumulation in two accession of *L. peruvianum* could be distinguished. Using digoxigenin-labeled DNA probe, begomovirus DNA was detected after two dilutions in the plant extract from tomatoes with symptoms. We were able to detect the virus accumulation in plant with no symptoms in our study, although the sensitivity of non radioactive label was relatively less than radioactive label. This result would indicate the potential application of nonradioactive DNA probe for determining actual response of plant genotypes to the virus infection.

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