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Transmission of *Tomato leaf curl begomovirus* by Two Different Species of Whitefly (Hemiptera: Aleyrodidae)

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Whitefly-transmitted geminiviruses (WTGs) are economically important pathogens causing serious damage on tomato and chilli pepper in Indonesia. Geminiviruses are readily transmitted by its insect vector, sweetpotato whitefly (*Bemisia tabaci*). However, greenhouse whitefly (*Trialeurodes vaporariorum*), another species of whitefly, is commonly found together with *B. tabaci* in the field. Incidence of yellow leaf curl disease in tomato and chilli pepper is probably correlated with the population of whitefly complex. It is becoming important to find the role of *T. vaporariorum* in the spread of the disease. Therefore, research is conducted to study the characteristic relationship between tomato leaf curl begomovirus (ToLCV) and two species of whitefly. The two species of whitefly, *B. tabaci* and *T. vaporariorum*, was capable to transmit ToLCV although it was evidenced that *B. tabaci* is more effective as insect vector of ToLCV in tomato and chilli pepper. A single *B. tabaci* was able to transmit ToLCV to tomato with a minimum acquisition and inoculation access period of 10 h. Transmission of ToLCV by *T. vaporariorum* required at least 10 insects per plant with a minimum acquisition and inoculation access period of 24 h. The transmission efficiency will increase with longer acquisition and inoculation access period of the insect and the higher number of insect per plant.

Keywords **:** *Bemisia tabaci*, *Trialeurodes vaporariorium*, geminivirus

Whitefly-transmitted geminiviruses (WTGs) (*Geminiviridae*, *Begomovirus*) are economically important pathogens causing serious losses in food crops globally. Agriculture in tropical and subtropical is most threatened, with crops such as beans, peppers, cucurbits, cassavas, and tomatoes particularly being affected (Varma and Malathi, 2003). In Indonesia, begomovirus infecting chilli pepper and tomato was first reported in early 2000 in West Java (Rusli et al., 1999; Sudiono et al., 2001). The diseases has occurred eversince, causing severe to complete loss especially on chilli pepper. Based on our observation on the incidence of the disease during the last 5 years, we can come up with a conclusion that the severity and incidence of the disease is highly correlated with the high population of whitefly in the field, especially during a long and hot dry season. Two dominant whitefly species was reported from previous study on whitefly population in the begomovirus-infected field, i.e. *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorium* (Westwood) (personal communication, Yuliani 2002 and Nurrohman 2003). The two species have always been regarded as pests to a large range of worldwide crops. Both species are capable of transmitting plant viruses, with *T. vaporariorium* being the vector of only a few 'clostero'-like viruses and *B. tabaci* the vector of plant viruses in several groups (Bock, 1982; Butter and Rataul, 1977; Polston and Anderson, 1997; Varma, 1963).

Transmission of begomoviruses from Indonesia by *B. tabaci* has been demonstrated earlier (Aidawati et al., 2002; Rusli et al., 1999; Sudiono et al., 2001). A single *B. tabaci* was able to transmit the virus in a persistent manner, but the virus is not transovarally transmitted. It is believed that specific relationship occurred between begomoviruses and *B. tabaci*, which make *B. tabaci* as the only insect vector for begomoviruses (Costa, 1969; Mehta et al., 1994). The co-existence of *T. vaporariorium* with *B. tabaci* in the begomovirus-infected field raised the question whether *T. vaporariorium* has capability to transmit the virus. This paper reports the transmission study of ToLCV in order to assess the capability of *T. vaporariorium* to transmit the virus in comparison to *B. tabaci*.

Materials and Method

Identification and Maintenance of Whiteflies. Two species of whiteflies were collected from different location in Bogor, West Java. *B. tabaci* were obtained from broccoli plants in Baranangsiang, Bogor, West Java and the insect were then reared on broccoli (*Brassica oleareceae* var. *Italica*) plants in whitefly-proof cages. The other whitefly species, *T. vaporariorium*, was collected from tomato

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growing area at Cisarua, Bogor, West Java and reared on tomato (*Lycopersicon esculentum* var. Arthaloka). Both whitefly species were identified based on pupal case morphology. Prepararion of microscope slide of pupal case and identification of whitefly species was done following procedures developed by Martin (1997).

Maintenance of Virus Source. Whitefly-transmitted tomato geminivirus (ToLCV) used in this study is originally collected from Kaliurang, D.I. Yogyakarta (collection of Virology Laboratory, Department of Plant Protection, Bogor Agricultural University). The virus was propagated and maintained in tomato (*L. esculentum* var. Arthaloka) through whitefly transmission as described previously (Aidawati et al., 2002).

Whitefly Transmission Assay. ToLCV transmission experiments with whiteflies were conducted using cylindrical cages with mesh tops which were inverted over individual leaves. Adults whiteflies were introduced into the cage through a hole which then was sealed. The insects were given access to ToLCV-infected tomato plants in separate whitefly-proof cages. After acquisition access period the whiteflies were re-collected individually using an aspirator and transferred to separately caged healthy plants for inoculation access periods. After inoculation access period, the whiteflies were removed, and the plants were sprayed with an insecticides and held for symptom development in an insect-proof screen house. The effect of vector number on the relative efficiency of virus transmission was determined by allowing 1, 5, or 10 adult whiteflies a 48-h inoculation access period on healthy tomato or chilli pepper plants after a 48-h acquisition access period on ToLCV-infected tomato plants. The minimum acquisition access period required for transmission of ToLCV was determined by allowing adult whiteflies access to ToLCV-infected tomato plants (10 adults per plant) for 10, 24, or 48 h before transferring them to healthy tomato or chilli pepper plants for a 48-h inoculation access period. To determine the minimum inoculation access period, adult whiteflies were given a 48-h acquisition access period on ToLCV-infected tomato plants and inoculation access periods of 10, 24, or 48 h on healthy tomato or chilli pepper plants (10 adults per plant). Ten plants were used for each experiment on vector number, acquisition and inoculation access period. Percentage of virus infection was calculated from plants showing TLCV symptoms up to 25 d.

Result

Whitefly Identification. Morphology characters of pupal case that was observed under microscope provides evidence

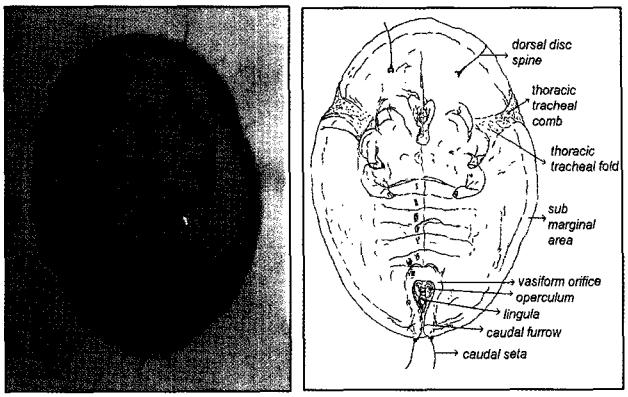

Fig. 1. Pupal case of *B. tabaci* (1:90 scale). A. Microscope slide of pupal case; B. Line drawing of pupal case

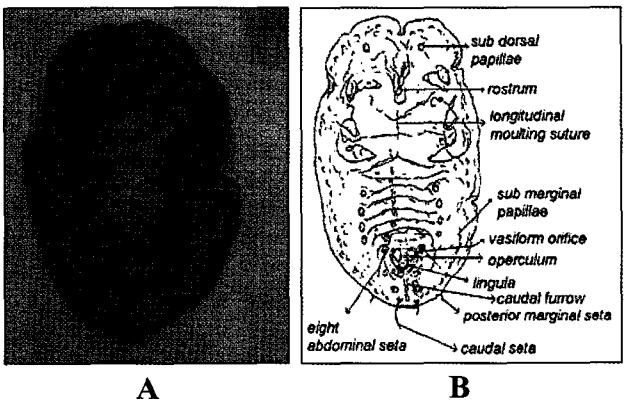

Fig. 2. Pupal case of *T. vaporariorum* (1:80 scale). A. Microscope slide of pupal case; B. Line drawing of pupal case

that whiteflies collected from two different locations are different whitefly species. Whitefly population collected from Baranangsiang is *B. tabaci*, whereas population from Cisarua is *T. vaporariorum*. Spesific characters of *B. tabaci* pupal case was shown by a firm caudal setae that has similar length with vasiform orifice, and there is not much variation among individuals. Vasiform orifice is longer than caudal furrow and almost straight on its side (Fig. 1). Morphology characters of *T. vaporarium* that was found involves among others : noticeable sub marginal papillae, lobular lingula head, big sub dorsal papillae, small and fine setae at the base of mesothoraxic and metathoraxic legs (Fig. 2).

Effect of the Number of Adults Whitefly on Transmission Efficiency. Adults *B. tabaci* was able to transmit ToLCV to tomato and chilli pepper with incubation period of 4-8.5 d (Table 1). Effectiveness of *B. tabaci* as a vector was shown by capability of single insect to transmit the virus to tomato and resulted in 40% transmission. Number

Table 1. The effect of number of insects on transmission and incubation period of ToLCV on tomato and chilli pepper after a 48-h acquisition feeding period and a 48-h inoculation feeding period

Number of insects	Number of infected plants (%)		Incubation period (days)[a]	
	Tomato	Chilli pepper	Tomato	Chilli pepper
B. tabaci				
1	40	0	8.5	NA
5	60	20	6	6
10	100	60	4.6	4
T. vaporariorium				
1	0	0	NA	NA
5	0	0	NA	NA
10	40	0	13	NA

[a]NA indicates that the plants did not show any visible symptoms during observation period

Table 2. The effect of various acquisition feeding periods of *B. tabaci* and *T. vaporariorium* (10 adults each plant) on transmission and incubation period of ToLCV on tomato and chilli pepper after a 48-h inoculation feeding period

Acquisition feeding period (h)	Number of infected plants (%)		Incubation period (days)[a)]	
	Tomato	Chilli pepper	Tomato	Chilli pepper
B. tabaci				
10	0	0	NA	NA
24	60	20	5	12
48	80	80	10	10
T. vaporariorium				
10	0	0	NA	NA
24	20	0	11	NA
48	60	0	9	NA

[a]NA indicates that the plants did not show any visible symptoms during observation period

of infected plants increased with bigger number of insects per plant. Transmission of ToLCV using *T. vaporarium* was only successful in tomato plant by 10 insects per plant (Table 1). Symptom development in tomato plants required long incubation period (13 d) and none of chilli pepper plants showed symptoms up to 25 d.

Acquisition Feeding Period. Transmission was not observed after a 10-h acquisition access period both for *B. tabaci* and *T. vaporarium* in tomato or chilli pepper plants (Table 2). After a 24-h acquisition access period, ten adults of *B. tabaci* were able to cause 60% and 20% transmission in tomato and chilli pepper plants, respectively. The virus required longer incubation period in chilli pepper plants than those in tomato plants for symptom development. The number of infected tomato and chilli pepper plants reached 100% as the acquisition-access period was lengthened to 48-h.

Table 3. The effect of various inoculation feeding periods of *B. tabaci* and *T. vaporariorium* (10 adults each plant) on transmission and incubation period of ToLCV on tomato and chilli pepper following a 48-h acquisition feeding period

Inoculation feeding period (h)	Number of infected plants (%)		Incubation period (days)[a)]	
	Tomato	Chilli pepper	Tomato	Chilli pepper
B. tabaci				
10	60	0	9	NA
24	60	60	6	11
48	80	80	10	12.75
T. vaporariorium				
10	0	0	NA	NA
24	20	0	12	NA
48	40	20	11	9

[a]NA indicates that the plants did not show any visible symptoms during observation period

Inoculation Feeding Period. Ten hour inoculation-access period following a 48-h acquisition-access period was sufficient to cause 60% transmission to tomato plants by ten *B. tabaci* adults. Transmission to chilli pepper plants requires longer inoculation-access period, i.e. minimum of 24-h. Longer inoculation-access period was also required for transmission using *T. vaporarium* both to tomato and chillipepper plants (Table 3). It is also observed that in general incubation period of the virus in chilli pepper plants was longer than those in tomato plants.

Discussion

Geminiviruses are single-stranded DNA plant viruses with one or two circular genome components of 2.7-3.0 kb in size, encapsidated in twinned particles. They are transmitted by whiteflies, leafhoppers or treehoppers. The whitefly species *B. tabaci* is the most efficient vector of members of the genus Begomovirus (Idris and Brown, 1998; van Regenmortel, 2000). Begomoviruses are currently emerging as a major threat in many tropical and subtropical regions worldwide (Varma and Malathi, 2003). Leaf curl diseases in chilli pepper and tomato associated with begomoviruses cause severe crop damage in Indonesia. Begomoviruses from infected chilli pepper and tomato was designated as *Pepper yellow leaf curl geminivirus* (PYLCV) and ToLCV, respectively (Hidayat et al., 2006; Sukamto et al., 2005; Kon et al., 2006). The two begomoviruses are transmitted readily by whiteflies, *B. tabaci*, to plants of among others tomato (*L. esculentum*), ageratum (*Ageratum conyzoides*), chilli pepper (*Capsicum annuum* L., and *C. frutescens*),

Nicotiana tabacum, and *Nicotiana benthamiana*, (Sudiono et al., 2001; Sulandari et al., 2005). This fact shows differences with earlier report on experimental inoculation of *tomato Australian leaf curl bigeminivirus* (http://image.fs.uidaho.edu/vide/descr822.htm) in which the chilli pepper was insusceptible host. The host range of begomoviruses may indicate divergence of the virus group. Begomoviruses associated with tomato leaf curl diseases in Java, Indonesia have been reported to have close relationship with *ageratum yellow vein virus* and *pepper yellow leaf curl Indonesia virus* with respect to amino acid identities of the N-terminal halves of the coat proteins (Sukamto et al., 2005).

Transmission of begomovirus by single *B. tabaci* has been reported previously such as for *cotton leaf curl virus* (Kirkpatrick, 1931), *tomato yellow leaf curl virus* (Mehta et al., 1994) and *tobacco leaf curl virus* (Aidawati et al., 2002). In most cases, the efficiency of transmission increased as the number of adults *B. tabaci* was increased. Similar result was achieved from this experiment when ToLCV was transmitted to tomato. Although showing similar trend, fewer disease incidence was observed when the virus was transmitted to chilli pepper. Based on this facts it can be proposed that host plants effect transmission efficiency of virus by insect vector. The ability of *B. tabaci* to transmit ToLCV is also affected by the inoculation and acquisition feeding period. Percent transmission increased as the inoculation and acquisition period was prolonged, and reached a maximum of 80% transmission after a 48-h inoculation and acquisition feeding period. Inoculation and acquisition feeding period required for ToLCV transmission explained the mechanism of WTGs transmission. Circulative non-propagative transmission of most WTGs involves the passages of the virus through the body of the insect and consists of two distinct phases; firstly acquisition during which the virus passes into the insect's body (probably via the gut wall) and secondly the inoculation of the virus back into the plant, which involves the passage of the virus from the blood into the salivary secretion (Hunter et al., 1998; Liu et al., 1997).

The transmission mechanism explained above required a specific relationship between begomovirus and *B. tabaci*. However, it has also reported that acquisiton of geminiviruses by *B. tabaci*, and some other whitefly species, is non-specific but that the inoculation stage is virus-vector specific (Cohen et al., 1989). Liu et al. (1997) demonstrated in squash-blot experiments the acquisition of cloned *African cassava mosaic virus* (ACMV-K) by *T. vaporarium*. However, they also showed that coat protein was essential for the acquisition process since viruses which were not encapsidated could not be acquired. Interesting evidence was observed in this research when transmission of ToLCV was accomplished using *T. vaporarium*, a non-vector species. The transmission efficiency by *T. vaporarium* is considered low which might indicate that although *T. vaporarium* was able to acquire the virus but it inoculated the virus back into the plant ineffectively. This findings may explain contribution of *T. vaporarium* in the spread of leaf curl disease in tomato growing area in Indonesia. High population of whitefly in the field, which may composed of *B. tabaci* and *T. vaporarium*, is correlated with disease incidence most of the time. Further investigations on ability of the insects collected from the infected field to acquire and inoculate the virus will allow us to better understand the role of whitefly species in the development and spread of the diseases.

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