Tomato Leaf Curl Disease in Taiwan and Breeding for Resistance Against it

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ABSTRACT

In the past few decades, whiteflies have become more important as agricultural pests in many areas. The whitefly-transmitted geminiviruses (begomoviruses) have also become major constraints to tomato production worldwide. A tomato-infecting begomovirus, *Tomato leaf curl Taiwan virus* (ToLCTWV), was first detected in Taiwan in 1981, and was endemic throughout the island by the early 1990's. Since then, three other tomato-infecting begomoviruses have been detected in Taiwan; *Ageratum yellow vein Hualien virus* (AYVHuV) and *Tomato leaf curl Hsinchu virus* (ToLCHsV) have been detected only occasionally from tomato, whereas *Tomato yellow leaf curl Thailand virus* (TYLCTHV), which was probably imported to Taiwan in about 2005, is now widespread throughout Taiwan. TYLCTHV is a bipartite and mechanically transmissible begomovirus, whereas ToLCTWV is monopartite and not mechanically transmissible. Through monitoring the begomoviruses in the tomato production areas over time, it is apparent that the introduced TYLCTHV is now displacing ToLCTWV in most areas. The tomato leaf curl resistance gene *Ty-2* was identified in Taiwan using ToLCTWV, and was incorporated into tomato cultivars to help manage the leaf curl disease. However, tomato cultivars carrying the *Ty-2* gene are not resistant to TYLCTHV which causes severe disease symptoms on them. The combining (pyramiding) of different *Ty* resistance genes in new tomato cultivars shows potential for resistance to leaf curl disease in Taiwan. The combined use of resistant cultivars and cultural methods to exclude vector whiteflies and prevent virus infection is the most effective and sustainable strategy to manage the tomato begomovirus complex in Taiwan and elsewhere.

Keywords: Begomovirus, Ty resistance, resistance breeding, disease management

INTRODUCTION

Over the last 30 years, the whiteflies (*Bemisia tabaci*) have emerged as major pests in agricultural areas worldwide, and as a consequence, plant diseases caused by whitefly-transmitted viruses such as the begomoviruses have also been increased [18]. In tropical and subtropical tomato production areas, begomoviruses often caused severe disease epidemics [25, 30, 37, 38, 40]. The disease are commonly known as tomato leaf curl or tomato yellow leaf curl disease as the major symptoms are leaf curling, interveinal and marginal yellowing, interveinal crinkling and internode shorting (Fig. 1) [40]. The disease can cause significant yield loss; up to 100% loss is frequent [29, 30, 37]. The tomato-infecting begomoviruses are genetically diverse, and so far more than 100 species have been delineated based on the International Committee on Taxonomy of Viruses (ICTV) species demarcation criteria [38, 39, 41]. This diversity compounds the difficulty in managing the diseases, especially where several different species are present at the same time. More than 12 tomato-infecting begomovirus species have been reported in China, five in Indonesia, four each in India, the Philippines and Taiwan, and at least two each in Bangladesh, Iran, Japan, Laos, Thailand and Vietnam [37, 38, 39, 41]. While some tomato-infecting begomovirus species are only present in small areas, others are more widely distributed across several countries. For example, *Tomato leaf curl Taiwan virus* (ToLCTWV) has been detected in China and Taiwan [26, 39], while *Tomato yellow leaf curl Thailand virus* (TYLCTHV) has been detected in China, Myanmar, Taiwan and Thailand [12, 13, 31, 33, 38]. The widely distributed *Tomato yellow leaf virus* (TYLCV) has spread from its likely origin in the middle East (Israel) to become the dominant tomato-infecting begomovirus in many countries including China, Dominican Republic, Israel, Italy, Japan, South Korea, Spain, USA and islands of the Indian Ocean [5, 6, 9, 30, 32, 41].

Tomato-infecting begomoviruses are monopartite with a single circular single-stranded DNA (ssDNA, DNA-A like) genome, or bipartite having a genome of two circular ssDNAs, DNA-A and DNA-B [21] (Fig. 2). The DNA-A or DNA-A like component is essentially for virus reproduction. They contain two open reading frames (ORFs; AV1 and AV2) in viral sense and four (AC1, AC2, AC3 and AC4) in complementary sense. Viral DNA-B component contains an ORF each in viral sense (BV1) and complementary sense (BC1). The AV1 encodes the coat protein (CP) and AV2 is associated with virus movement. The AC1 is the viral DNA replication associated protein. The AC2 protein is a transcriptional activator. AC3 protein enhances

viral DNA replication. The AC4 can determine symptom development. The BV1 and BC1 are the nuclear shuttle protein and the movement protein, respectively. AC2 and AC4 can also act as suppressors of gene silencing [4, 44].

The deployment of natural resistance is considered the most sustainable strategy for controlling tomato leaf curl disease. No effective resistance was discovered in the cultivated tomato (*Solanum lycopersicum*), so the tomato wild relatives such as *S. chilense*, *S. habrochaites* f. *glabratum*, and *S. peruvianum* have been screened for resistance [10]. Five resistance genes (designated *Ty-1*, *2*, *3*, *4* and *5*) have been identified and deployed to greater or lesser extents [1, 14, 16, 17, 45]. The first to be identified, *Ty-1,* originated from *S. chilense* (LA1969) and was mapped to chromosome 6 [45]. Next, *Ty-2* was derived from *S. habrochaites* (B6013) and mapped to chromosome 11 [14]. The alleles *Ty-3* and *Ty-3a* originated from *S. chilense* LA2779 and LA1932, respectively, and map to chromosome 6 [16]. *Ty-1* and *Ty-3* have recently been shown to be allelic [42]. Also from *S. chilense* LA1932, *Ty-4* has been mapped on chromosome 3, but is less effective than the other genes [17]. Recently, *Ty-5* was identified from breeding line TY172 (originating from *S. peruvianum*) and mapped to chromosome 4 [1]. In addition, resistances to the vector whiteflies have been identified in *S. pennellii* and *S. habrochaites* [2, 3, 27].

In this review, the tomato-infecting begomoviruses of Taiwan, as well as the possible effect the resistance breeding efforts have had on the population dynamics will be described.

The tomato-infecting begomovirus in Taiwan

In Taiwan, the tomato leaf curl disease was first observed in 1981 in Tainan County. Later on, the causal agent was confirmed as a begomovirus based on the observation of geminate particles with an electronmicroscope, and a positive reaction with an antibody against tobacco leaf curl virus, a begomovirus form Japan [11]. By the 1990's, the disease had spread throughout the island of Taiwan and was causing significant yield losses of up to 84% [34, 36]. In 1997, the causal virus was identified by viral genomic sequence as *Tomato leaf curl Taiwan virus* (ToLCTWV) based on the species delimitation criteria of the ICTV [21]. The host range of ToLCTWV was limited to tomato (*Solanum sp.*), *Datura stramonium*, *Lonicera japonica*, *Nicotiana benthamiana*, *Petunia hybrida*, *Physalis floridana* and *S. melongena* [11]. Later on, ToLCTWV was detected in Southeast China [26]. The ToLCTWV Taiwan isolates were confirmed as a

monopartite begomovirus since no DNA-B was detected in samples that tested positive for ToLCTWV alone [38]. Agroinoculation of tomato plants with an infectious clone of a ToLCTWV DNA-A like component alone also generated similar symptoms as a field isolate. The ToLCTWV Taiwan isolates were classified into 3 strains [38]. The strain A is commonly found throughout the country, whereas the strains B and C were restricted to East and West Taiwan, respectively.

In 2000, a second tomato-infecting begomovirus, *Tomato leaf curl Hsinchu virus* (ToLCHsV) was detected in Hsinchu area of Taiwan [38]. However, the distribution of ToLCHsV was limited to the Hsinchu area with low detection frequency; only four of 14 begomovirus-positive samples collected in 2000 and two of 39 samples in 2001. ToLCHsV was not detected after that in Taiwan. However, after ToLCHsV was detected in Taiwan, a high genetic identity virus was detected in plants of Ramie (*Boehmeria nivea* L.) in China, and hence the name Ramie mosaic virus (RamMV) was given. The ToLCHsV/RamMV in China was found to infect tobacco also, but has not been detected in tomato in the field in China. Since RamMV was found to infect ramie in many provinces of China, it may be that ramie represents the original host of ToLCHsV [23].

The third tomato-infecting begomovirus detected in Taiwan was *Ageratum yellow vein Hualien virus* (AYVHuV) **[38]**. The virus was only detected in two samples collected in Hsinchu area in 2003, and these isolates were identified as likely recombinants between ToLCTWV and AYVHuV. The virus background was AYVHuV ageratum isolate and the recombination region (607 nucleotides in length) including the complete AC4 ORF, the 5' half of AC1 (Rep) ORF and left part of the IR was most similar (92.8%) to the corresponding region of the ToLCTWV strain B tomato isolate.

The forth tomato-infecting begomovirus detected in Taiwan was *Tomato yellow leaf curl Thailand virus* (TYLCTHV). TYLCTHV had previously been detected in south China, Myanmar and Thailand **[12, 24, 33]**, but its first detection in Taiwan was in 2005 in Central West Taiwan suggesting it was only a recent introduction to Taiwan **[38]**. After 2005, TYLCTHV spread rapidly in West Taiwan and then in East Taiwan (Table 1). TYLCTHV Taiwan isolates was found to infect not only tomato but also pepper plants in the field **[35]**. This indicated pepper was a natural host for TYLCTHV, but not for ToLCTWV. TYLCTHV has been considered as a bipartite begomovirus since it is always detected as a combination of DNA-A and DNA-B, and it can be mechanical transmitted **[38]**. Although agroinoculation of an infectious clone of the DNA-A alone

results in development of symptoms; symptoms are mild and the development is much delayed in comparison to when both components are present, and infection with DNA-A alone is not mechanically transmissible.

Mixed infection of tomato plants with two begomoviruses was commonly detected in Taiwan, including with the less common species, AYVHuV or ToLCHsV. Because of the relatively high genetic diversity of ToLCTWV and its predominance before the emergence of TYLCTHV, it was considered to be the endemic tomato-infecting begomovirus in Taiwan [(38)]. However, since it was first detected in Western Taiwan in 2005, TYLCTHV rapidly spread to become prevalent across all tomato-growing regions of Taiwan. Virus survey results show that TYLCTHV is likely to displace ToLCTWV in many parts of Taiwan (Table 1). Whether this dynamic change of tomato-infecting begomovirus species affects the tomato production and breeding effort in Taiwan or vice-versa will be discussed below.

Resistance breeding against tomato-infecting begomoviruses in Taiwan

Since the emergence of tomato leaf curl disease caused by begomovirus in the early 1980s, the disease has become prominent and a major limiting factor for tomato production in Taiwan by the early 1990s. The reduction in tomato productivity in Taiwan from 35-52 tonnes/ha (1983-1992) to 24-26 tonnes/ha (after 2005) is attributed in part to the increased prevalence of leaf curl disease (Table 2). Since deployment of tomato cultivars carrying resistance is considered the most effective and sustainable method for managing tomato (yellow) leaf curl disease, especially for resource-poor growers in developing countries. AVRDC- the World Vegetable Center started to try to identify sources of resistance and include them in the tomato breeding program soon after leaf curl disease became a problem in Taiwan. Resistance to ToLCTWV was identified in accessions of *S. chilense*, *S. habrochaites* f. *glabratum*, *S. lycopersicum* and *S. peruvianum* [(10)]. Based on screening with ToLCTWV, leaf curl disease resistance identified from the tomato line H24, derived from *S. habrochaites* B6013, was included in the breeding program in 2000, and commercial varieties carrying this resistance were released in Taiwan in 2003. The gene responsible for resistance was subsequently mapped and named *Ty-2* [(14)].

When TYLCTHV was first detected in Taiwan in 2005, it caused severe disease symptoms in tomato lines carrying the *Ty-2* gene [(15)]. This indicates the *Ty-2* gene is not effective against TYLCTHV. All other resistance sources identified by screening with

ToLCTWV did not provide effective resistance to TYLCTHV. Recently, the tomato line FLA456 was identified as being able to provide a good level of tolerance to TYLCTHV, and two major and two minor QTLs were identified as involved in the resistance [20]. Pyramiding of several resistance genes in a variety can provide broader and more durable disease resistance in crops [19]. Combining two resistances from wild species provided increased resistance to TYLCV [43]. The pyramiding of resistance genes has been also tested for controlling tomato-infecting begomovirus disease in Taiwan. Tomato lines combining *Ty-2* and *Ty-1*/*Ty-3* were generated and identified as being able to provide the greatest resistance to leaf curl disease in the field in Taiwan where both ToLCTWV and TYLCTHV may present [28]. However, combining resistances to virus and its vector whitefly into tomato cultivars have the potential to generate more substantial resistance to tomato-infecting begomovirus disease in Taiwan. As well as resistance to the begomoviruses, resistances against the vector whitefly (*B. tabaci*) have been identified in accessions of *S. pennellii* and *S. habrochaites* [2, 3, 27]. Combining resistances to the virus and its vector in tomato cultivars has the potential to provide better leaf curl disease control and improve the durability of the resistance against the virus. Thus, AVRDC- the World Vegetable Center is also in the process of pyramiding begomovirus resistance with whitefly resistance into improved tomato breeding lines.

Management of tomato-infecting begomoviruses

The management of tomato leaf curl disease caused by begomoviruses is difficult and methods used are often unsuccessful. Various cultural practices have been used to control tomato leaf curl disease [27]. These include methods for reducing the sources of virus inoculum and reducing vector whitefly population. Virus inoculum can be reduced by good field sanitation including roguing diseased plants and cleaning the field of crop debris after final harvest. The whitefly population can be controlled by application of appropriate pesticide, deployment of sticky yellow traps, intercropping with non-host plant species, and using biological and physical barriers. Controlling the weeds, especially alternative hosts of the virus and/or whitefly, can also contribute to leaf curl disease management. Whiteflies transmit begomoviruses in the persistent manner; they remain viruliferous for life after virus acquisition. Since young tomato plants are a preferred host for whiteflies, protecting tomato seedlings, for example within a whitefly-proof net cage, will prevent them from becoming infected during this very vulnerable stage. However, it is difficult to convince growers to strictly keep the net

cages closed and thus keep the whiteflies out since this interferes with their routine cultural practices, and because it takes weeks for disease symptoms to develop after inoculation, so the link between whiteflies and leaf curl disease is not obvious. Tomato plants infected at the seedling stage will often not develop pronounced leaf curl symptoms until several weeks after transplanting, by which time the virus has been transmitted to neighbouring plants and it is no longer possible to control the disease.

Although the deployment of resistant or tolerant tomato cultivars is considered likely to be the most effective and sustainable means of controlling leaf curl disease, the strategy should be implemented with care to ensure that it really is effective and sustainable. Not all identified resistance is effective against all tomato-infecting begomovirus species [(10)]. Pyramiding many different resistance genes into a single tomato cultivar should provide protection against a wide range of begomovirus species, but runs the risk of selecting for virus, perhaps via recombination, that can overcome all the resistances included. A more sustainable approach might be to only deploy one or two resistances that are effective against the local begomoviruses, while retaining other resistances in reserve in case the deployed resistances are overcome. Use of good cultural management practices to prevent or at least delay infection with begomovirus will help increase the durability of deployed resistances. A field trial with seedling protection at AVRDC- the World vegetable Center in 2004 showed that 89 days after transplanting, there was less than 2.5% of a resistant/tolerance cultivar became infected under the net protection (60 mesh), whereas 47.5% of the same cultivar were infected in the open nursery (Fig. 3). In the same experiment, 77.1% of a susceptible variety raised under the net protection were infected 89 days after transplanting, but all of the similar plants raised in the open nursery were infected 42 days after transplanting. Combining the use of leaf curl disease resistant tomato cultivars with good plant protection such as the net protection from the seedlings stage is effective in controlling leaf curl disease in Taiwan and is likely to be effective elsewhere.

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Table 1. The percentage of ToLCTWV and TYLCTHV detected in begomovirus-positive tomato samples collected in Taiwan

Year	Virus detection[a]					
	Western Taiwan			Eastern Taiwan		
	TH	TW	TH+TW	TH	TW	TH+TW
2005	1%	99%	0%	0%	100%	0%
2007	35%	16%	49%	2%	84%	14%
2008-2010	50%	2%	48%	74%	15%	11%
2012-2013	54%	10%	33%	80%	14%	6%

[a]TW indicates percentage of samples only positive for ToLCTWV by PCR using specific primer pair TW1978/PAR1c715H. TH indicates percentage of samples only positive for TYLCTHV using specific primer pair TH1978/PAR1c715H. TW + TH indicate percentage of samples positive for both of ToLCTWV and TYLCTHV. Percentages indicate the proportions of viruses detected in the begomovirus-positive samples. Detailed information of primers was described previously (Tsai *et al*., 2011).

Table 2. The tomato production in Taiwan since 1980

Year	Planted area, ha	Yield, kg/ha	Production, m.t.	Year	Planted area, ha	Yield, kg/ha	Production, m.t.
1980	10,947	30,075	329,232	1997	4,090	29,157	118,818
1981	8,561	23,734	203,188	1998	3,798	25,690	96,875
1982	10,450	31,685	331,106	1999	3,831	28,398	108,554
1983	12,248	39,389	480,945	2000	4,392	28,692	124,727
1984	12,394	51,741	641,127	2001	4,459	26,197	116,171
1985	11,212	43,181	483,756	2002	5,200	29,461	153,081
1986	9,682	42,591	396,201	2003	5,128	27,858	142,703
1987	9,047	40,415	365,436	2004	5,043	28,532	143,889
1988	6,821	36,516	249,029	2005	4,762	24,906	118,422
1989	7,122	42,596	298,997	2006	4,597	25,975	119,275
1990	6,369	41,111	257,920	2007	3,936	24,627	96,841
1991	5,434	39,726	215,728	2008	4,535	24,428	110,662
1992	4,375	35,186	151,062	2009	4,104	24,264	99,491
1993	4,525	31,822	143,962	2010	4,734	24,520	116,034
1994	4,094	31,361	127,960	2011	4,817	25,524	122,870
1995	4,367	30,358	132,444	2012	4,501	24,751	111,361
1996	4,385	31,387	137,394				

Source: Agriculture and Food Agency, Council of Agriculture, Executive Yuan, Republic of China

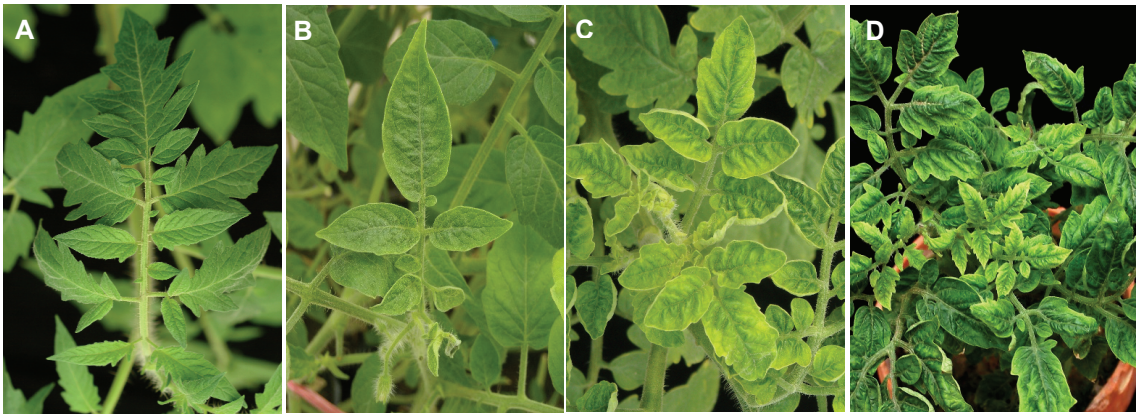


Fig. 1. Symptoms of tomato infected with begomoviruses on *Solanum lycopersicum*. A: symptomless healthy plant leaf; B: mild yellowing and leaf curling symptoms, C: yellowing and leaf curling symptoms, and D: severe yellowing, leaf curling and stunting symptoms.

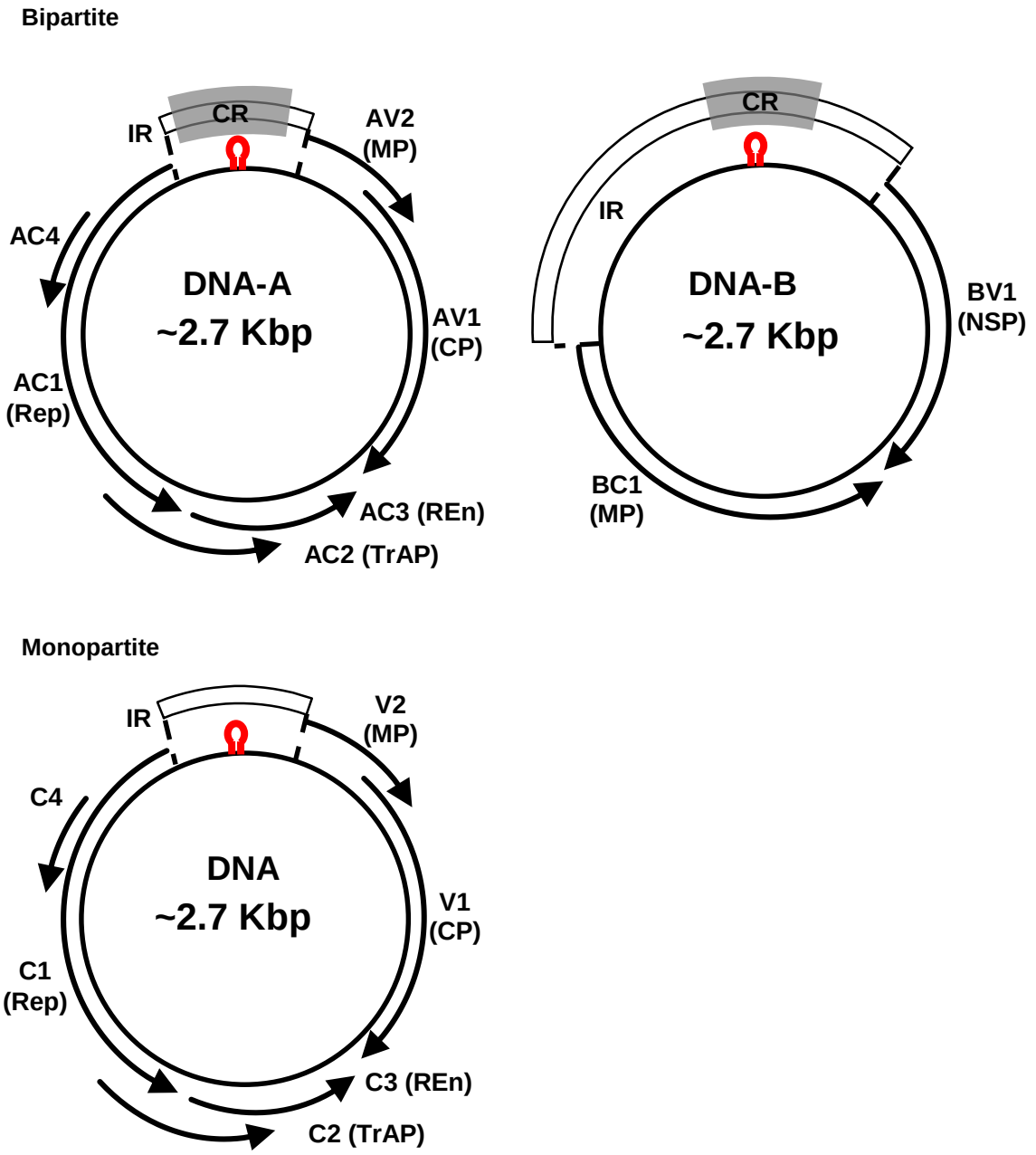


Fig. 2. Genome organization of tomato-infecting begomoviruses. The gray box indicated the common region (CR). The geminivirus conserved stem-loop structure has represented in red color. Arrows represent open reading frames of virus and complementary sense. IR: intergenic region; CP: capsid protein; Rep: replication-associated protein; TrAP: transcriptional activator protein; REn: replication enhancer; MP: movement protein; NSP: nuclear shuttle protein.

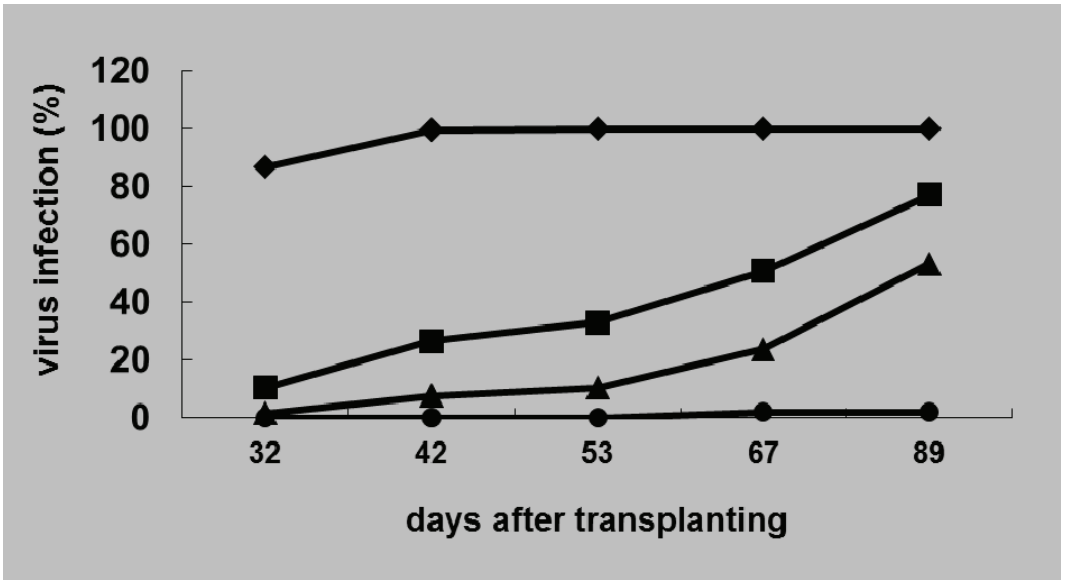


Fig. 3. Time course of begomovirus infection in resistant and susceptible tomato lines grown under the net (60 mesh) protection and in the open field prior to transplanting to the open field. ◆: susceptible tomato without net protection; ■: susceptible tomato with net protection; ▲: resistant tomato without net protection; ●: resistant tomato with net protection.