



Ty-1 confers resistance to geminivirus tomato curly top virus

Ken Matsuoka¹ · Soshi Kido² · Kojiro Kimura² · Nadya Syafira Pohan¹ · Sota Koeda^{1,2,3}

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Abstract

Tomato curly top virus (ToCTV), a geminivirus first isolated from tomato in Japan, shares approximately 86% nucleotide sequence identity with common bean curly stunt virus. Despite its isolation from tomato, previous attempts to infect tomato using an infectious clone were unsuccessful, and its potential impact on tomato production has remained unclear. In this study, we reconstructed an infectious clone of ToCTV and evaluated its pathogenicity and the effectiveness of *Ty-1*-mediated resistance. ToCTV infected *Nicotiana benthamiana* and *Nicotiana tabacum* at high rates and induced more severe symptoms than tomato yellow leaf curl virus (TYLCV) strains IL and Mld, indicating high virulence in *Nicotiana* species. We optimized an agroinoculation method to establish ToCTV infection in tomato. Although the infection rate in a geminivirus-susceptible tomato genotype was lower than that of TYLCV, infected plants developed severe leaf curling and growth suppression. In contrast, a *Ty-1*-bearing tomato genotype had no visible symptoms and significantly reduced viral DNA, even under high inoculum pressure via graft inoculation. These results demonstrate that *Ty-1*-mediated resistance effectively restricts ToCTV infection and symptom development, suggesting that current *Ty-1*-based cultivars are likely to remain protected against this geminivirus.

Keywords Geminivirus · Resistance breeding · Tomato curly top virus (ToCTV) · Tomato yellow leaf curl virus (TYLCV) · *Ty*-resistance genes

Introduction

The family *Geminiviridae* comprises 18 genera and 560 species, the most species of any plant virus family (ICTV 2025), and the genus *Begomovirus* accounts for the majority of species, which are persistently transmitted by the whitefly (*Bemisia tabaci*; Hemiptera: Aleyrodidae). Several begomoviruses cause tomato yellow leaf curl disease (TYLCD), posing a serious threat to global tomato production (Moriones and Navas-Castillo 2000). Tomato yellow leaf curl virus (TYLCV) is one of the causal agents of TYLCD, and among

its multiple strains, the Israel (IL) and Mild (Mld) strains have expanded their distribution worldwide (Mabvakure et al. 2016). In Japan, the introduction of both strains was first reported in 1996, and since then they have spread throughout the country, becoming a major constraint to tomato production (Kato et al. 1998; Koeda et al. 2015; Taniguchi et al. 2023; Ueda et al. 2004, 2005).

In 2009, tomato plants with symptoms distinct from those caused by TYLCV were found in Fukushima Prefecture, Japan, and the causal agent was subsequently isolated and named tomato curly top virus (ToCTV) (Kubota et al. 2023). ToCTV shares 86.1% nucleotide sequence identity with common bean curly stunt virus (CBCSV) (Zhang et al. 2020). These viruses were classified into a new genus *Cobecusvirus* of the family *Geminiviridae* in the ICTV Master Species List (MSL#41) released on March 20, 2026 (ICTV 2025). For CBCSV, the previously reported isolate was characterized solely based on sequence analysis from naturally infected plants, and no inoculation or pathogenicity tests were done. In contrast, an infectious clone was constructed for ToCTV and demonstrated to infect and induce symptoms in *Nicotiana tabacum*. However, infection has

✉ Sota Koeda
818sota@nara.kindai.ac.jp

¹ Graduate School of Agriculture, Kindai University, Nara 631-8505, Japan

² Faculty of Agriculture, Kindai University, Nara 631-8505, Japan

³ Agricultural Technology and Innovation Research Institute (ATIRI), Kindai University, Nakamachi, Nara 631-8505, Japan

not yet been achieved through direct inoculation of tomato using the infectious clone. Instead, symptom development in tomato was confirmed by grafting infected tobacco plants to tomato, thereby fulfilling Koch's postulates.

ToCTV was initially isolated from diseased tomato plants in Japan, and given its potential impact on tomato production, several concerns arise: ToCTV might directly cause economically significant losses, and recombination with TYLCV could give rise to more virulent variants, thereby exacerbating disease outbreaks. Important issues also remain unresolved. As mentioned earlier, despite its isolation from tomato, ToCTV failed to infect tomato plants when introduced via the infectious clone. Moreover, to properly assess the potential threat of ToCTV to commercial tomato production, it is essential to determine whether *Ty-1*-based resistance, which is most widely used in breeding programs (Koeda and Kitawaki 2024; Verlaan et al. 2013; Zamir et al. 1994), is effective against ToCTV. To address these questions, we redesigned the infectious clone of ToCTV and used it to evaluate the effectiveness of *Ty-1*-mediated resistance against ToCTV infection in tomato.

Materials and methods

Infectious clone construction and pathogenicity evaluation of ToCTV

Partial tandem repeats of ToCTV genome sequences (isolate JP-Fuk-K4-09, GenBank accession AB935398) (Kubota et al. 2023) were cloned into the binary vector pGreenII-p35S and introduced into *Agrobacterium tumefaciens* strain EHA105 as described by Koeda et al. (2017) (Fig. S1). The primers used for clone construction are listed in Table S1. Infectious clones of TYLCV-IL (isolate Japan:Itoman:T278, GenBank LC642631) and Mld (isolate Japan:Takatsuki, GenBank AB921568) have been described elsewhere (Koeda et al. 2015; Taniguchi et al. 2023). For mock inoculation, *Agrobacterium* EHA105 transformed with empty pGreenII-p35S was used.

Seeds of *Nicotiana benthamiana*, cv. Samsun NN (*Nicotiana tabacum*), tomato F₁ hybrid cultivars Momotaro (M: no *Ty*-genes, susceptible to geminiviruses) and TYLCV-resistant cultivar Momotaro Hope (MH) (*Ty-1/ty-1*) (Takii, Kyoto, Japan) were sown in a horticultural soil mix; individual seedlings were transplanted into separate plastic pots of the same mix. Momotaro is one of the most popular fresh tomato cultivars in Japan, and MH has the same genetic background as M. The abaxial side of the first and second leaves of 2-week-old *N. benthamiana* and *N. tabacum* plants were infiltrated with the infectious clone using a needleless 1-ml syringe (agroinfiltration). Tomato plants were

agroinoculated once using the colony inoculation method (Koeda et al. 2017; Pohan et al. 2024). In this inoculation method, *Agrobacterium* grown on solid LB medium were collected with a toothpick from a Petri plate, spread on the hypocotyl just below the apex of the seedlings before the first true leaf emerged, then an insect pin (No. 00; Shiga, Tokyo, Japan) was used to stab through the inoculum into the hypocotyl approximately 10 times. The plants were grown in a growth room at 25–30 °C day/23–25 °C night with 13 h light/11 h dark. Plants were checked for symptoms at 28 and 56 days post inoculation (dpi). Newly developed upper young leaves were collected and stored at –80 °C for later DNA extraction.

MH plants were also graft-inoculated with geminivirus. Plants were initially inoculated with TYLCV-IL, Mld, or ToCTV and used as the scion, then healthy MH plants were used as the rootstocks for grafting using the method of Koeda et al. (2018). We used virus-infected tomato plants as scions because several scions can be obtained from one plant. The grafted section was sealed with Parafilm (Bemis Company, Sheboygan Falls, WI, USA), and the plants were kept in humid conditions for approximately 2 weeks. The lateral shoots of the scion were then removed to promote the growth of a single lateral shoot from the rootstock. At 17 days after graft-inoculation (dpi), symptoms were evaluated, and new upper young leaves were collected to extract DNA.

Virus detection and quantification

Total DNA was extracted from all samples using a modified CTAB method (Permingeat et al. 1998). Virus-specific primers and EmeraldAmp MAX PCR Master Mix (Takara Bio, Kusatsu, Japan) were used for PCR with the primers and conditions for a GeneAtlas G02 Thermal Cycler (Astec, Fukuoka, Japan) listed in Table S1. PCR products were separated by electrophoresis in 1.0% (w/v) agarose gels.

Genomic DNA of TYLCV IL, Mld, and ToCTV was quantified by quantitative PCR (qPCR) as described by Koeda et al. (2021) using the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) and the primers and conditions listed in Table S1. Viral DNA levels were normalized using the tomato 25S ribosomal RNA gene amplified with primers 25S-rRNA 2F and 2R (Koeda et al. 2020). Samples were analyzed in duplicate to calculate the average threshold cycle. The $2^{-\Delta\Delta CT}$ comparative method (Livak and Schmittgen 2001) was used to calculate the relative quantity of each virus. Significant differences ($P < 0.05$) in mean values for viral DNA were compared using a Student's *t*-test in Excel (Microsoft, Redmond, WA, USA).

Table 1 Number and percentage of infected and symptomatic *Nicotiana* plants 56 days after agroinfiltration with the empty vector (Mock), tomato curly top virus (ToCTV) or tomato yellow leaf curl virus (TYLCV)

Plant species	Inoculated virus	Number of plants (%)		
		Inoculated	Infected ^a	Symptomatic ^b
<i>N. benthamiana</i>	Mock	12	0 (0)	0 (0)
	TYLCV-IL	12	12 (100)	12 (100)
	TYLCV-Mld	12	12 (100)	12 (100)
	ToCTV	24	23 (96)	23 (100)
<i>N. tabacum</i>	Mock	6	0 (0)	0 (0)
	TYLCV-IL	8	2 (25)	0 (0)
	TYLCV-Mld	8	2 (25)	0 (0)
	ToCTV	12	12 (100)	12 (100)

^a Viruses were detected using PCR. (Number of infected plants ÷ number of inoculated plants) × 100

^b (Number of symptomatic plants ÷ number of infected plants) × 100

Results

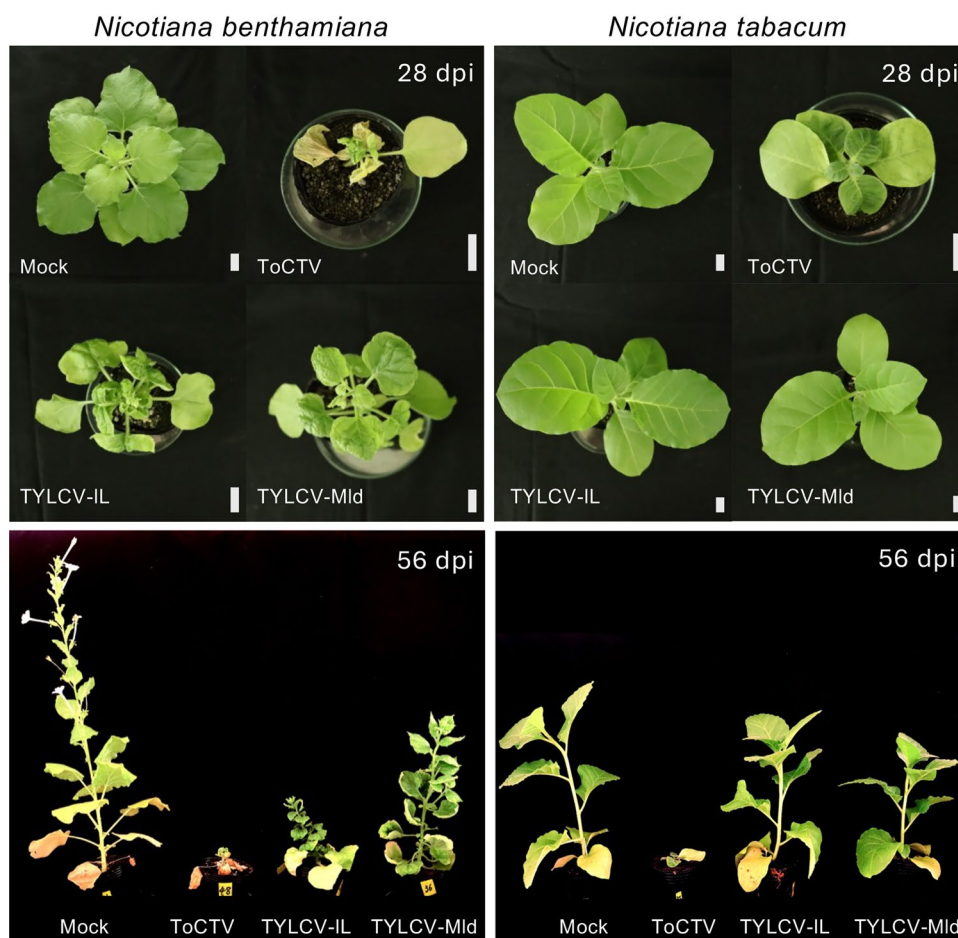
ToCTV is highly virulent against *Nicotiana* species compared to TYLCV

When we compared the virulence of ToCTV with that of

TYLCV-IL and TYLCV-Mld in *N. benthamiana* using infectious clones, more than 96% of the inoculated plants were infected with the respective clones (Table 1). Plants infected with TYLCV-IL had more severe leaf curling than those infected with TYLCV-Mld (Fig. 1). In contrast, ToCTV-infected plants developed markedly more severe symptoms, including pronounced leaf yellowing and curling and consistent severe stunting (Fig. 1). Thus, ToCTV is more virulent than TYLCV-IL or TYLCV-Mld in *N. benthamiana*.

When we similarly tested the virulence of the clones in *N. tabacum*, only two of the TYLCV-IL- and the TYLCV-Mld-inoculated plants were infected (25% infection), whereas all plants inoculated with ToCTV became infected (Table 1). No visible symptoms developed on TYLCV-IL- or TYLCV-Mld-infected plants, but ToCTV-infected plants exhibited leaf yellowing, curling, and severe stunting (Fig. 1). Collectively, these results demonstrate that ToCTV is more virulent than TYLCV-IL and TYLCV-Mld in two distinct *Nicotiana* species.

Fig. 1 Symptoms on *Nicotiana benthamiana* and *Nicotiana tabacum* at 28 and 56 days after agroinfiltration (dpi) with empty vector (Mock) or strains of tomato yellow leaf curl virus (TYLCV) Israel (IL), Mild (Mld), and tomato curly top virus (ToCTV). Scale bars = 2 cm



Ty-1-conferred resistance is effective against ToCTV

In tests to compare the virulence of ToCTV with that of TYLCV in the begomovirus-susceptible tomato cultivar M (no *Ty*-genes). PCR-based virus diagnosis confirmed that the infection rate of TYLCV-IL and TYLCV-Mld was 90% and 75%, respectively (Table 2). In contrast, the infection rate of ToCTV was markedly lower (14%) than that of the TYLCV strains. Symptom evaluation revealed that all TYLCV-IL- and TYLCV-Mld-infected plants developed typical yellow leaf curl symptoms, with more severe symptoms observed in TYLCV-IL-infected plants than in those infected with TYLCV-Mld (Fig. 2). In ToCTV-infected plants, characteristic upward leaf curling and growth suppression were observed, and these symptoms were more severe than those induced by the TYLCV strains. Collectively, these results indicate that although ToCTV has a lower infection rate than TYLCV in tomato M, it induces more severe symptoms.

To determine whether *Ty-1* confers resistance to ToCTV, we similarly inoculated the TYLCV-resistant tomato cultivar MH (*Ty-1/ty-1*) (Table 2). The infection rate of TYLCV-IL and TYLCV-Mld in MH was 100% and 78%, respectively, whereas the infection rate of ToCTV was 28%, again lower than those of the TYLCV strains. Notably, no visible symptoms were observed in MH plants infected with any of the viruses (Fig. 2A). These results demonstrate that *Ty-1*-mediated resistance is effective against ToCTV and can suppress symptom development even when infection occurs.

We used graft inoculations to further assess whether *Ty-1* resistance against ToCTV remains effective under high inoculum pressure. Tomato M plants infected with TYLCV or ToCTV were grafted onto healthy MH plants. We then assessed the virus resistance of the newly elongated lateral branches of the rootstock (Table 3). PCR-based diagnosis confirmed that no infection occurred in the mock treatment, whereas all plants in the TYLCV and ToCTV graft

treatments became infected. Nevertheless, no symptoms were observed in MH plants infected with either virus (Fig. 2A), indicating that *Ty-1* effectively suppresses disease development even under strong infection pressure.

To examine the relationship between symptom expression and viral DNA accumulation, we used qPCR to quantify viral DNA in M and MH plants inoculated with infectious clones via agroinoculation (Fig. 2B). For all viruses tested, MH had significantly less viral DNA than in M. These results confirm that *Ty-1*-mediated resistance effectively suppresses accumulation not only of TYLCV but also of ToCTV.

Discussion

The ToCTV isolate used in this study shares approximately 86% pairwise sequence identity with the closely related virus CBCSV (Kubota et al. 2023). These viruses were classified into the new genus *Cobecusvirus* of the family *Geminiviridae* in the ICTV Master Species List (MSL#41) released on March 20, 2026 (ICTV 2025). To align species demarcation criteria within most genera of the family *Geminiviridae*, a threshold of 78% pairwise nucleotide identity was proposed for *Cobecusvirus*. Based on this criterion, CBCSV and ToCTV isolates are considered to belong to a single species, designated *Cobecusvirus phaseoli*. However, because the latest ICTV Master Species List (MSL#41 v1) was released on March 20, 2026 during the final review stage of this manuscript, we used the name ToCTV throughout for consistency to avoid confusion. Studies on CBCSV have been limited to molecular analyses of viral sequences (Zhang et al. 2020); inoculation studies to characterize the virus had not been done. In this study, we used an infectious clone of ToCTV to characterize its infectivity and pathogenicity in *Nicotiana* species and tomato.

In a previous study, *N. tabacum* was infected with ToCTV through inoculation with an infectious clone, but tomato plants were not infected via similar inoculations (Kubota et al. 2023). Consistent with these findings, ToCTV infected *N. tabacum* and *N. benthamiana* at high rates in our study, and symptoms on the plants were severe, confirming that ToCTV is highly virulent in *Nicotiana* species. Moreover, we were also able to establish ToCTV infection in tomato plants, and a geminivirus-susceptible tomato genotype exhibited apparent disease symptoms, demonstrating that the newly constructed infectious clone and inoculation method were functional. In this study, we made several modifications to the method described by Kubota et al. (2023), including changes to the vector construct design, the *Agrobacterium* strain, and the inoculation method. For construct development (Fig. S1), the vector pBI121 was replaced with pGreenII, and the cauliflower mosaic virus 35S promoter

Table 2 Number and percentage of infected and symptomatic tomato plants 56 days after agroinoculation with the empty vector (mock), tomato curly top virus (ToCTV) or tomato yellow leaf curl virus (TYLCV)

Genotype	Virus	Number of plants (%)		
		Inoculated	Infected ^a	Symptomatic ^b
M (no <i>Ty</i> -genes)	Mock	10	0 (0)	0 (0)
	TYLCV-IL	10	9 (90)	9 (100)
	TYLCV-Mld	8	6 (75)	6 (100)
	ToCTV	57	8 (14)	8 (100)
MH (<i>Ty-1/ty-1</i>)	Mock	10	0 (0)	0 (0)
	TYLCV-IL	10	10 (100)	0 (0)
	TYLCV-Mld	9	7 (78)	0 (0)
	ToCTV	40	11 (28)	0 (0)

^a Virus infection was detected by PCR. (Number of infected plants ÷ number of inoculated plants) × 100

^b (Number of symptomatic plants ÷ number of infected plants) × 100

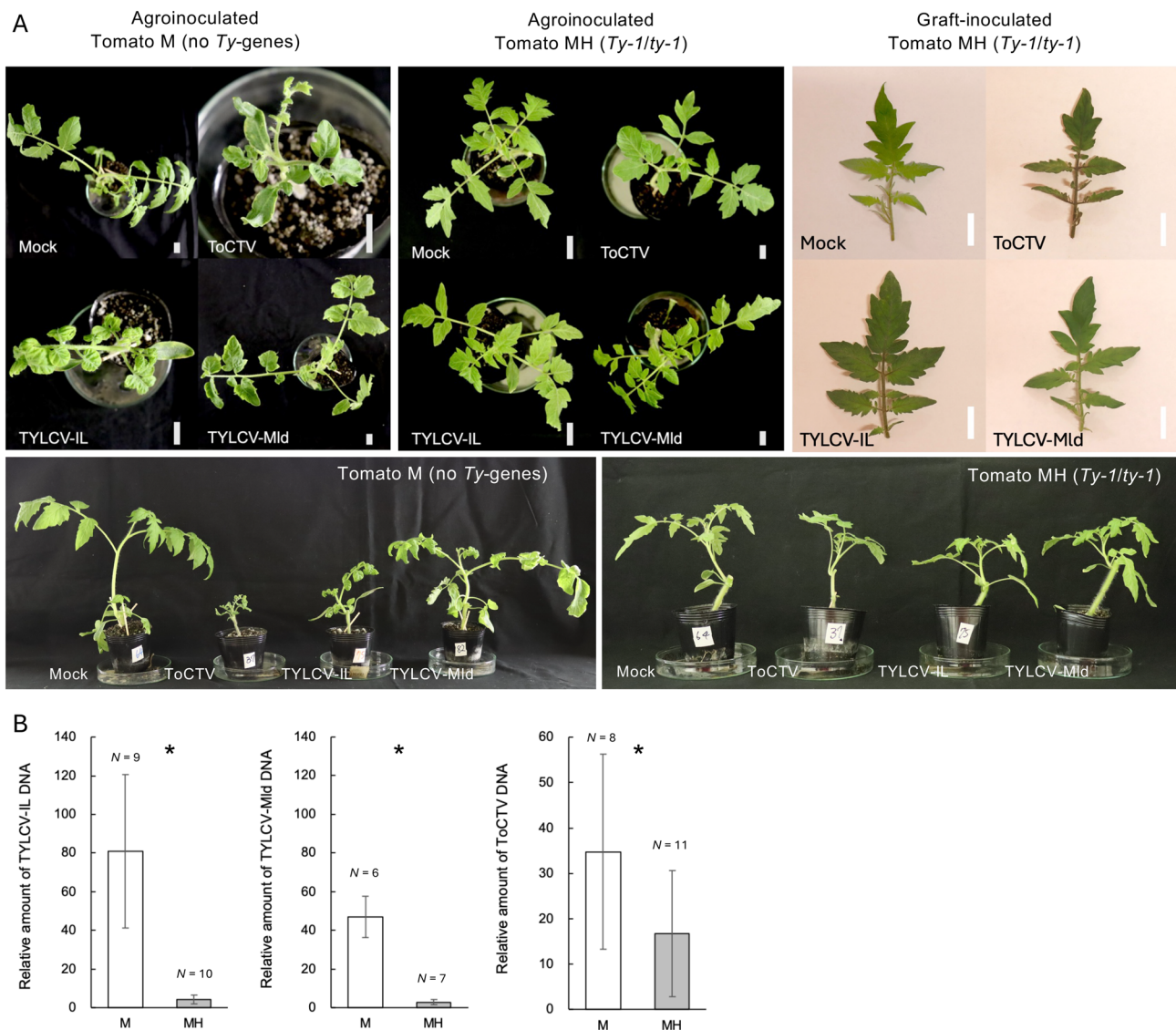


Fig. 2 Symptoms on susceptible tomato Momotaro (M) and resistant tomato Momotaro Hope (MH), which harbors *Ty-1*, and viral DNA levels after agroinoculation and graft-inoculation with empty vector (Mock) or strains of tomato yellow leaf curl virus (TYLCV) Israel (IL), Mild (Mld), and tomato curly top virus (ToCTV). **A** Symptoms

on plants at 56 days after agroinoculation and 17 days after graft-inoculation. Scale bars=2 cm. **B** Relative viral DNA in plants at 56 days after agroinoculation. Error bars indicate standard deviation. The asterisk indicates a significant difference between the means (Student's *t*-test, $P < 0.05$)

Table 3 Graft inoculation of tomato curly top virus (ToCTV) and tomato yellow leaf curl virus (TYLCV) to tomato MH plants bearing *Ty-1*

Tomato genotypes	Inoculated virus	Number of plants (%)		
		Grafted	Infected ^a	Symptomatic ^b
MH	Mock	7	0 (0)	0 (0)
<i>(Ty-1/ty-1)</i>	TYLCV-IL	9	9 (100)	0 (0)
	TYLCV-Mld	5	5 (100)	0 (0)
	ToCTV	9	9 (100)	0 (0)

^a Virus infection was detected by PCR. (Number of infected plants ÷ number of inoculated plants) × 100

^b (Number of symptomatic plants ÷ number of infected plants) × 100

(p35S) and NOS terminator (NOST) were introduced flanking the viral sequence insertion site. In addition, the full-length and partial sequences of ToCTV were cloned such that the C1 (Rep) gene was positioned relatively close to the p35S promoter and in the same transcriptional orientation. This design was based on the understanding that Rep is the only viral gene product required for replication and is likely the first viral protein expressed during the early stage of infection (Lazarowitz et al. 1989; Palmer and Rybicki 1998). In contrast, Kubota et al. (2023) did not include a p35S promoter and employed tandem repeats of the full-length ToCTV sequence cloned in the opposite orientation. In addition, they used *A. tumefaciens* strain GV2260,

whereas we used strain EHA105, which confers higher infectivity for geminivirus than GV2260 in tomato plants (Koeda et al. 2017). Our agroinfiltration method also differed; we pricked the hypocotyl with an insect pin instead of agroinfiltrating true leaves and petioles as done by Kubota et al. (2023). Although we are uncertain which of these modifications predominantly facilitated the infection, the infectivity of ToCTV is comparatively lower in tomato than that of TYLCV, aligning with the reported difficulty in establishing infection in tomato plants using infectious clones.

In tomato breeding programs worldwide, *Ty-1* is the most widely used resistance gene against TYLCV (Koeda and Kitawaki 2024; Lapidot et al. 2014). In MH tomato, *Ty-1* conferred disease tolerance to ToCTV-infected plants and significantly restricted viral DNA compared with the level in a geminivirus-susceptible genotype. Numerous studies have shown that *Ty-1* does not confer complete immunity to TYLCV; rather, it provides tolerance by limiting viral accumulation, thereby suppressing symptom development (Butterbach et al. 2014; Fortes et al. 2023; Koeda and Kitawaki 2024; Verlaan et al. 2013). In addition, *Ty-1* has been reported to confer resistance not only to TYLCV, but also to other geminiviruses, including curtoviruses (Voorburg et al. 2020). Our present results demonstrate that *Ty-1*-mediated resistance is also effective against *Cobecusvirus* suggesting that *Ty-1* may confer broad-spectrum resistance to viruses in the family *Geminiviridae*.

Since *Ty-1*-mediated resistance does not completely block viral infection but instead functions by suppressing viral accumulation, there is a concern that viral evolution or recombination could alter virulence and increase viral adaptation to *Ty-1*-based resistance (Koeda et al. 2024). In geminiviruses, recombination is known to occur frequently during a mixed infection (Lefeuvre and Moriones 2015). IS76, a recombinant of TYLCV and tomato yellow leaf curl Sardinia virus (TYLCSV), is more virulent and accumulates at higher levels in *Ty-1*-bearing tomato plants, resulting in a high selective advantage beyond that of its parental viruses (Belabess et al. 2016). Additionally, the recombinant ES421/99 of TYLCV and TYLCSV has a broader host range than its parent viruses (Monci et al. 2002). When ToCTV coexists with other geminiviruses such as TYLCV, there is also a potential risk for changes in virulence and host range. Therefore, although ToCTV is currently considered unlikely to cause serious damage to tomato plants when *Ty-1* is deployed, continuous monitoring of viruses infecting tomato plants is necessary. When ToCTV was first isolated in Fukushima Prefecture in 2009 (Kubota et al. 2023), *N. tabacum* was grown on 1,054 ha in the prefecture, ranking seventh nationwide for tobacco production area (National Tobacco Cultivators Association of Japan). Given that our present study demonstrated that ToCTV is more adapted to

tobacco than to tomato, ToCTV has the potential to be a more significant threat for tobacco production. Therefore, tobacco also needs to be monitored for the virus.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10327-026-01283-2>.

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Author contributions KM and SK designed the experiments, analyzed the data, and interpreted the results. KM, SK, and KK did the experiments. SK prepared the infectious clones. KM, NSP, and SK wrote the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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