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Full Length Article

Development of a Tobacco Rootstock-Based Graft Inoculation Method for the *Tomato Chlorosis Virus*

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Abstract

Tomato chlorosis virus (*ToCV*) spread by *Bemisia tabaci* has induced serious economic losses to the tomato industry around the world, and has aroused great attention from researchers. Due to the absence of a stable and efficient acquisition technology of *ToCV*, studies on the pathogenic mechanisms, interactions with hosts and the breeding of resistant varieties are greatly limited. In this study, we carried out relevant studies for the graft inoculation of *ToCV* on tobacco rootstock. An infectious cDNA clone of *ToCV* successfully infected *Nicotiana benthamiana* plants (the unnatural host), exhibiting typical symptoms, howevre, it could not make the tomato plants (the natural host) exhibit any symptom. Moreover, *B. tabaci* failed to infect by *ToCV* after feeding on the inoculated tomato plants. Grafting healthy tomato plants onto diseased *N. benthamiana* plants using different grafting methods all caused systemic infection of tomato plants. The incidence of *ToCV* in tomato plants grafted using cleft grafting was up to 79.08%, which was more suitable for the grafting of tomato plants onto *N. benthamiana* plants than approach grafting. In addition, the virus from the grafted tomato plants could be transmitted to healthy tomato plants by the insect vector, *B. tabaci*.

Keywords: *ToCV*; Infectious clone; Graft inoculation; *Bemisia tabaci*; *Nicotiana benthamiana*

Introduction

Tomato chlorosis virus (*ToCV*) is a newly-emerged virus that is transmitted by the whitefly. Once *ToCV* infects plant hosts, it causes a yellowing of plant leaves, resulting in the delay of fruit maturation, reduced total yields, and finally, causes serious economic losses to farmer households (Wintermantel and Wisler 2006). The disease was first discovered in tomato plantations of Florida in 1989, identified as *ToCV* in 1998, and then spread rapidly throughout the world (Wisler *et al*. 1998a; Navas-Castillo *et al*. 2011; Tzanetakis *et al*. 2013). With the exception of Antarctica and Oceania, *ToCV* has been found in more than 20 countries on the other five continents (Wei *et al*. 2018). In China, *ToCV* was detected in Taiwan for the first time in 2004 (Tsai *et al*. 2004), and was thereafter detected in tomato and pepper in greenhouse in Daxing province, near Beijing (Zhao *et al*. 2013). At present, the virus has been found in additional tomato-growing regions in different provinces, including Beijing, Shandong, Henan, Tianjin, Shanxi, Jiangsu, Liaoning, and Guangdong, among others, and has caused considerable economic damage (Zhao *et al*. 2014a; Gao *et al*. 2015; Hu *et al*. 2015; Zheng *et al*. 2016; Tang *et al*. 2017; Wang *et al*. 2017).

ToCV belongs to the genus *Crinivirus* in the family *Closteroviridae*, and the genome is composed of two single-stranded positive-sense RNA molecules, RNA1 (8594–8595 nt) and RNA2 (8242–8247 nt), which are separately encapsidated in linear virions (Wisler *et al*. 1998b; Liu *et al*. 2000; Martelli *et al*. 2002; Kataya *et al*. 2008; Albuquerque *et al*. 2013). RNA1 contains four open reading frames (ORFs) that encode proteins involved in virus replication, while RNA2 contains nine ORFs that encode proteins mainly involved in virus encapsidation, movement and vector transmission (Karate 2000; Martelli *et al*. 2002; Livieratos *et al*. 2004). Previous studies verified that only RNA1 and RNA2 simultaneously existed, the plant could be infected successfully by *ToCV* (Wisler *et al*. 1998b; Wintermantel *et al*. 2005; Wintermantel and Wisler 2006; Orílio *et al*. 2014). *ToCV*, like other viruses of the genus *Criniviruses*, is phloem-limited and not transmitted by mechanical inoculation or seeds (Wintermantel and Wisler 2006). Under natural conditions, *ToCV* can only be transmitted by whiteflies in a semi-persistent manner. *B.*

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tabaci, *Trialeurodes vaporariorum* and *T. abutilonea*, belonging to two genera, can spread the virus by biting and sucking the phloem of plants. All of whiteflies have effective transmission abilities in spite of different spreading efficiencies (Wisler *et al*. 1998a; Wintermantel and Wisler 2006; Shi *et al*. 2018).

At present, *ToCV* research is still in a primary stage, and only few studies have investigated nosogenesis, plant resistance, vector-virus-host interactions and the selection and breeding of resistant varieties. Therefore, it is necessary to obtain a single stable source of single infection of *ToCV* for further correlational research. In this study, we established the inoculation technology of *ToCV* by using the pathogenic *N. benthamiana* as rootstock and healthy tomato plant as scions, to provide technical support for *ToCV* research.

Materials and Methods

Insect rearing

B. tabaci MED (formerly Q biotype) was obtained from the Applied Insect Laboratory of Beijing Academy of Agriculture and Forestry Sciences, and raised on cotton plants at a temperature of 26 ± 1°C, with a relative humidity of 70 ± 5% and a photoperiod of 16: 8 h (L : D). The purity of *B. tabaci* MED was monitored regularly according to the mitochondrial cytochrome oxidase I gene (*mtCOI*) (Chu *et al*. 2012).

Plant cultivation

Tobacco seeds (*N. benthamiana*) were obtained from Shandong Agricultural University. The cotton seeds ('Jimian 616') were provided by Institute of Cotton, Hebei Academy of Agriculture and Forestry Sciences. The tomato seeds (Jinpeng No.1) were purchased from Xi'an Jinpeng seedlings Co. Ltd. The seeds of different plants were sown respectively in a plate with 75 holes, and incubated in a growth chamber at a temperature of 25~27°C. After five true leaves had grown, the plants were transferred to a pot with 12 cm diameter for the following experiments.

Inoculating infectious clone of *ToCV*

The infectious *ToCV* clone used in this study was provided by Zhou Tao of China Agricultural University. Thirty μL of *Agrobacterium tumefaciens* C58C1cintaining RNA1 and RNA2 clones were added to 30 mL Luria-Bertani (LB) broth l containing rifampicin (25 μg/mL) and kanamycin (50 μg/mL), and was oscillated at 28°C, 200 rpm/min for 12 h. The bacterial cells were collected by centrifugation, and suspended for 4 h in MMA buffer (10 m*M* $MgCl_2$, 10 m*M* MES, 200 μM As). Suspensions were then adjusted to a final $OD600_{nm}$ of 1.0. Next, the bacterial cells containing RNA1 and RNA2 were mixed in a 1:1 ratio. Each healthy plant with five true leaves was injected with about 0.5 mL of bacterial suspensions on the underside of leaves using a 1 mL syringe without a needle (Zhao *et al*. 2016). Plants were cultivated in a growth chamber under the same conditions described previously. After inoculation for 21 days, the infection rate was detected by reverse transcription-polymerase chain reaction (RT-PCR), and *Agrobacterium* with no plasmid pCY was used as the negative control.

Effects of different rootstock on virus accumulation

To determine the effects of different rootstock on accumulation of *ToCV*, two treatments were set. Treatment A refers to the grafting healthy tomato onto pathogenetic *N. Benthamiana* plants used as the rootstock. Treatment B refers to the grafting pathogenetic *N. Benthamiana* onto health tomato plants used as rootstocks. Each treatment used 30 pathogenetic *N. Benthamiana* plants and 30 healthy tomato plants, respectively (in triplicate). After grafting for 7, 14, 21 and 28 days, the tomato plant leaves were collected to detect virus accumulation by qRT-PCR and analyzed the difference between treatments.

Effect of different grafting methods on the transmission of *ToCV*

Here are two grafting methods, including approach grafting and cleft grafting. The Approach grafting method is shown in Fig. 1A. When the stem diameter of the *N. benthamiana* plant was up to 2 mm, the upper stem was cut off in the middle of the third and fourth true-leaf using a sterile blade at a cut angle of about 45 degrees. Then cut off diagonally the latter stem of the scion of tomato just keeping a heart and a leaf at the same cut angle with rootstock, aligned the two incisions, and fixed them with grafting clip. The cleft grafting is shown in Fig. 1B. When the stem diameter of the *N. benthamiana* plant was up to 2 mm, the upper stem was cut off in the middle of the third and fourth true leaves with a sterilizing blade, then cut vertically the latter stem with a 1.5 cm incision from top to bottom. The scion of tomato keeping a heart and a leaf was cut off partial stem, insert the incision of the *N. benthamiana* plant, and fixed them by grafting clip.

To determine the effect of different grafting method on the incidence of *ToCV*, we performed treatments A (approach grafting) and B (cleft grafting). Each treatment used 30 pathogenetic *N. benthamiana* plants and 30 healthy tomato plants respectively (in triplicate). The tomato plants were grafted onto *N. benthamiana* plants using different grafting methods under unified production management. We collected the leaves of the tomato plants 21 days post-grafting and detected the incidence and the accumulation of *ToCV* using PCR and qRT-PCR, respectively.

Transmission of *ToCV* in grafted tomato by *B. tabaci*

Newly emerged adults of *B. tabaci* MED were used for transmission assays. One hundred *ToCV*-free female *B. tabaci* populations were reared on the underside of true

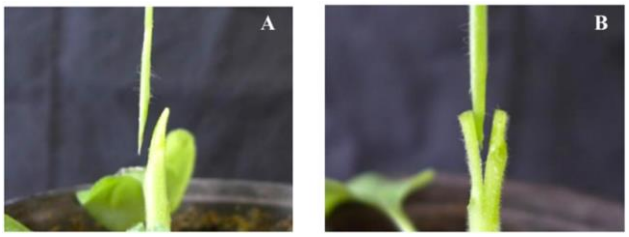

Fig. 1: Different grafting methods of tomato plants. (**A**) Approach grafting. (**B**) Cleft grafting

leaves (the third one numbered from bottom to top) of pathogenetic tomato in insect-proof cages. Thirty *B. tabaci* adults were collected after feeding for 48 h and stored at -80°C until experiments to detect the virus acquisition rate. Experiment had three replicates, 100 *B. tabaci* per replicate, and *ToCV*-free female *B. tabaci* as a negative control.

Five *ToCV*-infected female *B. tabaci* were reared on the leaves of *ToCV*-free tomato plants that were at the five true leaf stage and incubated in insect proof cages for 48 h. The leaves of tomato plants were collected to detect the incidence rate of *ToCV* by RT-PCR after cultivating for 21 days in insect proof greenhouse. This experiment was performed with three replicates and 30 tomato plants per replicate. *ToCV*-free tomato plants were used as a negative control.

Total RNA isolation and cDNA synthesis

Total RNA was extracted following the protocol of the RNA extraction kit (Sangon Biotech, Shanghai, China), and the quality was detected using a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Waltham, USA). Two hundred ng of total RNA (OD260/OD280: 1.80–2.10) per sample was used for subsequent cDNA synthesis. First-strand cDNA was synthesized using EasyScript First-Strand cDNA Synthesis SuperMix (Vazyme Biotech, Nanjing, China), according to the manufacturer's instructions. The synthesized cDNA was stored at −80°C until use.

Detection of the incidence of *ToCV* using PCR

Two pairs of gene-specific primers (shown in Table 1), including T6-R/F (designed based on RdRp of RNA1) and ToC5/ToC6 (Dovas and Katis 2002) were used for PCR. PCR reactions were carried out in a final volume of 20 μL, containing 10 μL 2× Taq Master Mix (Vazyme Biotech, Nanjing, China), 0.5 μL of each degenerate primer (10 μM, Sangon Biotech, Shanghai, China), 1 μL cDNA template and 8 μL RNase-free water. The PCR cycling conditions were as follows: 95°C for 3 min, 35 cycles of 95°C for 30 s, 50°C for 30 s, extension at 72°C for 50 s, and a final extension at 72°C for 4 min. PCR products were electrophoresed on a 1.0% agarose gel and visualized by GelRed staining. DNA fragment of the expected length were gel-purified and cloned into the pMD18-T vector (Takara Biotech, Dalian, China), and the constructs containing the target gene fragment were sequenced (BGI Genomics, Shenzhen, China).

Accumulation of *ToCV* by using a real-time fluorescent quantitative PCR (RT-qPCR)

The transcripts of *ToCV* were measured by using an Eppendorf Mastercycler ep realplex PCR Detection System (Eppendorf, Hamburg, Germany). Plasmids contain CP gene of *ToCV* diluted in 10 times for six gradients were used as the template to generate standard curve with which to normalize the accumulation of *ToCV*. The primers of the CP gene were designed using Primer Express 5.0 (Applied Biosystems, Carlsbad, CA) (Table 1). The qRT-PCR reactions were conducted in 20 μL reaction mixtures containing 10 μL of 2 × SYBR Premix Ex Taq (Vazyme Biotech, Nanjing, China), 0.3 μL of each primer (10 μM), 1 μL of cDNA, and 8.4 μL of sterilized H_2O. The RT-qPCR cycling conditions were as follows: 94°C for 30 s and 40 cycles of 94°C for 5 s, 63°C for 15 s and 72°C for 10 s. Melt curves stages included 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. Each sample included three biological and three technological replicates to ensure reproducibility.

Data analysis

Data from all experiments were analyzed using SPSS 19.0 (IBM SPSS Statistics, Chicago, I.L., U.S.A.). One-way ANOVA was used to analyse the variance of each treatment, while the Duncan's new complex range method was used for detecting significant differences between groups ($P = 0.05$).

Results

Infection of infectious *ToCV* clones in *N. benthamiana* and tomato plants

After the infectious *ToCV* clone invading *N. Benthamiana* and tomato plants for 21 days, the suspected DNA fragments of about 751 bp and 463 bp were detected both in plants using RT-PCR (shown in Fig. 2A). But the probability of detecting these two fragments simultaneously varied greatly, with *N. benthamiana* plants being 76.79% and tomato plants only 12.85%. After sequencing, the results showed that the target gene sequences were more than 99.0% consistent with the RNA1 and RNA2 sequences of *ToCV* in GenBank, and had high homologousness.

Compared with healthy *N. Benthamiana* plants in the control group, the *N. Benthamiana* plants in the treatment group showed typical symptoms after inoculated with infectious cDNA clone of *ToCV* (Fig. 2B). The upper leaves were yellow and the lower leaves showed obvious chlorotic areas with the crispy margins. However, the tomato plants inoculated with infectious *ToCV* clone showed no symptom of disease (Fig. 2C).

Table 1: Primer pairs used in study

Primer name	Primer sequence	Gene	PCR Amplicon size/bp
T6-R	TCGGTTGACCATTGAAGGTT	RdRp	751
T6-F	CAGGGTGCCGAGAGTTTCTA		
ToC5	GGTTTGGATTTTGGTACTACATTCAGT	Hsp70	463
ToC6	AAACTGCCTGCATGAAAAGTCTC		
qCP-2-R	TCTTATCTGTCATCGGGG	CP	123
qCP-2-F	GGAAATTGAAGGTACACTCC		

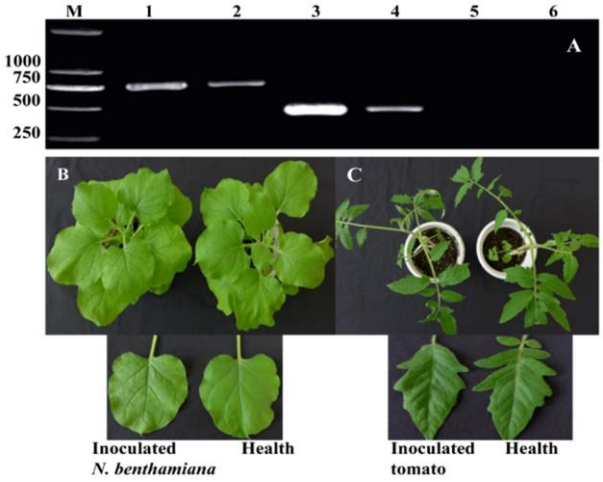

Fig. 2: Infection of infectious ToCV clones in *N. Benthamiana* and tomato plants. (**A**) Detection of plants inoculated with infectious clone of ToCV. Note, 1, 3 *N. benthamiana*; 2, 4, tomato; 5, 6 negative control. (**B**) Symptom of the *N. Benthamiana* plants inoculated with infectious clone of ToCV. (**C**) Symptom of the tomato plants inoculated with infectious clone of ToCV

Effects of different hosts on the survival rate and virus acquisition capability of *B. tabaci*

Newly emerged adults of *B. tabaci* were inoculated to *ToCV*-infected *N. benthamiana*, *ToCV*-free *N. benthamiana*, *ToCV*-infected tomato and *ToCV*-free tomato, respectively. The *B. tabaci* reared on both *ToCV*-infected and *ToCV*-free *N. benthamiana* began to die after feeding for 1 h, and masses of *B. tabaci* had died after feeding for 2 h. The death rate of *B. tabaci* in *ToCV*-free *N. benthamiana* even reached 59.37%, which was significantly higher than that of the *ToCV*-infected *N. benthamiana* and the two tomato groups. After feeding for 12 h, all *B. tabaci* fed on *ToCV*-free *N. benthamiana* had died, and that fed on *ToCV*-infected *N. benthamiana* had died after feeding for 24 h. However, *B. tabaci* grew well on the host of tomato (shown in Fig. 3A). *B. tabaci* growing on the *ToCV*-infected *N. benthamiana* and *ToCV*-infected tomato after feeding for 6 h and 24 h separately were detected by RT-PCR, and the results showed that *B. tabaci* populations were not infected by *ToCV* (Fig. 3B).

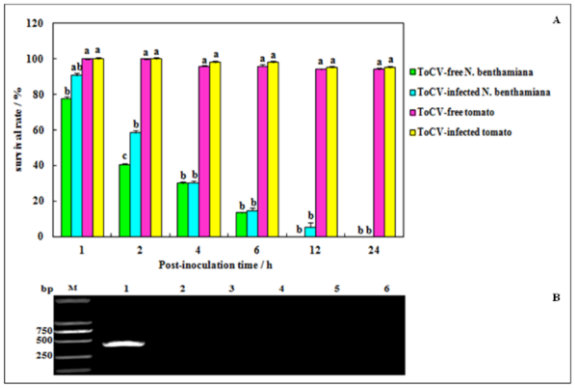

Fig. 3: Effects of different hosts on the survival rate and virus acquisition capability of *B. tabaci*. (**A**) Survival rate of *B. tabaci* inoculated to the *N. benthamiana* and tomato plants. (**B**) Detection of ToCV in *B. tabaci* feeding on the *N. benthamiana* and tomato plants. Note, 1, 2, 3 *N. benthamiana*; 4, 5 tomato; 6 negative control

Effects of different rootstock on virus accumulation in tomato plants

After grafting 7, 10, 14 and 21 days, the leaves of tomato plants from group A and B were collected to detect the accumulation of *ToCV* at different stages by RT-PCR. The results showed that *ToCV* CP gene was detected in the plants from treatment A after grafting for 10 days, and the viral load increased to maximum until grafting for 21 days. In the treatment B, *ToCV* was detected after 14 days, and the virus load also increased to maximum with the prolonging of time (shown in Fig. 4A). These results demonstrated that graft inoculation of *ToCV* was effective and the grafting inoculation with *N. benthamiana* as rootstock was more beneficial to the infection of *ToCV*.

Effects of different grafting methods on virus accumulation in tomato plants

We used two different methods to graft healthy tomato plants onto pathogenetic *N. benthamiana*. After grafting for 21 days, tomato plant leaves were collected for RT-PCR detection. It was found that there was no significant difference in virus accumulation between approach the grafting and cleft grafting methods (shown in Fig. 4B). However, the incidence of *ToCV* on tomato plants with different grafting methods exhibited an obvious difference. The incidence of *ToCV* of the cleft grafting method is as high as 79.08%, significantly higher than that of approach grafting method (shown in Table 2). Therefore, the cleft grafting method was more favourable for the graft-inoculation of *ToCV* with *N. benthamiana* as the rootstock.

Transmission of *ToCV* from grafting tomato by *B. tabaci*

The newly-emerged adults of *B. tabaci* populations were inoculated into grafting tomato to acquire *ToCV* in

Table 2: Morbidity rate of tomato plants with different grafting methods

Treatment	Grafting number/plant	Morbidity number/plant	Rate of morbidity/%
A	130	76	58.13 ± 3.99b
B	128	101	79.08 ± 2.48a

Different letters indicate significant difference ($P < 0.001$; N=30) by Duncan's new multiple range test

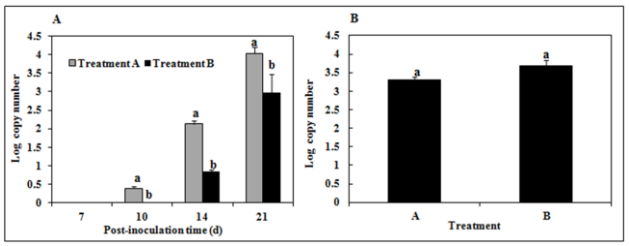

Fig. 4: Accumulation of ToCV in tomato plant. (**A**) Accumulation of ToCV of different rootstock. (**B**) Accumulation of ToCV of different grafting methods. Note, standard curves of ToCV is given by y = -3.4169x + 38.97. Efficiency is 96%, R^2 is 0.98. Data are mean ± SE. Different letters indicate significant difference ($P < 0.05$; N=4) by Duncan's new multiple range test

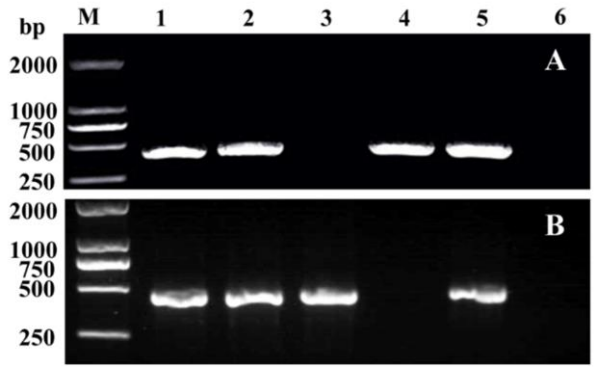

Fig. 5: Detection of ToCV in *B. tabaci* and grafted tomato plants. (**A**) Detection of ToCV in *B. tabaci*. (**B**) Detection of ToCV in tomato. Note, 1-5 samples; 6 negative control

insect-proof cages for 48 h. Following this, a single of *B. tabaci* was for RT-PCR detection. It was found that the *ToCV* fragment could be detected in 64.20% of *B. tabaci* populations (shown in Fig. 5A). In addition, veneniferous *B. tabaci* populations were inoculated on healthy tomato plants for 48 h, and the target fragment was detected in 85.98% of tomato plants with typical symptoms of *ToCV* (shown in Fig. 5B).

Discussion

The *ToCV* was initially detected in tomatoes and caused a huge loss in tomato production (Wisler *et al.* 1998a). According to the current literature, *ToCV* has been detected in tomato, pepper, eggplant, potato, lettuce, pumpkin, and even some types of weeds (Lozano *et al*. 2004; Morris *et al.* 2006; Barbosa *et al.* 2011; Solórzano-Morales *et al.* 2011; Fortes and Navas-Castillo 2012; Orfanidou *et al.* 2014; Zhou *et al*. 2015; Şahin-Çevik *et al*. 2019), and still expanded its hosts (Fiallo-Olivé and Navas-Castillo 2019). It would be detrimental, if *ToCV* continues to increase its number of hosts without effective control. Moreover, *ToCV* always infects plants together with multiple plant viruses, for example, *Tomato yellow leaf curl virus* (TYLCV), *Tomato infectious chlorosis virus* (TICV) in agricultural production (Zhao *et al*. 2014b; Dai *et al.* 2016; Ding *et al.* 2019). The co-infection of different viruses may be beneficial for breaking through host resistance mechanisms and leading to occurrence and epidemic of new diseases (Renteria-Canett *et al.* 2011) which aroused great attention from researchers. Now, researchers typically use *B. tabaci* to feed on infected plant in the laboratory to maintain *ToCV* for research purposes. As a vector of a variety of plant viruses, it is almost impossible to ensure that there is only one type of the viruses carried in *B. tabaci* (Andret-Link and Fuchs 2005; Hohn 2007). Moreover, inoculation using insect vector is always limited by sex, feeding conditions, inoculation technique, *etc*., and that is difficult to perform (Lapidot 2007; Polston and Capobianco 2013; Çevik *et al*. 2019).

Orílio *et al.* (2014) successfully constructed the infectious cDNA clone of *ToCV*, but the infectious clone could not effectively infect the natural host tomato, but could infect the non-natural host, *N. benthamiana*. The same situation occurred in research involving *Lettuce chlorosis virus* (LCY) and *Lettuce infectious yellows virus* (LIYV), which also belonged to the genus *Crinivirus* (Wang *et al*. 2009; Chen *et al*. 2012). In our study, we used the infectious clone of *ToCV* provided by Zhao to infect tomato and *N. benthamiana*. The results showed both RNA1and RNA2 could be detected in the tomato and *N. benthamiana* plants, however, the detection rate of natural host tomato was very low and lacked any symptom of *ToCV*. Meanwhile, the newly-emerged healthy *B. tabaci* populations feeding on the tomato plants infected by the infectious clone of *ToCV* failed to acquire the virus. It suggested that the infectious clone might not successfully infect the natural host tomato. Whether a plant virus can infect effectively a host depends on the result of confrontation between virus increment and host defense (Cleene and Ley 1976; Wroblewski *et al*. 2005; Cañizares *et al*. 2008). The reason for the failure to infect the natural host may be defense responses of the tomato plants caused by combinations of the *A. rhizogenes* strains C58C1 and *ToCV*, resulting in a low efficiency of transformation. We also found that all the *B. tabaci* feeding on the *ToCV*-free *N. benthamiana* plants died out within 6 h, while that feeding on the *ToCV*-infected *N. benthamiana* plants died out within 12 h. It might be because that the virus had inhibited the jasmonate (JA) -signalling pathway of *N. benthamiana* plants, thereby reduced the synthesis of terpenes, and improved the suitability of *B. tabaci* for *N. benthamiana* (Zhang *et al*. 2012; Luan *et al*. 2013; Fang *et al*. 2013).

Previous studies have demonstrated that after

infection by *ToCV*, the virions can transmit in both directions through the phloem of a plant (Wisler *et al*. 1998a; Dovas and Katis 2002). Lee *et al*. (2017) grafted *ToCV*-infected simple leaves into the phloem of tomato seedlings, with 87.8% of the plants being infected with virus. Similarly, transplanting a section of *ToCV*-infected tomato stem containing phloem tissue into the phloem of healthy tomato plants also caused systemic infection in tomato plants (Çevik *et al*. 2019). In general, virus transmission by grafting is performed by grafting infected plants onto healthy plants (Picó *et al*. 1996). In this study, by comparing the virus accumulation between tomato rootstock and tobacco rootstock, it was found that tobacco rootstock was more favorable to the virus accumulation in the tomato plants. We grafted healthy tomato onto *ToCV*-infected *N. benthamiana* by the approach grafting and cleft grafting methods. Both grafting methods obtained the *ToCV*-infected tomato plants, which had showed typical symptoms of *ToCV*. This indicated that viral progeny of the infection clone had owned the ability to increase in tomato plants after replicating in *N. benthamiana*. Additionally, the *ToCV* virion could be also transmitted effectively by *B. tabaci* inoculated on graft-inoculated tomato plants. The graft inoculation method is simple, efficient and easy to perform. Moreover, the method is not limited by season and temperature. In particular, cleft grafting is more suitable when *N. benthamiana* is the rootstock for grafting tomato plants, so it can be used as an effective method to obtain a single infection of *ToCV*. The graft inoculation of *ToCV* will quicken the studies concerning the selection and breeding of germplasm resources, the mechanism of virus-host interaction, *etc*., which have great significance for further researches.

Conclusion

The graft inoculation of *ToCV* on tobacco rootstock was established a stable and effective acquisition technology of *ToCV* from the root. The method could help to quicken the studies concerning the selection and breeding of germplasm resources, the mechanism of virus-host interaction, *etc*.

Acknowledgements

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