Successful generation of anti-ToCV and TYLCV transgenic tomato plants by RNAi

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Abstract

Tomato is an economically important vegetable. *Tomato chlorosis virus* (ToCV) and *Tomato yellow leaf curl virus* (TYLCV) are two major viruses that cause serious losses to tomato production. The effective method to control these two viruses is to breed antiviral species by genetic engineering techniques. In order to obtain the RNA interference (RNAi) expression vector of tomato, the *coat protein* (*CP*) genes of ToCV and TYLCV were selected in this study. The tandem sequences of the two *CP* genes were obtained using the recombinant PCR technique. Using Gateway cloning technology, the RNAi expression vector pRNAi-ToCV-TY including the two *CP* genes was constructed by attB×attP and attL×attR recombination reactions. Polymerase chain reaction and sequencing analysis confirmed that the vector was obtained successfully and contained the *ToCV* and *TYLCV CP* genes. The RNAi expression vector pRNAi-ToCV-TY was transformed into *Agrobacterium* strain GV3101. The RNAi vector was then used to transform tomato. The objective fragments were successfully transformed into a tomato by PCR identification. At the fourth-leaf stage, the positive transgenic plants were challenged with ToCV and TYLCV. Out of 15 transgenic plants, 33 % showed early symptoms within 4 weeks post-infection (WPI); 20 % showed delayed symptoms (5 - 7 WPI); and the remaining 47 % were symptomless even after 9 WPI. The untransformed control plants (90 %) showed severe symptoms within 2 - 4 WPI, whereas 10 % delayed symptoms.

Additional key words: *Agrobacterium*-mediated transformation, coat protein, *Solanum lycopersicum.*

Introduction

The tomato (*Lycopersicon esculentum* L. or *Solanum lycopersicum* L.) is an economically important vegetable. *Tomato chlorosis virus* (ToCV) and *tomato yellow leaf curl virus* (TYLCV) are the two major viruses that cause serious losses to tomato production (Jin *et al*. 2011, 2016, Pasev *et al*. 2012, Chinnaraja *et al*. 2016). The number of these two viruses has been increasing at a global scale and has become an important limiting factor for tomato production in many parts of the world.

The ToCV, belonging to the genus *Crinivirus* (*Closteroviridae*), is a whitefly-transmitted virus with bipartite RNA genomes RNA1 and RNA2. The RNA1 comprises 8 594 nucleotides with four open reading frames (ORFs). The RNA2 has 8 242 nucleotides and encodes nine ORFs including a heat shock protein 70 and the coat protein (CP) (Wisler *et al*. 1998). Infected tomato plants exhibit yellow thick leaves and dark veins, which greatly reduce the quality and yield of tomato.

The TYLCV belongs to the genus *Begomovirus* (*Geminiviridae*) and is a DNA virus that can be also transmitted by the whitefly. It consists of a single circular DNA molecule comprising 2 781 nucleotides and has six ORFs. The CP is encoded by the viral strand (Jin *et al*. 2011). The infected plants have yellow veins, curved and shriveled leaves, and a dwarf phenotype. The two viruses have recently often been observed to co-infect tomato, aggravating the disease and causing severe yield and quality declines (Zhao *et al*. 2014, Wu *et al*. 2016, Liu *et al*. 2018, Song *et al*. 2019). It is thus necessary to breed tomato cultivars that are resistant to ToCV and TYLCV.

The introduction of resistance genes found in wild tomato species into tomato cultivars has been employed in breeding for improving resistance to TYLCV (Pilowsky *et al*. 1990, Zakay *et al*. 1990). However, this method is ineffective in ToCV. To date, there have been no reports oncultivars resistant to ToCV (Liu *et al*. 2018). It is therefore urgent to obtain tomato genotype resistant to ToCV.

In recent years, RNA interference (RNAi) technology, which can silence foreign viruses, has become the most effective method for improving plant resistance. The double-stranded RNA (dsRNA) model proposed by Waterhouse *et al*. (1998) as a form of RNAi technology is currently accepted as a gene silencing mechanism. In the dsRNA-mediated resistance to viruses, an inverse repeat sequence derived from the virus is delivered to the plant. The inverse repeat sequence can be transcribed to dsRNA in plants, which induces gene silencing when the foreign virus infects the plant. The transgenic plant then becomes resistant to viruses (Baulcombe 1999, Stoutjesdijk *et al*. 2002). The dsRNA technology has been gradually applied in the prevention and control of plant viruses. For example, the polyprotein gene inverse repeat of the barley Yellow dwarf virus-PAV (BYDV-PAV) was transferred to barley, which enabled 9 out of 25 plants to be immune to the disease (Wang *et al.* 2000). The inverted repeat structure of the *Tomato mosaic virus* movement protein gene (ToMV-MP) was transformed into tobacco, and 23 out of 47 plants became immune to ToMV (Niu *et al*. 2003). The *CP* gene inverted repeat of papaya *Ringspot virus* (PRSV) was transferred into tobacco and *Arabidopsis* by an *Agrobacterium*-mediated method, resulting in resistant plants (Zhang and Jiang 2011). However, the transformation of tomato with the *CP* gene of TYLCV and the *CP* gene of ToCV has never been attempted to improve the resistance of tomato to TYLCV and ToCV.

The construction method of the homologous recombinant RNAi expression vector represented by Gateway is widely used for its high efficiency and rapidity (Oey *et al*. 2014, Salim *et al*. 2016, Marino *et al*. 2017). There have been many reports on the use of Gateway technology to construct anti-plant virus expression vectors (Tian and Niu 2013, Xu *et al*. 2011). Gateway technology is a DNA recombinant cloning technology that was developed based on the homologous recombination system of the phage. It is an improvement to the traditional plasmid construction model, and the target gene can be efficiently and rapidly constructed into an expression vector only by the attB×attP (BP) and attL×attR (LR) reactions, and the cloning step is greatly reduced. It guarantees an accurate reading framework and gene mapping (Hartley *et al.* 2000). Reverse repeats can be obtained directly using this technique, avoiding repeated digestion and ligation.

The CP is the conservative protein in TYLCV and ToCV. The homology of the TYLCV CP in Tianjin isolate (GU563330) compared to that of the most isolates in China and Korea, Japan, Jordan, Israel, America, and Mexico is over 99 % (Jin *et al*. 2016). The homology of the ToCV CP in Tianjin isolate (KP246844) to that of the most isolates in China and Korea, Japan, and America is over 99 % (Jin

et al. 2011). We thus aimed to construct a binary dsRNA expression vector of the *CP* genes of TYLCV and ToCV using Gateway technology, further transforming tomato to develop an antiviral material in order to regenerate transgenic plants resistant to TYLCV and ToCV.

Materials and methods

Nucleic acid extraction: Tomato leaves with typical TYLCV and ToCV symptoms were collected in Xiqing District, Tianjin. Total plant DNA was isolated from leaves of TYLCV-infected plants by cetyltrimethyl ammonium bromide (CTAB), as described by Wang and Fang (2002). Total RNA was extracted from the leaves of the ToCV-infected plants using an RNA extraction kit (*Takara*, Dalian, China).

Amplification of partial *ToCV* and *TYLCV CP* gene fragments: The primers TY-F/R (Table 1 Suppl.) were designed according to the sequence of the TYLCV Tianjin isolate (GU563330). The partial *TYLCV CP* fragment (1 to 405 in the *CP* gene) was amplified by the primers TY-F/R. In order to connect with *ToCV CP* fragment, the forward primer TY-F has an overlapping sequence with ToCV-R. The total PCR volume was 25 mm³, including 2.5 mm³ of 10× buffer (containing Mg²⁺), 0.5 mm³ deoxynucleotide triphosphate (dNTP) mixture (2.5 mM), 2.5 mm³ of forward primer TY-F (10 μM), 2.5 mm³ of reverse primer TY-R (10 μM), 0.5 - 2 mm³ of DNA, and 0.5 mm³ of DNA polymerase (5 U mm⁻³) (*Takara*), and the remainder was supplemented with water. The partial *ToCV CP* fragment (1 - 432 in the *CP* gene; 4332 - 4763 in RNA2) was obtained by reverse transcription PCR (RT-PCR). The primer ToCV-F/R (Table 1 Suppl.) was designed according to the sequence of the ToCV Tianjin isolate (KP246844). The first strand of cDNA was synthesized by *M-MLV* reverse transcriptase (*Takara*) with Oligo dT primer. The total PCR volume was 10 mm³, including 1 mm³ of RNA, 1 mm³ of oligo dT primers, and 0.5 mm³ of dNTP, and the remainder was supplemented with water. After denaturing at 70 °C for 10 min and incubating on ice for 2 min, the following reagents were added to the total volume: 5 mm³ × *M-MLV* buffer, 1 mm³ of *M-MLV*, and 0.5 mm³ of RNAase inhibitor. Then, it was incubated at 37 °C for 1 h. The total PCR volume was 25 mm³, including 2.5 mm³ of 10×buffer (containing Mg²⁺), 0.5 mm³ of dNTP mixture (2.5 mM), 2.5 mm³ of forward primer ToCV-F (10 μM), 2.5 mm³ of reverse primer ToCV-R (10 μM), 0.5 - 2 mm³ of cDNA, and 0.5 mm³ of DNA polymerase (5 U mm⁻³) (*Takara*), and the remainder was supplemented with water.

The PCR program consisted of initial denaturation (94 °C for 2 min) followed by 35 cycles of denaturation (94° C for 30 s), annealing (53 °C for 30 s), and extension (72 °C for 1.5 min), with final extension at 72 °C for 10 min. After amplification, the PCR products were recovered and sequenced.

Tandem gene acquisition of the partial *ToCV CP* and *TYLCV CP*: The obtained PCR products of the partial

TYLCV CP and *ToCV CP* as the template were mixed, and PCR was carried out using primers ToCV-F and TY-R (Table 1 Suppl.) to obtain a tandem gene fragment of partial *TYLCV CP* and *ToCV CP*. The total volume of the PCR mixture was 50 mm³, with 25 mm³ of 2× SG PCR *MasterMix* (*SinoGene Scientific Co.*, Beijing, China), 1 mm³ of (100 ng mm⁻³) of each template, 2.5 mm³ of forward primer (10 μM), and 2.5 mm³ of reverse primer (10 μM), and the remainder was supplemented with water. The PCR procedure included denaturation at 94 °C for 2 min; 94 °C for 30 s, 54 °C for 30 s, 72 °C for 1.5 min, 35 cycles; and 72 °C for 10 min. The PCR product was electrophoresed on 1 % (m/v) agarose gel, and the tandem gene fragment was recovered using a PCR purification kit (*Tiangen Biochemical Technology Co*., Beijing, China) according to the manufacturer's protocol. The tandem gene fragment was named ToCV-TY. ToCV-TY was then ligated into the pMD18-T vector (*Takara*) and named P-ToCV-TY.

Expression vector construction: Using the plasmid P-ToCV-TY as the template, PCR was performed with primers attB-ToCV-F and attB-TY-R (Table 1 Suppl.). The attB adapters were then added forward and reverse of the tandem gene. According to the instructions of the Gateway LR clonase enzyme mix kit (*Invitrogen*, Carlsbad, CA, USA), the PCR product with the attB adapters reacted with the entry vector pDONRTM 221 (*Invitrogen*) by BP reaction. The reaction product was transformed into DH5α by heat shock method and the transformed cells were uniformly spread on Luria-Bertani (LB) solid medium containing 50 μg cm⁻³ kanamycin (kan). After incubating at 37 °C for 16 h, PCR was carried out using the primers M13F/R. The positive clones were selected, and then sequenced. The correct clones were named pENTR-ToCV-TY.

Plasmids were extracted from positive clones to obtain entry vector pENTR-ToCV-TY. The vector was reacted with the expression vector pK7GWIWG2(I) (*SinoGene Scientific*) by LR reaction. The reaction product was transformed into DH5α, and the transformed cells were detected by the primers p35SP and TY-R. The colonies with the expected band were further sequenced with P35SP and T35SP to verify the insert direction. The positive clone in the correct direction was selected and named pRNAi-ToCV-TY (Fig. 1).

The expression vector (pRNAi-ToCV-TY) was transformed into *Agrobacterium* GV3101 (*Tiangen*) by freeze-thawing. Selected colonies were screened by PCR using the specific primers ToCV-F and TY-R and p35SP and TY-R. A positive colony containing the pRNAi-ToCV-TY construct (Fig. 1) was used for tomato transformation.

Genetic transformation of tomato and identification of transgenic plants: The tomato (*Solanum lycopersicum* L.) susceptible inbred line J501 was preserved at the Tianjin Research Center of Agricultural Biotechnology. Healthy tomato seeds of J501 were surface-sterilized in 10 % (m/v) commercial bleach (15 % hypochlorite) for 15 min, washed in sterile water, and set to germinate on half-strength

Murashige and Skoog (MS) medium. Cotyledon explants of two-week-old seedlings were used, and the transformation was performed as described previously (McCormick *et al.* 1986, 1991). The cotyledons of the explants were soaked for 5 min with *Agrobacterium* liquid medium (absorbance at 60 nm of 0.5). The blot-dried explants were replaced on the same plate and incubated in the dark at 25 °C for 3 d. After co-cultivation, the cotyledonary leaf discs were transferred onto selection medium [MS containing 2.0 mg dm⁻³ of 6-benzylaminopurine (6-BA) and 0.2 mg dm⁻³ of indole-3-acetic acid (IAA), supplemented with 100 mg dm⁻³ of kan, and 500 mg dm⁻³ of cefotaxime]. The explants were removed, and the residual bacterial solution was blotted on sterile filter paper and transferred to MS medium containing IAA (0.2 mg dm⁻³) and 6-BA (2.0 mg dm⁻³) for 3 d. Following this, it was transferred to a screening medium containing cephalosporin (500 mg dm⁻³) and kan (50 mg dm⁻³), and cultured three weeks. The plates were kept at 25 °C, a 16-h photoperiod, and an irradiance of 45 μmol m⁻² s⁻¹ in the culture room. Sub-culturing was performed at 2- to 3-week intervals, and explants showing shoot regeneration were subsequently transferred to the same medium but with a different phytohormone (1.0 mg dm⁻³ of IBA) for shoot development. The roots were developed and transplanted into the soil. The established plants were designated as T0-generation plants.

To confirm the presence of the *ToCV-TY* gene in the T0 generation plants, total DNA was isolated from all transformed and untransformed (negative control) plant samples. The DNA was subjected to PCR using the primers TY-R and P35SP. The total PCR volume was 25 mm³, including 2.5 mm³ of 10× buffer (containing Mg²⁺), 0.5 mm³ of dNTP (2.5 mM) mixture, 2.5 mm³ of forward primer (10 μM), 2.5 mm³ of reverse primer (10 μM), 0.5 - 2 mm³ of DNA, and 0.5 mm³ of DNA polymerase (5 U mm⁻³), and the rest was supplemented with water. The PCR program consisted of initial denaturation (94 °C for 2 min) followed by 35 cycles of denaturation (94 °C for 30 s), annealing (53 °C for 30 s), and extension (72 °C for 1.5 min), with final extension at 72 °C for 10 min.

Identification of resistance: The group of whiteflies (*Bemisia tabaci*) carrying TYLCV and ToCV were maintained on tomato seedlings in the Tianjin Agricultural Biotechnology Research Center. The positive transgenic plants were planted into an insect-proof greenhouse. At the fourth-leaf stage, they were challenged with ToCV and TYLCV using 10 - 20 viruliferous whiteflies and maintained in an insect-proof glasshouse to observe the development of disease symptoms for nine weeks post-inoculation (WPI) during the winter season (October 2018 to December 2018). A set of untransformed control plants was also challenged with viruliferous whiteflies. The viruses of challenged transgenic and control plants were detected at 2 WPI and 9 WPI by PCR and RT-PCR, respectively. The DNA was isolated to detect *TYLCV CP* gene (144 - 716 bp) by PCR using primer shanghaiF/R (Table 1 Suppl.), and the RNA was isolated to detect *ToCV CP* gene (4 301 – 5 147 bp in RNA2) by RT-PCR with

primer ToCVCP-F/R (Table 1 Suppl.).

Results

The RT-PCR and PCR were performed using the total RNA and total DNA of plants infected with the ToCV and TYLCV viruses, respectively. Two fragments of 432 and 405 bp were obtained (Fig 2*A*,*B*). The sequencing results of TYLCV and ToCV showed that the homology in the Tianjin isolates (TYLCV was GU563330; ToCV was KP246844) was 100 %.

Overlapping PCR was conducted to gain ToCV and TYLCV interference segments using ToCV-F and TY-R as the primers, and the mixture of the ToCV CP and TYLCV CP PCR products as the template. The length of the interference segment was 837 bp (Fig. 2*C*). The PCR product was cloned into the pMD-18T vector and named P-ToCV-TY.

The entry vector pENTR-ToCV-TY was constructed by BP reaction with the ToCV-TY tandem fragment and pDONR221 vector, and the positive recombinant clone was confirmed by PCR using primers M13F/R. A fragment of 1 100 bp was amplified, which was significantly larger than the insert fragment (837 bp), indicating that the tandem gene fragment ToCV-TY was ligated into the vector (Fig. 2*D*).The entry vector was obtained, which was named pENTR-*TOCV*-TY.

The obtained entry vector was subjected to LR reaction with the expression vector pK7GWIWG2(I) to obtain an expression vector, which was transformed into DH5a and named pRNAi-ToCV-TY. Colony PCR was performed using the primers ToCV-F and TY-R, and the 837 bp band was amplified (Fig. 2*E*), which was consistent with the size of the target fragment. The forward and reverse sequencing were performed using P35SP and T35SP, and the results showed that the direction and sequencing were correct. This verified that pRNAi-ToCV-TY was successfully transferred into DH5a.

The plasmid was extracted from DH5a and transformed into *Agrobacterium* strain GV3101. The target bands were amplified with primers ToCV-F and TY-R and P35SP and TY-R. The ToCV-F and TY-R primers amplified an 837 bp band (Fig. 2*F*), which was consistent with the size of the target fragment. The PCR product of the P35SP and TY-R primers was approximately 1100 significantly longer than the insert fragment (Fig. 2*G*). This indicated that the expression vector had been successfully transferred into

Table 1. The degree of resistance observed after virus challenge in T0 generation transgenic plants

	Total	Resistant	Tolerant	Susceptible
Transgenic plants	15	7 (47 %)	3 (20 %)	5 (33 %)
Untransformed control plants	10	0	1 (10 %)	9 (90 %)

GV3101.

A total of 30 regenerated plants (Fig. 4*A*) were obtained by *Agrobacterium*-mediated transformation of tomato J501. Control and regenerated plant genomic DNA were extracted, and PCR was performed using the p35SP and TY-R primers. The results showed that a band of about 1 100 bp was amplified in 15 of the plants (Fig. 3*A*), while the control plants had no amplified bands, indicating that the target gene had been integrated into the tomato genome.

In this study, 15 positive T0 transgenic tomato plants were challenged with ToCV and TYLCV using viruliferous whiteflies (*Bemisia tabaci*), and the results showed that the resistance of plants to ToCV and TYLCV was significantly improved compared to the untransformed control plants (Table 1).

Untransformed control plants and transgenic plants showed no symptoms at 3 WPI, and no bands were amplified by PCR. At 4 WPI, the T0-generation transgenic plants showed variable degrees of resistance/tolerance/susceptibility against TYLCV and ToCV compared to the untransformed control plants (Table 1). Nine of the untransformed control plants grew slowly, the old leaves turned yellow, and the new leaves were wrinkled and yellowed. Five transgenic plants (4, 9, 10, 11, and 13) also showed the same symptoms. There were three transgenic plants (6, 7, and 15) and one untransformed control plant that also showed obvious symptoms at 5 - 7 WPI. The other seven transgenic plants (1, 2, 3, 5, 8, 12, and 14) showed no symptoms until 9 WPI (Figs 4*B*,*C*).

Out of 15 transgenic plants, 5 (33 %) showed early symptoms within 4 WPI, 3 (20 %) showed delayed symptoms (5 - 7 WPI), and the remaining 7 (47 %) were symptomless even after 9 WPI. Nine (90 %) of the untransformed control plants showed severe symptoms within 4 WPI, while one (10 %) showed delayed symptoms.

The transgenic plants and untransformed control plants were further tested by PCR and RT-PCR, respectively. The specific primer shanghaiF/R for detecting the *TYLCV CP* gene and a specific primer ToCV CP-F/R for detecting
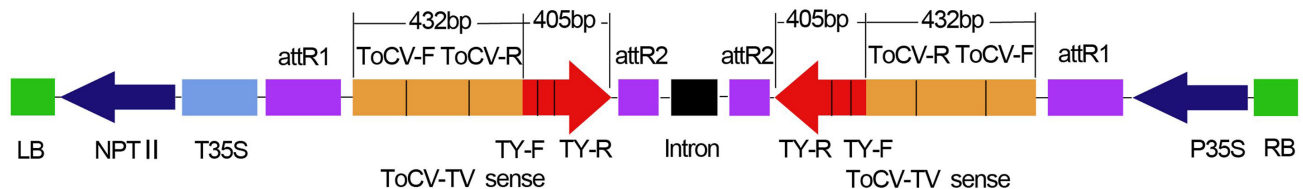

Fig. 1. The scheme of the recombinant expression vector pRNAi-ToCV-TY. The interference segment was cloned in a pK7GWIWG2(I) vector under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The interference segment consisted of two ToCV-TY sequences and an intron. Two ToCV-TY sequences were connected in the antisense orientation, and there was an intron between them. The vector features kanamycin resistance due to the *neomycin phosphotransferase II* (*NPTII*) gene.

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the *ToCV CP* gene were used. The results showed that the target bands of about 573 bp (TYLCV) and 847 bp (ToCV) could be amplified in all plants, which were susceptible
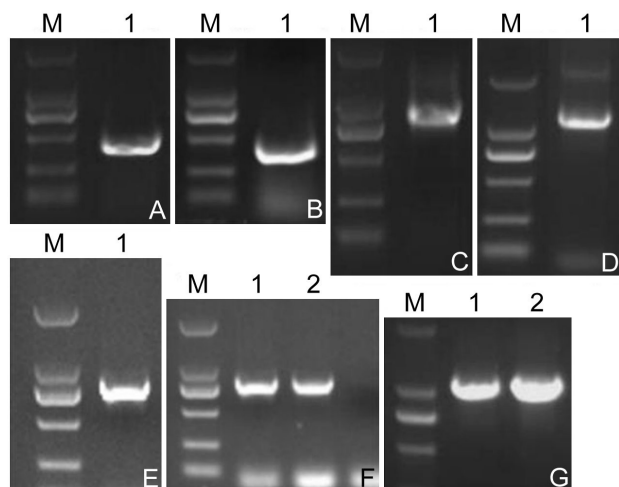

Fig. 2. Identification of gene fragments, an entry vector, and clones by PCR. *A - ToCV CP* fragment (1), *DL2000* (M); *B - TYLCV CP* fragment (1), *DL2000* (M); *C - ToCV-TY* gene fragment obtained using primers *ToCV-F* and *TY-R* (1), *DL2000* (M); *D* - entry vector obtained using primers *M13F/M13R* (1), *DL2000* (M); *E* - clones of *pRNAi-ToCV-TY* transformed into DH5α obtained using primers *ToCV-F* and *TY-R* (1), *DL5000* (M); *F,G* - clones of *pRNAi-ToCV-TY* transformed into *Agrobacterium* strain GV3101 obtained using primers *ToCV-F* and *TY-R* (*F*, 1,2) and *P35SP* and *TY-R* (*G*, 1,2), *DL2000* (M).

and showed delayed symptoms (Fig. 3*B,C*). However, no bands could be amplified in these asymptomatic plants (1, 2, 3, 5, 8, 12, and 14) indicating no accumulation of the challenged virus in these resistant transgenic plants.

Discussion

Two approaches, the restriction enzyme ligation approach and Gateway cloning technology, can be used to construct an expression vector, which utilizes the bacteriophage lambda site-specific recombination system. However, the restriction enzyme ligation approach has some disadvantages. It usually requires multiple digestions and ligations, making it time-consuming and laborious (Karimi *et al.* 2002).

In this study, Gateway technology was used to construct the expression vector of the *TYLCV* and *ToCV* partial coat protein genes, which greatly reduces the number of ligation and transformation steps and decreases the probability of a base mutation. It also eliminates the limitations of DNA restriction enzymes and the restriction sites of the genes themselves, resulting in greatly improved efficiency.

The *CP* gene has become a well-studied gene in plant antiviral research. Many reports suggest that resistance to plant viruses is improved by transforming the *CP* gene. For example, Kunik *et al.* (1994) demonstrated that tomatoes transformed with *TYLCV CP* became resistant to TYLCV. The *CP* gene of *Tobacco mosaic virus* (TMV) was used to construct an RNAi vector that was transformed into tobacco,
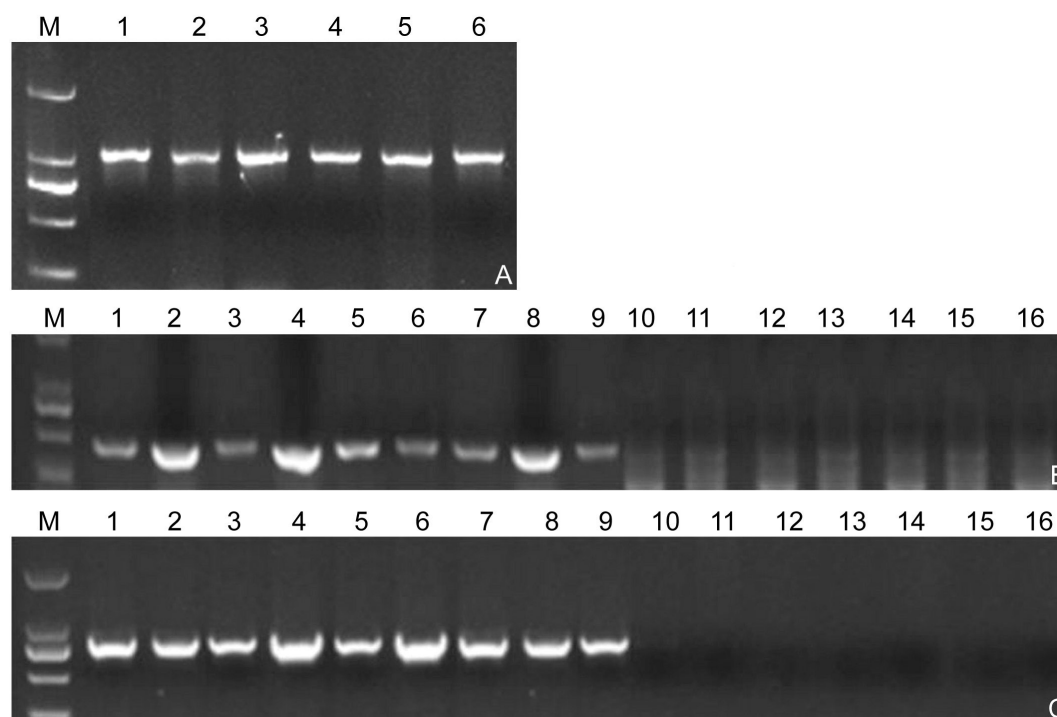

Fig. 3. Detection of transformed tomato plants by PCR. M - DL2000, 1 to 6 - products obtained using primers *p35SP* and *TY-R* (*A*). Detection of TYLCV and ToCV using primers *shanghaiF/R* (*B*) and *ToCV CP-F/R* (*C*); M - DL2000, 1 - untransformed control plants, 2 to 9 - transgenic plants Nos 4, 9, 10, 11, 13, 6, 7, and 15; 10 to 16 - transgenic plants Nos 1, 2, 3, 5, 8, 12, and 14.

and the transgenic plants showed resistance to TMV (Yan *et al*. 2007). The *CP* of the *Cucumber green mottle mosaic virus* (CGMMV) was transformed into *Nicotiana benthamiana*, and the transgenic plants became resistant to CGMMV (Kamachi *et al*. 2007). *Agrobacterium*-mediated tomato transformation and regeneration of transgenic lines expressing the tomato leaf curl virus (TLCV) *CP* gene resulted in resistance against TLCV infection (Raj *et al*. 2005). There are also some reports where the *CP* genes of two or three viruses were fused to construct an expression vector and carry out genetic transformation. For example, a binary RNAi expression vector was constructed with the conserved sequence of the *CP* gene of *Grapevine leaf roll virus* (GLRaV) and *Grapevine fan leaf virus* (GFLV) using Gateway techno1ogy (Tian and Niu 2012). In addition, a *Lily symptomless virus* and *Lily mottle virus* binary plant RNAi vector was also constructed using Gateway technology (Xu *et al.*, 2011). These studies have provided a good foundation for the control of plant viruses using the *CP* gene. Co-infection by TYLCV and ToCV is common in the field, and thus the construction of a vector against only a single virus is insufficient. In this study, the *CP* genes of TYLCV and ToCV were ligated together, and the RNAi binary expression vector was successfully constructed and transformed into a tomato.

We found that when the 15 transgenic plants were challenged by ToCV and TYLCV, the resistance of the plants to ToCV and TYLCV differed. This may be due to the number of copies of exogenous genes in the genome and the insert position. It is thus also necessary to detect the copy number and integration sites on the transgenic-positive plants.
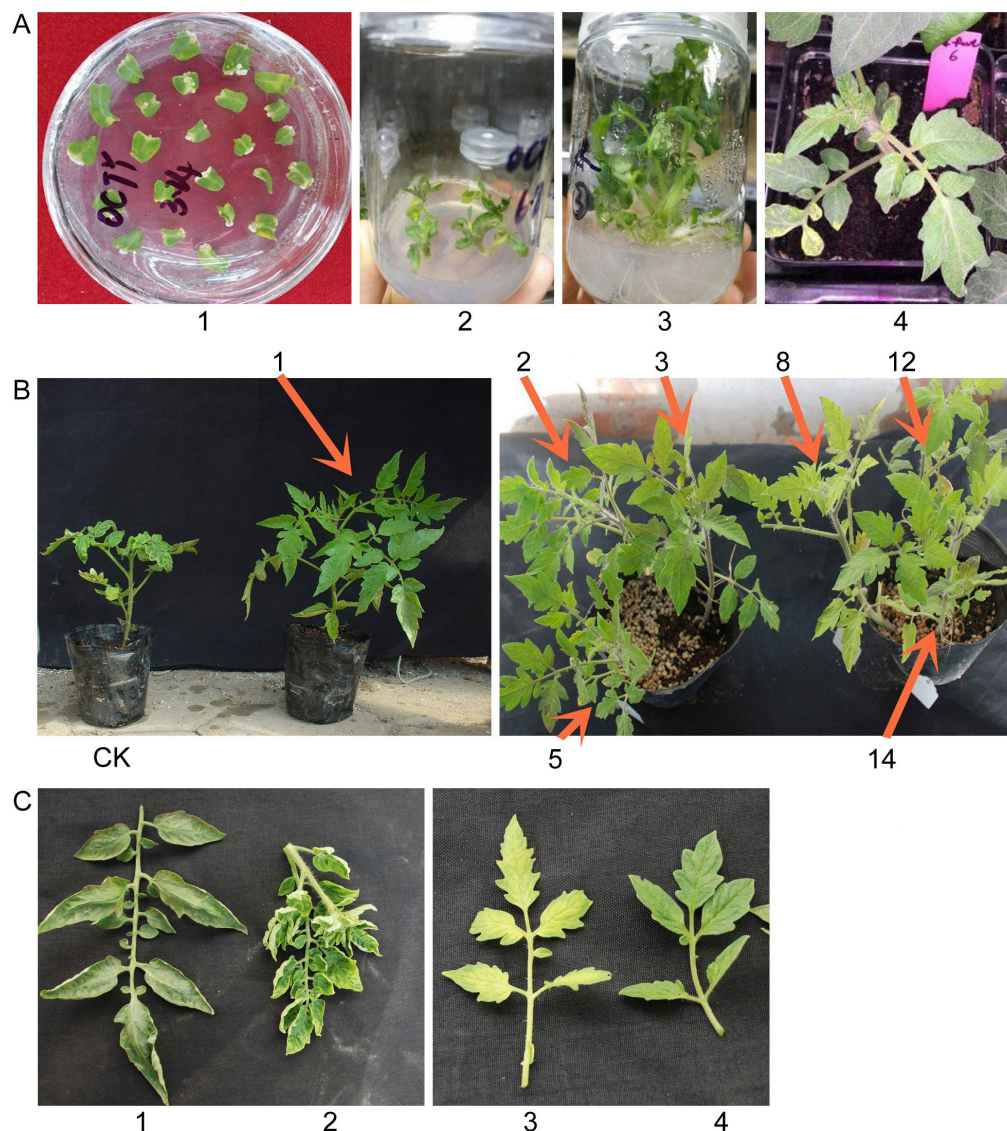

Fig. 4. *A* - Tomato transformed by *Agrobacterium*; 1 - transformed cotyledons, 2 - induction of buds; 3 - induction of roots, 4 - regenerated plants; *B* - untransformed control plant (CK), transgenic plants Nos 1, 2, 3, 5, 8, 12, and 14; *C* - new leaves of transgenic plant No. 1 (1), new leaves of untransformed control plants (2), old leaves of untransformed control plants (3), old leaves of transgenic plant No. 1 (4).

Our results provide a preliminary approach for achieving resistance against ToCV and TYLCV infection in transgenic tomato plants. The method developed for tomato transformation has a wide scope for the generation of transgenic plants using other genes of interest.

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