



Asian Journal of Plant Sciences

ISSN 1682-3974

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

The Infectious Clone Construction of Tomato Yellow Leaf Curl Virus Isolate from Tianjin

¹Feng-Mei Jin, ¹Jun Xue, ¹Zhong-Qi Liu and ²Jian-Jiang Wang

¹Tianjin Research Center of Agricultural Biotechnology, Tianjin 300192, China

²The Vegetable Research Institute of Xinjiang Shihezi, Shihezi 832000, China

Abstract: Tomato Yellow Leaf Curl Disease (TYLCD) is a serious disease that affects tomato production worldwide. Breeding resistant cultivar is an effective method to avoid this disease. In the program of breeding, the inoculation method to identify the plant resistance is particularly important. In this study, the infectious clone of TYLCV Tianjin isolate (TYLCV-TJ) was constructed. The inoculation test showed the infectious clone was effective in inoculated *Solanum lycopersicum*, typical downward yellow leaf curl was observed at 30 dpi.

Key words: Tomato yellow leaf curl virus, infectious clone, Tianjin isolate construction

INTRODUCTION

Tomato Yellow Leaf Curl Disease (TYLCD) is the most destructive disease of tomato caused by Tomato Yellow Leaf Curl Virus (TYLCV). It can lead to serious loss and have become the main limiting factor in tomato (*Solanum lycopersicum*) production throughout the world. In the late 1930s, TYLCD was first found in Israel and led to severe outbreaks on tomato plants in the 1960s in the Mediterranean basin (Antignus and Cohen, 1994), then spread to the Middle East, Southeast Asia, East Asia, Africa and many other countries and regions (Czosnek and Laterrot, 1997; Delatte *et al.*, 2005; Pico *et al.*, 1996; Polston and Anderson, 1997; Bhyan *et al.*, 2007; El-Din *et al.*, 2005). In recent years, TYLCV has become the one of the most economically damaging plant pathogens in tomato with yield losses of up to 100% in Guangxi, Guangdong, Yunnan, Hainan, Jiangsu, Anhui, Shandong and other provinces in China (Cai *et al.*, 2006; Wang *et al.*, 2006; Gui *et al.*, 2009).

Tomato Yellow Leaf Curl Virus (TYLCV) is a member of the genus *Begomovirus*, family Geminiviridae, it can be transmitted by whitefly *Bemisia tabaci* exclusively (Navot *et al.*, 1991; Hussain *et al.*, 2008; Mazyad *et al.*, 2007). In many begomoviruses, there is a bipartite genome named DNA-A and DNA-B, both of them are required for systemic infection. However, some begomoviruses have only a single genomic component of DNA-A, which is sufficient for inducing the disease (Harrison and Robinson, 1999).

So far, the research of this disease was mostly focused on the identification of pathogen and gene sequence analysis. The domesticated tomato *Solanum lycopersicum* is susceptible to the virus and the effect of conventional measures on prevention and treatment of the disease is not obvious so breeding

resistant cultivar is an effective method. The inoculation method to identify the plant resistance is particularly important in the program of breeding. It is reported TYLCV can be transmitted by whitefly but not via mechanical inoculation. But the method by whitefly has some drawbacks. For example, the number of whitefly can not be guaranteed in different years so the results are quite different (Ye *et al.*, 2009). The technology of infectious clone provides an effective mean to identify the plant resistance. The infectious clone has been successfully constructed and used for identification of disease resistance in ramie mosaic virus, sweet potato leaf curl virus and other virus (Yang, 2009).

In this study, TYLCV was identified in tomato showing leaf curl disease in Tianjin of China and an infectious clone of TYLCV Tianjin isolate (TYLCV-TJ) was constructed and its infectivity in *Solanum lycopersicum* was examined.

MATERIALS AND METHODS

Virus sources: The naturally infected tomato plants showing yellow curl leaf and stunt symptoms were collected from fields in Tianjin China.

Total plant DNA was extracted from leaves of naturally infected symptomatic plants as described (Wang and Fang, 2002).

The primers design: The degenerate primer pair CoPR and AV494 was used to amplify an approximate 575 bp fragment of begomoviral DNA-A as described (Yu, 2008). The amplified fragments were purified, cloned and sequenced. The sequence was compared by Blast to identity whether it was the isolate of TYLCV. Based on the determined sequence, the primer pair BamHIup and BamHIdown was designed and used to amplify the full

length genomic DNA-A (TYLCV-TJ), the size was about 2.7 kb. The restriction site was induced in the designed primers.

Clone of full length genome and 0.4 Å fragment of isolate TYLCV: The full length genome of isolate TYLCV-TJ was amplified using primers BamHIup and BamHIdown (Table 1) and cloned into pMD-19T vector (Takara) to produce the clone 1.0 Å TJ-pMD. The 0.4 Å fragment (573 bp) was amplified using the primers BamR and 2356F, cloned into pMD-19T vector to produce the clone 0.4 Å TJ-pMD.

Construction of express vector: The 2.7 kb band, released by digestion with *BamHI* from the recombinant vector 1.0 Å TJ-pMD, was subsequently cloned at the same restriction sites of vector 0.4 Å TJ-pMD. This clone was named 1.4 Å TJ-pMD. Insert integrity and orientation of the dimeric clones of DNA-A were confirmed by PCR using primer pair PMD19F and BamR. At the next step, the clone of 1.4 Å TJ-pMD and the plant express vector pRI 101-An was digested with *Knpl* and *Sall*. The corresponding fragments were recycled by Kit (Takara). The fragment of 1.4 Å-TJ and the linearised vector pRI 101-An were ligated with T4 ligase and then mobilized into *Escherichia coli* DH5α. The vector was named 1.4 Å TJ-pRI. The plasmid of 1.4 Å TJ-pRI was extracted from the DH5α by MiniBEST Plasmid Purification Kit Ver.2.0 (Takara). After identification by digesting with *BamHI*, the plasmid of 1.4 Å TJ-pRI mobilized into *Agrobacterium tumefaciens* strain LBA4404 by electroporation. The *Agrobacterium* cells containing the plasmid of 1.4 Å TJ-pRI were cultured in YEB medium for 48 h at 28°C.

Sequencing and analysis: The clone was sequenced by the TaKaRa Biotechnology (Dalian) Co., Ltd. Sequencing data was processing with DNAMAN Version 6.0 (Lynnon Biosoft, Quebec, Canada). Similarity research was performed using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>).

Table 1: PCR primers used for cloning and construction

Primers	Sequence(5'-3')	Position in DNA-A
BamHIup ^a	G TGGGATCCAC TTCTAAATG	150-170
BamHIIdown ^a	GGATCC CATATGCAAGACAGACTAC	131-150
2356F ^b	GGATGGAAATGTGCTGACCT	2356-2376
BamR ^b	GTGGATCCACATATTGCAA	128-148
PMD19F ^c	CGCC AAG CTT GCA TGCCG	A fragment of PMD19
BamR ^c	GTGGATCCACATATTGCAA	141-160

F: Upstream primer, R: Downstream primer, a: Amplify the full length genomic DNA, underline is *BamHI* site, The *BamHI* site in primer of BamHIIdown was introduced when designed, b: Amplify the 0.4 Å viral DNA-underline is *BamHI* site. The fragment covers the part of intergenic region (IR) (2446-148 bp), c: Detect the positive insertion, the product size is 2.3 kb

Inoculation of tomato plants with infectious clone and field test: The *Agrobacterium* cells containing the plasmid of 1.4 Å TJ-pRI were cultured in Yeast Extract-Manitol (YEM) medium (pH 6.8) including kanamycin (50 µg mL⁻¹) and rifampicin (20 µg mL⁻¹) for 48 h at 28°C before being inoculated to healthy test plants at the four to six-leaf stage. The bacteria were injected into the plant phloem with 1 mL syringes using a needle. A total of 20 plants (15 plants in flowering stage; 5 plants in flowering stage) were inoculated. Plants were kept at 25°C with 70% relative humidity and 16 h day⁻¹ light in an insect-free greenhouse and symptom development was examined daily.

Analysis of viral DNA in inoculated plants: Total nucleic acids were extracted from systemically infected tomato leaves 30 days post-inoculation (dpi) as described (Wang and Fang, 2002) and electrophoresis was performed on 1% agarose gels in TE buffer (90 mM Tris-Borate, 2 mM-EDTA). The fragment of TYLCV-TJ was amplified by PCR using the primers CoPR and AV494.

RESULTS

Construction of express vector and identification with restriction enzyme and sequencing: The plasmid of 1.4 Å TJ-pMD was digested with *Sall* and *Knpl*, then produce two fragments the one size was 3.3 kb and the other size was 2.7 kb. The fragment of 3.3 kb (2.8+0.5 = 3.3 kb) size was the 1.4 Å TJ, the 2.7 kb size fragment was the clone vector pMD-19T (2.7 kb). The fragment of 3.3 kb size was send to sequence. The sequence was same with the DNA of isolate from Tianjin (GU563330) (Jin *et al.*, 2011). The result showed that the 1.4 Å dimeric clones of TYLCV-TJ was successfully cloned into the clone vector.

The 3.3 kb size fragment was recycled by Kit and ligated to the linearised express vector pRI101-AN (Fig. 1)

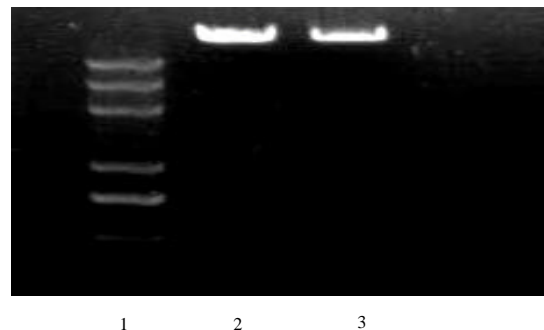


Fig. 1: The digestion of pRI101-AN by *Sall* and *Knpl*. 1: MD5000, 2-3: pRI101-AN vector

which digested with *Sall* and *Knpl*. The product transformed into DH5 α . The plasmid was purified and digested with *Sall* and *Knpl* (Fig. 2) then produced two fragments the one size was 3.3 kb and the other size was 10 kb (pRI101-AN size is 10 kb).

Agroinoculation assays with TYLCV-TJ infectious clone:
The fragment of 575 bp size was amplified (Fig. 3) from the

leaves that inoculated after 30 days using the primers CoPR and AV494. The 1.4 A clone of TYLCV-TJ was shown to be infectious in inoculated *S. lycopersicum*. In *S. lycopersicum*, typical downward yellow leaf curl was observed at 30 dpi (Fig. 4). The TYLCV symptoms were observed in the four-leaf to six-leaf stage tested host plants 30 days after inoculation. However the test plants which was inoculated in flowering stage could not be

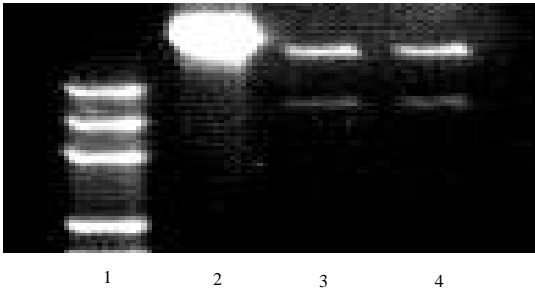


Fig. 2: The digestion of pRI101-AN-1.4A-TYLCV-TJ by *Sall* and *Knpl*. 1: M5000, 2: Plasmid, 3-4: The products of digestion

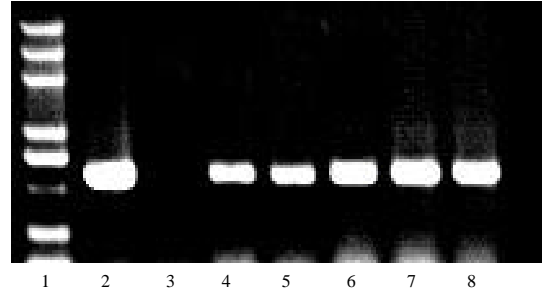


Fig. 3: PCR analysis of inoculated plants, 1: 5000 D Marker 2: CK+, 3: CK-, 3-8: Test plants



Fig. 4(a-d): The symptoms of inoculated plants, (a) The inoculated plant, (b) The healthy plant, (c) The leaf of inoculated plant and (d) The leaf of healthy plant

amplified the fragment of 575 bp size and no typical symptoms (data not published). The reason maybe is the seedling is sensitive to the infectious clone.

DISCUSSION

The method of *Agrobacterium* inoculation to identify the plant resistance to virus has been developed in recent years. After inoculating on the plant the viral DNA can copy and transport, then induce symptoms in plants. The first successful application of the method was on polio virus (poliovirus) (Ahlquist *et al.*, 1984). Since then a lot of infectious clone of plant viruses have been successfully constructed. Zhang *et al.* (2009, 2010) had constructed the infectious clones of Papaya leaf curl China virus and YLCV Shanghai isolate, respectively. Tomato leaf curl virus of eggplant and tomato leaf curl Palampur virus was also constructed (Pratap *et al.*, 2011; Malik *et al.*, 2011). The method can induce symptoms was proved. We construct infectious clone of TYLCV Tianjin isolate and inoculated tomato plant. The symptom was induced after about 1 month.

There is an Intergenic Region (IR) between the first Open Reading Fragment (ORF) in the encoding strand and complementary strand. Because the intergenic region can start viral strand and complementary strand transcript, this region is also known as bi-directional promoter. To date, a lot of *Geminivirus* promoters have been identified and used for construction of infectious clone (Guan and Zhou, 2005; Zhan *et al.*, 1991; Xu *et al.*, 1998; Haley *et al.*, 1992). It is difference from the other infectious clone (Yang, 2009; Zhang *et al.*, 2009) we insert the fragment containing IR into the end of full-length genome. The strategy was proved successful to promote the viral gene transcript and express.

TYLCV isolate in Tianjin was identified at molecular level and the genome sequence of DNA-A has been cloned and analyzed (Jin *et al.*, 2011). The results showed Tianjin isolate distinguished from the isolates in Shanghai and Shandong province. Two bases mutate in the open reading frame which encode AC2 protein and lead to the 90th amino acid of AC2 protein E G and the 104th H Y. AC2 gene code transcriptional activator protein. We speculate the two mutations may enhance the pathogenicity of Tianjin isolate. According to the previous reports the outbreak of tomato yellow leaf curl virus occurred mainly in the tropical and subtropical regions (Valizadeh *et al.*, 2011), so it is inconceivable that the virus can spread so quickly in the temperate region of China. Especially, it is found in Tianjin, a typical temperate region where the winter is relatively cold. Maybe the virus mutation can explain this.

ACKNOWLEDGMENT

This work was supported by The Production and Construction Corps support Xinjiang project. The project number is 2011AB002.

REFERENCES

- Ahlquist, P., R. French, M. Janda and L.S. Loesch-Fries, 1984. Multicomponent RNA plant virus infection derived from cloned viral cDNA. *Proc. Natl. Acad. Sci.*, 81: 7066-7070.
- Antignus, Y. and S. Cohen, 1994. Complete nucleotide sequence of an infectious clone of a mild isolate of Tomato Yellow Leaf Curl Virus (TYLCV). *Am. Phytopathol. Sci.*, 84: 707-712.
- Bhyan, S.B., M.A.H. Chowdhury, M.M. Alam and M.S. Ali, 2007. Incidence and severity of tomato yellow leaf curl virus under phytopesticidal management. *Int. J. Agric. Res.*, 2: 590-598.
- Cai, J.H., B.X. Qin, G.N. Zhu, F.X. Huang, Y.H. Chen and H. Li, 2006. The reasons and control strategies of tomato yellow leaf curl virus disease outbreak in Guangxi. *China Vegetables*, 7: 47-48.
- Czosnek, H. and H. Laterrot, 1997. A worldwide survey of tomato yellow leaf curl viruses. *Archives Virol.*, 142: 1391-1406.
- Delatte, H., D.P. Martin, F. Naze, R. Goldbach, B. Reynaud, M. Peterschmitt and J.M. Lett, 2005. South West Indian ocean islands tomato *Begomovirus* populations represent a new major monopartite begomovirus group. *J. Gen. Virol.*, 86: 1533-1542.
- El-Din, E., K.M., F.M. Abo El-Abbas, N.M.A. Aref and N.A. Abdallah, 2005. A new whitefly-transmitted geminiviruses infecting tomato plants in Egypt. *Int. J. Virol.*, 1: 29-29.
- Guan, C.P. and X.P. Zhou, 2005. The identification of Bi-directional promoter of China tomato yellow leaf curl virus. *Prog. Nat. Sci.*, 15: 937-942.
- Gui, Y.W., T.M. Zhao and M.L. Yang, 2009. PCR detection and sequence analysis of whitefly-transmitted geminiviruses virus on cultivated tomato in Shandong and Anhui provinces. *J. Agric. Sci.*, 4: 2-6.
- Haley, A., X. Zhan, K. Richardson, K. Head and B. Morris, 1992. Regulation of the activities of African cassava mosaic virus promoters by the AC1, AC2 and AC3 gene products. *Virology*, 188: 905-909.
- Harrison, B.D. and D.J. Robinson, 1999. Natural genomic and antigenic variation in whitefly-transmitted Geminiviruses (Begomoviruses). *Annu. Rev. Phytopathol.*, 37: 369-398.

- Hussain, A.F., G.H. Anfoka and D.S. Hassawi, 2008. Transformation of tomato with TYLCV gene silencing construct using optimized *Agrobacterium*-mediated protocol. *Biotechnology*, 7: 537-543.
- Jin, F.M., J. Xue, Y.H. Jia, M.X. Zhou, J.J. Wang and Z.Q. Liu, 2011. Cloning and sequence analysis of TYLCV DNA-A in Tianjin. *Acta Agric. Boreali-Sin.*, 20: 223-226.
- Malik, A.H., R.W. Briddon and S. Mansoor, 2011. Infectious clones of *Tomato leaf curl Palampur virus* with a defective DNA B and their pseudo-recombination with *Tomato leaf curl New Delhi virus*. *Virology J.*, Vol. 8.
- Mazyad, H.M., E.M. Khalil, A.A. Rezk, M.A. Abdel-Hakem and A.E. Aboul-Ata, 2007. Genetic studies on Tomato Yellow Leaf Curl begomovirus (TYLCV) resistance in Egypt: Six-population analysis. *Int. J. Virol.*, 3: 88-95.
- Navot, N., E. Pihersky, M. Zeidan, D. Amir and H. Czosneck, 1991. *Tomato yellow leaf curl virus*: A whitefly-transmitted *Geminivirus* with a single genomic component. *Virology*, 185: 151-161.
- Pico, B., M.J. Diez and F. Nuez, 1996. Viral diseases causing the greatest economic losses to the tomato crop. II. The tomato yellow leaf curl virus-a review. *Sci. Hortic.*, 67: 151-196.
- Polston, J.E. and P.K. Anderson, 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the Western hemisphere. *Plant Dis.*, 81: 1358-1369.
- Pratap, D., A.R. Kashikar and S.K. Mukherjee, 2011. Molecular characterization and infectivity of a *Tomato leaf curl New Delhi virus* variant associated with newly emerging yellow mosaic disease of eggplant in India. *Virol. J.*, Vol. 8. 10.1186/1743-422X-8-305
- Valizadeh, M., J. Valizadeh and M. Jafari, 2011. Identification, distribution and incidence of important tomato and cucurbits viruses in Southeast of Iran. *Am. J. Plant Physiol.*, 6: 242-251.
- Wang, D.S., K.Y. Kuang, H. Zhang, F.M. Dai, L.Y. Li, P.R. Qu, Y.K. Ch and C.M. Wang, 2006. Tomato yellow leaf curl virus disease occurrence and prevention in Shanghai Greenhouse. *Cultivation Plant Prot.*, 10: 25-26.
- Wang, G.L. and H.J. Fang, 2002. *Plant Genetic Engineering*. 2nd Edn., Science Press, Beijing, China.
- Xu, Y.Q., X.C. Zhan and B. Morris, 1998. The activity and regulation of bi-directional promoter in tobacco yellow dwarf virus. *J. Virol.*, 14: 68-74.
- Yang, C.X., 2009. Molecular identification of six begomoviruses in fujian and selection the interactive proteins of RaMoV NSP. Ph.D. Thesis, Fujian Agriculture and Forest University, Fujian, China.
- Ye, Q.J., Y.J. Yang, R.Q. Wang, Z.M. Li, M.Y. Ruan, G.Z. Zhou and Z.P. Yao, 2009. Progress in research on TYLCD-resistant breeding of tomato. *Sci. Agric. Sin.*, 42: 1230-1242.
- Yu, L., 2008. The research on tomato yellow leaf curl virus of Shanghai. M.Sc. Thesis, Yangzhou University, China.
- Zhan, X.C., A. Haley, K. Richardson and B. Morris, 1991. Analysis of the potential promoter sequences of African cassava mosaic virus by transient expression of the β -glucuronidase. *J. Gen. Virol.*, 72: 2849-2852.
- Zhang, H., H. Gong and X. Zhou, 2009. Molecular characterization and pathogenicity of tomato yellow leaf curl virus in China. *Virus Genes*, 39: 249-255.
- Zhang, H., X.Y. Ma, Y.J. Qian and X.P. Zhou, 2010. Molecular characterization and infectivity of *Papaya leaf curl China virus* infecting tomato in China. *J. Zhejiang Univ. Sci. B*, 11: 109-114.