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Original Contribution

EXPRESSION OF THE TOMATO YELLOW LEAF CURL VIRUS MOVEMENT PROTEIN GENE IN *ESCHERCHIA COLI*

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ABSTRACT
Tomato yellow leaf curl virus (TYLCV) is destructive pathogen of tomato (*Solanum lycopercicum*) and causes annual damage in tomato crops of many tropical and subtropical regions worldwide. Due to distribution of this pathogen in Iran, detection of this virus is necessary for controlling the damages caused by this pathogen. As enzyme-linked immunosorbent assay (ELISA) is a convenient method for the detection of viruses, preparing a source of antigen for immunization process is essential. In this study, the movement protein gene of TYLCV-Ir2 was amplified by polymerase chain reaction (PCR) and cloned in the expression vector pET26. It was transformed into *Escherchia coli* BL21 competent cells. The identity of the clone and the fidelity of the PCR product were verified by sequence analysis. The expression of recombinant TYLCV movement protein was induced by IPTG. The results indicated that the TYLCV movement protein gene was expressed in *Escherchia coli* and can be used as a source of antigen for producing antibodies against it.

Key words: TYLCV, polymerase chain reaction, gene expression, movement protein

INTRODUCTION

Tomato yellow leaf curl virus (TYLCV) is destructive pathogen of tomato *(Solanum lycopercicum)* and causes annual damage in tomato crops of many tropical and subtropical regions worldwide (1). Tomato yellow leaf curl virus (TYLCV) is a species of the genus Begomovirus in the family Geminiviridae (2). The family *Geminiviridae* is composed of very important plant viruses which cause an economically important disease in the tropical and subtropical regions. The virus particles of the family *Geminiviridae* are geminates particle of approximately 20-30 nm in size and containing genomes consisting of one or two molecules of circular single- stranded DNA (3). The *Geminiviridae* exhibits considerable diversity in terms of their genome structure, sequence, host range, tissue tropism and insect vectors. Based on these properties, geminiviruses have been classified into four genera, *Begomovirus, Curtovirus, Mastrevirus* and *Topocuvirus* (4).

The genus *Begomovirus* is the largest genus of the family which transmitted by whiteflies (*Bemisia tabaci* Genn.) and infected dicotyledonous plants (3). TYLCV causes an economically important disease in the major tomato growing regions in Iran (5). The TYLCV-Ir2 has a genome of 2776 nt which codes for two (V1 and V2) open reading frames (ORFs) and its complementary sense codes for four (C1 to C4) ORFs. The V2 ORF codes for a cell to cell movement protein (2).

During last four decades, several variations of the serological techniques have used widely by pathologists and have increased tremendously the ability of plant pathologists to detect and study plant viruses (6,7). As the coat proteins of the begomoviruses have amino acid sequence similarity and they are serologicaly related

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when tested with antibodies prepared to coat proteins of purified begomo-virues these methods have not been as common for routine detection and identification of begomoviruses (8; 9; 10). In this study, the TYLCV-Ir2 cell to cell movement protein was expressed in *E. coli* BL21 strain to prepare a source of antigen for immunization process.

MATERIALS AND METHODS

Virus, bacterial strain and plasmids:

The TYLCV-Ir2 isolate used in this study was collected from a infected tomato field of Bandar-Abbas province, south of Iran and kindly supplied by A. Azizi (accession EU085423 in NCBI gene bank). *E. coli* strain DH5α was used for cloning while *E. coil* strains BL 21 was used as hosts for the expression vector.

E. coli expression vector pET-26b(+) was chosen for the expression of movement protein in *E. coli*. The vector pET 26b(+) contains T7 *lac* promoter for higher transcription, kanamycin resistance marker for plasmid stability and pelB signal sequence for periplasmic expression.

Oligonucleotide primers

Oligonucleotides for TYLCV-Ir2 movement protein gene amplification were designed by Primer 3 software and according to the sequence of TYLCV-Ir2 (accession EU085423 in NCBI gene bank) amplifying a 350 bp fragment. Primers TYLCV-MP-F (5′CGGAATTCGATGTGGGACCCACTT C 3′) consisted of 25 nucleotides identical to nucleotides 129-145 with a *Eco*RI restriction site (underlined) at the 5′-end and TYLCV-MP-R (5′CGGTCGACTCAGGGCTTCGATACA TTC 3′) consisted of 27 nucleotides, complementary to nucleotides 457-479 with a *Sal*I restriction site (underlined) at the 5′-end, that designed according to the sequence of TYLCV-Ir2 (EU085423). Oligonucleotides were purchased from CinnaGen, Iran.

Polymerase Chain Reaction (PCR)

Total viral DNA was extracted from second apex leaf as described by Dellaporta (11), and used as templates for amplification by PCR. A 350 bp viral DNA fragment was amplified by means of primers TYLCV-MP-F and TYLCV-MP-R. The PCR amplification was performed in a 25 µl reaction volume containing 50 ng of extracted template DNA, 2.5 µl PCR buffer (10×, CinnaGen, Iran), 2 mM MgCl2, 2 mM deoxynucleotide triphosphates , 10 µM of each TYLCV-F and TYLCV-R primers, 5 µM of each 18S1 and 18S2 primers and 1 U *Taq* polymerase (CinnaGen, Iran). PCR reactions were performed in a thermocycler (Eppendorf, Germany). PCR conditions were as follows: initial denaturation at 94 ºC for 4 min, followed by 30 cycles of denaturation at 94 ºC for 30 S, annealing at 60 ºC for 45 S, extension at 72 ºC for 1 min and a final extension at 72 ºC for 10 min. The PCR reaction products were analyzed by electrophoresis on a 1% agarose gel in 1×TBE buffer (90 mM Tris-borate, 2 mM EDTA) followed by staining with ethidium bromide (0.5 µg mL^{-1}). DNA molecular weight markers (GeneRuler™ 1 kb DNA ladder, Fermentas) were used to determine the size of the amplified fragments.

The PCR products from the samples were purified using the gel extraction (QIAGEN). The purified PCR products generated from primer set were digested with *Eco*RI, and SalI.

Cloning and DNA sequencing

Restricted vector (20-50 ng) was ligated with the DNA fragment to be cloned in molar ratios 3:1, 2:1 and 1:1 (vector:insert) respectively. The ligation was carried out in a volume of 20 µl containing 50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 10mM dithiothreitol, 0.5 mM ATP and T4 DNA ligase (CinnaGen, Iran). The mixture was incubated either at room temperature for 4h or at 4°C overnight. The ligated material was used for the transformation of *E. coli* according to the standard protocol (12).

Several transformed colonies were selected randomly, and were used as template instead of extracted plasmids for screening by colony PCR (12), using the primers TYLCV-MP-F and TYLCV-MP-R. To make sure that the amplification and construction processes did not affect the base sequence of TYLCV-Ir2 movement protein gene; the desired recombinant plasmids were sequenced by Macrogen (South Korea).

Expression of the recombinant TYLCV movement protein

An overnight culture of *E. coli* cells containing the pTYLCV-MP construct was diluted 10 times into 20 ml of Luria-Bertain (LB) medium containing ampicilin (100 mg/ml) and incubated at 37°C for 1h before adding isopropyl-β−D thiogalactopyranoside (IPTG) to 0.2 mM. After further incubation for

3h and centrifuged, cell pellets were resuspended in SDS-loading buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 0.1% SDS) and proteins separated by 12% SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). After staining with Coomassie brilliant blue R250 (0.25% w/v in 40% methanol and 10% acetic acid) and destained in 12% methanol and 7% acetic acid, a band corresponding to the recombinant TYLCV movement protein was observed in the gel.

RESULTS AND DISCUSSION

Tomato yellow leaf curl disease (TYLCD) causes devastating annual damage in tomato crops of many tropical and subtropical regions of the world (1). The disease is caused by several whitefly *(Bemisia tabaci)* transmitted geminiviruses, all of which are referred to as tomato yellow leaf curl virus (TYLCV) (2). During three last decades, the Tomato yellow leaf curl disease became the most important disease of the tomato in south of Iran. Since now, there is no efficient strategy for combating against the disease, so developing of an efficient, economical, and simple method for distinguishing of infected plants is very important. Serological methods such as ELISA have proved their great worth in detection of plant disease. Toward this aim and for efficient and simple detection of infected plants, present study describes cloning and expression of MP gene of TYLCV in bacteria cell. Traditional approach for preparation of antibody against TYLCV is based on immunizing animal with purified virus. This approach resulted in antisera with relatively low specific titer, contamination with plant-derived immunogens and occurring cross reactions with healthy crude extract (13). The cloning and expression of the MP gene of TYLCV in *E. coli* and purification of the protein are proposed that help in advancement of our ability to overcome these limitations. In this study, V2 ORF of TYLCV-Ir2 was amplified by PCR from a tomato infected using primers TYLCV-MP-F and TYLCV-MP-R **(Figure 1)**. The amplified TYCV-Ir2 movement protein gene was purified from agarose gel and ligated into the *Eco*RI and *Sal*I sites of the pET-26b(+) vector and used to transform *E. coli* DH5α to check the sequence. Colony PCR screening showed that nine out of eleven transformed colonies had the insert **(Figure 2)**. The orientation of the insert in pET26b(+) was determined by restriction enzyme analysis and recombinant plasmid was designated pTYLCV-MP. The identity of the clone and the fidelity of the PCR product were verified by sequence analysis. The V2 ORF was linked in-frame to encoding sequence of pET26b(+). The resulting cDNA clone, pTYLCV-MP, was used to the expression of the movement protein product of the V2 in *E. coli* BL21 starin.

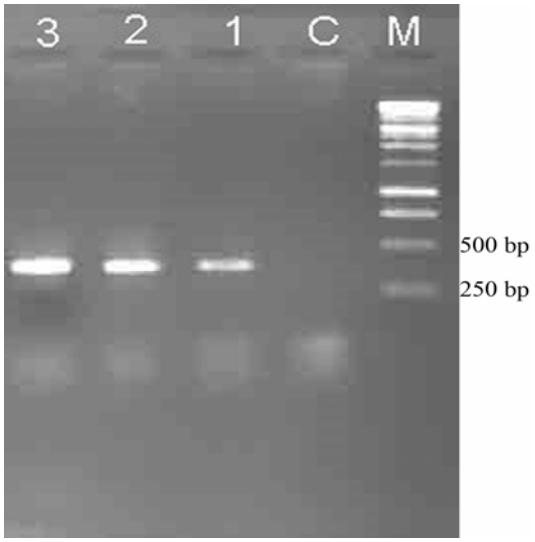

Figure 1. PCR amplification of TYLCV-Ir2 movement protein gene (V2) using TYLCV-MP-F and TYLCV-MP-R primers from inoculated tomato plant, electrophoresis on a 1% agarose gel and staining by ethidium bromide, M, SM0338 (Fermentas), size marker, C, Control, 1, 2, and 3, annealing temperature of 55, 63 and 70°C, respectively.

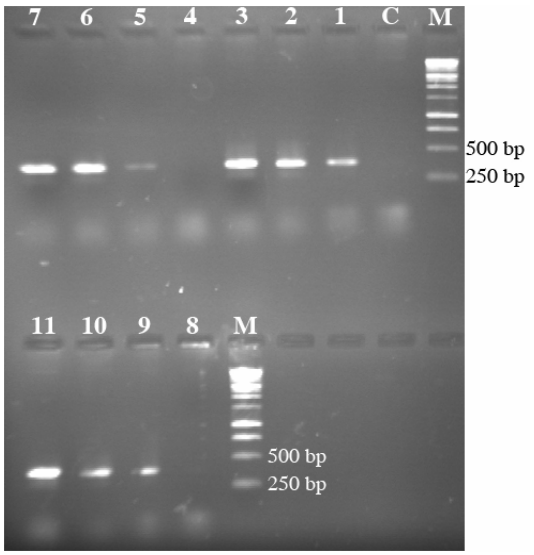

Figure 2. PCR amplification of TYLCV-Ir2 movement protein gene using TYLCV-MP-F and TYLCV-MP-R primers from transformed colonies, electrophoresis on a 1% agarose gel and staining by ethidium bromide, M, SM0338 (Fermentas), size marker, C, Control, 1-11, transformed colonies.

A variety of expression conditions were examined. Post-induction incubation temperature (27°C and 37°C), final IPTG concentration (0.2 mM and 1 mM), media (LB, 2YT, and FTB), host strain (DH5α and BL 21) and length of incubation following induction (0, 2, 3,4 and 4 h) were the varied parameters. As a result, the best expression of the TYLCV movement protein was successfully obtained at 37°C, 0.2 mM of IPTG, LB media, BL 21 host strain and three hours incubation after induction.

The total bacterial protein extract was electrophoresed on a 12% SDS-PAGE gel; a 13.3 kDa recombinant protein was detected in the induced control but was absent in the non-induced control **(Figure 3)**. The quantity of expressed protein is another challenge. In our study, we used a pET system for bacterial expression, which is one of the most powerful systems that has been developed for the cloning and expression of recombinant proteins in *E. coli* and movement protein gene was cloned under control of strong bacteriophage T7 expression signals. Expression of recombinant viral proteins were successfully used as source for producing antibodies in several plant viruses such as Barley yellow dwarf virus (14), Citrus tristeza virus (15), Odontoglossum ringsopt Tobamovirus (16), Potato mop top virus (17), Prune dwarf virus (18), Tuberose mild mosaic virus (19) and Rupestris stem pitting associated virus (20).

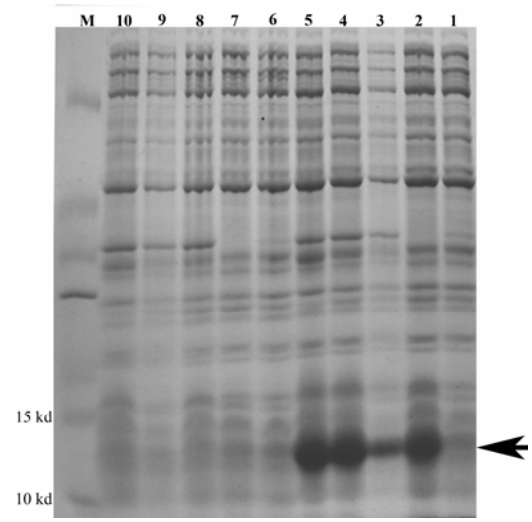

Figure 3. Expression of the TYLCV-Ir2 movement protein in *E. coli* cell line Bl21 bacterial culture of pTYLCV-MP and empty pET 27b(+) vector transformants grown and induced with IPTG at 0.2 mM final concentration. The total protein extract prepared from the cultures were electrophoresed on a 12% acrylamide and stained with 0.2% Coomassie blue. pTYLCV-MP 0-4 h incubation after induction (1-5), pETb(+) vector 0-4 h incubation after induction (6-10), M3913, molecular weight protein maker.

CONCLUSION

As the tomato yellow leaf curl disease causes destructive damage in tomato crops in south of Iran, developing of an efficient, economical, and simple method for detection of infected plants is very important. In this article, for the first time we have produced TYLCV-Ir2 recombinant movement protein expressed in *E. coli.* This recombinant protein can be used as an imunogen to produce antibody against it.

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REFERENCES

1. Moriones E., Navas-Castillo J., Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Research*, 71: 123-134, 2000.
2. Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M., Zhou, X., Geminivirus strain demarcation and nomenclature. *Archives of Virology*, 153, 783-821, 2008.
3. Cohen, S., Antignus, Y., Tomato yellow leaf curl virus, a whitefly-borne geminivirus of tomatoes. *Advance in Disease Vector Research*, 10, 259-288, 1994.
4. Rojas, M.R., Hagen, C., Lucas, W.J., Gilbertson, R.L., Exploiting chinks in the plants armor: evolution and emergence of geminiviruses. *Annual Review of Phytopathology*, 43, 361–394, 2005.
5. Azizi, A., Mozafari, J., Shams-Bakhsh, M., Phenotypic and molecular screening of tomato germplasm for resistance to Tomato yellow leaf curl virus. *Iranian Journal of Biotechnology*, 6, 199-206, 2008.
6. Hull R., Matthews' Plant Virology. 4th Ed., Academic Press, New York. 2002.
7. Agrios G. N., Plant Pathology. 5th ed. Academic Press, New York. 2005.
8. Roberts, I. M., Robinson, D. J., and Harrison, B. D., Serological relationships and genome homologies among geminiviruses. *Journal of General Virology*, 65:1723-1730, 1984.
9. Harrison, B. D., Swanson, M. M., McGrath, P., and Fargette, D., Patterns

of antigenic variation in whitefly-transmitted geminiviruses. *Annual Report of Scottish Crop Research Institute*, 1990:88-90, 1991.
10. Harrison, B. D., and Robinson, D. J., Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (Begomoviruses). *Annual Review of Phytopathology*, 37:369- 398, 1999.
11. Dellaporta, S.L., Wood, J., and Hicks, J.B., A plant DNA minipreparation: version P. *Plant Molecular Biology Report*, 1, 19-21, 1983.
12. Sambrook J, Fritsch E, and Maniatis T., Molecular Cloning: a laboratory manual, 2nd edition, Cold Spring Harbor Laboratory, NewYork, 1989.
13. Wei, W., Kakizawa, S., Jung, H.-Y., Suzuki, S., Tanaka, M., Nishigawa, H., Miyata, S., Oshima, K., Ugaki, M., Hibi, T., and Namba, S., An antibody against the SecA membrane protein of one phytoplasma reacts with those of phylogenetically different phytoplasmas. *Phytopathology, 94*, 683-686, 2004.
14. Shams-bakhsh, M. and Symons, R. H., Cloning and expression of the coat protein of *Barley yellow dwarf Virus*-PAV in *Escherichia coli. Iranian Journal of Biotechnology*, 2(2):84-89, 2004.
15. Nikolaeva O.V., Karasev A.V., Gumpf D.J., Lee R.F. and Garnsey, S. M., Production of polyclonal antisera to the coat protein of *Citrus tristeza virus* expressed in *Escherichia coli*, application for immunodiagnosis. *Phytopathology*, 85: 691-694, 1995.
16. Isomura, Y, Matumoto, A., Murayama, a., Chatani, M., Inouye, N. and Ikegami, M., Molecular cloning, sequencing and expression in *Escherichia coli* of the *Odontoglossum ringsopt Tobamovirus* coat protein gene. *Journal of General Virology*, 72(9):2247-2249, 1991.
17. Crovska, N., Moravek, T., Rosecka, P., Dedic, P. and Filigarrova, M., Production of polyclonal antibodies to recombinant coat protein of potato mop-top virus. *Journal of Phytopathology*, 151:195-200, 2003.
18. Abou- Jawdah, Y., Soubra, K. H., Shebaro, H., Cordahi, N., Kawtharani, H., Nemer, G., Maxwell, D. P., and Nakhla, M K., Immunodiagnosis of *Prune dwarf virus* using antiserum production to its recombinant coat protein. *Journal of Virological Methods*, 121:31-38, 2004.
19. Chen C.C., Hsiang T., Chiang F.L. and Chang C.A., Molecular characterization of *Tuberose mild mosaic virus* and preparation of its antiserum to the coat protein expression in bacteria. *Botanical Bulletin of Academia Science*, 43:13-20, 2002.
20. Petrovic, N., Meng, B., Ravnikar, M., Mavric, I., and Gonsalves, D., First detection of *Rupestris stem pitting associated virus* particles by antibody to a recombinant coat protein. *Plant Disease*, 87:510-514, 2003.