

CONSTRUCTION AND DEMONSTRATION OF INFECTIVITY OF THE INFECTIOUS CLONE OF THE BIPARTITE GENOME OF TOMATO LEAF CURL PALAMPUR VIRUS-IRANIAN ISOLATE

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Abstract

Among begomoviruses infecting tomato, Tomato leaf curl Palampur virus-IR (ToLCPMV-IR) is the first bipartite begomovirus reported in Iran. To demonstrate infectivity of the bipartite genome and identify the experimental host range of the ToLCPMV-IR, dimer construct for each genomic component A and B was designed and constructed. The resulting clones were separately ligated to binary vector pGreen0029 and used simultaneously with pSoup (helper plasmid) to transform *Agrobacterium tumefaciens* strain C58. Agroinoculation with the cloned DNA of the ToLCPMV-IR genome resulted in the efficient infection of several experimental hosts such as cucumber, squash, tomato and three tobacco species but not watermelon. The virus was transmitted from agroinoculated plants to healthy squash seedlings by whitefly *Bemisia tabaci*, the natural vector of ToLCPMV-IR. Based on the results of this study, we demonstrate infectivity of the bipartite cloned genome of ToLCPMV-IR. The constructed infectious clone can be used for evaluation of the reaction of host plant cultivars and analysis the genes function.

Keywords: Geminivirus, Begomovirus, Tomato leaf curl Palampur virus, Infectious clone, Cucumber.

See Persian text for figures and tables (Pages ۴۰۳-۴۰۹).

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References

- ALI, I., MALIK, H. and MANSOOR, S. 2010. First Report of Tomato leaf curl Palampur virus on Bitter Gourd in Pakistan. **Plant Dis.** 94: 276.
- BANANEJ, K., KHEYR-POUR, A., SALEKDEH, GH. and AHOONMANESH, A. 2004 Complete nucleotide sequence of Iranian tomato yellow leaf curl virus isolate: further evidence for natural recombination amongst begomoviruses. **Arch. Virol.** 149:1435–1443.
- BEHJATNIA, S.A.A., IZADPANA, K.A., DRY, I. and REZAIAN, M.A. 2003 Molecular characterization and taxonomic position of the Iranian isolate of Tomato leaf curl virus. **Iran. J. Plant Pathol.** 40: 77–94. (In Farsi With English Summary).
- BROWN, J.K., FAUQUENT, C.M., BRIDDON, R.W., ZERBINI, M., MORIONES, E. and NAVAS-CASTILLO, J. 2012. Family *Geminiviridae*. Pp. 351-373, In: A. M. Q. King, M. J. Adama, E. B. Carstens and E. J. Lefkowitz (Eds.), **Virus Taxonomy: The Ninth Report of International Committee on Taxonomy of Viruses**. Academic Press, New York.
- FAZELI, R., HEYDARNEJAD J., MASSUMI H., SHAABANIAN M. and VARSANI A. 2009. Genetic diversity and distribution of tomato-infecting begomoviruses in Iran. **Virus Genes** 38: 311–319.
- GRIMSLEY, N., HOHN, B., HOHN, T. and WALDEN, R. 1986. Agroinfection, an alternative route for viral-infection of plants by using the Ti plasmid. **Proc. Natl. Acad. Sci. U S A** 83:3282-3286.
- HELLENS, R.P., EDWARDS, E.A., LEYLAND, N.R., BEAN, S. and MULLINEAUX, P.M. 2000. pGreen: a versatile and flexible binary Ti vector for Agrobacterium-mediated plant transformation. **Plant Mol. Biol.** 42:819-832.
- HAJIMORAD, M., KHEYRPOUR, A., ALAVI, V., AHOONMANESH, A., BAHAR, M., REZAIAN, M.A. and GRONENBORN, B. 1996. Identification of whitefly transmitted tomato yellow leaf curl geminivirus from Iran and a survey of its distribution with molecular probes. **Plant Pathol.** 45: 418–425.
- HEYDARNEJAD, J., HESARI, M., MASSUMI, H. and VARSANI, A. 2013. Incidence and natural hosts of Tomato leaf curl Palampur virus in Iran. **Aust. Plant Pathol.** 42:195-203.
- HEYDARNEJAD, J., MOZAFFARI, A., MASSUMI, H., FAZELI, R., GRAY, A., MEREDITH, S., LAKAY, F., SHEPHERD, D.N., MARTIN, D.P. and VARSANI, A. 2009. Complete sequences of Tomato leaf curl Palampur virus infecting tomatoes and cucurbits in Iran. **Arch. Virol.** 154: 1015–1018.
- KUMAR, Y., HALLAN, V. and ZAIDI, A.A. 2009. Molecular characterization of a distinct bipartite begomovirus species infecting tomato in India. **Virus Genes** 37: 425–431.
- MALIK, A.H., BRIDDON R.W. and MANSOOR M. 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*. **Virol. J.** 8:173.
- MORALES, F.J. 2011. Interaction between *Bemisia tabaci*, begomoviruses, and plant species in Latin America and the Caribbean. Pp. 14-49, In: W.M.O. Thompson (Ed.), **The Whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants**, Springer Science, New York.
- PAKNIAT, A., BEHJATNIA, S.A.A., KHARAZMI, S., SHAHBAZI, M. and IZADPANA, K. 2010. Molecular Characterization and construction of an infectious clone of a new strain of Tomato yellow leaf curl virus in southern Iran. **Iran. J. Plant Pathol.** 46: 101-115. (In Farsi With English Summary).
- SAEED, M., ZAFAR, Y., RANGLES, J.W. and REZAIAN, M. A. 2007. A monopartite begomovirus-associated DNA β satellite substitutes for the DNA B of a bipartite begomovirus to permit systemic infection. **J. Gen. Virol.** 88:2881-2889.
- SHEPHERD, D.N., MARTIN, D.P., LEFEURVE, P., MONJANE, A.L., OWOR, B., RYBICKI, E.P. and VARSANI, A. 2008. A protocol for the rapid isolation of full geminivirus genomes from dried plant tissue. **J. Virol. Methods** 149: 97–102.
- ZHANG, Y. P., UYEMOTO, J. K. and KIRKPATRICK, B. C. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. **J. Virol. Methods** 71: 45-50.