The Construction of Infectious Tomato Yellow Leaf Curl Virus(TYLCV) and the Analysis of Resistance on Transgenic ...

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ABSTRACT

Tomato yellow leaf curl virus(TYLCV) is a member of the Begomovirus in the Geminiviridae family. TYLCV contains a monopartite genome and is transmitted by whitefly Bemisia argentifoli and results in severe losses in tomato productions. In this study, naturally infected tomato samples were collected from eight tomato fields in Changhua County and polymerase chain reaction (PCR) were used for the detection of TYLCV using primers pair that is specific to TYLCV genome. Five in the forty samples from Sihu and one in eight samples collected from Yongjin, Dacun, and Yuanlin, respectively, showed positive in PCR analyses. The average infection rate is 12.5%. The PCR products were cloned and sequenced. Sequences alignment with the C1 gene and the full-length genome revealed that TYLCV Sihu isolate is most closely related to a TYLCV Thailand strain, with the nucleotide sequence identity of 98%. By the concept of parasite-derived resistance (PDR), a part of TYLCV replicase gene (C1) was used to engineer into three different transgene constructs for plant transformation, which include the TYLCV C1gene arranged in a sense, antisense and double antisenses manners. These three constructs were transformated into tobacoo (Nicotiana benthamiana) by Agrobacterium tumefaciens. The putative transgenic tobacco plants were selected in Kanamycin medium and confirmed by PCR with the C1 and NPTII specific primer pairs. Ten to fifteen putative transgenic lines were obtained from the transformed of each sense, antisense, and double antisenses constructs. Since it was not possible to analyses the resistant ability of the TYLCV transgenic tobacco by mechanical inoculation of natural TYLCV. An infectious TYLCV clones was constructed. Three overlapping DNA fragment that covered the full-length of TYLCV genome of Sihu isolate were obtained from PCR amplification. The TYLCV clone contains approximately 3.5 Kb that is about 1.27 times longer than virus genome unit size were inoculated into non-transgenic tobacco by argoinoculation. The mild mosaic and less leaf curve symptom were observed 14 days post inoculation. The ability of the infectivity in the inoculated plants were further confirmed by PCR amplification and sequencing. The TYLCV infectious clone was then used as a virus source for challenge into transgenic tobacco and the resistant ability of the transgenic is under investigation. The study of the resistant ability of TYLCV transgenic tobacco can provide the information for the engineering of TYLCV transgenic tomato in the future

Keywords : Tomato yellow leaf curl virus(TYLCV) ; transformation ; Agrobacterium tumefaciens ; polymerase chain reaction

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REFERENCES

1. Ahlquist, P. (2002). RNA-dependent RNA polymerases, viruses, and RNA silencing. Science 296, 1270-1273. 2. Ait-Si-Ali, S., Guasconi, V., and Harel-Bellan, A. (2004).
[RNA interference and its possible use in cancer therapy]. Bull Cancer 91, 15-18. 3. Akad, F., Dotan, N., and Czosnek, H. (2004). Trapping of Tomato yellow leaf curl virus (TYLCV) and other plant viruses with a GroEL homologue from the whitefly Bemisia tabaci. Arch. Virol. 149, 1481-1497. 4. Akad, F., Eybishtz, A., Edelbaum, D., Gorovits, R., Dar-Issa, O., Iraki, N., and Czosnek, H. (2007). Making a friend from a foe: expressing a GroEL gene from the whitefly Bemisia tabaci in the phloem of tomato plants confers resistance to tomato yellow leaf curl virus. Arch. Virol. 7, 1323-1339. 5. Andrade, E.C., Manhani, G.G., Alfenas, P.F., Calegario, R.F., Fontes, E.P., and Zerbini, F.M. (2006). Tomato yellow spot virus, a tomato-infecting begomovirus from Brazil with a closer relationship to viruses from Sida sp., forms pseudorecombinants with begomoviruses from tomato but not from Sida. J. Gen Virol. 87, 3687-3696. 6. Antignus, Y., Lapidot, M., Hadar, D., Messika, Y., and Cohen, S. (1998). Ultravioletabsorbing screens serve as optical barriers to protect crops from virus and insect pests. J.Econ. Entomol. 91, 1401-1405. 7. Aragao, S.G., Ribeiro, L.M.G., Barros, A.C.M., Brasileiro, D.P., Maxwell, E.L., and Rech, J.C. (1998). Transgenic beans (Phaseolus vulgaris L.) engineered to express viral antisense RNAs showed delayed and attenuated symptoms to bean golden mosaic geminivirus. Molecular Breeding 4, 491-499. 8. Asad, S., Haris, W.A.A., Bashir, A., Zafar, Y., Malik, K.A., Malik, N.N., and Lichtenstein, C.P. (2003). Transgenic tobacco expressing geminiviral RNAs are resistant to the serious viral pathogen causing cotton leaf curl disease Arch. Virol. 148, 2341-2352. 9. Batuman, O., Mawassi, M., and Bar-Joseph, M. (2006). Transgenes consisting of a dsRNA of an RNAi suppressor plus the 3' UTR provide resistance to Citrus tristeza virus sequences in Nicotiana benthamiana but not in citrus. Virus Genes. 33, 319-327. 10. Bernstein, E., Denli, A.M., and Hannon, G.J. (2001). The rest is silence. RNA 7, 1509-1521. 11. Berthome, R., Teycheney, P.Y., Renou, J.P., Okada, Y., and Tepfer, M. (2000). Expression of a yeast RNase III gene in transgenic tobacco silences host nitrite reductase genes. Plant Mol. Biol. 44, 53-60. 12. Bonfim, K., Faria, J.C., Nogueira, E.O., Mendes, E.A., and Aragao, F.J. (2007). RNAi-mediated resistance to Bean golden mosaic virus in genetically engineered common bean (Phaseolus vulgaris). Mol. Plant Microbe Interact. 20, 717-726. 13. Bosco, D., Mason, G., and Accotto, G.P. (2004). TYLCSV DNA, but not infectivity, can be transovarially inherited by the progeny of the whitefly vector Bemisia tabaci (Gennadius). Virology 323, 276-283. 14. Briddon, R.W., Bedford, I.D., Tsai, J.H., and Markham, P.G. (1996). Analysis of the nucleotide sequence of the treehopper-transmitted geminivirus, tomato pseudo-curly top virus, suggests a recombinant origin. Virology 219, 387-394. 15. Brown, J.K., Lambert, G.M., Ghanim, M., Czosnek, H., and Galbraith, D.W. (2005). Nuclear DNA content of the whitefly Bemisia tabaci (Aleyrodidae: Hemiptera) estimated by flow cytometry. Bull Entomol. Res. 95, 309-312. 16. Brunetti, A., Tavazza, M., Noris, E., Tavazza, R., Caciagli, P., Ancora, G., Crespi, S., and Accotto, G.P. (1997). High expression of truncated viral Rep protein confers resistance to tomato yellow leaf curl virus in transgenic tomato plants. Mol. Plant-Microbe Interact. 10, 571-579. 17. Bull, S.E., Briddon, R.W., and Markham, P.G. (2003). Universal primers for the PCR-mediated amplification of DNA 1: a satellite-like molecule associated with begomovirus-DNA beta complexes. Mol. Biotechnol. 23, 83-86. 18. Carvalho, M.F., Turgeon, R., and Lazarowitz, S.G. (2006). The geminivirus nuclear shuttle protein NSP inhibits the activity of AtNSI, a vascular-expressed Arabidopsis acetyltransferase regulated with the sink-to-source transition. Plant Physiol. 140, 1317-1330. 19. Cohen, S., and Harpaz. (1964). Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (Bemisia tabaci Gennadius). Exp. Appl. 7, 155-166. 20. Cui, X., Tao, X., Xie, Y., Fauquet, C.M., and Zhou, X. (2004). A DNAbeta associated with Tomato yellow leaf curl China virus is required for symptom induction. J. Virol. 78, 13966-13974. 21. Czosnek, H., and Laterrot, H. (1997). A worldwide survey of tomato yellow leaf curl viruses. Arch. Virol. 142, 1391-1406. 22. Czosnek, H., Kheyr-Pour, A., Gronenborn, B., Remetz, E., Zeidan, M., Altman, A., Rabinowitch, H.D., Vidavsky, S., Kedar, N., and Gafni, Y. (1993a). Replication of tomato yellow leaf curl virus (TYLCV) DNA in agroinoculated leaf discs from selected tomato genotypes. Plant Mol. Biol. 22, 995-1005. 23. Czosnek, H., Kheyr-Pour, A., Gronenborn, B., Remetz, E., Zeidan, M., Altman, A., Rabinowitch, H.D., Vidavsky, S., Kedar, N., Gafni, Y., and et al. (1993b). Replication of tomato yellow leaf curl virus (TYLCV) DNA in agroinoculated leaf discs from selected tomato genotypes. Plant Mol. Biol. 22, 995-1005. 24. Duan, Y.P., Powell, C.A., Purcifull, D.E., Broglio, P., and Hiebert, E. (1997). Phenotypic variation in transgenic tobacco expressing mutated geminivirus movement/pathogenicity (BC1) proteins. Mol. Plant Microbe Interact. 10, 1065-1074. 25. Everett, K.D., Thao, M., Horn, M., Dyszynski, G.E., and Baumann, P. (2005). Novel chlamydiae in whiteflies and scale insects: endosymbionts 'Candidatus Fritschea bemisiae' strain Falk and 'Candidatus Fritschea eriococci' strain Elm. Int. J. Syst Evol. Microbiol. 55, 1581-1587. 26. Fagoaga, C., Lopez, C., de Mendoza, A.H., Moreno, P., Navarro, L., Flores, R., and Pena, L. (2006). Post-transcriptional gene silencing of the p23 silencing suppressor of Citrus tristeza virus confers resistance to the virus in transgenic Mexican lime. Plant Mol. Biol. 60, 153-165. 27. Fauquet, C.M., Bisaro, D.M., Briddon, R.W., Brown, J.K., Harrison, B.D., Rybicki, E.P., Stenger, D.C., and Stanley, J. (2003). Revision of taxonomic criteria for species demarcation in the family Geminiviridae, and an updated list of begomovirus species. Arch. Virol. 148, 405-421. 28. Fontenelle, M.R., Luz, D.F., Gomes, A.P., Florentino, L.H., Zerbini, F.M., and Fontes, E.P. (2007). Functional analysis of the

naturally recombinant DNA-A of the bipartite begomovirus Tomato chlorotic mottle virus. Virus Res. 126, 262-267. 29. Frischmuth, S., Wege, C., Hulser, D., and Jeske, H. (2007). The movement protein BC1 promotes redirection of the nuclear shuttle protein BV1 of Abutilon mosaic geminivirus to the plasma membrane in fission yeast. Protoplasma 230, 117-123. 30. Fulton, T.M., Chunwongse, J., and Tanksley, S.D. (1995). Microprep Protocol for Extraction of DNA from Tomato and other Herbaceous Plants. Plant Mol. Biol. Rep. 13 207-209. 31. Furner, I.J., Sheikh, M.A., and Collett, C.E. (1998). Gene silencing and homology-dependent gene silencing in Arabidopsis: genetic modifiers and DNA methylation. Genetics 149, 651-662. 32. Gafni, Y., and Epel, B.L. (2002). The role of host and viral proteins in intra and inter-cellular trafficking of geminiviruses. Physiol. Mol. Plant Pathol. 60, 231-241. 33. Gamborg, O.L. (1968). Nutr ient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50, 151-158 34. Ge, L., Zhang, J., Zhou, X., and Li, H. (2007). Genetic structure and population variability of tomato yellow leaf curl China virus. J. Virol. 81, 5902-5907. 35. Gutierrez, C. (1999). Geminivirus DNA replication. Cell Mol. Life Sci. 56, 313-329. 36. Gutierrez, C., Ramirez-Parra, E., Mar Castellano, M., Sanz-Burgos, A.P., Luque, A., and Missich, R. (2004). Geminivirus DNA replication and cell cycle interactions. Vet. Microbiol. 98, 111-119. 37. Hammond, S.M., Bernstein, E., Beach, D., and Hannon, G.J. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 404, 293-296. 38. Hehnle, S., Wege, C., and Jeske, H. (2004). Interaction of DNA with the movement proteins of geminiviruses revisited. J. Virol. 78, 7698-7706. 39. Herr, A.J., and Baulcombe, D.C. (2004). RNA silencing pathways in plants. Cold Spring Harb Symp. Quant Biol. 69, 363-370. 40. Hileman, L.C., Drea, S., Martino, G., Litt, A., and Irish, V.F. (2005). Virus-induced gene silencing is an effective tool for assaying gene function in the basal eudicot species Papaver somniferum (opium poppy). Plant J. 44, 334-341. 41. Hong, Y., Saunders, K., Hartley, M.R., and Stanley, J. (1996). Resistance to geminivirus infection by virus-induced expression of dianthin in transgenic plants. Virology 220, 119-127. 42. Ketting, R.F., Fischer, S.E., Bernstein, E., Sijen, T., Hannon, G.J., and Plasterk, R.H. (2001). Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev. 15, 2654-2659. 43. Khey-Pour, A., Bananej, K., Dafalla, G.A., Caciagli, P., Noris, E., Ahoonmanesh, A., Lecoq, H., and Gronenborn, B. (2000). Watermelon chlorotic stunt virus from the Sudan and Iran: sequence comparisons and identification of a whitefly-transmission determinant. Phytopathology 90, 629-635. 44. Kon, T., Kuwabara, K., Hidayat, S.H., and Ikegami, M. (2007). A begomovirus associated with ageratum yellow vein disease in Indonesia: evidence for natural recombination between tomato leaf curl Java virus and Ageratum yellow vein virus-[Java]. Arch. Virol. 152, 1147-1157. 45. Kong, L.J., and Hanley-Bowdoin, L. (2002). A geminivirus replication protein interacts with a protein kinase and a motor protein that display different expression patterns during plant development and infection. Plant Cell 14, 1817-1832. 46. Kunik, T., Salomon, R., Zamir, D., Navot, N., Zeidan, M., Michelson, I., Gafni, Y., and Czosnek, H. (1994). Transgenic tomato plants expressing the tomato yellow leaf curl virus capsid protein are resistant to the virus. Biotechnology (NY) 12, 500-504. 47. Leshkowitz, D., Gazit, S., Reuveni, E., Ghanim, M., Czosnek, H., McKenzie, C., Shatters, R.L., Jr., and Brown, J.K. (2006). Whitefly (Bemisia tabaci) genome project: analysis of sequenced clones from egg, instar, and adult (viruliferous and non-viruliferous) cDNA libraries. BMC Genomics. 7, 79. 48. Liang, P., Cui, J.Z., Yang, X.Q., and Gao, X.W. (2007). Effects of host plants on insecticide susceptibility and carboxylesterase activity in Bemisia tabaci biotype B and greenhouse whitefly, Trialeurodes vaporariorum. Pest Manag. Sci. 63, 365-371. 49. Mansoor, S., Briddon, R.W., Bull, S.E., Bedford, I.D., Bashir, A., Hussain, M., Saeed, M., Zafar, Y., Malik, K.A., Fauquet, C., and Markham, P.G. (2003). Cotton leaf curl disease is associated with multiple monopartite begomoviruses supported by single DNA beta. Arch. Virol. 148, 1969-1986. 50. Matsumoto, T. (1946). Tabacco disease in Formosa. Mem Fac. Agric.Taiwan Univ. 1, 1-26. 51. Miller, E.D., and Hemenway, C. (1998). History of coat protein-mediated protection. Methods Mol. Biol. 81, 25-38. 52. Moffat, A.S. (1999). Geminiviruses emerge as serious crop threat. Science 286, 1835. 53. Morilla, G., Krenz, B., Jeske, H., Bejarano, E.R., and Wege, C. (2004). Tete a tete of tomato yellow leaf curl virus and tomato yellow leaf curl sardinia virus in single nuclei. J. Virol. 78, 10715-10723. 54. Moriones, E., and Navas-Castillo, J. (2000). Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res. 71, 123-134. 55. Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plantarum 15 473-497. 56. Navas-Castillo, J., Sanchez-Campos, S., and Diaz, J.A. (1999). Tomato Yellow Leaf Curl Virus-Is Causes a Novel Disease of Common Bean and Severe Epidemics in Tomato in Spain. Plant Dis. 83, 29-32. 57. Noris, E., Accotto, G.P., Tavazza, R., Brunetti, A., Crespi, S., and Tavazza, M. (1996). Resistance to tomato yellow leaf curl geminivirus in Nicotiana benthamiana plants transformed with a truncated viral C1 gene. Virology 224, 130-138. 58. Noris, E., Vaira, A.M., Caciagli, P., Masenga, V., Gronenborn, B., and Accotto, G.P. (1998). Amino acids in the capsid protein of Tomato yellow leaf curl virus that are crucial for systemic infection, particle formation, and insect transmission. J. Virol. 72, 10050-10057. 59. Parrella, G., Scassillo, L., Crescenzi, A., and Nappo, A.G. (2006). Typing of tomato yellow leaf curl viruses and their vector in Italy. Commun Agric. Appl Biol. Sci. 71, 1229-1236. 60. Ploeg, A.T., Mathis, A., Bol, J.F., Brown, D.J., and Robinson, D.J. (1993). Susceptibility of transgenic tobacco plants expressing tobacco rattle virus coat protein to nematode-transmitted and mechanically inoculated tobacco rattle virus. J. Gen. Virol. 74 (Pt 12), 2709-2715. 61. Polston, J.E., and Anderson, P.K. (1997). The emergence of whitefly-transmitted geminiviruses in tomato in the western hemisphere. Plant Dis. 81, 1358-1369. 62. Polston, J.E., McGovern, R.J., and Brown, L.G. (1999). Introduction of tomato yellow leaf curl virus in Florida and implications for the spread of this and other geminiviruses of tomato. Plant Dis. 83, 984-988. 63. Powell-Abel, P., Nelson, R.S., De, B., Hoffmann, N., Rogers, S.G., Fraley, R.T., and Beachy, R.N. (1986). Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232, 738-743. 64. Raj, S., Singh, R., Pandey, S., and Singh, B. (2003). Association of a Geminivirus with a Leaf Curl Disease of Sunn Hemp (Crotalaria juncea) in India. Eur. J. Plant Pathol. 109, 467-470. 65. Ruiz-Medrano, R., Guevara-Gonzalez, R.G., Arguello-Astorga, G.R., Monsalve-Fonnegra, Z., Herrera-Estrella, L.R., and Rivera-Bustamante, R.F. (1999). Identification of a sequence element involved in AC2-mediated transactivation of the pepper huasteco virus coat

protein gene. Virology 253, 162-169. 66. Sanderfoot, A.A., Ingham, D.J., and Lazarowitz, S.G. (1996). A viral movement protein as a nuclear shuttle. The geminivirus BR1 movement protein contains domains essential for interaction with BL1 and nuclear localization. Plant Physiol. 110, 23-33. 67. Sanford, J.C., and Johnson, S.A. (1985). The concept of parasite-derived resistance: deriving resistance genes from the parasites own genome. J. Theor. Biol. 115, 395-405. 68. Saunders, K., Salim, N., Mali, V.R., Malathi, V.G., Briddon, R., Markham, P.G., and Stanley, J. (2002). Characterisation of Sri Lankan cassava mosaic virus and Indian cassava mosaic virus: evidence for acquisition of a DNA B component by a monopartite begomovirus. Virology 293, 63-74. 69. Settlage, S.B., Miller, A.B., and Hanley-Bowdoin, L. (1996). Interactions between geminivirus replication proteins. J. Virol. 70, 6790-6795. 70. Settlage, S.B., See, R.G., and Hanley-Bowdoin, L. (2005). Geminivirus C3 protein: replication enhancement and protein interactions. J. Virol. 79, 9885-9895. 71. Settlage, S.B., Miller, A.B., Gruissem, W., and Hanley-Bowdoin, L. (2001). Dual interaction of a geminivirus replication accessory factor with a viral replication protein and a plant cell cycle regulator. Virology 279, 570-576. 72. Steenberg, T., and Humber, R.A. (1999). Entomopathogenic potential of Verticillium and Acremonium species (Deuteromycotina: Hyphomycetes). J. Invertebr Pathol. 73, 309-314. 73. Sunter, G., and Bisaro, D.M. (1992). Transactivation of geminivirus AR1 and BR1 gene expression by the viral AL2 gene product occurs at the level of transcription. Plant Cell 4, 1321-1331. 74. Tsai, M.C., Liu, C.S., and Su, H.J. (1997). Poinsettia leaf curl, a new disease caused by a geminivirus. J. Phytophthology 145, 347-350. 75. Unseld, S., Hohnle, M., Ringel, M., and Frischmuth, T. (2001). Subcellular targeting of the coat protein of African cassava mosaic geminivirus. Virology 286, 373-383. 76. Varma, A., and Malathi, V.G. (2003). Emerging geminivirus problems: A serious threat to crop production. Ann. Appl. Biol. 142, 145-164. 77. Vaucheret, H. (2005). MicroRNA-dependent trans-acting siRNA production. Sci STKE. 2005, pe43. 78. Vincent, N., Fondong, R.V., Chowda Reddy, Lu, C., Hankoua, B., Felton, C., Czymmek, K., and Achenjang, F. (2006). The consensus N-myristoylation motif of a geminivirus AC4 protein is required for membrane binding and pathogenicity. Mol. Plant Microbe Interact. 20, 380 – 391. 79. Yang, Y., Polston, J.E., Patte, C.P., Hiebert, E., and Sherwood, T.A. (2004). Use of Tomato yellow leaf curl virus (TYLCV) Rep gene sequences to engineer TYLCV resistance in tomato. APS. 94, 490-496. 80. Yin, Q., Yang, H., Gong, Q., Wang, H., Liu, Y., Hong, Y., and Tien, P. (2001). Tomato yellow leaf curl China virus: monopartite genome organization and agroinfection of plants. Virus Res. 81, 69-76. 81. Zilberman, D., and Henikoff, S. (2004). Silencing of transposons in plant genomes: kick them when they're down. Genome Biol. 5, 249. 82. Zrachya, A., Kumar, P.P., Ramakrishnan, U., Levy, Y., Loyter, A., Arazi, T., Lapidot, M., and Gafni, Y. (2007). Production of siRNA targeted against TYLCV coat protein transcripts leads to silencing of its expression and resistance to the virus. Transgenic Res. 16, 385-398.