A field survey, phylogenic analysis and infectious clone construction of papaya leaf-distortion mosaic virus taiwan stra

許婷婷、江主惠

E-mail: 321901@mail.dyu.edu.tw

ABSTRACT

Papaya ringspot virus (PRSV) causes the most economy loss in papaya production of Taiwan. Recently, the Papaya leaf-distortion mosaic virus (PLDMV) was also found. The infection rate of both viruses on papaya are uncertain. In this study, we investigated the PRSV and PLDMV distribution in Taiwan. Total 768 samples of papaya were collected from net-houses and field in papaya growth area, including MiaoLi, Changhua, Yunlin, Nantou, Chia-yi, Tainan, Kaohsiung, Pingtung, Taitung, and Hualien. According to Enzyme-Linked Immunosorbent Assay (ELISA), 53% of samples were only infected with PRSV, 4% were only infected with PLDMV, 17% were co-infected with PRSV and PLDMV, and 30% were undetectable. Multiplex RT-PCR for the specific detection of PRSV and PLDMV was used to identify the ELISA uncertain samples. The amplified DNA fragments of 378 bp and 564 bp indicated the infection of PRSV and PLDMV, respectively. The coat protein genes of 15 PLDMV samples that amplified by RT-PCR were further cloned. Sequence analysis of these CP genes showed 97.7 to 99.7% of nucleotide identities with each other, and 94.9-99% identity with other Taiwan isolates（TW-KS, TW-DL, and TW-TD. Moreover, these CP genes showed 93.7-95.2% identity with Japan strain（J56P and J179P）and much lower sequence identity（85.1-86.4%）with J199C strain which only infected cucurbit specise. Phylogenetic tree of 15 PLDMV CP gene is shown in differen clade of TW-TD、TW-KS and Japan isolates. To determine if the PLDMV-P Taiwan isolates could infect cucumber plants, two PLDMV isolates were inoculated into Cucumis metuliferus Acc.2459 and Cucumis sativus and the infectivity of the virus was detected by ELISA and western blotting analysis. Only 8.8% of symptomless C. metuliferus showed PLDMV infection. In previous study, the full-length PLDMV DL sequence was complete except for the nucleotides near the 5' end. 5'RACE was performed to obtain the 5'end sequence. Comparison of the full-length sequence of DL isolate with PLDMV TW-KS and J56P showed 94.5% and 94.6% of nucleotide identity, respectively. When comparing with the 5'UTR region, the identity to PLDMV TW-KS and J56P was 87.4% and 84.4%, respectively. With the PLDMV-DL complete sequence, an infectious transcript can be constructed. Seven PLDMV clones, including p35S-5'426, pPL5'-2764, pPL2680NarI-4361, pPL4247-6604, pPL5761-8298, pPL8150-9810, and pPL9528-3'NotI, with overlapping DNA fragments to each other were obtained by RT-PCR. These PLDMV DNA fragments were used to replace the sequence in 35S-PRSV infectious clone. A plasmid p35SPL2711-HA-9779NotI was obtained that contained the 35S promoter sequence followed by the 5'end of PLDMV nt 1 to 2711 and the 3'end of nt 9779 to the poly(A) tail. Finally, by introducing the PLDMV DNA fragment of nt 2680 to 9810 into p35SPL2711-HA-9779NotI, the full-length genomic sequence of PLDMV will be complete.

Keywords : Papaya leaf-distortion mosaic virus (PLDMV)、infectious transcript、ELISA、multiplex RT-PCR

Table of Contents

REFERENCES

梁心玥 (2009). 應用Real-Time RT-PCR技術研究木瓜輪點病毒在木瓜主體內的分佈、移動以及增殖動態. 臺灣大學植物病理與微生物學研究所碩士論文。 廖翊廷 (2006). 台灣木瓜畸葉嵌紋病毒全長度基因體序列之解讀. 大葉大學生物產業科技學系碩士班。 Adams, M. J. &Beaudoin, F. (2005). overview and analysis of the polyprotein leavage sites in the family potyviridae. Mol. Plant Path. 6: 471-487. Ahlquist, P. &M.Janda. (1984). cDNA cloning and in vitro transcriptions of the complete brome mosaic virus grmone. Mol.Cell.Biol. 4: 2879-2882. Arazi, T., Slutsky, S. G., Shiboleth, Y. M., Wang, Y. Z., Rubinstein, M., Barak, S., Yang, J. &Gal-On, A. (2001). Engineering zucchini yellow mosaic potyvirus as a non-pathogenic vector for expression of heterologous proteins in cucurbits. J. Biotechnol. 87: 67-82. Baltimore, D. (1970). Viral RNA-dependent DNA polymerase:RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature 226: 1209-1211. Bau, H. J. (2000). Studues on the resistance of transgenic papaya conferred by the coat protein gene of papaya ringspot virus. Doctoral dissertation,National Chung Hsing University. Bau, H. J., Cheng, Y. H., Yu, T. A., Yang, J. S., Liou, P. C., Hsiao, C. H., Lin, C. Y. &Yeh, S. D. (2004). Field evaluations of transgenic papaya lines carrying the coat protein gene of Papaya ringspot virus in Taiwan. Plant Dis. 88: 594-599. Bau, H. J., Cheng, Y. H., Yu, T. A., Yang, J. S. &Yeh, S. D. (2003). Broad-Spectrum Resistance to Different Geographic Strains of Papaya ringspot virus in Coat Protein Gene Transgenic Papaya. Phytopathology 93: 112-120. Bau, H. J., Kung, Y. J., Raia, J. A. J., Chan, S. J., Chen, K. C., Chen, Y. K., Wu, H. W. &Yeh, S. D. (2008). Potential Threat of a New Pathotype of Papaya leaf distortion mosaic virus Infecting Transgenic Papaya Resistant to Papaya ringspot virus. Virology 98: 848-856. Bencharki, B., Boissinot, S., Revollon, S., Ziegler-Graff, V., Erdinger, M., Wiss, L., Dinant, S., Renard, D., Beuve, M., Lemaitre-Guillier, C. &Brault, V. (2010). Phloem protein partners of Cucurbit aphid borne yellows virus: possible involvement of phloem proteins in virus transmission by aphids. Mol Plant Microbe Interact 23: 799-810. Berger, P. H., Adams, M. J., Barnett, O. W., Brunt, A. A., Hammond, J., Hill, J. H., Jordan, R. L., Kashiwazaki, S., Rybicki, E., Spence, N., Stenger, D. C., Ohki, S. T., Uyeda, I., van Zaayen, A., Valkonen, J. &Vetten, H. J. (2005). Potyviridae. Academic Press: 819-841. Boyer, J. C. &Haenni, A. L. (1994). Infectious transcripts and cDNA clones of RNA viruses. Virology 198: 415-426. Breiteneder, H., Krebitz, M., Wiedermann, U., Wagner, B., Essl, D., Steinkellner, H., Turpen, T. H., Ebner, C., Buck, D., Niggemann, B. &Scheiner, O. (2001). Rapid production of recombinant allergens in Nicotiana benthamiana and their impact an diagnosis and therapy. Int. Arch. Allergy Immunol 124: 48-50. Bujarski, J. J. & Miller, W. A. (1992). Use of in vitro transcription to study gene expression RNA plant viruses. In Wilson TMA, Davies JW (Eds):Genetic Engineering with plant viruses. Boca Raton CRC Press,pp.: 115-147. Chen, K. C., chiang, C. H., Raja, J. A. J., Liu, F. L., Tai, C. H. & Yeh, S. D. (2007). A single amino acid of nlapro of papaya rinspot virus determines host specificity for infection of papaya. MPMI 21: 1046-1057. Cheng, Y. H., Yang, J. S. & Yeh, S. D. (1996). Efficient transformation of papaya by coat protein gene of Papaya ringspot virus mediated by Agrobacterium following liquid-phase wounding of embryogenic tissues with Carborundum. Plant Cell Rep. 16: 127-132. Chiang, C. H. & Yeh, S. D. (1997). Infctivity assays of in vitro and in vivo transcripts of papaya ringspot potyvirus. Bot. Bull. Acad.Sin. 38: 153-163. Conover, R. A. & Litz, R. E. (1978). Progress in breeding papaya with tolerance to Papaya ringspot virus. Proc. Fla. State Hortic. Soc. 91: 182-184. Dirnberger, D., Steinkellner, H., Abdennebi, L., Remy, J. J. &van de Wiel, D. (2001). Secretion of biologically active glycoforms of bovine follicle stimulating hormone in plants. Eur. J. Biochem 268: 4570-4579. Fakhfakh, H., Vilaine, F., Makni, M. &Robaglia, C. (1996). Cell-free cloning and biolistic inoculation of an infectious cDNA of potato virus Y. J. Gen. Virol. 77 : 519-523 Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.. Ferreira, S. A. &Pitz, K. Y. (2002). Virus coat protein transgenic papaya provides practical control of Papaya ringspot virus in Hawaii. Plant Dis. 86: 101-105. Fitch, M. M. M., Manshardt, R. M., Gonsalves, D., Slightom, J. L. &Sanford, J. C. (1990). Stable transformation of papaya via microprojectile bombardment. Plant Cell Rep. 8: 189-194. Flora S?鴨chez, David Mart?瀋ez-Herrera, Aguilar, I. &Ponz, F. (1998). Infectivity of turnip mosaic potyvirus cDNA clones and transcripts on the systemic host Arabidopsis thaliana and local lesion hosts. Virus Res.55: 207-219. Franconi, A., Roggero, P., Pirazzi, P., Arias, F. J., Desiderio, A., Bitti, O., Pashkoulov, D., Mattei, B., Bracci, L., Masenga, V., Milne, R. G. &Benvenuto, E. (1999). Functional expression in bacteria and plants of an scFv antibody fragment against tospoviruses.J . Immunoly Methods. 4:

189-201. Fromm, M., Taylor, L. P. &Walbot, V. (1985). Expression of genes transferred into monocot and dicot plant cells by electroporation. Proc. Natl. Acad. Sci. U S A 82(17): 5824-5828. Gal-On, A., Meiri, E., Huet, H., Hua, W. J., Raccah, B. & Gaba, V. (1995). Particle bombardment drastically increases the infectivity of cloned DNA of Zucchini yellow mosaic potyvirus. J. Gen. Virol. 76: 3223-3227. Giddings, G. (2001). Transgenic plants as protein factories. Curr. Opin. Biotechnol. 12: 450-454. Gil, F., Brun, A., Wigdorovitz, A., Catala, R., Martinez-Torrecuadrada, J. L., Casal, I., Salinas, J., Borca, M. V. &Escribano, J. M. (2001). High-yield expression of a viral peptide in transgenic plans. FEBS Lett. 488: 13-17. Grill, L. K., Palmer, K. E. & Pogue, G. P. (2005). Use of plant viruses for production of plant-derived vaccines. Crit. Rev. Plant Sci. 24: 309-323. Hammond, R. W. (2005). OBPC Symposium:Maize 2004 & beyond-plant virus-based vectors in agriculture and biotechnology. In vitro Cell DEV-PL 41: 405-410. Harpster, M. H., Townsend, J. A., Jones, J. D., Bedbrook, J. &Dunsmuir, P. (1988). Relative strengths of the 35S cauliflower mosaic virus, 1', 2', and nopaline synthase promoters in transformed tobacco sugarbeet and oilseed rape callus tissue. Mol. Gen. Genet. 212: 182-190. Hefferon, K. L. &Dugdale, B. (2003). Independent expression of Rep and RepA and their roles in regulating bean yellow dwarf virus replication. J.Gen.Virol 84: 3465-3472. Hood, E. E., Woodard, S. L. & Horn, M. E. (2002). Monoclonal antibody anufacturing in transgenic plants--myths and realities. Curr. Opin. Biotechnol. 13: 630-635. Hsu, Y. C., Yeh, T. J. & Chang, Y. C. (2005). A new combination of RT-PCR and reverse dot blot hybridization for rapid detection and identification of potyviruses. J. Virol. Methods 128: 54-60. Inouye, N. & Hakkaart, F. A. (1980). Preliminary description of a potyvirus from Vallota speciosa. EJPP 86: 265-275. Kawano, S. & Yonaha, T. (1992). The occurrence of papaya leaf distortion mosaic virus in Okinawa. Technical Bulletin of Food and Fertilizer Technology Center, Taipei, Taiwan 132: 13-23. Kiritani, K. & Su, H. J. (1999). Papaya ringspot, banana bunchy top, and citrus greening in the Asia and Pacific region: Occurrence and control strategy. Jpn. Agric. Res.Q. 33: 23-30. Krebitz, M., Wiedermann, U., Essl, D., Steinkellner, H., Wagner, B., Turpen, T. H., Ebner, C., Scheiner, O. &Breiteneder, H. (2000). Rapid production of the major birch pollen allergen Betv 1 in Nicotiana benthamiana plants and its immunological in vitro and in vivo characterization. FASEB J. 14: 1279-1288. Kumagai, M. H., Turpen, T. H., Weinzettl, N., Della-Cioppa, G., Turpen, A. M., Donson, J., Hilf, M. E., Grantham, G. L., Dawson, W. O., Chow, T. P., Piatak, M. &Grill, L. K. (1993). Rapid, high-level expression of biologically active α-trichosanthin in transfected plants by an RNA viral vector. Proc. Natl. Acad. Sci. U S A 90: 427-430. Lawrence, S. D. & Novak, N. G. (2001). A rapid method for the production and characterization of recombinant insecticidal proteins in plants. Mol. Breeding 8: 139-146. Maia, I. G., Haenni, A. L. & Bernardi, F. (1996). Potyvirus HC-Pro: a multifunctional protein J.Gen.Virol. 77: 1335-1341. Maiss, E., Timpe, U., Brisske-Rode, A., Lesemann, D. E. &Casper, R. (1992). Infectious in vivo transcripts of a plum pox potyvirus full-length cDNA clone containing the cauliflower mosaic virus 35S RNA promoter. J. Gen. Virol. 73: 709-713. Maoka, T. & Hataya, T. (2005). The complete nucleotide sequence and biotype variability of papaya leaf distortion mosaic virus. Phytopathology 95: 128-135. Maoka, T., Kashiwazaki, S., Tsuda, S., Usugi, T. &Hibino, H. (1996). Nucleotide sequence of the capsid protein gene of papaya leaf distortion mosaic potyvirus. Arch. Virol. 141: 197-204. Matsuo, K., Hong, J. S., Tabayashi, N., Ito, A., Masuta, C. &Matsumura, T. (2007). Development of Cucumber mosaic virus as a vector modifiable for different host species to produce therapeutic proteins. Planta 225: 277-286. McInerney, T. L., Brennan, F. R., Jones, T. D. & Dimmock, N. J. (1999). Analysis of the ability of five adjuvants to enhance immune responses to a chimeric plant virus displaying an HIV-1 peptide. . Vaccine 17: 1359-1368. McKinney, H. H. (1929). Mosaic diseases in the Canary Islands, West Africa and Gibraltar. J. Agric. Res. 39: 557-578. Mekako, H. U. & Nakasone, H. Y. (1975). Interspecific hybridization among six Carica species. J. Am. Soc. Hortic. Sci. 100: 294-296. Odell, J. T., Nagy, F. & Chua, N. H. (1985). Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313: 810-812. Ow, D. W., Jacobs, J. D. & Howell, S. H. (1987). Functional regions of the cauliflower mosaic virus 35S RNA promoter determined by use of the firefly luciferase gene as a reporter of promoter activity. Proc. Natl. Acad. Sci. U S A 84: 4870-4874. Petrzik, K. & Franova, J. (2006). Complete genome sequence of Daphne mosaic virus - a potyvirus from an ornamental shrub related to papaya leaf distortion mosaic virus. Arch Virol 151: 1461-1465. Pink, D. A. C. & Walkey, D. G. A. (1990). Resistance to turnip mosaic virus in white cabbage Euphytica 51: 101-107. Pogue, G. P., Lindbo, J. A., Dawson, W. O. & Turpen, T. H. (1998). Tobamovirus transient expression vectors: tools for plant biology and high-level expression of foreign proteins in plants. In Gelvin SB, Schilperoot RA (Eds): Plant Molecular Biology Manual. Kluwer, Dordrecht pp: 1-27. Purcifull, D. E., Edwardson, J. R., Hiebert, E. & Gonsalves, D. (1984). Papaya ringspot virus. Descriptions of Plant Viruses. (Commonw. Mycol. Inst., Kew, England). Sanford, J. C. & Johnson, S. A. (1985). The concept of parasite-derived resistance:deriving resistance genes from the parasites own genome. J.Theor.Biol. 115: 395-405. Scholthof, H. B., Scholthof, K. B. G. & Jackson, A. O. (1996). Plant virus gene vectors for transient expression of foreign proteins in plants. Annu. Rev. Phytopathol. 34: 299-323. Shiboleth, Y. M., Arazi, T., Wang, Y. Z. & Gal-On, A. (2001). A new approach for weed control in a cucurbit field employing an attenuated potyvirus-vector for herbicide resistance. J. Biotechnol. 92: 37-46. Takahashi, Y., Takahashi, T. & Uyeda, I. (1997). A cDNA clone to clover yellow vein potyvirus genome is highly infectious. Virus Genes 14: 235-243. Taylor, D. R. (2001). Virus disease of carica papaya in Africa their distribution, importance, and control. PVSSA 25-32. Temin, H. M. & Mizutani, S. (1970). Viral RNA-dependent DNA polymerase:RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature 226: 1211-1213. Urcuqui-Inchima, S., Haenni, A. L. & Bernardi, F. (2001). Potyvirus proteins: a wealth of functions. Virus Res. 74(1-2): 157-175. Walmsley, A. M. & Arntzen, C. J. (2000a). Plant for delivery of edible vaccines. Curr.Opin. Biotechnol. 11: 126-129. Walmsley, A. M. & Arntzen, C. J. (2000b). Plants for delivery of edible vaccines. Curr. Opin. Biotechnol. 11: 126-129. Wang, H. L., Wang, C. C., Chiu, R. J. & Sun, M. H. (1978). Preliminary study on Papaya ringspot virus in Taiwan. Plant Prot. Bull. 20: 133-140. Yonaha, T., Yonemori, S. & Tamori, M. (1976). Relation between the flight occurrence of alate aphids and the spread of papaya virus disease in the field. Okinawa Agric. 14: 7-15. Zhang, C. & Ghabrial, S. A. (2006). Development of Bean pod mottle virus-based vectors for stable protein expression and sequence-specific virus-induced

gene silencing in soybean. Virology 344: 401-411. Zhou, F. Y., Wang, M. L., Albert, H. H., Moore, P. H. & Zhu, Y. J. (2006). Efficient transient expression of human GM-CSF protein in Nicotiana benthamiana using potato virus X vector. Appl. Microbiol. Biotechnol 72: 756-762.