木瓜畸葉嵌紋病毒台灣品系病害調查、親緣性分析與具感染力轉錄體之構築

許婷婷、江主惠

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

摘 要

木瓜輪點病毒(Papaya ringsport virus，PRSV)為主要危害台灣木瓜栽種的病毒，造成嚴重的經濟損失，近年來在田間也發現有木瓜畸葉嵌紋病毒(Papaya leaf distortion mosaic virus，PLDMV)之感染，所以本研究針對 PRSV 及 PLDMV 在台灣的感染情形進行調查。採集來自10個縣市之木瓜樣本，包含來自網室及非網室材料共768個樣本，利用酵素免疫分析法進行病毒偵測，其中有374個樣本(49%)單獨感染 PRSV、27個樣本(4%)單獨感染 PLDMV、134個樣本(17%)為複合感染，剩餘233個樣本(30%)為未感染此兩種病毒之材料。當以酵素免疫分析無法判定其是否感染病毒時，利用多重引子對反轉錄聚合?○s鎖反應方式 (multiplex RT-PCR) 進行偵測，即可依增幅出來的 DNA 片段大小判定其感染病情形。另外，本研究也選殖了15個來自不同地區之 PLDMV 分離株的病毒鞘蛋白基因，利用 NCBI 進行比對，此15個 PLDMV 鞘蛋白選殖株彼此間的核?・藹　C與台灣其他分離株 (TW-DL、TW-TD及TW-WF) 比較有很高的相同度，為94.9%-99%；與日本分離株 (J56P 及J179P)有93.7-95.2%相同度，但與瓜類分離株 J199C 相比只有85.1-86.4%。根據 PLDMV 鞘蛋白核?・藹　C進行演化樹分析，則可發現本次所選殖得到的台灣分離株和 TW-TD、TW-KS 及日本之 PLDMV 歸屬於不同群組。另外，為了測試台灣 PLDMV 木瓜分離株是否也能感染瓜類，分別將來自農試所試驗田及大里之 PLDMV 接種於胡瓜及刺角瓜中，並以酵素免疫分析法及西方墨點法偵測病毒感染，結果發現 PLDMV 均無法感染胡瓜，感染刺角瓜的比例只有8.8%且無明顯病徵。之前本實驗室已將大部分 PLDMV 大里分離株 (TW-DL)的基因體解序完成，但最5'端之序列仍然未知，因此本研究再利用5'RACE 來獲得 PLDMV-DL 5'端序列，並將其全長度及5'UTR 之核?・藹　C與網路 PLDMV TW-KS 及 J56P 進行比對，結果全長度基因體之相同度約為95%，而5'UTR 序列為84%-87%。在 PLDMV 具感染力轉錄體的構築方面，選殖七個互相重疊可以涵蓋整個 PLDMV 基因體的質體，分別為 p35S-5PL426、p5PL-2764、pPL2680NarI-4361 、pPL4247-6604 、pPL5761-8298、pPL8150-9810及 pPL9528-PolyA-NotI。目前已完成 p35SPL2711-9779NotI 之構築，此質體中的35S 啟動子後面接有 PLDMV 第1到第2711個核?・薹P第9779至3'端 poly A 的 DNA 片段，只需再將第2680-9810的片段剪切黏合，即可完成 PLDMV 全長度之構築。

關鍵詞 : 木瓜畸葉嵌紋病毒、感染力轉錄體、多重引子對反轉錄聚合?連鎖反應、酵素免疫分析法

目錄

化關係分析54 圖9、以西方墨點法偵測接種在瓜類上的 PLDMV 病毒55 圖10、含 PLDMV DNA 序列的質體，以 HindIII 及 EcoRI 剪 切後之電泳圖，以確認其選殖之正確性56 表目錄 表1、構築含35S 啟動子及涵蓋全長度 PLDMV 基因體時所使 用的引子對57 表2、台灣田間網室之木瓜 PRSV 及PLDMV 病毒病害調查統 計58 表3、台灣各鄉鎮之田間非網室木瓜病害調查統計59 表4、本研究選殖之15個 PLDMV 分離株之鞘蛋白與日本分離 株及其他台灣分離株之核?・藹　C比對63 表5、台灣 isolate PLDMV-DL 與其他來源的 PLDMV 核?・ 序列相同度之比對64 表6、大里 isolate (TW-DL)及農試所試驗田分離之 PLDMV 機 械接種65

參考文獻

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.