

西瓜銀斑病毒核鞘蛋白與矮南瓜黃化嵌紋病毒及木瓜輪點病毒西瓜系統鞘蛋白轉機因西瓜之構築

李青梅、余聰安

E-mail: 9417458@mail.dyu.edu.tw

摘要

中文摘要 台灣全年氣候適合瓜類栽植，栽培面積廣大且種類繁多，其中以西瓜和甜瓜為大宗。病毒危害目前仍無任何化學藥劑可防治，因此常造成瓜類嚴重的經濟損失，其中以西瓜銀斑病毒 (Watermelon silver mottle virus ; WSMoV)、矮南瓜黃化嵌紋病毒(Zucchini yellow mosaic virus ; ZYMV)及木瓜輪點病毒西瓜系統(Papaya ringspot virus W type ; PRSV-W)為危害西瓜最嚴重之病毒種類。目前為止在西瓜轉基因植物研究方面上，仍無與抗病毒相關之報告，這可能與西瓜基因轉殖困難有關。本研究方向是希望建立一套適合本土西瓜栽培品種之組織培養再生與基因轉殖系統，分別構築具WSMoV 病毒之核鞘蛋白或ZYMV 及PRSV-W 病毒之鞘蛋白轉基因西瓜，期望能得到抗病毒的植株。種子經由不同天數的前處理後，將子葉切成約1.5 mm × 1.5 mm片段大小，與含nptII和不同病毒鞘蛋白基因之農桿菌進行基因轉殖，探討最適合基因轉殖之條件。結果顯示經3天的前處理，培植體癒合組織形成率達96.9 %，當培養在1.5 mg I-1 BA中芽體再生率可達42.9 %，並且發現子葉不同區域片段之再生率也不同，靠近胚軸的區域再生分化的能力較好，可高達78.9 %。目前經抗生素篩選經PCR分析結果，已確定轉殖成功有56個轉基因株系，分別為45個轉殖WSMoV 病毒之核鞘蛋白轉基因株系及11個轉殖ZYMV 及PRSV-W 病毒之鞘蛋白轉基因株系，在轉WSMoV 之核鞘蛋白基因轉基因西瓜方面，經南方點漬法及西方點漬法結果證實外來基因確實有併入染色體內並表現不同程度之蛋白，在ZYMV 及PRSV-W 鞘蛋白轉基因方面，經由南方點漬法證實外來基因確實有併入染色體內，而初步之溫室接種發現部份轉基因株系對ZYMV及PRSV-W病毒表現抗性。關鍵字：農桿菌基因轉殖、再生、轉基因西瓜、抗病分析

關鍵詞：農桿菌基因轉殖；再生；轉基因西瓜；抗病分析

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參考文獻

參考文獻 余聰安。2001。木瓜微體繁殖與營養器官基因轉殖。中興大學植物學系博士論文。陳冠君。2001。木瓜輪點病毒西瓜型生體外具感染力載體之構築及感染木瓜寄主專一性基因之分析。國立中興大學植物病理學系碩士論文。陳富永。1993。胡瓜嵌紋病毒鞘蛋白基因及番茄斑萎病毒核鞘蛋白基因轉型植物之構築。國立中興大學植物病理學系碩士論文。陳慶忠。2000。台灣番茄斑萎病之發生與防治。台中區農業技術專刊 155 期。趙佳鴻。2000。西瓜銀斑病毒。植物防疫專欄。第十四期。蔡竹固、陳瑞祥。2000。本省瓜類作物之重要病害及其管理。農業世界雜誌。200 : 12-19. Baulcome, D. C. 1996. Mechanisms of pathogen-derived resistance to viruses in transgenic plant. Plant Cell 8 : 1833-1944. Bald, J. G. and Samuel, G. 1931. Investigations on "spotted wilt" of tomatoes. II. Australian Commonw. Coun. Sci. Ind. Res. Org. Bull. 54. Bollag, D. M. and Edelstein, S. J. 1991. Protein Methods. (WILEYLISS, A John Wiley and Sons, INC), pp. 155. Cabrera - Ponce, J. L., Vegas - Garcia, A. and Herrera - Estrella, L 1995. Herbicide resistant transgenic papaya plants produced by an efficient particle bombardment transformation method. Plant Cell Rep 15 : 1-7. Cai, W., Goncalves, C., Tennant, P., Fermin, G., Souza, M., Sarinud, N., Jan F. J., Zhu, H. Y. and Gonsalves, D. 1999. A protocol for efficient transformation and regeneration of *Carica papaya* L. In Vitro Cell. Dev. Biol. 35 : 61-69. Chang, Y. M., Hsiao, C. H., Yang, W. Z., Hseu, S. H., Chao, Y. J. and Huang, C. H. 1987. The occurrence and distribution of five cucurbit viruses on melon and watermelon in Taiwan. J. Agri. Res. China 36 : 389-397. Chen, C. C., Shy, J. F. and Yeh, S. D 1990. Thrips transmission of tomato spotted wilt virus from watermelon. Plant Prot. Bull. 32:331-332. Compton, M. E. and Gray D. J. 1993. Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid, and tetraploid watermelon. J Am Soc Hortic Sci 118 : 151 – 157. Choi, P. S., Soli, W. Y., Kim, Y. S. Yoo, O. J. and Liu, J. R. 1994. Genetic transformation and plant regeneration of watermelon using *Agrobacterium tumefaciens*. Plant Cell Rept. 13 : 344-348. Cooper, B., Lapidots, M., Heick, J. A., Dodds, J. A. and Beachy, R. N. 1995. A defective movement protein of MVT in transgenic plant confer resistance to multiple viruses whereas the function analog increase susceptibility. Virology 206 : 307. Davis, R. F. 1986. Partial characterization of zucchini yellow mosaic virus isolated from squash in Turkey. Plant Dis. 70 : 735-738. Dong, J. Z. and Jia, S. R. 1991. High efficiency plant regeneration from cotyledons of watermelon (*Citrullus vulgaris* Schrad.). Plant Cell Rep. 9 : 559 – 562. Dougherty, W. G., Lindbo , J. A., Parks, T. D., Swaney, S., and Proebsting, W. M. 1994. RNA-mediated virus resistance in transgenic plant : Exploitation of a cellular pathway possibly involved in RNA degradation. Mol. Plant-Microbe Interact. 7 : 544-552. Ellul, P., Rios, G., Atare, A., Roig, L. A., Serrano, R. and Moreno, V. 2003. The expression of *Saccharomyces cerevisiae* HAL1 gene increases salt tolerance in transgenic watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Theor Appl Genet. 107:462-469. Fromm, M. E., Taylor, L. P. and Walbot, V. 1986. Stable transformation of maize after gene transfer by electroporation. Nature 319 : 791-793. Fulton, T. M. Chunwongse J, and Tanksley S. D. 1995. Microprep Protocol for Extraction of DNA from Tomato and other Herbaceous Plants. Plant Molecular Biology Reporter 13 : 207-209. Gamborg, O.L., Miller, R. A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell. Res. 50 : 151-158. Gelvin, S. B. 2000. Agrobacterium and Plant Genes involved in T-DNA transfer and integration. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51 : 223-256. Grant, S. R. 1999. Dissecting the mechanism of posttranscriptional gene silencing : divide and conquer. Cell 96 : 303-306. Griesbach, F. 1995. Protoplast microinjection. Plant Mol. Bio. Rep. 1 : 32-37. Grumet, R. 1994. Development of virus resistant plant via genetic engineering. Plant Breed. Rev. 12 : 47-79. Hollings, M. and Brunt, A. A. 1981. Potyvirus group. CMI/AAB Descriptions of plant viruses no. 245. Kew, Surrey. Hseu, S. H., Wang, H. L. and Huang, C. H. 1985. Identification of a zucchini yellow mosaic virus from *Cucumis sativus*. J. Agri. Res. China 34 : 87-95. Hseu, S. H., Huang, C. H., Chang, C. A., Yang, W. Z., Chang, Y. M. and Hsiao, C. H. 1987. The occurrence of five viruses in six cucurbits in Taiwan. Plant Prot. Bull. (Taiwan) 29 : 233-244. Huang, C. H., Chang, L. and Tsai, J. H. 1993. The partial characterization of melon vein-banding mosaic virus, a newly recognized virus infecting cucurbits in Taiwan. Plant Pathol. 42 : 100-107. Klein, T. M., Wolf, E. D., Wu, R. and Sanford, J. C. 1987. High velocity microprojectiles for delivery of nucleic acids into living cell. Nature 327 : 70-73. Lecoq, H., Lisa, V. and Dellavalle, G. 1983. Serological identity of Muskmelon yellow stunt and Zucchini yellow mosaic viruses. Plant Dis. 67 : 824-825. Lisa, V. and Lecoq, H. 1984. Zucchini yellow mosaic virus. CMI/AAB Description of Plant Virus, No. 282. Kew, Surrey. Lisa, V., Boccardo, G., D'Agostino, G., Dellavalle, G. and d'Aquilio, M. 1981. Characterization of a potyvirus that causes Zucchini yellow mosaic. Phytopathology 71 : 667-672. Lovisolo, O. 1981. Virus and viroid disease of cucurbits. Acta Horticulturae. 88 : 33-82. Luo, Z. and Wu, R. 1989. A simple method for the transformation of rice via the pollen-tube pathway. Plant Mol. Biol. Rep. 7: 69-77. Mahgoub, H. A., Desbiez, C., Wipf-Scheibel, C., Dafalla, G. and Lecoq, H. 1997. Characterization and occurrence of zucchini yellow mosaic virus in Sudan. Plant Pathol. 46 : 800-805. Milne, K. S., Grogan, R. G. and Kimble, K. A. 1969. Identification of viruses infecting cucurbits in California. Phytopathology 59 : 819-828. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497. Nameth, S. T., Dodds, J. A., Paulus, A. O. and Laemmlen, F. F. 1986. Cucurbit viruses of California : An ever-changing problem. Plant Dis. 70 : 8-11. Nameth, S. T., Dodds, J. A., Paulus, A. O. and Laemmlen, F. F. 1986. Cucurbit viruses of California : An ever-changing problem. Plant Dis. 70 : 8-11. Pirinc, V., Onay, V.,

Yildirim, H., Adiyaman, C., Isikalan, C. and Basaran, D. 2003. Adventitious Shoot Organogenesis and Plant Regeneration from Cotyledons of Diploid Diyarbakir Watermelon (*Citrullus lanatus* cv. "Surme") Turk J Biol. 27 : 101-105. 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 plant that express the tobacco mosaic virus coat protein gene. Science 232 : 738-743. Provvidenti, R. 1986. Viral disease of cucurbits and sources of resistance. Food & Fertilizer Technology Center. Technical bulletin. No. 93. Provvidenti, R., Gonsalves, D. and Humaydan, H. S. 1984. Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New-York, Florida, and California. Plant Dis. 68 : 443-446. Purcifull, D. E., Edwardson, J. R., Hiebert, E. and Goncalves, D. 1984. Papaya ringspot virus. CMI/AAB Description of Plant Virus. No. 292. Reddy, D. V. R. and Wightman, J. A. 1988. Tomato spotted wilt virus: Thrips transmission and control. Adv. Dis. Vector Res. 5:203-220. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Analysis and cloning of eukaryotic genomic DNA.. In Molecular cloning. 2nd. vol. 2 : 9.34-9.45 Cold Spring Harbor Laboratory Press. Sanford, J. C. and Johnston, S. A. 1985. The concept of parasite-derived resistance genes from the parasite's own genome. J. Theor. Biol. 113 : 395-405. Schenk, R. U. and Hildebrandt, A. C. 1972. Medium and Techniques for Induction and Growth of Monocotyledonous and Dicotyledonous Plant Cell Cultures, Can. J. Bot. 50 : 199-204. Srivastava, D. K., Andrianov, V. M. and Piruzian, E. S. 1991. Regeneration and genetic transformation studies in watermelon (*Citrullus vulgaris* L. cv. melitopolski). In: Prakash J, Pierik RLM (eds) Horticulture – new technologies and applications. Kluwer, Dordrecht, pp 127 – 130. Srivastava, D. K., Andrianov, V. M. and Piruzian, E. S. 1989. Tissue culture and plant regeneration of watermelon. Plant Cell Report. 8 : 300-302. Tabei, Y., Yamanaka, H. and Tsuguo, K. 1993. Adventitious shoot induction and plant regeneration from cotyledons of mature seed in watermelon (*Citrullus lanatus* L.). Plant Tissue Culture Letters. 10(3) : 235-241. Tomlinson, J. A. 1987. Epidemiology and control of virus disease of vegetables. Ann. Appl. Biol. 110 : 661-681. van den Boogaart, T., Lomonssoff, G. P. and Davies, J. W. 1998. Can we explain RNA-mediated virus resistance by Homology-dependent gene silencing? Mol. Plant-Microbe Interact. 11 : 717-723. Vaucheret, H., Christophe, B., Elmayan, T., Feuerbach, F., Godon, C., Morel, J. B., Mourrain, P., Palauqui, J. C. and Vernhettes, S. 1998. Transgene-induced gene silencing in plants. Plant J. 16 : 651-659. Wassenegger, M. and Pelisserier, T. 1998. A model for RNA-mediated gene silencing in higher plant. Plant Mol. Biol. 37 : 349-362. Yashida, K., Goto, T., Nemoto, M. and Tsuchizaki, T. 1980. Ripe viruses isolated from melon (*Cucumis melo* L.) in Hokkaido. Ann. Phytopath. Soc. Japan. 46 : 339-343. Yeh, S. D., Jan, F. J., Chiang, C. H., Doong, T. J., Chen, M. C., Chung, P. H. and Bau, H. J. 1992. Complete nucleotide sequence and genetic organization of Papaya ringspot virus RNA. J. Gen. Virol. 73 : 2531-2541. Yeh, S. D., Lin, Y. C., Cheng, Y. H., Jih, C. L., Chen, M. J. and Chen, C. C. 1992. Identification of tomato spotted wilt-like virus on watermelon in Taiwan. Plant Dis. 76:835-840. Yeh, S. D. and Chang, T. F. 1995. Nucleotide sequence of the N Gene of watermelon silver mottle virus, a proposed new member of the genus Tospovirus. Phytopathology 85:58-64. Zupan, J. R. and Zambryski, P. 1995. Transfer of T-DNA Agrobacterium to the plant cell. Plant Physiol. 107 : 1041-1047.