

抗矮南瓜黃化嵌紋病毒及木瓜輪點病毒之轉基因西瓜株之後代抗病分析

陳郁文、余聰安

E-mail: 345456@mail.dyu.edu.tw

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以防治，因此造成瓜類嚴重的經濟損失，其中以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 Type-W; PRSV-W)為危害西瓜最嚴重之病毒種類。本研究之前已構築出11個具有ZYMV-PRSV-W複合鞘蛋白之轉基因株系。由結果發現ZW10在接種後，利用ELISA、western blotting及northern blotting分析中，均偵測不到ZYMV及PRSV-W兩病毒的存在。因此，將ZW10歸類為免疫性(immunity)，且ZW10在Southern blotting分析後，發現為單一個copy併入西瓜之染色體中。ZW10接種前，可偵測出Small interfering RNA (siRNA)累積，顯示對病毒抗性機制為RNA媒介的後轉錄基因沉默(post-transcriptional gene silencing)。ZW10 自交子代利用康徽素篩選顯示符合孟德爾遺傳分離律。

關鍵詞：轉基因西瓜、病毒鞘蛋白、後轉錄基因沉默

目錄

封面內頁 簽名頁 中文摘要 英文摘要 誌謝 目錄 圖目錄 表目錄 符號說明 1. 前言 1.1 西瓜的特色及所面臨的問題 1.2 矮南瓜黃化嵌紋病毒之發生及特性 1.3 木瓜輪點病毒西瓜系統之發生及 1.4 轉基因瓜類對抗病毒之研究 1.5 轉基因西瓜之分子分析 研究 1.6 前人研究 2. 材料和方法 2.1 實驗材料 2.1.1 研究材料 2.1.2 基本培養基 2.1.3 植物生長調節劑的母液配置 2.1.4 抗生素母液之配製 2.2 實驗方法 2.2.1 轉基因植物之髮根及馴化處理 2.2.2 轉基因植物之自交育種 2.2.3 轉基因株系子代取得 2.3 轉基因子代株系之分子分析 2.3.1 植物總 DNA 抽取法 2.3.2 聚合鏈鎖反應 (Polymerase chain reaction) 2.3.3 南方點漬法 (Southern blotting) 2.3.4 植物總 RNA 抽取法 2.3.5 植物siRNA抽取法 2.3.6 北方點漬法 (Northern blotting) 2.3.7 植物siRNA偵測 2.3.8 轉基因子代接種試驗與生長觀察 3. 結果 3.1 南方點漬法分析 3.2 轉基因植物之自交育種 3.3 轉基因植株子代篩選 3.4 轉基因西瓜後代之分子分析 3.5 北方點漬法及植物SIRNA偵測 3.6 轉基因子代接種試驗與生長觀察 4. 結論 參考文獻

參考文獻

1. 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。
2. 吳鳳儀、許秀惠、黃秋雄，1994。台灣瓜類作物之病毒。瓜類作物保護技術研討會專刊:159-167。
3. 趙佳鴻、陳慶忠、黃秋雄，1993。瓜類兩種屬馬鈴薯Y 群病毒之傳播。
4. 彭瑞菊、陳昇寬、鄭安秀。2009。洋香瓜病毒病害及防治方法簡介。台南區農業專訊。69:9-14。
5. 行政院農委會統計室。2009。農業統計年報。
6. 林俊雄、陳?澍。2008。作物抗病育種之現況與展望。節能減碳與作物病害管理研討會專刊: 13-59。
7. 施純堅、韓青梅。2004。黃皮西瓜一代雜交種『澎湖5號』之育成。高雄區農業改良場研究彙報。36:36-48。
8. 陳起祥、方怡丹。2009。臺灣西瓜產業發展現況。國際西瓜產業發展與利用研討會專刊。24-30。
9. Bateson, M. and Dale, J. 1992. The nucleotide sequence of the coat protein gene and 3' untranslated region of papaya ringspot virus type W (Aust). Arch Virol. 123(1-2): 101-9.
10. Baulcome, D. C. 1996. Mechanisms of pathogen-derived resistance to viruses in transgenic plant. Plant Cell 8: 1833-1944.
11. Bonfim, K., Faria, J. C., Nogueira, E. O. P. L., Mendes, E. A and Aragao, F. J. L. 2007. RNAi-mediated resistance to Bean golden mosaic virus in genetically engineered common bean (*Phaseolus vulgaris*). Mol Plant Microbe Interact. 20 (6):717-726.
12. 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.
13. Cho, M. A., Moon, C. Y., Liu, J. R and Choi P. S. 2008. Agrobacterium mediated transformation in *Citullus lanatus*. Biol Plant. 52:365-369.
14. 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.
15. Cooper, B., Lapidot, M., Heick, J. A., Dodds, J. A. and Beachy, R. N. 1995. A defective movement protein of TMV in transgenic plant confer resistance to multiple viruses whereas the function analog increase susceptibility. Virology 206: 307.
16. Davis, R. F. 1986. Partial characterization of zucchini yellow mosaic virus isolated from squash in Turkey. Plant Dis. 70: 735-738.
17. 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.
18. 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 [*Citullus lanatus* (Thunb.) Matsum. & Nakai.]. Theor Appl Genet. 107: 462-469.
19. Faria, J. C., Albino, M. M. C., Dias, B. B. A., Cancado, L. J., Cunha, N. B., Silva, L. M., Vianna, G. R., and Aragao, F. J. L. 2006. Partial resistance to Bean golden mosaic virus in a transgenic

common bean (*Phaseolus vulgaris*) line expressing a mutant rep gene. *Plant Sci.* 171:565-571. 20. 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.

21. 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. 22. Gonsalves, D. and Ishii, M. 1980. Purification and serology of papaya ringspot virus. *Phytopathology* 70: 1028-1032. 23. Grant, S. R. 1999. Dissecting the mechanism of posttranscriptional gene silencing : divide and conquer. *Cell* 96: 303-306. 24. Grumet, R. 1994. Development of virus resistant plant via genetic engineering. *Plant Breed. Rev.* 12: 47-79. 25. Hollings, M. and Brunt, A. A. 1981. Potyvirus group. CMI/AAB Descriptions of plant viruses no. 245. Kew, Surrey. 26. Hsu, 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. 27. Hsu, S. H., Wang, H. L. and Huang, C. H. 1985. Identification of a zucchini yellow mosaic virus from *Cucumis astivus*. *J. Agri. Res. China* 34: 87-95. 28. Jan, F. J., Pang, S. Z., Tricoli, D. M. and Gonsalves, D. 2000. Evidence that resistance in squash mosaic comovirus coat protein-transgenic plants is affected by plant developmental stage and enhanced by combination of transgenes from different lines. *Journal of General Virology.* 81, 2299 – 2306. 29. Lecoq, H., Lisa, V. and Dellavalle, G. 1983. Serological identity of Muskmelon yellow stunt and Zucchini yellow mosaic viruses. *Plant Dis.* 67: 824-825.

30. Liao, L. J., Pan, I. C., Chan, Y. L., Hsu, Y. H., Chen, W. H. and Chan, M. T. 2004. Transgene silencing in phalaenopsis expressing the coat protein of Cymbidium Mosaic Virus is a manifestation of RNA-mediated resistance. *Molecular Breeding.* 13: 229-242. 31. Lisa, V. and Lecoq, H. 1984. Zucchini yellow mosaic virus. CMI/AAB Description of Plant Virus. No. 282. Kew, Surrey. 32. Lisa, V., Boccoardo, G., D'Agostino, G., Dellavalle, G. and d'Aquilio, M. 1981. Characterization of a potyvirus that causes Zucchini yellow mosaic. *Phytopathology.* 71: 667-672.

33. Lovisolo, O. 1981. Virus and viroid disease of cucurbits. *Acta Horticulturae.* 88: 33-82. 34. 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. 35. Milne, K. S., Grogan, R. G. and Kimble, K. A. 1969. Identification of viruses infecting cucurbits in California. *Phytopathology.* 59: 819-828.

36. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497. 37. 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. 38. Niu, W. Q., Lin, S. S., Reyes, J. L., Chen, K. C., Wu, H. W., Yeh, S. D., and Chua, N. H. 2006. Expression of artificial microRNAs in transgenic *Arabidopsis thaliana* confers virus resistance. *Nat Biotechnol.* 24 : 1420-1428. 39. Park, S.M., Lee, J.S., Jegal, S., Jeon, B.Y., Jung, M., Park, Y.S., Han, S.L., Shin, Y.S., Her, N.H., Lee, J.H., Lee, M.Y., Ryu, K.H., Yang, S.G., and Harn, C.H. 2005. Transgenic watermelon rootstock resistant to CGMMV (cucumber green mottle mosaic virus) infection. *Plant Cell Rep.* 24: 350-356. 40. 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. 41. Provvidenti, R. 1986. Viral disease of cucurbits and sources of resistance. Food & Fertilizer Technology Center. Technical bulletin. No. 93. 42. 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. 43. Purcifull, D. E., Edwardson, J. R., Hiebert, E. and Gonsalves, D. 1984. Papaya ringspot virus. CMI/AAB Description of Plant Virus. No. 292. 44. Quemada, H., Hostis, B., Gonsalves, D., Reardon, I. M., Heinrichson, R., Hiebert, E. L., Sieu, L. C. and Slightom, J. L. 1990. The nucleotide sequences of the 3' -terminal regions of papaya ringspot virus strains W and P. *J. Gen. Virol.* 71: 203-210. 45. 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. 46. 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.

47. 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. 48. Strange, E. B., Guner, N., Pesic-VanEsbroeck, Z. and Wehner, T. C. 2002. Screening the Watermelon Germplasm Collection for Resistance to Papaya Ringspot Virus Type-W. *Crop Sci.* 42: 1324-1330. 49. Thomson, K. G., Dietzgen, R. G., Gibbs, A. J., Tang, Y. C., Liesack, W., Teakle, D. S. and Stackerbandt, E. 1995. Identification of Zucchini yellow mosaic potyvirus by RT-PCR and analysis of sequence variability. *J. Virol. Meth.* 55: 83-96. 50. Tomlinson, J. A. 1987. Epidemiology and control of virus disease of vegetables. *Ann. Appl. Biol.* 110: 661-681. 51. van den Boogaart, T., Lomonosoff, 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. 52. Vaucheret, H., Beclin, C., and Fagard, M. 2001. Post-transcriptional gene silencing in plants. *Journal of Cell Science.* 114: 3083-3091. 53. Vaucheret, H., Christophe, B., Elmayan, T., Feuerbach, F., Godon, C., Morel, J. B., Mourrain, P., Palauqui, J. C. and Vernhetts, S. 1998. Transgene-induced gene silencing in plants. *Plant J.* 16 : 651-659. 54. Wang, C. H., Bau, H. J and Yeh, S. D. 1994. Comparison of the Nuclear Inclusion b Protein and Coat Protein Genes of Five Papaya Ringspot Virus Strains Distinct in Geographic Origin and Pathogenicity. *Phytopathology* 84:1205-1210. 55. Wassenegger, M. and Pelissier, T. 1998. A model for RNA-mediated gene silencing in higher plant. *Plant Mol. Biol.* 37: 349-362. 56. Wisler, G. C., Purcifull, D. E. and Hiebert, E. 1995. Characterization of Yashida, K., Goto, T., Nemoto, M. and Tsuchizaki, T. 1980. Rive viruses isolated from melon (*Cucumis melo* L.) in Hokkaido. *Ann. Phytopath. Soc. Japan.* 46: 339-343. 57. Yashida, K., Goto, T., Nemoto, M. and Tsuchizaki, T. 1980. Rive viruses isolated from melon (*Cucumis melo* L.) in Hokkaido. *Ann. Phytopath. Soc. Japan.* 46 : 339-343. 58. 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. 59. Yeh, S. D. and Gonsalves, D. 1984. Purification and immunological analysis of cylindrical-inclusion protein induced by papaya ringspot virus and watermelon mosaic virus 1. *Phytopathology* 74: 1273-1278. 60. Yeh, S. D. and Gonsalves, D. 1985. Translation of papaya ringspot virus RNA in vitro: detection of a possible polyprotein that is processed for capsid protein,

cylindrical-inclusion protein and amorphous-inclusion protein. *Virology* 143: 260-271. 61. Yu, T. A., Chiang, C. H., Wu, H. W., Li, C. M., Yang, C. F., Chen, J. H., Chen, Y. W and Yeh, S. D. 2011. Generation of transgenic watermelon resistant to Zucchini yellow mosaic virus and Papaya ringspot virus type W. *Plant Cell Rep.* 30: 359-371. 62. Zupan, J. R. and Zambryski, P. 1995. Transfer of T-DNA Agrobacterium to the plant cell. *Plant Physiol.* 107: 1041-1047.