Construction of transgenic watermelons expressing the nucleocapsid protein of Watermelon Silver Mottle Virus or the doub

李青梅、余聰安

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

ABSTRACT

ABSTRACT Watermelon is an economically important crop of the tropic and subtropic regions. Virus disease often causes serious economic loss of watermelon and there is still no chemicals for prevention and therapy of virus damage. Watermelon silver mottle virus (WSMoV), Zuchini yellow mosaic virus (ZYMV) and Type W stain of Papaya ringspot virus (PRSV-W) are the most hazardous species among all kinds of viruses infected in cucurbit. There is still no paper about transgenic watermelon related to anti-virus. It might be due to the difficulty of watermelon gene transfer. This investigation tried to set up a system that is fit for our local watermelon regeneration and an approach of Agrobacterium-mediated transformation of watermelon carrying with NP gene of WSMoV or double fusion CP gene of ZYMV and PRSV-W. Seed is through pretreatment for different days, and cotyledons are cut into 1.5 mm × 1.5 mm segments as explants. The explants co-cultured with Agrobacterium cell for 4 days and then transferred to the selection medium. The results display the callus of growing explants reaches 96.9 % when the seed through pretreatment for 3 days. The bud-regeneration rate can reach 42.9 % when explants develops in SH basal medium containing 1.5 mg l-1 BA, we also find the bud-regeneration ability near hypocotyls is better and reaches 78.9 %. Eleven putative ZYMV-PRSV-CP transgenic lines and 45 putative WSMoV NP transgenic lines were obtained. PCR and Southern blot analysis confirmed that the foreign gene was incorporated into the genomic DNA of the regenatants. Western blot analysis indicated the WSMoV NP transgenic lines displays N protein in different levels. Three ZYMV-PRSV-CP transgenic lines expressed resistance to infection by both viruses under greenhouse conditions. Key Words: Agrobacterium-mediated transformation, regeneration, transgenic watermelon, resistant assay

Keywords : Agrobacterium-mediated transformation ; regeneration ; transgenic watermelon ; resistant assay

Table of Contents

REFERENCES

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 trandgenic 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., Gonalves, 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., Hsaio, C. H., Yang, W. Z., Hseu, S. H., Chao, Y. J. and Huang, C. H. 1987. The occurrence and distribution of five cucurbit vir uses 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 intransgenic 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 astivus. 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 vai 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 desease 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 & Tertilizer 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 Gonaslves, 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. 2 nd.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. Adventitous 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 gnen 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 Vernhetts, 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. Rive 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 mumber of the genes 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.