抗真菌轉機因甜瓜之構築Construction of transgenic melon(Cucumis melo L.cv.Sliver Light)

張燕玲、余聰安

E-mail: 9417457@mail.dyu.edu.tw

摘 要

中文摘要 甜瓜 (Cucumis melo L.)，屬於葫蘆科 (Cucurbitaceae) 蔓性一年生草本作物，原產地起源於亞洲近中東地區。台灣熱帶及亞熱帶的氣候下非常適合種植，由於全年盛行種植的結果，病蟲害的發生率也相對提高，其中微生物引起的病害中80 %以上是經由真菌所引起，一般藉由噴灑大量的農藥或抑菌劑來克服此病害，因考慮到農藥對環境的危害及殘留的疑慮，希望能藉由遺傳工程的方式，將抗真菌蛋白基因導入植物體內，期望可達到防治效果。 抗真菌蛋白基因Bo-AFP3-HB-GFP與Cp-AFP3-HB-GFP，由中央研究院 蕭介夫博士提供，經胺基酸序列比對，發現其結構類似於植物防禦素(plant defensins)中的AFP3，屬於cysteine-rich protein，包括4個雙硫鍵，由青花菜及木瓜中篩選出，故命名為Bo-AFP3-HB-GFP及Cp-AFP3-HB-GFP，比較推演出來的胺基酸序列，發現兩者具有95.92 %的相似性。 本研究主要將Bo-AFP3-HB-GFP及Cp-AFP3-HB-GFP基因以農桿菌轉殖的方式，將此抗真菌蛋白基因送入植物體內，並進行相關的抗菌分析，期望抗真菌蛋白基因在甜瓜中大量表現，減低真菌所帶來的危害，目前已構築出23株來自Bo-AFP3-HB-GFP的轉基因甜瓜及6株來自Cp-AFP3-HB-GFP的轉基因甜瓜，經由聚合酵素鏈鎖反應、南方點漬法及反轉錄聚合酵素鏈鎖反應證明抗真菌蛋白基因確實併入甜瓜染色體中。 關鍵字：甜瓜，抗真菌蛋白，農桿菌，基因轉殖

關鍵詞 : 甜瓜 ; 抗真菌蛋白 ; 農桿菌 ; 基因轉殖

目錄

參考文獻

參考文獻 行政院農委會。民93。農業統計之農作物產銷統計。 http://www.coa.gov.tw/8/195/202/894/894.html 余聰安。2001。木瓜微體繁殖與營養器官基因轉殖。國立中興大學植物學系博士論文。 林宗俊。2000。丁香及其主成分防治甘藍苗立枯病的功效。國立中興大學植物病理學系碩士論文。 陳彥良。2002。木瓜之抗真菌蛋白基因。私立東海大學食品科學系。 廖家德 (1994) 臺灣立枯絲核菌(Rhizoctoniasolani Kuhn) 第四融合群菌株質體狀去氧核醣核酸的歧異性及其核酸定序。國立中興大學植物病理學研究所碩士論文。 賴宣妤。2002。青花菜之抗真菌蛋白基因。私立東海大學食品科學系。 蔡尚光。1995。設施洋香瓜與胡瓜的高品質生產。P14-23。淑馨出版社。 蔡竹固譯。1997。瓜類作物病害的田間診斷。嘉農植保 8:6-11。 蔡雲鵬編。1991。台灣植物病害名彙 修訂3版。頁419-420。中華植物保護學會,中華民國植物病理學會印。604頁。 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。 薛聰賢編著。2000。蔬香果樂。頁71-72。 蘇宗振。1999。植物基因轉殖之研究。科學農業47 (3, 4) : 112 – 119 。 Ainsworth, G: (ed.). 1973. The fungi, an advance treatise. Academic Press, New York, N.Y. Akasaka - Kennedy, Y., Tomita, K. O. and Ezura, H. 2004 . Efficient plant regeneration and Agrobacterium - mediated transformation via somatic embryogenesis in melon (Cucumis melo L.). Plant Science 166 : 763-769. Almeida, M.S., K. M. Cabral, R. B. Zingali, and E. Kurtenbach. (2000). Characterization of two novel defense peptides from pea (Pisum sativum) seeds. Arch. Biochem. Biophys. 378, 278 – 286. Benhamou, N., Broglie., K., Chet, I., and Broglie, R. 1993. Cytology of infection of 35S-bean chitinase transgenic canola plants by Rhizoctonia solani； cytochemical aspects of chitin breakdown in vivo. Plant J. 4:295-305. Broekaert, W. F., Terras, F.R., Cammue, B.P. and Osborn, RW. 1995. Plant defensins: novel antimicrobial peptides as components of host defense system. Plant Physiol. 108:1353 – 1358. Broglie, K., Chet, I., Holiday, M., Cressman,R., Biddle, P., Knowlton, S., Mauvais, C. J., and Broglie, R. 1991.Transgenic plants with enhanced resistance to the fungal pathogen Rhyzoctonia solani. Science 254:1194-1197. Bull, J., Mauch, F., Hertig, C., Regmann, G. and Dudler, R. 1992. Sequence and expression of a wheat gene that encodes a novel protein associated with pathogen defense. Mol. Plant Microbe Interact. 5: 516 – 519. Cabrera-Ponce, J. S., 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. Coca, M., Bortolotti, C., Rufat, M., Penas, G.,Eritja,R.,Tharreau,D.,Martinez del Pozo,A.,Messeguer,J. and San Segundo, B. 2004. Transgenic rice plants expressing the antifungal AFP protein from Aspergillus giganteus show enhanced resistance to the rice blast fungus Magnaporthe grisea. Plant Molecular Biology 54:245-259. Durner, J., Shah, J. and Klessig, D. F. 1997. Salicylic acid and disease resistance in plants. Trends Plant Sci 2: 266-274. Ezura H. 2001. Genetic engineering of melon (Cucumis melo L.). Plant Biotechnology. 18 : 1-6. Fang, G. and Grumet, R. 1990. Agtobacterium tumefaciens mediated transformation and regeneration of muskmelon plants. Plant Cell Rep. 9 : 160-164. Fant, F., W. Vranken, W. Broekaert, and F. Borremans. (1998). Determination of the three-dimensional solution structure of Raphanus sativus antifungal protein 1 by 1H NMR. J. Mol. Biol. 279, 257 – 270. 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 SD. 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. Grenier, J., Potvin, C. and Asselin, A. 1993. Barley pathogenesis-related proteins with fungal cell wall lytic activity inhibit the growth of yeasts. Plant Physiol. 103: 1277 – 1283. Griesbach, R.J. 1983. Protoplast microinjection. Plant Mol.Biol.Rep. 1 : 32-37. Gun Lee, D., S. Y. Shin, C. Y. Maeng, Z. Z. Jin, K. L. Kim, and K. S. Hahm. (1999). Isolation and characterization of a novel antifungal peptide from Aspergillus niger. Biochem. Biophys. Res. Commun. 263, 646 – 651. Guis, M., Amor, M. B., Latche, A., Pech, J-C., and Roustan J-P. 2000. A reliable system for the transformation of cantaloupe charentais melon (Cucumis melo L. var. cantalupensis) leading to majority of diploid regenerants. Scientia Horticulturae 84 : 91-99. Hopkins、W. L. 1996. Global Fungicide Directory. 148pp. AG Chem Information Services. USA. Howie, W., Joe, L., Newbigin, E., Suslow, T., and Dunsmuir, P. 1994. Transgenic tobacco plants which express the chiA gene from Serratia marcescens have enhanced tolerance to Rhizoctonia solani. Transgenic Res. 3: 90-98. Kitajima, S. and Sato, F. 1999. Plant pathogenesis-related proteins: molecular Klein, T. M., Wolf, E. D., Wu, R. and Sanford, J. C. 1987. High velocity microprojectiles for delivery of nucleic acids into living cells. Nature 327: 70-73. Klement, Z. 1982. Hypersensitivity. In phytopathogenic prokaryotes, volume 2 (Mount MS and Lacy GH) New York: Academic Press, pp. 149-177. Kombrink, E. and Somssich, I. E. 1995. Defence responses of plants to pathogens. Adv Bot Res 21: 1-34. Kragh, K.M., J. E. Nielsen, K. K. Nielsen, S. Dreboldt, and J. D. Mikkelsen. (1995.). Characterization and localization of new antifungal cysteine-rich proteins from Beta vulgaris. Mol. Plant Microbe Interact. 8, 424 – 434. Kristensen, A.K., J. Brunsted, J. W. Nielsen, J. D. Mikkelsen, P. Roepstorff, and K. K. Nielsen. (1999). Processing, disulfide pattern, and biological activity of a sugar beet defensin, AX2, expressed in Pichia pastoris. Protein Expr. Purif. 16, 377 – 387. Ku, H.M & Tsay, H.S. 1994a .Effect of medium composition on the vitrification of carnation plantlets cultureed in vitro. Jour.Agric.Res.China. 43 (1) : 51-62. Ku, H.M & Tsay, H.S. 1994b .Influence of subculture generation on the vitrification of carnation plantlets culture in vitro. Jour.Agric.Res.China. 43 (3) : 308-319. Lacadena, J., A. Martinez del Poxo, M. Gasset, B. Patino, R. Campos-Olivas, C. Vazquez, A. Martinez-Ruiz, J. M. Mancheno, M. Onaderra, and, and Gavilanes., J.G. (1995). Characterization of the antifungal protein secreted by the mould Aspergillus giganteus. Arch. Biochem. Biophys. 324, 273 – 281. Lamberty, M., S. Ades, S. Uttenweiler-Joseph, G. Brookhart, D. Bushey, J. A. Hoffmann, and P. Bulet. (1999). Insect immunity. Isolation from the lepidopteran Heliothis

virescens of a novel insect defensin with potent antifungal activity. J. Biol. Chem. 274, 9320 – 9326. Landon, C., Pajon, A., Vovelle, F. and Sodano, P. 2000. The active site of drosomycin, a small insect antifungal protein, delineated by comparison with the modeled structure of Rs-AFP2, a plant antifungal protein. J. Pept. Res. 56:231 – 238. Lin, W., Anuratha, C. S., Datta, K., Potrykus, I., Muthukrishnan, S., and Datta, S.K. 1995. Genetic engineering of rice for resistance to sheath blight. Bio/Technology 13: 686-691. Lipke, P. and Ovalle, R. 1998. Yeast cell walls: new structures, new challenges. J. Bacteriol. 180: 3735 – 3740. Liu, Y., J. Luo, C. Xu, F. Ren, C. Peng, G. Wu, and J. Zhao. (2000). Purification, characterization, and molecular cloning of the gene of a seedspecific antimicrobial protein from pokeweed. Plant Physiol. 122, 1015 – 1024. Lucca, D. A. J. and Walsh, T. J. 1999. Antifungal peptides: novel therapeutic compounds against emerging pathogens. Antimicrob. Agents Chemother. 43: 1 – 11. 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. Mehdy, M. C. 1994. Active oxygen species in plant defense against pathogens. Plant Physiol 105: 467-47. Martinez-Ruiz, A., Martinez del Pozo, A., Lacadena, J., M.Mancheno,J., Onaderra,M. and G.Gavilanes, J. 1997. Characterization of a nature larger form of the antifungal protein(AFP) from Aspergillus giganteus. Biochemica et Biophysica Acta 1340:81-87. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497. Nawrath, C. and Metraux, J. 1999. Salicylic acid induction-deficient mutants of Arabidopsis express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. Plant Cell 11: 1393 – 1404. Nauerby, B., Billing, K. and Wyndaele, R. 1997. Influence of the antibiotic timentin on plant regeneration compared to carbenicillin and cefotaxime in concentrations suitable for elimination of Agrobacterium tumefaciens. Plant Sci. 123 : 169-177. Ryals, J., Uknes, S. and Ward, E. 1994. Systemic acquired resistance. Plant Physiol 104: 1109-1112. Ryals, J.A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H. Y. and Hunt, M.D. 1996. Systemic acquired resistance. Plant Cell 8: 1809-1819. Salzman, R. A., Tikhonova, I., Bordelon, B. P. P., Hasegawa, M. and Bressan, R. A. 1998. Coordinate accumulation of antifungal proteins and hexoses constitutes adevelopmentally controlled defense response during fruit ripening in grape. Plant Physiol. 117: 465 – 472. Sambrook, J., Fritsch E.F., Maniatis T. (1989). Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press. New York. Segura, A., Moreno, M., Molina, A. and Garcia-Olmedo, F. 1998. Novel defensin subfamily from spinach (Spinacia oleracea). FEBS Lett. 435: 159 – 162. Selitrennikoff, C. P. 2001. Antifungal protein. Applied and Environmental MicroBiology. p: 2883-2894. Terras, F. R., Eggermont, K., Kovaleva, V., Raikhel, N. V., Osborn, R. W., Kester, A., Rees, S. B., Torrekens, S., Van Leuven, F. and Vanderleyden, J. 1995.Small cysteine-rich antifungal proteins from radish: their role in host defense. Plant Cell 7: 573 – 588. Shao, F., Y. M. Xiong, Q. Z. Huang, C. G. Wang, R. H. Zhu, and D. C. Wang. (1999). A new antifungal peptide from the seeds of Phytolacca americana: characterization, amino acid sequence and cDNA cloning. Biochim. Biophys. Acta 1430, 262 – 268. Sneh, B., Burbee, L., and Ogoshi, A. 1991. Identification of Rhizoctonia species.133pp. ASP press. Terras, F.R.G., Eggermont,K., Kovaleva,V., Raikhel,N.V., Osborn,R.W., Kester,A., Rees,S.B., Torrekens,S., van Leuven,F., Vanderleyden,J., Cammue,B.P.A. and Broekaert,W.F. (1995). Small cysteine-rich antifungal proteins from radish: their role in host defense. Plant Cell 7 (5), 573-588. Thevissen, K., Ghazi, A., Samblanx, D. G. W., Brownlee, C., Osborn, R. W. and Broekaert, W. F. 1996. Fungal membrane responses induced by plant defensins and thionins. J. Biol. Chem. 271: 5018 – 15025. Thevissen, K., Osborn, R. W., Acland, D. P. and Broekaert, W. F. 1997. Specific, high affinity binding sites for an antifungal plant defensin on Neurospora crassa hyphae and microsomal membranes. Biol. Chem. 272: 32176 – 32181. Thevissen, K., Osborn, R. W., Acland, D. P. and Broekaert, W. F. 2000. Specific binding sites for an antifungal plant defensin from Dahlia (Dahlia merckii) on fungal cells are required for antifungal activity. Mol. Plant Microbe Interact. 13:54 – 61. Thevissen, K., Terras, F. T. and Broekaert, W. F. 1999. Permeabilization of fungal membranes by plant defensins inhibits fungal growth. Appl. Environ. Microbiol. 65: 5451 – 5458. Tulasi, R.B., and S. K. Nadimpalli. (1997). Purification of a-mannosidase activity from Indian lablab beans. Biochem. Mol. Biol. Int. 41, 925 – 931. Tzfira, T & Citovsky, V. 2002 . Partners-in-infection:host proteins involved in the transformation of plant cells by Agrobacterium. Trends in Cell Biology 12 (3) : 121-129. Valles, M.P. & Lasa. J. M. 1994. Agrobacterium-mediated transformation of commercial melon (Cucumis melo L., Amarillo Oro). Plant Cell Rep. 13 : 145-148. Vandenmoortele, J. L. 1999 . A procedure to prevent hyperhydrcicty in cauliflower axillary shoots. Plant Cell Tiss. Org.Cult. 56 : 85-88. Vila, L., Lacadena, V., Fontanet, P.,Martinez del Pozo, A. and San Segundo, B. 2001. A Protein from the Mold Aspergillus giganteus Is a Potent Inhibitor of Fungal Plant Pathogens.The American Phytopathological Society 14:1327-1331. Waniska RD, V.R., Chandrashekar A, Krishnaveni S, Bejosano FP, Jeoung J, Jayaraj J, Muthukrishnan S, Liang GH. (2001). Antifungal proteins and other mechanisms in the control of sorghum stalk rot and grain mold. J Agric Food Chem. 49(10), 4732-4742. Zupan, J. R. and Zambryski, P. 1995. Transfer of T-DNA Agrobacterium to the plant cell. Plant Physiol. 107: 1041-1047.