Solution for the watermelon hyperhydricity during tissue culture and generation of multiple pathogen resistance in trans

黃穎芝、陳明造、余聰安

E-mail: 324918@mail.dyu.edu.tw

ABSTRACT

Watermelon is an important economically fruit in tropic and subtropic areas. Virus and fungal diseases often cause serious economic loss of watermelon, however there are still no chemicals for prevention the virus damage. Watermelon silver mottle virus (WSMoV), Zuchini yellow mosaic virus (ZYMV), Papaya ringspot virus type W (PRSV-W) and fungal disease damage the cucurbits. So far, by convential breeding, this is no watermelon variety confers multiple disease resistance. Hyperhydricty is a severe problem in tissue culture watermelon, resulting lower multiplication rate, necrosis, and poor quality shoots. In our results, Murashige and Skoog (MS) salts containing Schenk and Hildebrandt (SH), vitamins and thimeine-HCl reduced the hyperhydric rate. Moreover, the WSMoV, ZYMV, PRSV-W viral CP gene and a chitinase genes (CHI) for against Rhizoctonia solani were transferred into watermelon via Agrobacterium-mediated transformation. The individual transgenic watermelons were obtained and inoculated with R. solani in vitro. The transgenic lines expressing CHI-ZW gene had a better level of resistance than single CP lines that expressed CpCHI gene. There transgenic lines were analyzed by PCR and Southern blot for confirming the inserting of transgenes. When the transgenic lines were inoculated with ZYMV, and PRSV-W and R. solani respectivity, the results showed different levels of resistance for the two viruses and the fungus, including immunity. Western blot analysis indicated that the transgenic lines express chitinase protein in different levels and that a high level of chitinase expression in transgenic plants confers higher levelsresistance to the fungal pathogen. Our results indicated that the construsture combing gene silencing and gene expression can confer viral and fungal resistance in the some time.

Keywords : WSMoV、ZYMV、PRSV-W、chitinase、coat protein

Table of Contents

REFERENCES

1. Ainsworth, G. C., Sparrow, F. K. and Sussman, A. S. (Eds.). 1973. The Fungi:An Advanced Treatise. p.87-132. Academic Press, New York, N.Y. 2.Akanda, A. M. 1991. Studies on the virus and mycoplasma disease of crops in Bangladesh. A thesis submitted to the Faculty of Agriculture, Kyushu University, Japan for the partial fulfillment of Doctor of agriculture:181. 3.Anghel, I. and Rosu, A. 1985. In vitro morphogenesis in diploid, triploid and tetraploid genotypes of watermelon-Citullus lanatus (Thumb.). Mansf Rev Roum Biol Vega 30:43-55. 4.Asao, H., Nishizawa, Y., Arai, S., Sato, T., Hirai, M., Yoshida, K., Shinmyo, A. and Hibi, T. 1997. Enhanced resistance against a fungal pathogen Sphaerotheca humuli in transgenic strawberry expressing a rice chitinase gene. Plant Biotechnol 14:145-149. 5.Babaeizad, V., Imani, J. G., Kogel, K. H., Eichmann, R. and Huckelhoven, R. 2009. Over-expression of the cell death regulator BAX Inhibitor-1 in barley confers reduced or enhanced susceptibility to distinct fungal pathogens. Theoretical and Applied Genetics 118:455-463. 6.Banerjee, D. and Slack, F. 2002. Control of developmental timing by small temporal RNAs: a paradigm for RNA-mediated regulation of gene expression. Bioessays 24:119-129. 7.Baulcombe, D. 2004. RNA silencing in plant. Nature 431:356-363. 8.Benhamou, N., Broglie, K., Chet, H. and Broglie, R. 1993. Cytology of infection of 35S-bean chitinase trandgenic canola plants by Rhiaoctonia solani: cytochemical aspects of chitin breakdown in vivo. The plant journal 4:295-305. 9.Boughalleb, N. and Mahjoub, M. E. 2006. Watermelon sudden decay in tunisia :identification of pathogenic fungi and determination of primary agents. Pakistan Journal of Biological Sciences 9:1095-1103. 10.Boyhan, G. J., Morton, D., Jacobsen, B. J. and Abrahams, B. R. 1992. Evaluation of watermelon and related germ plasm for resistance to zucchini yellow mosaic virus. Plant Disease 76:251-252. 11.Brand, M. H. 1993. Agar and ammonium nireate influence hyperhydricity, tissue nitrate and total nitrogen content of serviceberry(Amelanchier arborea)shoots in vitro. Plant Cell Tissue and Orgran culture 35:203-209. 12.Broglie, K., Chet, I., Holiday, M., Cressman, R., Biddle, P., Knowlton, S., Mauvais, C. J. and Broglie, R. 1991. Transgenic plants with enhanced reisitance to the fungal pathogen Rhyzoctonia solani. Science 254:1194-1197. 13.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. Molecular Plant-Microbe Interactions 5:516-519. 14.Cai, W. Q., Gonsalves, C., Tennant, P., Fermin, G., Souza, M., Sarindu, N., F.J., J., H.Y., Z. and Gonsalves, D. 1999. A protocol for efficient transformation and regeneration of Carica papaya L. In Vitro Cellular & Developmental Biology-Plant 35:61-69. 15.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 viruses on melon and watermelon in Taiwan. Journal of Agricultural Research of China 36:389-397. 16.Chattopadhyay, A., Bhatnagar, N. B. and Bhatnagar, R. 2004. Bacterial Insecticidal Toxins. Critical Reviews in Microbiology 30:33-54. 17.Chen, C. C., Ho, H. M., Chang, T. F., Chao, C. H. and Yeh, S. D. 1995. Characterization of a topovirus-like virus isolated from was gourd. Plant Protection Bulletin 37:117-131. 18.Chen, T. C., Lu, Y. Y., Cheng, C. A. and Yeh, S. D. 2008. Melon yellow spot virus in watermelon: a first record from Taiwan. Plant Pathology 57:765. 19.Chen, T. C., Huang, C. W., Kuo, Y. W., Liu, F. L., Hsuan Yuan, C. H., Hsu, H. T. and Yeh, S. D. 2006. Identification of Common Epitopes on a Conserved Region of NSs Proteins Among Tospoviruses of Watermelon silver mottle virus Serogroup. Phytopathology 96:1296-1304. 20.Chen, U. C., Shiau, Y. J., Lai, C. C. and Tsay, H. S. 1998. Effects of composition and vessel closure on the hyperhydricity and rooting of carnation in vitro culture. Journal of Agricultural Research of China 47:364-376. 21.Chen, Y. T., Hsu, L. H., Huang, I. P., Tsai, T. C., Lee, G. C. and Shaw, J. F. 2007. Gene cloning and characterization of a novel recombinant antifungal chitinase from papaya (Carica papaya). Journal of Agricultural and Food Chemistry 55:714-22. 22.Cho, M. A., Moon, C. Y., Liu, J. R. and Choi, P. S. 2008. Agrobacterium-mediated transformation in citullus lanatus. Biologia plantarum 52:365-369. 23.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 Reports 13:344-348. 24.Choi, S. K., Yoon, J. Y., Ryu, K. H., Choi, J. K. and Park, W. M. 2002. First Report of Zucchini yellow mosaic virus on Hollyhock (Althaea rosea). Plant Pathology 18:121. 25.Chu, F. H., Choa, C. H., Chung, M. H., Chen, C. C. and Yeh, S. D. 2001. Completion of the genome sequence of watermelon silver mottle virus and utilization of degenerate primers for detecting tospoviruses in five serogroups. Phytopathology 88:361-368. 26.Collinge, D. B., Kragh, K. M., Mikkelsen, J. D., Nielsen, K. K., Rasmussen, U. and Vad, K. 1993. Plant chitinases The Plant Journal 3:31-40.

27.Compton, M. E. and Gray, D. J. 1993a. Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid, and tetraploid watermelon. Journal of the American Society for Horticultural Science 118:151-157. 28.Compton, M. E. and Gray, D. J. 1993b. Somatic embryogenesis and plant regeneration from immature cotyledons of watermelon. Plant Cell Reports 12:61-65. 29.Compton, M. E., Gray, D. J. and Gaba, V. P. 2004. Use of tissue culture and biotechnology for the genetic improvement of watermelon. Plant Cell Tissue and Organ Culture 77:231-243. 30.Cost, A. S. and Muller, G. W. 1980. Tristeza control by cross protection: A U. S.-Brazil cooperative success. Plant Disease 64:538-451. 31.Debergh, P., Aitken-Christie, J., Cohen, D., Grout, B., von Amold, S., Zimmerman, R. and Ziv, M. 1992. Reconsideration of the term 'vitrification' as used in micropropagation. plant Cell Tissue and Organ Culture 30:135-140. 32.Desbiez, C. and Lecoq, H. 1997. Zucchini yellow mosaic virus. Plant Pathology 46:809-829. 33.Dong, J. Z. and Jia, S. R. 1991. High efficiency plant regeneration from cotyledons of watermelon (Citrullus vulgaris Schrad.). Plant Cell Reports 9:559-562. 34.Dougall, D. K. 1972. Nutrition and metabolism. In: DH Rothblat and VJ Christofalo (Eds) Growth, Nutrition and Metabolism of Cells in Culture 2:371-406. 35.Down, R. E., Ford, L., Bedford, S. J., Gatehouse, L. N., Newell, C., Gatehouse, J. A. and Gatehouse, A. M. 2001. Influence of plant development and environment on transgene expression in potato and consequences for insect resistance. Transgenic Research 10:223-236. 36.Durner, J., Shah, J. and Klessig, D. F. 1997. Salicylic acid and disease resistance in plants. Trends in Plant Science 2:266-274. 37.Edreva, A. 2005. Pathogenesis-related proteins:research progress in the last 15 years. General and Applied Plant Physiology 31:105-124. 38.Ellul, P., Rios, G., Atare, A., Roig, L. A. and Serrano, R. 2003. The expression of Saccharomyces cerevisiae HAL1 gene increases salt tolerance in transgenic watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai.].Moreno V Theoretical and Applied Genetics 107:462-469. 39.Fire, A., Xu, S. Q., Montgomery, M. k., Kostas, S. A., Driver, S. E. and Mello, C. C. 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806-811. 40.Fitchen, J. H. and Beachy, R. N. 1993. Genetically engineered protection against viruses in transgenic plants. Annual Review of Microbiology 47:739-763. 41.Fletcher, J. T. 1978. The use of avirulent virus strains to protect plants against the effects of virulent strains. Annals of Applied Biology 89:110-114. 42.Fromm, M. E., Taylor, L. P. and Walbot, V. 1986. Stable transformation of maize after gene transfer by electroporation. Nature 319:719-793. 43.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. 44.Gaba, V., Zelcer, A. and Gal-On, A. 2004. cucurbit biotechnology-the importance of virus resistance. In Vitro cell dev boil plant 40:346-358. 45.Gamborg, O. L., Miller, R. A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research 50:151-158. 46.Gibson, G. E. and Zhang, H. 2002. Interactions of oxidative stress with thiamine homeostasis promote neurodegeneration. Neurochem International 40:493-504. 47.Gillaspie, A. G. and Wright, J. M. 1993. Evaluation of Citrullus sp.germplasm for resistace to watermelon mosaic virus 2. Plant Disease 77:352-354. 48.Gonsalves, D. 1998. Control of papaya ringspot virus in papaya: A case study. Annual Review of Phytopathology 36:415-437. 49.Grieshbach, R. J. 1983. Protoplast microinjection. Plant Molecular Biology Reporter 1:32-37. 50.Guis, M., Roustan, J. P., Dogimont, C., Pitrat, M. and Pech, J. C. 1998. Melon biotechnology. Biotechnol & Genetic Engineering Reviews 15:289-311. 51.Guner, N., Strange, E. B., Wehner, T. C. and Pesic-VanEsbroeck, Z. 2002. Methods for screening watermelon for resistance to Papaya ringspot virus type-W. Scientia Horticulturae 94:297-307. 52.Guo, S. and Kemphues, K. 1995. par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell 81:611-620. 53.Hamel, F., Boivin, R., Tremblay, C. and Bellemare, G. 1997. Structural and evolutionary relationships among chitinases of flowering plants. Journal of Molecular Evolution 44:614-624. 54.Hamilton, A. J. and Baulcombe, D. C. 1999. A species of small antisense RNA in posttranscriptional gene silencing in plant. Science 286:950-952. 55.Hollings, M. and Brunt, A. A. 1981. Potyvirus group. CMI/AAB Descriptions of plant viruses 245. 56.Howard, J. B. and Glazer, A. N. 1967. Studies of the physicochemical and enzymatic properties of papaya lysozyme. The Journal of Biological Chemistry 242:5715-5723. 57.Howard, J. B. and Glazer, A. N. 1976. Studies of the physicochemical and enzymatic properties of papaya lysozyme. The Journal of Biological Chemistry 242:5715-5723. 58.Hseu, S. H., Wang, H. L. and Huang, C. H. 1985. Identification of a zucchini yellow mosaic virus from Cucumis astivus. Journal of Agricultural Research of China 34 87-95. 59.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 Protection Bulletin (Taiwan) 29:233-244. 60.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 Pathology 42:100-107. 61.Ivanova, M. and Van Staden, J. 2009. Nitrogen source, concentration,and NH4+:NO3- ratio influence shoot regeneration and hyperhydricity in tissue cultured Aloe polyphylla. Plant Cell Tissue and Organ Culture 99:167-174. 62.Jausoro, V., Berta, E. L. and Apostolo, N. M. 2010. Structural differences between hyperhydric and normal in vitro shoots of Handroanthus impetiginosus (Mart. ex DC) Mattos (Bignoniaceae). Plant Cell Tissue and Organ Culture 101:183-191. 63.Jayaraj, J. and Punja, Z. K. 2007. Combined expression of chitinase and lipid transfer protein genes in transgenic carrot plants enhances resistance to foliar fungal pathogens. Plant Cell Reports 26:1539-1546. 64.Jones, D. R. 2005. Plant viruses transmitted by thrips. European Journal of Plant Pathology 113:119-157. 65.Jones, J. D. G. 1988. Expression of bacterium chitinase protein in tobacco leaves using two photosynthetic gene promoters. Molecular Genetics and Genomics 212:536-542. 66.Kadota, M., Imizu, K. and Hirano, T. 2001. Double-phase in vitro culture using sorbitol increases shoot proliferation and reduces hyperhydricity in Japanese pear 89 : 207-215. Scientia Horticulturae 89. 67.Kalpana, K., Maruthasalam, S., Rajesh, T., Poovannan, K., Kumar, K. K., Kokiladevi, E., Raja, J. A. J., Sudhakar, D., Velazhahan, R., Samiyappan, R. and Balasubramanian, P. 2006. Engineering sheath blight resistance in elite indica rice cultivars using genes encoding defense proteins. Plant Science 170:203-215. 68.Kamath, R. S., Fraser, A. G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., Welchman, D. P., Zipperlen, P. and Ahringer, J. 2003. Systermatic functional analysis of the Caenorhabditis elegans genome using RNAi. Nature 421:231-237.

69.Kishimoto, K., Nishizawa, Y., Tabei, Y., Hibi, T., Nakajima, M. and Akutsu, K. 2002. Detailed analysis of rice chitinase gene expression in transgenic cucumber plants showing different levels of disease resistance to gray mold (Botrytis cinerea). Plant science 162:655 - 662. 70.Kitajima, S. and Sato, F. 1999. Plant pathogenesis-related proteins: molecular mechanisms of gene expression and protein function. Journal of Biochemistry 125:1-8. 71.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. 72.Klement, Z. 1982. Hypersensitivity. In phytopathogenic prokaryotes (Mount MS and Lacy GH) .p.149-177. Academic Press, New York, USA. 73.Knorr, D. 1984. Use of chitinous polymers in food-a challenge for food research and development. Food Technology 38:85-97. 74.Koga, D., Sueshige, N., Utsumi, T. and Ide, A. 1989. Kinetics of chitinase fromyam, Dioscorea oppsita thumb. Agricultural and Biological Chemistry 53:3121-3127. 75.Kombrink, E. and Somssich, I. E. 1995. Defence responses of plants to pathogens. Advances in Botanical Research 21:1-34. 76.Krubphachaya, P., Juricek, M. and Kertbundit, S. 2007. Induction of RNA-mediated Resistance to Papaya Ringspot Virus Type W. Journal of Biochemistry and Molecular Biology 40:404-411. 77.Kubota, M. and Abiko, K. 2001. Induced resistance in cucumbers against Fusarium oxysporum f. sp. cucumerinum and Rhizoctonia solani AG2-2 by infection of the cotyledons. Journal of Phytopathology 149:297-300. 78.Kulchetscki, L., Harry, I. H. and Yenug, E. C. 1995. In vitro regeneration of Pacific silver fir (Abies amabilis) plantlets and histological analysis of shoot formation. Thorpe TA Tree Physiology 15:727-738. 79.Lacadena, J., Martinez, d. P. A., Gasset, M., Patino, B., Campos-Olivas, R., Vazquez, C., Martinez-Ruiz, A., Mancheno, J. M., Onaderra, M. and Gavilanes, J. G. 1995. Characterization of the antifungal protein secreted by the mould Aspergillus giganteus. Archives of Biochemistry and Biophysics 324:273-281. 80.Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriaphage T-4. Nature 227:680-685. 81.Lamberty, M., Ades, S., Uttenweiler-Joseph, S., Brookhart, G., Bushey, D., Hoffmann, J. A. and Bulet, P. 1999. Insect immunity. Isolation from the lepidopteran Heliothis virescens of a novel insect defensin with potent antifungal activity. Journal of Biological Chemistry 274:9320-9326. 82.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. Journal of Peptide Research 56:231-238. 83.Lin, S. S., Hou, R. F., Houng, C. H. and Yeh, S. D. 1998. Characterization of Zucchini yellow mosaic virus (ZYMV) isolates collected from Taiwan by host reactions, serlolgy, and RT-PCR. Plant Protection Bulletin 40:163-176. 84.Lin, S. S., Henriques, R., Wu, H. W., Niu, Q. W., Yeh, S. D. and Chua, N. H. 2007. Strategies and mechanisms of plant virus resistance. Plant Biotechnol Report 1:125-134. 85.Ling, K. S., Harris, K. R., Meyer, J. D. F., Levi, A., Guner, N., Wehner, T. C. W., Bendahmane, A. and Havey, M. J. 2009. Non-synonymous single nucleotide polymorphisms in the watermelon eIF4E gene are closely 5 associated with resistance to Zucchini yellow mosaic virus. Theoretical and Applied Genetics 120:191-200. 86.Link, A. J., Philops, D. and Church, G. 1997. Methods for generating precise deletions and insertions in genome of wild-type Escherichia coli: Application to open reading frame characterization. Journal of Bacteriology 179:6228-3267. 87.Lisa, V. and Lecoq, H. 1984. Zucchini yellow mosaic virus. CMI/AAB Description of Plant Virus 282:Kew, Surrey. 88.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. 89.Loesch-Fries, L. S., Merlo, D., Zinnen, T., Burhop, L., Hill, K., Krahm, K., Jarvis, N. S., Nelson, S. and Halk, E. 1987. Expression of alfalfa mosaic virus RNA 4 in transgenic plants confers virus resistance. EMBO Journal 6:1845-1851. 90.Lovisolo, O. 1981. Virus and viroid disease of cucurbits. Acta Horticulturae 88:33-82. 91.Lucca, D. A. J. and Walsh, T. J. 1999. Antifungal peptides: novel therapeutic compounds against emerging pathogens. Antimicrobial Agents and Chemotherapy 43:1-11. 92.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 Pathology 46:800-805. 93.Maine, M. E. 2000. A conserved mechanism for post-transcriptional gene silencing? Biology 1:1018.1-1018.4. 94.Mamta, R., Shah, P., Mukherjee, K. and Eapen, S. 2010. Expression of a fungal endochitinase gene in trandgenic tomato and tobacco results in enhanced tolerance to fungal pathogens. Physiology and Molecular Biology of Plants 16:39-51. 95.Mauch, F., Hadwiger, L. A. and Boller, T. 1984. Ethylene:Symptom, not signal for the induction of chitinase and s-1,3-glucanase in pea pods by pathogens and elicitors. Plant Physiology 76:607-611. 96.Mehdy, M. C. 1994. Active oxygen species in plant defense against pathogens. Plant Physiology 105:467-47. 97.Milne, K. S., Grogan, R. G. and Kimble, K. A. 1969. Identification of viruses infecting cucurbits in California. Phytopathology 59:819-828. 98.Molano, J., Polacheck, I., Duran, A. and Cabib, E. 1979. An endochitinase from wheat germ. Activity on nascent and preformed chitin. Journal of Biological Chemistry 254:4901-4907. 99.Munger, H. M. 1993. Breeding for viral disease resistance in cucurbits. In: Kyle MM ed Resistance to viral diseases of vegetables:. Genetics and Breeding. Portland, OR: Timber Press:44-60. 100.Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology 15:473-497. 101.Nameth, S. T., Dodds, J. A., Paulus, A. O. and Laemmlen, F. F. 1986. Cucurbit viruses of California : An ever-changing problem. Plant Disease 70:8-11. 102.Nauerby, B., Billing, K. and Wyndaele, R. 1997. Influence of the antibiotic timentin on plant regeneration compared to carbenicicllin and cefotaxime in concentrations suitable for elimination of Agrobacterium tumefeciens Plant Science 123:169-177. 103.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. 104.Nelson, R. S., Mccormick, S. M., Delanny, X., Dube, P., Layton, J., Anderson, E. J., Kaniewska, M., Proksch, R. K., Horsch, r. b., rOGERS, S. G., Fraley, R. T. and Beachy, R. N. 1988. Virus tolerance, plant growth and field perormance of transgenic tomato plants expressing coat protein from tobacco mosaic virus. Bio/Technology 6:403-409. 105.Niu, Q. W., 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 Nature Biotechnology 24:1420-1428. 106.Ntui, O. V., Thirukkumaran, G., Azadi, P., Khan, R. S., Nakamura, I. and Mii, M. 2010. Stable integration and expression of wasabi defensin gene in "Egusi" melon (Colocynthis citrullus L.) confers resistance to Fusarium wilt and Alternaria leaf spot. Plant Cell Reports 29:943-954 107.Pang, S., Jan, F., Tricoli, D., Russell, P., Carney,

K., Hu, J., Fuchs, M., Quemada, H. and Gonsalves, D. 2000. Resistance to Squash mosaic comovirus in transgenic squash plants expressing its coat protein genes. Molecular Breeding 6:87-93. 108.Papp, I., Mette, M. F., Aufsatz, W., Daxinger, L., Schauer, S. E., Ray, A., van der Winden, J., Matzke, M. and Matzke, A. J. M. 2003. Evidence for nuclear processing of plant micro RNA and short interfering RNA precursors. Plant Physiology 132:1382-1390. 109.Park, S. M., Lee, J. S., Jenal, S., Jeon, S. L., Shin, Y. S., Her, N. H., Lee, J. H., Lee, M., 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 Reports 24:350-356. 110.Pegg, G. F. and Young, D. H. 1981. Changes in glycosidase activity and their relationship to fungal colonization during infection of tomato by Verticillium albo-atrum Physiol Plant Pathology 19:3711-382. 111.Powell-abel, P., R.S. Nelson, B. De, N. Hoffmann, .G. Rogers, Rogers, R.T.Fraley and R.N.Beachy. 1986. Delay of sisease development in transgenic plants that express the tabacco mosaic virus coat protein gene. Science 232:738-743. 112.Prins, M. and Goldbach, R. 1998. The emerging problem of tospovirus infection and nonconventional methods of control. Trends in Microbiology 6:31-35. 113.Provvidenti, R. 1986. Viral disease of cucurbits and sources of resistance. Food & Tertilizer Technology Center. Technical Bulletin:93. 114.Provvidenti, R. 1987. Inheritance of resistance to strain of Zucchini yellow mosaic virus in cucumber. HortScience 22:102-103. 115.Provvidenti, R. 1991. Inheritance of resistance to the Florida strain of Zucchini yellow mosaic virus in watermelon. . HortScience 26:407-408. 116.Provvidenti, R. 1993. Resistance to viral diseases of cucurbits. In: Kyle MM (ed) Resistance to viral diseases of vegetables. Timber Press, Portland:8-43. 117.Provvidenti, R. and Gonsalves, D. 1982. Resistance to the Florida strain of Zychini yellow mosaic virus in cucumis metuliferus and its relationship to Watermelon mosaic virus 1. Journal of Heredity 73:239-240 118.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 Disease 68: 443-446. 119.Purcifull, D. E., Edwardson, J. R., Hiebert, E. and Gonaslves, D. 1984. Papaya ringspot virus. CMI/AAB Description of Plant Virus. 292. 120.Rane, K. K. and Latin, R. X. 1992. Bacterial fruit blotch of watermelon association of the pathogen with seed. Plant Disease 76:509-512 121.Restrepo, M. A., Feed, D. D. and Carrington, J. C. 1990. Nuclear transport of plant potyviral proteins. Plant Cell 2:987-998. 122.Rezende, J. A. M. and Pacheco, D. A. 1998. Control of Papaya ringspot virus- type W in zucchini squash by protection in Brazil. Plant Disease 82:171-175. 123.Roger, S. G., Horsch, R. B. and Fraley, R. T. 1986. Gene transfer in plants: production of transformed plants using Ti plasmid vectors. Methods in Enzymology 118:627-640. 124.Rohini, V. K. and Sankara, R. K. 2001. Transformation of peanut (Arachis hypogaea L.) with tobacco chitinase gene: variable response of transformants to leaf spot disease. Plant Science 160:889-898. 125.Rongchuang, Y., Helin, X., Mingsheng, L., Wengui, Y., Chungui, L., Guang, W., Naisui, P. and Zkhangliang, C. 1995. Expression of cucumber mosaic virus coat protein confers cross-protection in transgenic tomato plants. Acta Horticulturae 402:179-184. 126.Ryals, J., Uknes, S. and Ward, E. 1994. Systemic acquired resistance. Plant Physiology 104:1109-1112. 127.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. 128.Saher, S., Piqueras, A., Hellin, E. and Olmos, E. 2005a. Prevention of hyperhydricity in micropropagated carnation shoots by bottom cooling:implications of oxidative stress. Plant Cell Tissue and Organ Culture 81:149-158. 129.Saher, S., Fernandez-Garcia, N., Piqueras, A., Hellin, E. and Olmos, E. 2005b. Reducing properties, energy efficiency and carbohydrate metabolism in hyperhydric and normal carnation shoots cultured in vitro: a hypoxia stress? . Plant Physiology and Biochemistry:573-582. 130.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 Physiology 117:465-472. 131.Sambrook, J., Fritschi, E. F. and Maniatis, T. 1989. Molecular cloning: a laboratorymanual, Cold Spring Harbor Laboratory Press, New York. 132.Sanford, J. C. and Johnston, S. A. 1985. The concept of parasite-derived resistance. Journal of Theoretical Biology 113:395-405. 133.Schaffrath, U., Mauch, F., Freydl, E., Schweizer, P. and Dudler, R. 2000. Constitutive expression of the defense-related Rir1b gene in transgenic rice plants confers enhanced resistance to the rice blast fungus Magnaporthe grisea. Plant Molecular Biology 43:59-66. 134.Schenk, R. U. and Hildebrandt, A. C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Canadian Journal of Botany 50:199-204. 135.Schepers, U. and Kolter, T. 2001. RNA interference : a new way to analyze protein function Angewandte Chemie International Edition 40:2437-2439. 136.Schlumbaum, A., Mauch, F., Vogeli, U. and Boller, T. 1986. Plant chitinases are potent inhibitors of fungal growth. Nature 324:365-367. 137.Segura, A., Moreno, M., Molina, A. and Garcia-Olmedo, F. 1998. Novel defensin subfamily from spinach (Spinacia oleracea). FEBS Letters 435:159-162. 138.Selitrennikoff, C. P. 2001. Antifungal proteins. Applied and Environmental Microbiology 67:2883-2894. 139.Seta-Buurlage, M. B., Ponstein, A. S., Bres-Vloemans, S. A., Melchers, L. S., M., V. d. E. P. J. and Cornelissen, B. J. C. 1993. Only specific tobacco (Nicotiana tabacum) chitinases and β-1.3-glucanases ex-hibit antifungal activity. Plant Physiology 101:857-863. 140.Sharma, H. C., Sharma, K. K., Seetharama, N. and Ortiz, R. 2000. Prospects for using transgenic resistance to insects in crop improvement. Journal of Biotechnology 3:1-20. 141.Sijen, t., Wellink, J. B. and Van Kammen, A. 1996. RNA-mediated virus resistance: role of repeated transgenes and delineation of targeted regions. Plant Cell 8:2277-2294. 142.Sivanesan, I., Song, J. Y., Hwang, S. J. and Jeong, B. R. 2010. Micropropagation of cotoneaster wilsonii Nakaia rare endemic ornamental plant. Plant Cell Tissue and Organ Culture. 143.Srivastava, D. K., Andrianov, V. M. and Piruzian, E. S. 1989. Tissue culture and plant regeneration of watermelon. Plant Cell Reports 8:300-302. 144.Sticher, L. B., Mauch-Mani, J. and Metraux, P. 1997. Systemic acquired resistance. Annual Review of Phytopathology 35:235-270. 145.Strange, E. B., Gumer, N., Pesic-Van., Esbroeck, Z. and Wehner, T. C. 2002. Screening the watermelon gernplasm collection from to Papaya ringspot virus type-W. Crop Science 42 1324-1330. 146.Suslow, T. V., Matusbara, D., Jones, J., Lee, R. and Dunsmuir, P. 1988. Effect of expression of bacterial chitinase on tobacco susceptibility to leaf brown spot. Phytopathology 78:1566. 147.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 10:235-241. 148.Tabei, Y., Kitade, S., Nishizawa, Y., Kikuchi, N., Kayano, T., Hibi, T. and Akutsu, K. 1998. Transgenic cucumber plants harboringa rice chitinase gene exhibit enhanced resistance to gray mold (Botrytis cinerea). Plant Cell Reports 17:159-164. 149.Taji, A. M. and Williams, R. R. 1991. Effect of temperature, gel concentration and cytokinins on vitrification of Olearia microdisca (J.M. Black) in vitro shoot cultures Plant Cell Tissue and Organ Culture 26:1-6. DOI: 10.1007/BF00116601 150.Tepfer, M. 1993. Viral genes and transgenic plants. Biotechnology 11:1125-1132. 151.Terakawa, T., Takaya, N., Horiuchi, H., Koike, M. and Takagi, M. 1997. A fungal chitinase gene from Rhizopus oligosprus confers antifungal activity to transgenic tobacco. Plant Cell Reports 16:439-443. 152.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. 153.Thevissen, K., Terras, F. T. and Broekaert, W. F. 1999. Permeabilization of fungal membranes by plant defensins inhibits fungal growth. Applied and Environmental Microbiology 65:5451-5458. 154.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. The Journal of Biological Chemistry 272:32176-32181. 155.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. Molecular Plant-Microbe Interactions 13:54-61. 156.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. Journal of Biological Chemistry 271:5018-5025. 157.Thomas, P., Mythili, J. B. and Shivashankara, K. S. 2000. Explant, medium and vessel aeration affect the incidence of hyperhydricity and recovery of normal plantlets in triploid watermelon. Journal of Horticultural Science and Biotechnology 75 19-25. 158.Thomma, B. P., Cammue, B. P. and Thevissen, K. 2002. Plant defensins. Planta 216:193-202. 159.Thorpe, T. A. and Patel, K. R. 1984. Clonal propagation: adventitious buds. In: IK Vasil (ed). Cell Culture and Somatic Cell Genetics of Plants. Vol. 1. Laboratory Procedures and Their Applications. Academic Press, New York,:49-58. 160.Tomlinson, J. A. 1987. Epidemiology and control of virus disease of vegetables. Annals of Applied Biology 110:661-681. 161.Tougou , M., Yamagishi, N., Furutani, N., Shizukawa, Y., Takahata, Y. and Hidaka, S. 2007. Soybean dwarf virus-resistant transgenic soybeans with the sense coat protein gene. Plant Cell Reports 26:1967-1975. 162.Trudel, J., Grenier, J., C., P. and Asselin, A. 1998. Several thaumatin-like proteins bind to β-1,3-glucans. Plant Physiology 118:1431-1438. 163.Tsay, J. G. and Tung, B. K. 1995. The occurrence of Monosporascus root rot/vine decline of muskmelon in Taiwan. Plant Pathollogy Bulletin 4:25-29. 164.Vad, K., Mikkelsen, J. D. and Colling, D. B. 1991. Purification and Characterization of Chitinase Isolated from Pea Leaves Inoculated with Ascochyta pisi. Planta 184:24-29. 165.Van Dun, C. M. P., Bol, J. F. and Van Vloten-Doting, L. 1987. Expression of alfalfa mosaic virus and tobacco rattle coat protein genes in transgenic tobacco plants. Virology 159:299-305. 166.Vandenmoortele, J. L. 1999. A procedure to prevent hyperhydricity in cauliflower axillary shoots. Plant Cell Tissue and Orgran culture 56:85-88. 167.Vaucheret, H., Beclin, C., Ellmayan, T., Feuerbach, F., Godan, C., Morel, J. B., Mourrain, P., Palauqui, J. C. and Vernhettes, S. 1998. Transgenic-induced gene silencing in plant. Plant Journal 16:651-659. 168.Vellicce, G. R., Ricci, J. C., Hernandez, L. and Castagnaro, A. P. 2006. Enhanced resistance to Botrytis cinerea mediated by the transgenic expression of the chitinase gene c