

Evaluation of the progeny from transgenic watermelon resistance to Zucchini yellow mosaic virus and p

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ABSTRACT

Zucchini yellow mosaic virus (ZYMV) and Papaya ringspot virus type W (PRSV W) are major limiting factors for production of watermelon in Taiwan. For the effective control of these two viruses by transgenic resistance, an untranslatable chimeric construct containing truncated ZYMV coat protein (CP) and PRSV W CP genes was transferred to commercial watermelon cultivars by *Agrobacterium*-mediated transformation. Greenhouse evaluation of the selected 10 transgenic lines of 'Feeling' cultivar revealed that two immune lines conferred complete resistance to ZYMV and PRSV W, from which virus accumulation were not detectable by western blotting four weeks after inoculation. The transgenic transcript was not detected but small interfering RNA (siRNA) was readily detected from the two immune lines and T1 progeny of line ZW 10 before inoculation, indicating that RNA-mediated post-transcriptional gene silencing (PTGS) is the underlying mechanism for the double-virus resistance. The segregation ratio of T1 progeny of the immune line ZW10 indicated that a single inserted transgene is nuclearly inherited and associated with the phenotype of double-virus resistance as a dominant trait.

Keywords : transgenic watermelon、coat protein、PTGS

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REFERENCES

- 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、陳淵。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., Lapidots, 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 [*Citrullus 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. 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.

27. 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.

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., 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.

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., Heinrikson, 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., Lomonsoff, 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. Five 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. Five 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

number of the genes 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.