Plant Pathology Bulletin 3: 72-77, 1994

Satellite Defense as a Control Strategy for Cucumber Mosaic Virus

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Accepted for publication: March 25, 1994

ABSTRACT

Hsu, Y. H., Wu, C. W., Lee, C. W., Hu, C. C., and Lin, F. Z. 1994. Satellite defense as a control strategy for cucumber mosaic virus. Plant Pathol. Bull. 3:72-77.

Cucumber mosaic virus (CMV) causes severe losses in many economically important crops worldwide. Breeding of CMV-resistant plant species has been hampered by lack of suitable natural resistance sources. Recent advances in the molecular biology of CMV have made it possible to device control strategies based on virus related genes. Some virus strains contain a satellite RNA, which depends on the helper virus for replication and shares no significant sequence similarity with the virus genome. Some of the satellite RNAs of CMV are capable of affecting symptoms induced by CMV in certain host plants. Those satellites which decrease the severity of symptoms have been used as a biocontrol agent for CMV disease. Transgenic plants expressing satellite sequence also show tolerance to CMV infection. Our laboratory has investigated the incidence of CMV satellite RNAs in Taiwan. Satellite RNAs associated with mungbean and cucumber isolates have been obtained with ability to ameliorate the symptoms. They were characterized by their nucleotide sequences, biological activities, and symptom determinants. CMV-tolerant transgenic tobacco cultivar Van-Hicks expressing M-satellite was produced. The effectiveness and risk of using satellite transgenic plants in control of CMV disease are discussed.

Key words: cucumber mosaic virus, satellite RNA, biocontrol, transgenic plant.

INTRODUCTION

Cucumber mosaic virus (CMV) has been found most predominantly in countries in the temperate zones, but its increasing prevalence in some tropical countries has recently been reported (28). CMV has the broadest host range among all plant viruses. More than 775 plant species in 365 genera from 85 families were recorded as hosts for CMV (5). Transmission by some 75 species of aphids (30) in a non-persistent manner in nature is another factor responsible for the prevalence of CMV. In the last decade, CMV has caused severe losses to several major crops, including tobacco, banana, passionfruit, vegetable and floral crops in Taiwan. The existence of numerous strains has been reported (16). It is nearly impossible to grow a virus-free crop in Taiwan due to the geographic location and the multiple croping system being practiced. Traditional plant breeding for CMV-resistance has been hampered by lack of suitable natural resistance sources. Recent advances in the molecular biology of plant viruses coupled with the development of techniques for plant genetic transformation have made it possible to produce transgenic plants that are resistant or tolerant to specific plant viruses. Four different types of CMV-related genes, including the viral coat protein, viral satellite RNA, antisense RNA and defective polymerase, have been engineered into plants to confer CMV tolerance (40). This paper reviews the use of CMV satellite RNA for the control of CMV diseases.

CMV GENOME

Complete nucleotide sequences have been determined for four CMV strains: CMV-Q (4,9,31,32), CMV-Fny (27,33,34), CMV-Y (20,21,25), and CMV-O (12,13, Hase et al., 1992, unpublished data taken from genebank). The genome of CMV is divided among three RNAs designated as RNA 1 (3.4 kb), RNA 2 (3.0 kb), and RNA 3 (2.1 kb). These RNAs encode nonstructural proteins 1a (111 kd), 2a (97-94 kd), and 3a (30 kd), respectively. RNA 3 also encodes the virus

coat protein (24 kd) that is translated from a subgenomic RNA 4 (1.0 kb). RNAs 1, 2, 3 are required for infecting plants systemically (29). The function of protein 3a is believed to be the potentiation of movement of virus from cell to cell (4,23). RNAs 1 and 2 together can replicate in tobacco protoplasts (26). Protein 1a and 2a, which have been identified in the RNA-dependent RNA polymerase (14), contain sequence motifs characteristic of nucleic acid helicase and polymerase, respectively (8,10).

CMV SATELLITE RNA

Several isolates of CMV, but not all, also contain a satellite RNA (6,28). The satellite RNA has little sequence homology with CMV RNAs and is entirely dependent on CMV for its replication and encapsidation. Over 25 different CMV satellite RNAs have been characterized. They are all linear ssRNA molecules of approximately 334-386 nucleotides and are highly homologous.

CMV satellite RNAs have attracted attention because of their abilities to modulate disease expression of helper viruses in certain host plants. Kaper and Waterworth (1977) demonstrated that a CMV satellite and not CMV per se' was the causal agent of an outbreak of lethal tomato necrosis. Since then, numerous studies on structural and biological properties of CMV and its satellite have been reported. In addition to the disease exacerbation, many natural CMV satellites have the opposite effect in that the disease symptoms are greatly attenuated (18,28). Disease modulation is usually depending on the trilateral interaction of satellite RNA, helper virus and host plant. The satellite RNA seems to function as molecular parasites, interfering with helper virus replication. This property has become the basis for experimental and practical field strategies for the control of CMV infection.

SATELLITE-MEDIATED PROTECTION

Two different strategies have so far been used to protect plants against CMV infection with satellite. Vaccinations of plant seedlings with CMV amended with the benign satellite RNAs have been successfully applied in the field tests to tomato and pepper plants (7,24,36,39,43). The use of attenuative satellite RNA as a transgene is an alternative approach. Baulcombe et al. (1986) first reported the transformation of tobacco plants with DNA copies of CMV satellite RNA, the expression of satellite sequence in transgenic plants and the acquisition of satellite RNA by CMV infection. CMV replication is greatly decrease and symptom development largely suppressed in these transgenic plants following challenge inoculation with a satellite-free CMV isolate (11,17,35,42). These results indicate that genetically engineered protection by virus satellite RNA is a feasible strategy for enhancing the virus resistance of crop plants.

INCIDENCE of CMV SATELLITE RNAs and THEIR POTENTIAL for FIELD APPLICATION in TAIWAN

Among 23 CMV isolates collected in Taiwan, 19 contain small RNAs of about 0.1×10^6 daltons encapsidated in virions (15,16). These small RNAs were identified as satellite RNAs by Northern hybridization showing their sequences unrelated to CMV genomic RNAs and by their dependency on helper virus for replication. None of them causes lethal necrosis in tomato and so far no satellite of necrogenic-type has been found. The satellite RNAs M and C (M- and C-sat RNAs), associated with mungbean isolate CMV-M48 and cucumber isolate CMV-C respectively, are two satellites most extensively characterized (Hsu *et al.*, unpublished). Neither has an exacerbation effect on symptom expression of their helper CMV in tobacco and tomato plants. However, both satellite RNAs attenuate the symptom caused by another CMV isolate NT9, a most severe and satellite-free isolate in our collection. The nucleotide sequences of M- and C-sat RNAs were determined by dideoxy sequencing of cDNA and direct RNA sequencing. The molecules are 334 nucleotides long and share more than 90% homologies with most CMV satellites so far reported. By field evaluations, the cross protection effect by mild CMV strains amended with these two satellite RNAs ranges between 46%–91% (Fig. 1).

CMV-TOLERANT TRANSGENIC VAN-HICKS TOBACCO EXPRESSING M-SATELLITE RNA

Complementary DNA of M-sat RNA was constructed in a plant expression vector and transferred to tobacco cultivar Van-Hicks by *Agrobacterium*-mediated transformation (22). Transgenic plants were screened in kanamycin-containing medium and those expressing satellite RNAs were further selected by the Southern and Northern hybridization analyses. The stability of satellite sequence in the F1 progenies was analyzed by dsRNA detection and tissue printing hybridization after F1 plants were inoculated with CMV-NT9. The results showed that the ratio of the genotype of F1 with satellite RNA to the total number of test plants was 3/4, which followed the mendelian law. Ten F1 plants transformed with satellite RNA were self-pollinated to gain the F2 progenies. From latter, we

selected five seedings which have a genotype homozygousfor satellite RNA. After challenge inoculation with 100 μg/ml of CMV-NT9, plants were scored for disease symptoms. The accumulation of viral RNA and satellite RNA was examined by Northern hybridization and of virions by ELISA. These transgenic tobacco plants homozygous for satellite RNA suppressed the development of NT9 symptoms (Fig. 2).

Fig. 1. Field evaluation of protection effect of C-sat RNA against CMV-NT9 in tomato. Left lane, plants were vaccinated with C-sat RNA amended CMV during cotyledon stage. Right lane, unprotected plants. All plants were challenge-inoculated with CMV-NT9 3 weeks after vaccination and were transplanted from greenhouse to the field. Photo was taken 2 months later.

Fig. 2. Symptom development in M-sat transformed (left) and non-transformed (right) tobacco plants challenge-inoculated with 100 μg/ml CMV-NT9. Photo was taken 45 days post-inoculation.

Virus concentration in Sat-1 transgenic tobacco plants following inoculation with NT9 was about ten times lower than that in the control plants.

CONCLUSIONS

Satellite-mediated biocontrol has been investigated extensively in Mainland China (38,39,41), Japan (36,43), the United States (7,24), and Italy (3,7) and has proved useful in protecting plants from CMV infection. Transgenic plants that express CMV satellite RNA sequence can tolerate crop diseases induced by the helper virus. This has been demonstrated in tobacco (11, 17,38,42) and tomato (35,38). The apparent advantages of satellite mediated tolerance are that it is not strain-specific as coat protein mediated tolerance does, it is not necessary for the transgenic plants to produce new protein and because the expressed satellite RNA is replicated by CMV, there is effective resistance even when the inoculum is very concentrated, or when the satellite RNA itself is expressed at a low level. However, there are two potential drawbacks in the use of satellite-mediated protection: the high genetic variability of CMV satellite RNA results in very heterogeneous populations and the danger of a necrogenic satellite RNA may emerge or mutate from the vaccine components (1,28,37,38,40). The probability of such a mutation occuring and eventually predominating in the infection needs more experiments to address.

ACKNOWLEDGEMENTS

This research was supported in part by grants from Council of Agriculture (82-ST-1.1-F-56-5) and National Science Council (82-0418-B-005-091-B03).

LITERATURE CITED

1. Aranda, M. A., Fraile, A., and Garcia-Arenal, F. 1993. Genetic variability and evolution of the satellite RNA of cucumber mosaic virus during natural epidemics. J. Virol. 67:5896-5901.
2. Baulcombe, D. C., Saunders, G. R., Bevan, M. W., Mayo, M. A., and Harrison, B. D. 1986. Expression of biologically active viral satellite RNA from the nuclear genome of transformed plants. Nature 221:446-449.
3. Crescenzi, A., Barbarossa, L., Gallitelli, D., and Martelli, G. P. 1993. Cucumber mosaic cucumovirus populations in Italy under natural epidemic conditions and after a satellite-mediated protection test. Plant Dis. 77:28-33.
4. Davies, C., and Symons, R. H. 1988. Further implication for the evolutionary relationships between tripartite plant viruses based on cucumber mosaic virus RNA 3. Virology 165:216-224.
5. Douine, L., Quiot, J. B., Marchoux, G., and Archange, P. 1979. Recensement des especes vegetales sensibles au virus de la mosaique du concombre (CMV). Etudes biolographique. Ann. Phytopathol. 11:439-475.
6. Francki, R. I. B. 1985. Plant virus satellites. Annu. Rev. Microbiol. 39:151-174.
7. Gallitelli, D., Vovlas, C., Martelli, G., Montasser, M. S., Tousignant, M. E., and Kaper, J. M. 1991. Satellite-mediated protection of tomato against cucumber mosaic virus: II. Field test under natural epidemic conditions in southern Italy. Plant Dis. 75:93-95.
8. Gorbalenya, A. E., and Koonin, E. V. 1989. Viral proteins containing the purine NTP-binding sequence pattern. Nucleic Acid Res. 17:8413-8440.
9. Gould, A. R., and Symons, R. H. 1982. Cucumber mosaic virus RNA 3. Determination of the nucleotide sequence provides the amino acid sequences of protein 3A and viral coat protein. Eur. J. Biochem. 126:217-226.
10. Habili, N., and Symons, R. H. 1989. Evolutionary relationship between leuteoviruses and other RNA plant viruses based on sequence motifs in their putative RNA polymerases and nucleic acid helicases. Nucleic Acids Res. 17:9543-9555.
11. Harrison, B. D., Mayo, M. A., and Baulcombe, D. C. 1987. Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. Nature (London) 328:799-802.
12. Hayakawa, T., Mizukami, M., Nakamura, I., and Suzuki, M. 1989. Complete nucleotide sequence of RNA 3 from cucumber mosaic virus (CMV) strain O: comparative study of nucleotide sequences and amino sequences among CMV strains O, Q, D, and Y. J. Gen. Virol. 70:499-504.
13. Hayakawa, T., Mizukami, M., Nakamura, I., and Suzuki, M. 1989. Cloning and sequencing of RNA-1 cDNA from cucumber mosaic virus strain O. Gene 85:533-540.
14. Hayes, R. J., and Buck, K. W. 1990. Complete replication of a eukaryotic virus RNA in vitro by a purified RNA-dependent RNA polymerase. Cell 63:363-368.
15. Hsu, Y. H., Hu, C. C., Lin, N. S., and Chiu, R. J. 1988. Symptom determinant of two Taiwan strains of cucumber mosaic virus is on RNA 3. Bot. Bull. Acad. Sin. 29:231-237.
16. Hsu, Y. H., Lin, F. Z., Hu, C. C., and Yin, S. C. 1989. Host reaction, serology and RNA pattern of cucumber mosaic virus isolates. Plant Prot. Bull. 31:51-59.
17. Jacquemond, M., Amselem, J., and Tepfer, M. 1988. A gene coding for a monomeric form of cucumber mosaic virus satellite RNA confers tolerance to CMV. Mol. Plant-Microbe Interact. 1:311-318.

18. Kaper, J. M., and Collmer, C. W. 1988. Modulation of viral plant diseases by secondary RNA agents. Pages 171-193 *in*: RNA Genetics Vol. 3. E. Domingo, J. J. Holland and P. Ahlquist eds. CRC Press, Boca Raton, Florida.
19. Kaper, J. M., and Waterworth, H. E. 1977. Cucumber mosaic virus associated RNA 5: causal agent for tomato necrosis. Science 196:429-431.
20. Kataoka, J., Masuta, C., and Takanami, Y. 1990. Complete nucleotide sequence of RNA 2 of cucumber mosaic virus Y strain. Ann. Phytopathol. Soc. Jpn. 56:495-500.
21. Kataoka, J., Masuta, C., and Takanami, Y. 1990. Complete nucleotide sequence of RNA 1 of cucumber mosaic virus Y strain and evolutionary relationships among genome RNAs of the virus strains. Ann. Phytopathol. Soc. Jpn. 56:501-507.
22. Lee, C. W. 1993. Establishment and Resistance Assay of Transgenic Tobacco Plants Expressing Cucumber Mosaic Virus Satellite RNA. Master Thesis, Institute of Plant Pathology, National Chung Hsing University, Taichung, Taiwan. (in Chinese).
23. Mackenzie, D., and Tremaine, J. H. 1988. Ultrastructural localization of non-structural protein 3A of cucumber mosaic virus in infected tissue using monoclonal antibodies to a cloned chimeric fusion protein. J. Gen. Virol. 69:2387-2395.
24. Montasser, M. S., Tousignant, M. E., and Kaper, J. M. 1991. Satellite-mediated protection of tomato against cucumber mosaic virus: I. Greenhouse experiments and simulated epidemic conditions in the field. Plant Dis. 75:86-92.
25. Nitta, N., Masuta, C., Kuwata, S., and Takanami, Y. 1988. Comparative studies on the nucleotide sequence of cucumber mosaic virus RNA 3 between Y strain and Q strain. Ann. Phytopathol. Soc. Jpn. 54:516-522.
26. Nitta, N., Takanami, Y., Kuwata, S., and Kubo, S. 1988. Inoculation with RNAs 1 and 2 of cucumber mosaic virus induces viral RNA replicase activity in tobacco mesophyll protoplasts. J. Gen. Virol. 69:2695-2700.
27. Owen, J., Shintaku, M., Aeschleman, P., Ben Tahar, S., and Palukaitis, P. 1990. Nucleotide sequence and evolutionary relatioships of cucumber mosaic virus (CMV) strains: CMV RNA 3. J. Gen. Virol. 71:2243-2249.
28. Palukaitis, P., Roossinck, M. J., Dietzgen, R. G., and Francki, R. I. B. 1992. Cucumber mosaic virus. Adv. Virus Res. 41:281-348.
29. Peden, K. W. C., and Symons, R. H. 1973. Cucumber mosaic virus contains a funtionally divided genome. Virology 53:487-492.
30. Quiot, J. B., Labonne, G., and Marrou, J. 1982. Controlling seed and insect-borne viruses. Pages 95-122 *in*: Pathogens, Vectors and Plant Disease: Approaches to Control. K. F. Harris and K. Maramorosch eds., Academic Press, New York.
31. Rezaian, M. A., Williams, R. H. V., Gordon, K. H. J., Gould, A. R., and Symons, R. H. 1984. Nucleotide sequence of cucumber mosaic virus RNA 2 reveals a translation product significantly homologous to corresponding proteins of other viruses. Eur. J. Biochem. 143:277-284.
32. Rezaian, M. A., Williams, R. H. V., and Symons, R. H. 1985. Nucleotide sequence of cucumber mosaic virus RNA 1. Presence of a sequence complementary to part of the viral satellite RNA and homologies with other viral RNAs. Eur. J. Biochem. 150:331-339.
33. Rizzo, T. M., and Palukaitis, P. 1988. Nucleotide sequence and evolutionary relationships of cucumber mosaic virus (CMV) strains: CMV RNA 2. J. Gen. Virol. 69:1777-1787.
34. Rizzo, T. M., and Palukaitis, P. 1989. Nucleotide sequence and evolutionary relationships of cucumber mosaic virus (CMV) strains: CMV RNA 1. J. Gen. Virol. 70:1-11.
35. Saito, Y., Komari, T., Masuta, C., Hayashi, Y., Kumashiro, T., and Takanami, Y. 1992. Cucumber mosaic virus-tolerant transgenic tomato plants expressing a satellite RNA. Theor. Appl. Genet. 83:679-683.
36. Sayama, H., Sato, T., Kominato, M., Natsuaki, T., Kaper, J. M. 1993. Field testing of a satellite-containing attenuated strain of cucumber mosaic virus for tomato protection in Japan. Phytopathology 83:405-410.
37. Sleat, D. E., and Palukaitis, P. 1990. Site-directed mutagenesis of plant viral satellite RNA changes its phenotype from ameliorative to necrogenic. Proc. Natl. Acad. Sci. USA 87:2946-2950.
38. Tien, P., and Wu, G. 1991. Satellite RNA for the biocontrol of plant disease. Adv. Virus Res. 39:321-339.
39. Tien, P., Zhang, X., Qiu, B., Qin, B., and Wu, G. 1987. Satellite RNA for the control of plant diseases caused by cucumber mosaic virus. Ann. Appl. Biol. 111:143-152.
40. Wilson, T. M. A. 1993. Strategies to protect crop plants against viruses: Pathogen-derived resistance blossoms. Proc. Natl. Acad. Sci. USA 90:3134-3141.
41. Wu, G., Kang, L., and Tien, P. 1989. The effect of satellite RNA on cross-protection among cucumber mosaic virus strains. Ann. Appl. Biol. 114:489-496.
42. Yie, Y., Zhao, F., Zhao, S. Z., Liu, Y. Z., Liu, Y. L., and Tien, P. 1992. High resistance to cucumber mosaic virus conferred by satellite RNA and coat protein in transgenic commercial tobacco cultivar G-140. Mol. Plant-Microbe Interact. 5:460-465.

43. Yoshida, K., Goto, T., and Iizuka, M. 1985. Attenuatedisolates of cucumber mosaic virus produced by satellite RNA and cross-protection between attenuated isolates and virulent ones. Ann. Phytopathol. Soc. Jpn. 51:238-242.

摘　　要

徐堯煇、吳靖薇、李青蔚、胡仲祺、林芳如．1994．利用衛星核酸防治胡瓜嵌紋病毒之策略．植病會刊3:72-77．(台中市　國立中興大學遺傳工程中心)

胡瓜嵌紋病毒(CMV)在世界各地均造成許多經濟作物的嚴重損失，由於作物大都不具抗CMV之遺傳特性，傳統抗病育種進展甚緩。相反的，近年由於CMV分子生物學知識的累積，利用病毒相關基因設計的抗病策略，相繼展現了潛力。有些CMV系統攜帶有衛星核酸，此核酸片段與輔助病毒並無核酸序列同源性，但其複製完全依賴後者。有些衛星核酸系統具有減輕CMV病徵的效力，已被開發爲防治CMV病害的生物製劑，此類衛星核酸的轉殖植物亦表現對CMV的耐病性。本實驗室曾檢測本省CMV分離株所攜帶的衛星核酸，並自綠豆分離株(M)及胡瓜分離株(C)，分離出兩具抑病性的衛星核酸系統，並比較其核苷酸序列、生物特性及影響病徵的序列因子。M-衛星核酸轉殖的萬國士菸草，對CMV具抗病性。許多實驗已顯示利用衛星核酸防治CMV病害具良好效果，但其風險仍需評估。

關鍵詞：胡瓜嵌紋病毒、衛星核酸、生物防治、轉殖植物。