Biological interactions of two satellites RNAs with some cucumber mosaic cucumovirus

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Key words: tomatoes, chillies, cucumber mosaic cucumovirus, satellite RNAs

Introduction

Cucumber mosaic virus (CMV) satellite-RNA (sat-RNA) often interferes with the replication of the helper virus genome and affects viral concentration and symptom expression (Roossinck *et al.*, 1992). The association of sat-RNA with CMV generally reduced the virus accumulation (Mossop and Francki 1979; Takanami , 1981; Gonsalves *et al.*1982). The alteration of CMV symptoms by sat-RNA could be either an attenuation or an exacerbation. Waterworth *et al.* (1979) reported that Tabasco pepper infected with CMV-WT strain free from sat-RNA developed a severe mosaic, stunting and necrosis of leaves, petioles and stem. The association of sat-RNA with this strain resulted in mild chlorosis to non-infection to this cultivar. The CMV disease symptoms in tomato could be attenuated by sat-RNA (Yoshida *et al.*, 1985). Masuta *et al.* (1988) reported that sat-RNA of Y strain attenuated the CMV disease in several plant species. The sat-RNA had been used to protect pepper plants against a virulent strain of CMV (Tien *et al.*, 1987; Wu *et al.*, 1989). The most prevalent example of disease exacerbation was the systemic necrosis of tomato which lead to collapse of the infected plants (Kaper and Waterworth, 1977; Waterworth *et al.*, 1978; Nakashima and Ehar, 1989; Masuta *et al.*, 1990; Kaper *et al.*, 1995; White *et al.*, 1995). Particular sat-RNA sometimes had different disease expression with different strains of CMV. Masuta *et al.* (1988) reported that the association of sat-RNA Y with CMV-Y strain induced systemic necrosis on tomato but no necrosis occurred when it was associated with CMV-O strain.
Two sat-RNAs were found to be associated with CMV-6 (from infected purple cleome in Selangor) and CMV-7 (from infected tobacco in Kelantan). They were designated as sat-RNA6 and sat-RNA7, respectively (El-Sanousi *et al.* 1994; 1996; 1998). The interaction of these sat-RNAs with their helper viruses and two other isolates and CMV-3 and CMV-4 are presented here by carrying out artificial introduction of the sat-RNA into each of the four CMV isolates.

Materials and Methods

Extraction of Viral RNA Four virus isolates, CMV-3, CMV-4, CMV-6 and CMV-7, were purified from infected *N. tabacum* cv. White Burley leaves by the method of Mossop *et al.* (1976). The viral RNAs of each virus were isolated from purified virus using the proteinase K and phenol-SDS procedure as described by Sambrook *et al.* (1989). In each eppendorf tube 200 µl (1 µg/µl) of virus was mixed with an equal volume of extraction buffer(0.03 M of KCl, 3 mM $MgCl_2$, 0.01 M of SDS, 0.02 M Tris HCl) and 50 µl of proteinase K (2 mg/ml) and incubated for 20 min at 50° C. Then 80 µl of 1 M NaCl and one volume of equilibrated phenol (50° C) were added. The preparation was mixed vigorously and incubated for 5 min at 50° C. After centrifugation at 12,000 g for 5 min, the aqueous phase was removed and subjected to another phenol extraction. The aqueous phase was removed and one volume of chloroform isoamyl alcohol (24:1) was added and centrifuged at 12,000 g for 10 min at 4° C. The aqueous phase was removed and subjected to two further chloroform isoamylalcohol extractions. The final aqueous phase was removed and two volume of ethanol and one tenth volume of 3 molar sodium acetate were added and placed at -70° C. The precipitate was collected by centrifugation at 12,000 g for 15 min and washed once in 70 % ethanol dried and suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.3) and stored at -20° C.
Recovery of Sat-RNA from the Gel: After electrophoresis, (sat-RNA) bands of CMV-6 and CMV-7 were cut out from the gel, and placed in sterile dialysis bags with 1-2 ml of TBE buffer. The current was run at 70 V for 1 hr; the polarity was reversed for 1 min and the eluted sat-RNAs were reconcentrated by extraction with an equal volume of 2-butanol until 0.5 ml of buffer phase was obtained. The RNA was finally precipitated with two volumes of ethanol as previously described for the virus RNA . The precipitate was collected by centrifugation at 12,000 g for 15 min and washed once in 70% ethanol, dried, suspended in TE buffer and stored at -20° C.
Inoculation with Sat-RNAEach sat-RNA was adjusted to 20 µg/ml, mixed and inoculated with the virus genome of each CMV isolate to *N. tabacum* cv. White Burley. The presence of sat-RNA in each isolate was tested by further virus

purification followed by RNA extraction and RNA electrophoretic analysis as previously described. The response of each isolate to the sat-RNA was determined by hosts inoculation. The inoculum was prepared by homogenization of infected White Burley tobacco leaves in the presence of phosphate buffer.

Elimination of Sat-RNA from Genomic RNA: To eliminate the sat-RNA from CMV-6 and CMV-7 the genomic RNAs 1-3 were cut and recovered from gels by electroelution, reconcentration and precipitation as described previously in recovery of sat-RNA from the the gel band except the current was run at 70 vol for 2 hrs. The genomic RNAs were then inoculated into the local lesion host, *C. amaranticolor*. Single local lesion from *C. amaranticolor* was then inoculated to individual White Burley tobacco plant. The satellite free genomic RNAs were then tested against tomato seedlings by inoculation and further purified and isolated.

Results and Discussion

Although there was no difficulty in introducing sat-RNA into the CMV isolates, but there was difficulty in eliminating these sat-RNAs from their natural CMV isolates. The attempts to free CMV isolates from sat-RNA was successful with CMV-7 but not with CMV-6. The association of both sat-RNAs with the four CMV isolates reduced the virus concentration in White Burley tobacco by ten times.

The interference of the sat-RNAs on the symptoms induced by CMV isolates on different hosts showed that the association of sat-RNA6 with the CMV-3, -4 and -7 isolates lead to severe systemic necrosis in *L. esculetnum* cv. Eggtomato and local necrosis in *N. tabacum* cv. White Burley . Although sat-RNA6 did not interfere with its natural isolate (CMV-6) in infection of MC4 chilli but it prevented infection by CMV-7 .

CMV-7 free of sat-RNA produced mosaic symptoms in MC4 chilli. The association of sat-RNA7 with this isolate resulted in non-infection of MC4 chilli. Association of sat-RNA7 with CMV-3 and CMV-4 resulted in reduction of the number of MC4 chilli plants infected by these isolates and to severe lethal necrosis in tomato. The results also showed that sat-RNA7 attenuated the symptom caused by CMV-4 from severe mosaic to mosaic in White Burley tobacco.

The combination of CMV-3, CMV-4, CMV-7 and CMV-6 with sat-RNA6 showed a much severe symptoms than sat-RNA7 with the respective CMV isolates in inducing necrosis in tomato. The artificial introduction of sat-7 into CMV-3 and CMV-4 induced a more severe tomato necrosis as compared by its natural host, CMV-7 which caused a systemic necrosis to very few numbers of inoculated tomato plants. Sat-RNA7 with CMV -3, -4 and CMV-7 showed more reduction in numbers of infected plants of chilli MC4 than sat-RNA6 with the respective CMV isolates. The results also showed that the prevention of infection of MC4 chilli by CMV in the presence of sat-RNA was isolate dependent, since CMV-7 infection to MC4 chilli prevented by the presence of either sat-RNA-6 or -7

Conclusions

The presence of satellite RNA with the helper virus, CMV, showed mixed reaction in the tomato varieties tested. The satellite RNA-mediated resistance was only effective in certain varieties. Four of the varieties were resistant to ToMV. Only one variety showed positive satellite RNA-mediated resistance and also resistant to ToMV.

Benefits from the study

Production system and proper management of virus diseases for tomato growing in the tropical regions.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals

1. Abdul-Samad, N., M. Singh and K. Yusoff. 1996. Detection of tomato mosaic tobamovirus from Malaysia. Pertanika J. Trop. Agric. Sci. 19, 1-6.

2. Abdul-Samad, N., T.K.Teh and K. Yusoff. 1996. Detection of tomato mosaic tobamovirus infection by double stranded RNA analysis in infected plants. Malaysian Applied Biology 25,115-117.

3. El-Sanousi, O., C.A. Ong, K. Yusoff, S. Napis and N. Abdul-Samad. 1998. Properties of four cucumber mosaic virus isolates from West Malaysia. Asia Pacific Journal of Molecular Biology & Biotechnology 6,131-137

4. El-Sanousi. O., C.A. Ong, K. Yusof, S. Napis and N. Abdul-Samad. 2003. Biological interaction of two satellite RNAs with cucumber mosaic cucumovirus. Asia Pacific Journal of Molecular Biology & Biotechnology 10, 5-9

Project Publications In Conference Proceedings

1. El-Sanousi, O., C.A Ong, S. Napis, K.Yusoff and N. Abdul-Samad. 1996. Serology and PCR identification of several isolates of cucumber mosaic cucumovirus. Proc: Ninteeth Symposium of Malaysian Microbiolgy Society, Trengganu.

2. El-Sanousi, O., A.A. Ong and N. Abdul-Samad. 1997. Satellite RNA from cucumber mosaic cucumovirus. Proc: Sixteenth ASIAN-Pacific Weed Science Society Conference. Kuala Lumpur.

3. El-Sanousi, O. 1997. Differentiation and genetic studies of several isolates of cucumber mosaic virus. PhD Thesis, Universiti Putra Malaysia, Serdang.

4 Quah, J.L. and N. Abdul-Samad. 1998. Infectivity studies in tomatoes on the effect of cucumber mosaic virus in the presence and absence of satellite RNA. Proc: Malaysian Society of Applied Biology Conference, Kuala Lumpur.

5. El-Sanousi, O., C.A. Ong, K. Yusoff and N. Abdul-Samad. 1999. Properties of two satellite RNAs in cucumber mosaic cucumovirus isolates. Proc: Fifth International Conference on Plant Protection in the Tropics, Kuala Lumpur.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Omar El-Sanousi	Differentiation and genetic studies of several isolates of cucumber mosaic virus	Virology	PhD	1997

IRPA Project number: 01-02-04-0445
UPM Research Cluster:AFF