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Biological Control of a Severe Viral Strain Using a Benign Viral Satellite RNA Associated with *Cucumber mosaic virus*

Magdy Shaban Montasser*, Bader Al-Hamar and Radhika Guleri Bhardwaj

Department of Biological Sciences, College of Science, University of Kuwait, Safat 13060, State of Kuwait

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Two strains of *Cucumber mosaic virus* (CMV) isolated in Kuwait were confirmed their infectivity based on symptomatology and host range on different cultivars of tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum L.*) and squash (*Cucurbita pepo*). The pattern of symptoms differed for the two CMV strains in tomato and tobacco, showing severe stunting and mosaic symptoms with one strain designated KU2, and almost symptomless with the other strain designated KU1. A satellite RNA 5 (sat-RNA) was found to be associated with the KU1 strain and was characterized as a benign viral satellite RNA. Using reverse transcription and polymerase chain reaction (RT-PCR) with sat-RNA specific primers, an amplified PCR product of about 160bp was determined and analyzed by gel electrophoresis. This naturally occurring benign viral satellite RNA was successfully used as a biological control agent to protect tomato plants against the severe KU2 strain. Tomato plants grown in plant-growth chambers, were preinoculated with KU1 containing the benign viral satellite and then challenge inoculated with the severe KU2 strain at different time intervals. All plants challenged three weeks after preinoculation showed nearly complete protection from subsequent infection by the severe strain. This biological control technology using plant viruses was found protective and could be successfully established sooner after the pre-inoculation.

Keywords : *Cucumber mosaic virus*, RT-PCR, satellite RNA

Biological control technology based on viral satellite mediated protection is a novel concept of the viral disease control strategies. Plant virus diseases cause severe constraints on the productivity of wide range economically important crops worldwide (Dasgupta et al., 2003). Among these plant viruses, *Cucumber mosaic virus* (CMV) is considered one of the most economically damaging viruses among field grown vegetables (Allam and Abou-El-Ghar, 1970; Dodds and Lee, 1984; Gallitelli et al., 1988; Gonsalves et al., 1982; Tomlinson, 1987). This virus infects over 1000 species of host plants belonging to 85 plant families, making it the broadest host range virus known (Roossinck, 2001). Outbreaks of diseases incited by CMV have caused large losses of tomato and pepper crops in several Mediterranean (Gallitelli et al., 1988; Jorda et al., 1992; Marrou and Duteil, 1974) and Asian countries (Sayama et al., 1993; Tien and Wu, 1991). In the United States, high incidences of CMV have been reported in several states (Daniels and Campbell, 1992; Gonsalves et al., 1982; Kearney et al., 1990).

CMV can harbor molecular parasites known as satellite RNAs that dramatically alter the symptom phenotype induced by the virus (Roossinck, 2001). Over 40 CMV satellite RNA isolates have been identified and sequenced, most reduce the viral disease to a virtually asymptomatic condition, while relatively few increase the severity of virus symptoms (Collmer and Howell, 1992; Kaper, 1995; Roossinck et al., 1992). Thus, satellites have been referred to as "natural inhibitors of crop damaging viruses" (Courtice, 1987).

These satellites RNAs are small nucleic acids whose nucleotide sequences are unrelated to, but are replicatively dependent upon the viral genome; they have a molecular parasitic relationship (Montasser et al., 1998). According to Kaper and Waterworth (1977), the satellite RNA of CMV which was designated as CMV-associated RNA 5 (CARNA 5) or satRNA (Francki, 1985) modulated the symptom expression of its helper CMV. The satellite parasitizes the virus at molecular level by competing with latter for the host plant's enzymatic machinery and replicative enzymes that are essential in virus multiplication and infection. The satellite's competition literally causes the virus to starve and serves us the conceptual basis for experiments on biological control of CMV in the field conducted in China (Tien et al., 1987). The attenuating properties of some satellite RNAs can be exploited to control viral diseases by preinoculating crops with an attenuating satellite RNA variant combined with virus (McGarvey et al., 1994). This technique can effectively protect against CMV disease in the field (Gallitelli et al.,

*Corresponding author.
Phone) +965-498-5657, FAX) +965-484-7054
E-mail) m_montasser@hotmail.com

1991; Montasser et al., 1991; Sayama et al., 1993; Tien and Wu, 1991; Tien et al., 1987). Field tests had been carried out in 1988 on tomato, in 1989 on peppers and in 1990 on cucurbits in U.S. (Montasser et al., 1991, 1998). The vaccine was also applied in some commercial fields of tomatoes in Italy in 1989 and 1990 (Gallitelli et al., 1991) but information regarding this in Kuwait is still insufficient.

Recently, three CMV strains were isolated and showed the presence of satellite RNA in two of the CMV strains isolated in Kuwait (Montasser et al., 2006). Our previous work suggested possible application of the CARNA 5 as a biological control agent if proved to ameliorate the severe symptoms of the isolated strains. The main objective of this work is first to study the feasibility of using the benign viral satellite RNA that has been characterized in an endogenous KU1 strain of CMV isolated in Kuwait. Second, is to determine the efficacy of this CARNA 5 on tomato protection against the other virulent KU2 strain of the same virus.

Materials and Methods

Virus sources. Plant samples were collected from farms in Wafra, Abdally, Omariya and Khaldiya, Kuwait. Two different CMV strains, KU1 and KU2, identified from the collected plant samples were tested for host range and symptomatology.

Test and indicator plants. Experiments were conducted on different test plants *Lycopersicon esculentum* cvs. UC82B and Supermarmande, *Nicotiana tabacum L.*, *Cucurbita pepo* cvs. White Bush F_1 hybrid, Carmelita F_1 hybrid and Boma. *Vigna unguiculata* cv. Azmerly and *Chenopodium morale* were selected as indicator plants for local symptoms.

Host range and symptomatology. Host range and symptomatology were observed to check the disease causing efficiency of cucumber mosaic viral strains on different hosts. The symptomatology was checked by inoculating both KU1 and 2 CMV strains on test plants by mechanical sap transmission (Montasser, 1999). Inocula were prepared by grinding CMV infected tissues (1 g/9 ml) in 0.01 M potassium phosphate buffer (pH 7.1). The leaves of the young test plants were dusted with 600-mesh carborundum and then with a cotton swab, ground CMV infected tissues were rubbed over those leaves. Immediately after inoculation, sterilized distilled water was sprayed on the leaves. CMV infected tissues were rubbed on the cotyledonary leaves of 8-10 days old tomato (*Lycopersicon esculentum*), 5-7 days old squash (*Cucurbita pepo*) and 4-6 days old cowpea (*Vigna unguiculata*). Tobacco (*Nicotiana tabacum L.*) and Chenopodium (*Chenopodium morale*) were inoculated at young stages. Virus inoculated plants were kept in growth chambers (at 20, 22, and 24°C for 8 hrs each and 16 hrs/8 hrs, light/dark periods) in 5.0 inch diameter pots containing a mixture of soil and peat potting substratum (1:2 V/V) provided with a complete fertilizer mix. Symptoms on test plants were observed till 30 days after inoculation. Once symptoms appeared on the test plants, the respective test plants were back inoculated as CMV inocula for virus transmission in other test plants.

Total nucleic acid extraction. Total nucleic acids (TNAs) were extracted by powdering 0.25 g of infected tissue in liquid nitrogen in a sterile pre-cooled mortar and pestle (Montasser, 1999). Fine powdered tissues were transferred to a 50 ml conical centrifuge tube with the help of a sterile brush. To this added, 3 ml of each of 1x extraction buffer (0.1 M glycine, 0.01 M EDTA, 0.1 M Nacl, pH 9.0 with 10% Sodium dodecyl sulfate (SDS) and 10% *n*-lauryl sarcocine), water saturated phenol (saturated with 10 mM Tris HCl and 1 mM EDTA) and chloroform. The homogenate was vortexed vigorously for 45 seconds and was centrifuged for 10 minutes at 5000 rpm at 4°C, then kept on ice. The nucleic acids present in the upper aqueous layer were precipitated by adding 3 volumes of 95% ethanol in a 10 ml polypropylene copolymer tube and kept at -70°C for 20 minutes. The precipitate was collected by centrifugation at 6500 rpm for 20 minutes. The pellet obtained was dissolved in 200 µl of 1x extraction buffer and shifted to 1.5 ml eppendorf tube followed by precipitation with three volumes of 95% ethanol at –70°C for 15-20 minutes and then micro centrifugation at 10000 rpm for 10 minutes. The pellet obtained after decanting was dried in a speedvac and then dissolved in 200 µl of sterile distilled water. This total nucleic acid suspension was stored in the freezer at -70°C.

Total nucleic acid inoculations. TNAs extracted from virus infected plant tissues were further inoculated on test plants to check the viability of CMV viral strains as TNA inoculums. Extracted nucleic acid preparations were directly applied with glove-covered finger on plant leaves dusted with 600-mesh carborundum. Sterile distilled water was sprayed after TNA inoculum application.

Gel electrophoreses of TNAs. Polyacrylamide gel electrophoresis (PAGE) described by Montasser (1999) was used for the detection of satellite RNA and the other viral RNA molecules. TNA extracts were heated in a 50°C water bath for 5 minutes, followed by quick cooling in crushed ice, then analyzed by electrophoresis on 6% polyacrylamide gels (39:1 acrylamide : bis acrylamide) in Tris borate EDTA (TBE) buffer, at 200V for 2.5 hr and 300V for 2 hr. The gel was stained with ethidium bromide and exposed to UV light for photograph. The size of nucleic

acids appeared on the gels were further determined by using SynGene Gene Tools Analysis Software.

Detection of CARNA 5. For the detection of CARNA 5 at the molecular level, molecular biological techniques were used as follows:

(1) Designing of CARNA 5 specific primers. The primers were designed using Primer Express Software v 2.0 from Applied Biosystems, USA. Designing of CARNA 5 specific primers was based on the CARNA 5 sequences of the known S strain of CMV (Avila-Rincon et al., 1986). The forward primer of 21 bases (5' CTC AGC ACC ACG CAC TCA TTT 3') corresponded to nucleotides 147 to 167 and the reverse primer of 22 bases, (3' CGG AGA TCG GCA TGA CCT TAT A 5') corresponded to nucleotides 289 to 310 bases of the viral satellite RNA of 339 bases. The pair of primers was used to give RT- PCR product of 164bp.

(2) SDS removal and TNA estimations. Before carrying out the PCR amplifications, SDS was removed from the TNAs extracted from healthy and CMV infected plant samples (Montasser, 1999) and then the nucleic acid contents were checked. TNAs from the SDS free nucleic acid samples were estimated in "Gene Quant pro" machine from Amersham Biosciences. Working samples with known nucleic acid concentrations were prepared from estimated samples for carrying out RT-PCR.

(3) RT-PCR for CARNA 5 detection. Reverse transcription and PCR amplifications were performed in one reaction mix in a single tube by Perkin Elmers 9600 thermal cycler using single step cMaster RT-PCR kit (Eppendorf, Germany). The final 50 µl RT-PCR volume was prepared by adding 10 µl of mastermix 2 containing TNA sample with viral RNA and RNase free water to 40 µl of mastermix 1 containing 1x RT_{plus}PCR buffer with 2.5 mM Mg^{2+}, 200 µM of dNTP mix, 0.15 U/µl of cMaster RT enzyme, 0.05 U/µl of cMaster PCR enzyme mix, 0.01 U/µl of prime RNase inhibitor solution, 20-25 pmoles of forward and reverse primers along with RNase free water. Reverse transcription and PCR amplification was carried out with one cycle at 50°C for 30 minutes, another cycle at 94°C for 2 minutes followed by 35 cycles at 94°C for 15 seconds, 55°C for 20 seconds and 68°C for 30 seconds. Different working samples were applied by dilutions to determine the sensitivity for detecting CARNA 5 by RT-PCR. The RT-PCR products were first fractioned by 1% agarose gel electrophoresis in 1x Tris acetate EDTA (TAE) buffer and then on 5% DNA polyacrylamide gel in Tris borate EDTA (TBE) buffer.

Preinoculation and challenge inoculation experiment. The effect of plant age on the efficacy of the viral satellite RNA (that is, optimum length of time for the satellite RNA-inoculum development in test plants) was studied in the growth chambers by mechanically inoculating the cotyledonary leaves of squash (*Cucurbita pepo* cv. White Bush F_1 hybrid) with crude sap inoculum containing KU1 genomic RNA and CARNA 5 (the vaccine). Tomato (*Lycopersicon esculentum* cv. UC82B) plants selected for further preinoculation and challenge inoculations were divided into three groups. Group 1 represented untreated control plants, group 2 plants were preinoculated with KU1 total RNA and group 3 plants were inoculated with severe strain KU2. The preinoculated plants of group 2 were further divided into 4 subgroups, where subgroup 1 was kept without challenge inoculations as the preinoculated control and remaining 3 subgroups (subgroups 2, 3 and 4) were mechanically challenged with severe virus strain, KU2 at the age of 1, 2 and 3 weeks of preinoculations, respectively. Similarly, group 1 of healthy control plants was also divided into 4 subgroups, where subgroup 1 was uninoculated protected healthy plants, whereas remaining 3 subgroups (that is, subgroups 2, 3 and 4) were challenge inoculated with severe strain KU2 at the ages of 1, 2 and 3 weeks, respectively. Test plants were kept in the plant growth chamber (at 20, 22, and 24°C temperatures for 8 hrs each and 16 hrs/8 hrs, light/dark periods) in 5.0-inch diameter pots containing a mixture of soil and peat potting substratum (1:2 V/V) provided with a complete fertilizer mix (Montasser et al., 1998).

Results

Host range and symptomatology. Mechanical sap inoculations with KU1 and KU2 strains of CMV in test plants *Cucurbitaceae* and *Solanaceae* families and in indicator plants *Leguminosae* and *Chenopodiaceae* families showed the susceptibility of plants to both viral strains infection (Table 1). Tomato plants cvs. UC82B and Supermarmande were symptomless in KU1 inoculation. This particular strain showed mild mosaic like symptoms on very young leaves of tobacco, which vanished with the age of plant making the plant symptomless. One peculiar feature of this strain was observed in squash cv. White Bush F_1 hybrid plant showing mild chlorotic local lesions on cotyledonary leaves along with severe systemic symptoms on the rest of the plant leaves. Mosaics were visualized in the KU1 infected squash leaves as shown in Fig. 1A. The KU2 strain resulted in severe symptoms on test and indicator plants (Table 1). Local chlorotic lesions (Fig. 1B) and severe systemic symptoms were observed in squash cv. White Bush F_1 hybrid. Severe symptoms like stunting, chlorosis and mosaics on tomato leaves (Fig. 2A) along with severe mosaics on tobacco leaves (Fig. 2B) were also noticed.

Table 1. Host range and symptomatology of CMV strains KU1 and KU2

Test / Indicator plants	Symptoms[a]			
	KU1		KU2	
	Local symptoms	Systemic symptoms	Local symptoms	Systemic symptoms
Cucurbitaceae				
Cucurbita pepo cv. White Bush F1 hybrid	M, Llc	S, Mo, CS, B,VC, Mal	S, Llc	Mal, Mo, B, CS, VC
C. pepo cv. Carmelita F_1 hybrid	–	M, CS, Mo, B	–	M, CS, B
C. pepo cv. Boma	–	S, Mo, CS, B,VC, Mal	–	Mal, Mo, B, VC
Solanaceae				
Lycopersicon esculentum cv. UC82B	–	NS	–	S, SP, C, Mo
L. esculentum cv. Supermarmande	–	NS	–	S, SP, C
Nicotiana tabacum L.	–	M, Mo on young leaves NS on old leaves	–	S, Mo
Leguminosae				
Vigna unguiculata cv. Azmerly	M, Lln	–	S, Lln	–
Chenopodiaceae				
Chenopodium morale	M, Llc	–	S, Llc	–

[a]B, Blistering; C, Chlorosis; CS, Chlorotic spots; Llc, Local chlorotic lesions; Lln, Necrotic local lesions; M, Mild symptoms; Mal, Malformation; Mo, Mosaics; NS, No symptoms; S, Severe symptoms; SP, Stunted plants; VC, Vein clearing; –, Not applicable.

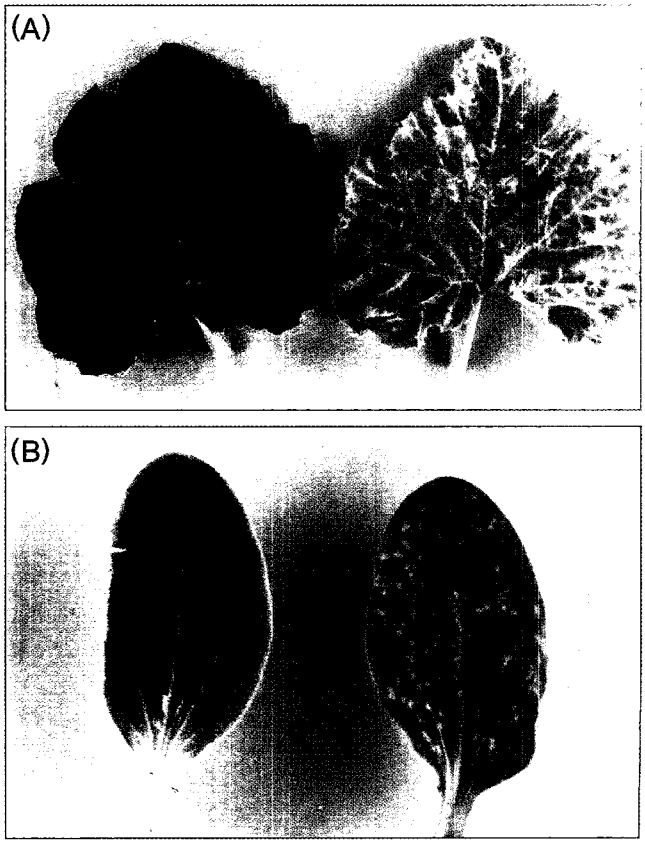

Fig. 1. Squash leaf infected with *Cucumber mosaic virus* (CMV) strain KU1, showing characteristic symptoms of mosaics, blistering, vein clearing (right) in comparison to a healthy (left) control leaf (A). Appearance of chlorotic local lesions on cucumber mosaic virus strain KU2 infected cotyledonary leaf of squash (right) in comparison to a healthy (left) control (B).

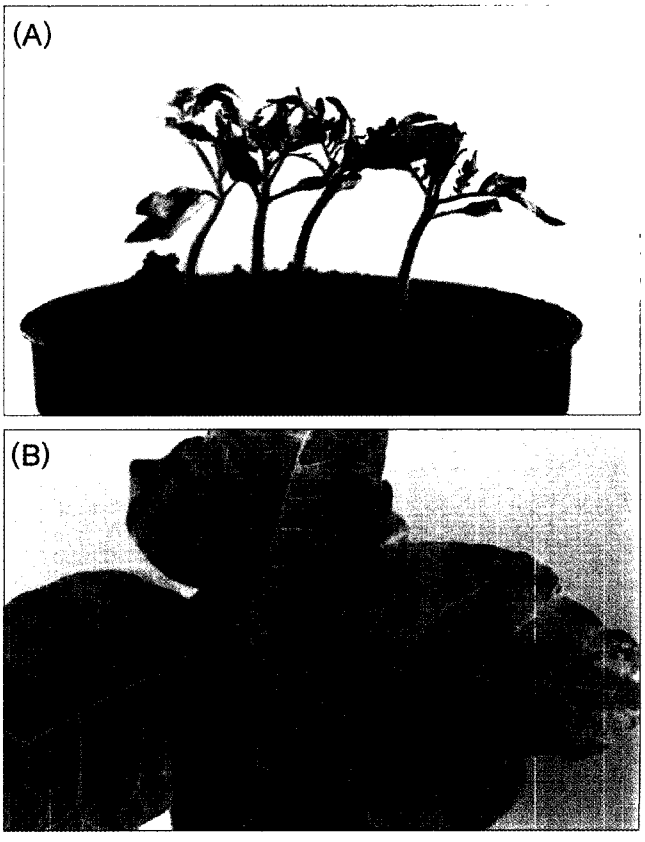

Fig. 2. Stunted tomato plants with chlorosis of leaves (A) and appearance of mosaics on tobacco leaves (B) caused by infection with KU2 strain of CMV .

Table 1 explaines that mild local symptoms were developed on *Leguminosae* and *Chenopodiaceae* families inoculated

Table 2. Viability and infectivity of CMV strains using extracted nucleic acids as inocul[a]

Test plants	KU2		KU1	
	Local symptoms	Systemic symptoms	Local symptoms	Systemic symptoms
Cucurbitaceae				
Cucurbita pepo cv. White Bush F_1 hybrid	Llc[a]	CS	M, Llc	M, CS
Solanaceae				
Lycopersicon esculentum cv. UC82B	–	S, SP, C, Mo on leaves	–	NS

[a]C, Chlorosis; CS, Chlorotic spots; Llc, Local chlorotic lesions; M, Mild symptoms; Mo, Mosaics; NS, No symptoms; S, Severe symptoms; SP, Stunted plants; –, Not applicable.

with KU1 strain contrast to same plants inoculated with KU2 strain showing severe symptoms.

TNA inoculations. Nucleic acids extracted from plants inoculated with KU1 and KU2 were tested for their viability and infectivity by inoculating on squash cv. White Bush F_1 hybrid of *Cucurbitaceae* and tomato cv. UC82B plants of *Solanaceae* families (Table 2). Tomato plants showed severe infections when inoculated with TNA inoculums of KU2 infected tissues, but were symptomless in the inoculation with TNA from KU1 infected plants. Chlorotic spots and local chlorotic lesions were observed on squash cv. White Bush F_1 hybrid inoculated with TNAs from either KU1 or KU2 infected plants.

Gel electrophoretic analyses. Average molecular weights of 3.3 kb and 1.0 kb were noticed in KU1 and KU2 infected squash TNA extracts, by analyzing on a 6% gel at 200 V, while no band at that distance in the healthy were observed (Fig. 3A). Nucleic acid bands of 374 bp and 400 bp appeared in KU1 infected plants (Fig. 3A). Similarly, KU1, KU1 challenged with KU2 extracts and KU2 alone infected tomato extracts showed a 3.5 kb band when they were analyzed on a 6% gel at 300V (Fig. 3B). There were some extra bands of 292 bp and 330 bp in KU1 and KU1 challenged with KU2 extracts but these bands were missing in healthy and KU2 alone infected extracts (Fig. 3B).

RT-PCR for detection of CARNA 5. The designed primers specific to S-CARNA 5 of CMV successfully generated a PCR product of about 160 bp from the viral RNA in total nucleic acid samples with 100 ng and 200 ng concentrations, respectively. RT-PCR products from healthy and KU1 infected strains fractioned on an 1% agarose electrophoresis. Fig. 4A showed the appearance of amplified bands of cDNA adjacent to 174 bp band of DNA micromarker in KU1 infected tomato (UC82B) and squash cv. White Bush F1 hybrid RT-PCR products. No amplifications were observed in healthy tomato and squash. Same products analyzed on 5% DNA polyacrylamide gel for

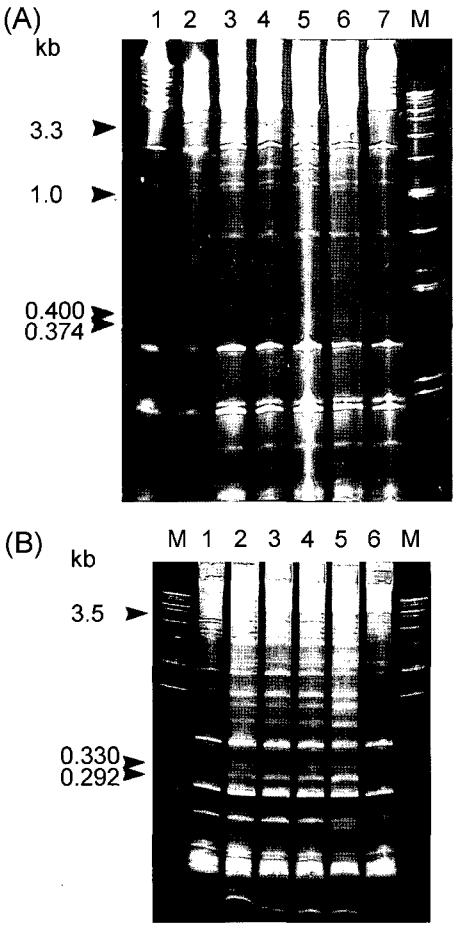

Fig. 3. Ethidium bromide stained 6% polyacrylamide gels showing nucleic acid bands from squash cv. White Bush F_1 hybrid (A) and tomato cv. UC82B (B). Photo (A): Lanes 1 and 2, KU2 infected squash; Lanes 3-6, KU1 infected squash; Lane 7, healthy (uninfected squash); M, 1 kb DNA marker. Photo (B): Lanes M, M, 1 Kb DNA markers; Lane 1, healthy (uninfected) tomato; Lane 2, KU1 infected tomato; Lanes 3, 4 and 5, KU1 infected tomato challenged with KU2 after 1 wk, 2 wk and 3 wk of pre-inoculation, respectively; Lane 6, KU2 infected plants.

better resolution, showed the presence of amplified DNA fragment of about 160 bp in KU1 infected tomato and squash plants but no such bands in healthy plants (Fig. 4B). No such amplifications were detected in KU2 infected RT-

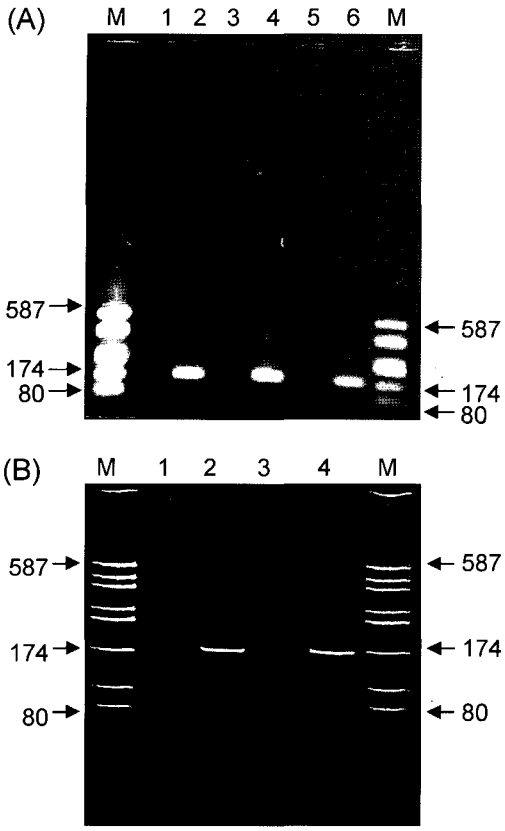

Fig. 4. Agarose (1%) gel (A) and polyacrylamide (5%) gel (B) electrophoretic analyses of RT-PCR amplified cDNA products from KU1 strain of CMV in tomato cv. UC82B and squash cvs. Boma and White Bush F_1 hybrid. Photo (A): Lanes M, M, DNA micromarker; Lane 1, healthy tomato cv. UC82B; Lane 2, KU1 infected tomato cv. UC82B; Lane 3, healthy squash cv. Boma; Lane 4, KU1 infected squash cv. Boma; Lane 5, healthy squash cv. White Bush F1 hybrid; Lane 6, KU1 infected squash cv. White Bush F1 hybrid. Photo B: Lanes M, M, DNA micromarker; Lane 1, healthy tomato cv. UC-82B; Lane 2, KU1 infected tomato cv. UC-82B; Lane 3, healthy squash cv. White Bush F_1 hybrid; Lane 4, KU1 infected squash cv. White Bush F_1 hybrid.

PCR products.

Preinoculation and challenge inoculations in growth chambers. The best time for challenge inoculation of tomato cultivar was determined by conducting experiment of preinoculation and challenge inoculations in the growth chambers. In comparison to healthy and KU1 infected symptomless tomato plants, KU2 infected plants were found stunted with chlorosis and mosaics on leaves (Fig. 5A). Maximum growth with minimum disease incidence was visualized in preinoculated tomato plants challenged after 3 weeks followed by 2 weeks and then 1 week after preinoculation (Fig. 5B). The satellite RNA protection of preinoculated tomato plants against the challenge of the severe virus strain KU2 in the growth chambers is shown in

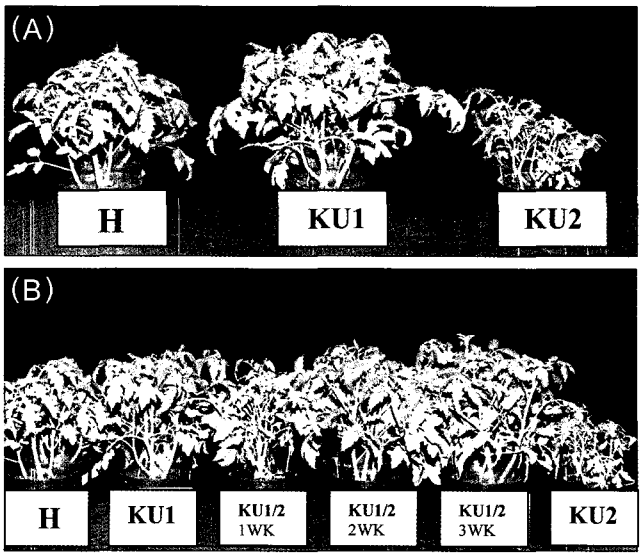

Fig. 5. Comparison of KU1 infected tomato cultivar UC82B plants (KU1) with KU2 infected stunted (KU2) and uninfected healthy control (H) plants (A). Preinoculation and challenge inoculation experiment in growth chambers to determine the length of time after preinoculation required to render tomato plants most resistant to challenge by severe strain KU2 (B). Tomato plants cv. UC82B from left: healthy (H); pre-inoculated with KU1 + KU1-CARNA 5 (KU1); challenge inoculated with KU2, 1, 2 and 3 weeks after preinoculation with KU1 + KU1-CARNA 5 (KU1/2); and inoculated only with the severe strain KU2 (KU2).

Fig. 5B.

Discussion

This study confirmed the fact that CMV is a wide host range virus, differing in symptom appearance pattern according to the viral strain used. In the present investigation, test plants selected for experiment were susceptible to viral infection by both strains KU1 and KU2 of CMV (Al-Sharidah, 1996; Montasser et al., 1991). Our results showed that the strains differed from each other, in the way of appearance of symptoms on different hosts and depending upon that were considered as mild strain KU1 and severe strain KU2 of CMV. The severe KU2 strain showed stunting, chlorosis and mosaic symptoms on tomato and severe mosaics on squash as well as on tobacco leaves. However, KU1 strain was found as a mild strain showing mosaic on squash leaves but symptomless on tomato (Montasser et al., 1991). Similarly, in tobacco plants also, this KU1 strain showed mild mosaics on very young leaves but later the plants were found symptomless. One peculiar feature observed for the first time during this investigation was the appearance of local chlorotic spots on the cotyledonary leaves of cultivar White Bush F_1 hybrid of squash

along with the systemic symptoms on the rest of plant leaves. These local symptoms were more severe in plants inoculated with the KU2 strain. Extracted CMV nucleic acid were also viable and effective in inoculation test (Montasser et al., 2006). This was proved by back inoculating the test plants with extracted nucleic acids of virus-infected plants.

According to Roossinck (2001), three genomic viral RNAs, designated RNA1 (3.3 kb), RNA2 (3.0 kb) and RNA3 (2.2 kb) are packaged in individual particles along with subgenomic RNA, RNA4 (1.0 kb), which is packed with genomic RNA 3. Nucleic acid band within the range of 339-347 bp was designated as RNA 5 or CARNA 5. Thus during our gel electrophoretic analyses, nucleic acid bands appeared near or equal to these size could be expected as viral RNAs and satellite RNA bands observed. The presence of satellite RNA was proved by undergoing RT-PCR and a DNA fragment of about 160 bp was consistently amplified from KU1 infected tomato and squash plants. No such amplifications were observed in healthy and KU2 infected plants. As the primer pair designed was able to amplify cDNA fragmented in KU1 infected plants, confirmed the presence of viral satellite RNA in this mild strain of CMV (Crescenzi et al., 1993; Finetti Sialer et al., 1999; Singh et al., 1995; White and Kaper, 1989). The detection of viral satellite in mild KU1 strain led us to use this as a biological control agent against the other severe strain (KU2) detected in Kuwait.

Preliminary level preinoculation and challenge inoculation experiment showed a complete protection in plants challenged 3 weeks after preinoculation, followed by 2 weeks and least prevention in plants challenged 1 week after preinoculation. The results demonstrate that introduction of a non-necrogenic CMV satellite via preinoculation or "vaccination", into tomato plants will prevent severe disease following later infection by a severe strain of CMV. The preinoculation technique has the advantage of providing a rapid response to viral epidemics (Montasser et al., 1998) and the satellite mediated protection of tomato (Montasser et al., 1991; Yoshida et al., 1985) and pepper (Montasser et al., 1998; Wu et al., 1989) against CMV infection has been tested. This technology is now being applied commercially in Japan for processing and market tomatoes and cucurbits and investigations are in progress in Indonesia too (Montasser et al., 1998). However, in China, where this technology has been applied since the 1980s, Tien and Wu (1991) reported no new virus diseases and no CMV satellite RNA induced tomato necrosis from preinoculated fields.

Our research studies lead towards the development of a technology for better and a suitable "vaccine" production by using viral satellite of mild strain as biological control agent to protect economically important tomato crops against other severe viral diseases. Thus the demonstration of the effectiveness of preinoculation or "vaccination" against a natural challenge infection emphasizes the usefulness of the method of satellite mediated biological control of CMV.

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