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Cucumber Mosaic Cucumovirus-CARNA5 Causing Bud Necrosis on Table Tomato

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Virus disease occurred up to 62% in average in the greenhouse production of table tomato Seokwang in Suwon, Korea. From symptomatic transition of the labeled tomatoes, two different symptoms, mosaic and bud necrosis, were developed independently. *Cucumber mosaic virus* necrosis strain (CMV-N) was isolated from table tomato showing bud necrosis symptoms. The isolate caused the bud necrosis on four tomato cultivars and locally infected *Chenopodium* spp. and *Vicia faba* by mechanical inoculation. The 5th RNA segment, satellite RNA, was identified from CMV-N-infected plants by dsRNA analysis. Crystals of virus particles were observed in cytosols and vacuoles. The virus particles of CMV-N presented abundantly in xylem vessel.

Keywords **:** bud necrosis, CMV-CARNA5, tomato.

Cucumber mosaic virus (CMV) is a member of genus *Cucumovirus* and a small icosahedral plant virus consisting of three genomic and one subgenomic RNAs (Peden and Symons, 1973). The RNAs were designated as RNA 1 to RNA 4 in decreasing order of molecular weight.

Tomato necrosis disease has devastated tomato crop in Alsace, France in 1972 (Kaper and Waterworth, 1977), Basiticata, Italy in 1988 (Gallielli et al., 1991), and eastern Spain in 1989 (Escriu et al., 2000). Nowadays the disease is reported to occur worldwide. CMV-associated RNA 5 (CARNA5) is a causal agent of the necrosis disease and its nucleotide sequences are different from those of the 4 genomic RNAs. The CARNA 5 is categorized as a satellite RNA (Kaper and Waterworth, 1977) and plays a role as a regulator of disease expression in viral pathogenesis (Waterworth et al., 1979).

Necrosis disease could not be induced by the kinds of host or temperature treatments on plants infected with CMV having Y satellite RNA (Wu et al., 1993). Variation of sequences among the satellite RNAs induced different biological symptoms (Mossop and Francki, 1978; Kaper et al., 1981). Some satellite RNAs that alleviated disease symptoms in tomato plants (Mossop and Francki, 1978) were used to prevent severe diseases by cross-protection (Gallitelli et al., 1991; Montasser et al., 1991; Yoshida et al., 1985; Wu et al., 1989).

In Korea, four viruses, namely, CMV, *Tomato mosaic virus*, *Tobacco mosaic virus* and *Potato virus Y*, have been reported in tomato crop (Choi et al., 1997). In this experiment, a new CMV strain with a satellite RNA was identified from table tomato showing bud necrosis symptoms including necrotic leaf spots and stem streaks, and eventually plant death.

Materials and Methods

Disease investigation. The incidence of virus disease on table tomato 'Seokwang' was investigated in 6 plastic houses at Suwon area in the spring of 1997. Sixty-three plants of table tomato per house were labeled for the visual inspection of symptom expression. Symptom expression of the labeled tomatoes was investigated visually every 14 days for 5 times after transplanting.

Biological tests. Seven samples were chosen by the different symptom development including mosaic at initial to bud necrosis at final stage. The selected specimens were inoculated mechanically to 9 different indicator hosts including *Chenopodium quinoa*. Pure isolation of virus isolates was conducted biologically. The pure isolates were used in host range and symptom studies. Inoculum was made by maceration with mortar in a 4 volumes of 0.01 M sodium phosphate buffer, pH 7.0. After mechanical inoculation, indicators were washed immediately with tap water.

Electron microscopy. Leaf tissues from plants artificially infected with the virus isolate and from field samples with typical symptoms were fixed overnight in Karnovsky's fixative in 0.2 M cacodylate buffer, pH 7.2, and post-fixed with 1% osmium tetroxide for 2 hrs. The tissues were dehydrated with ethanol, 50% to 100%, in six steps and embedded with Epon 812. The specimens

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were sectioned in 80 nm thickness and stained with uranyl acetate and lead citrate for 10 min and 5 min, respectively, before electron microscopy.

Analysis of dsRNAs. Five grams of fresh leaf tissue showing necrosis symptom was macerated in a mortar with liquid nitrogen. The powdered leaf tissues added with 10 ml of ice-cold 2X tris-sodium EDTA (STE) buffer containing 1.0 ml sodium dodecyl sulfate (10%), 10 mg bentonite and 10 ml STE-saturated phenol were clarified by low centrifugation. The aqueous phase was purified further with Whatman CF11 cellulose. The dsRNA was purified finally from the two cycles of ethanol precipitation. The purified 20 μl dsRNA was analyzed by electrophoresis on 5.0% polyacrylamide or 1.0% agarose gel. The nucleic acid was stained with ethidium bromide (10 ng/ml) in electrophoresis buffer for 15 min (Dodds, 1993; Valverde et al., 1990).

Results

Disease occurrence. Average infection rate of the disease was 32.1% in the first observation to 61.7% in the fifth reading (Table 1). The disease incidence varied depending upon the greenhouses. The average infection rate was increased up to 2 times for 60 days of investigation period.

Symptomatology. Symptoms observed from diseased tomatoes were grouped into two: mosaic/yellow mosaic, and bud necrosis/necrotic spots (Fig. 1). Mosaic or yellow mosaic symptoms were developed in plants with no symptom or mild mosaic and yellow mosaic in the initial stage. Meanwhile bud necrosis was developed from the labeled plants showing distinct mosaic and yellow mosaic in the initial stage. Different increase patterns were noticed in the two symptom groups, showing rapid increase of necrosis compared with slow increase of mosaic (Fig. 2).

Cucumber mosaic virus-necrosis strain. Out of 23 indicator plants, 14 plants including cucumber were infected systemically when inoculated mechanically with CMV-necrosis (CMV-N) strain (Table 2). The strain locally infected *Chenopodium* spp. and *Tetragonia expansa*, while it could not infect other six indicators including *Vigna sesquipedalis*. It also caused bud necrosis in cherry tomato 'Minicarol' and 3 cultivars of table tomato. Necrosis was induced initially on newly developed leaves and then spread to main stem (Fig. 3).

Table 1. Seasonal occurrence of virus disease on table tomato

Replication	% Infection rate[a]				
	1st	2nd	3rd	4th	5th
1	65.1	69.8	71.7	75.0	80.0
2	34.9	49.2	63.5	68.3	74.6
3	30.2	36.5	41.9	46.8	58.1
4	27.4	32.3	42.6	49.2	54.1
5	25.4	37.1	37.1	41.9	53.2
6	9.5	14.3	12.9	22.6	50.0
Average	32.1	39.9	45.0	50.6	61.7

[a] Infection rate of table tomato 'Seokwang' was judged visually in greenhouses at Suwon area. Disease investigation was done five times in every 14 days from August 11 in 1996.

Fig. 1. Bud necrosis on the table tomato 'Seokwang'.

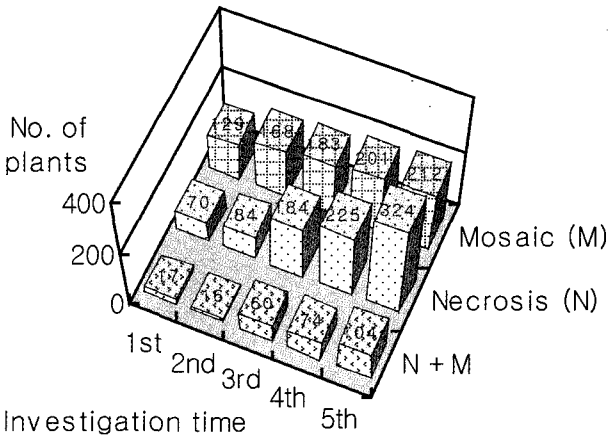

Fig. 2. Symptom development of virus disease in table tomato 'Seokwang' in greenhouses.

dsRNA analysis of necrosis strain. dsRNA patterns were compared between CMV-N induced bud necrosis and CMV-M induced mosaic symptom. CMV-N showed the fifth band in addition to 4 bands of CMV-M (Fig. 4).

Ultrastructures. Virus particles of CMV made circular or

Table 2. Reactions of cucumber mosaic virusnecrosis strain isolated from table tomato on the indicator plants

Indicator plants	Reaction[a]
Chenopodium quinoa	NL/–
C. amaranticolor	NL/–
Gomphrena globosa	NL/SM
Nicotiana glutinosa	NL/SM,FL
N. rustica	NL/M
N. benthamiana	NL/SM
N. clevelandii	NL/SM,FL
N. tabacum 'Ky-57'	NL/M
N. tabacum 'Xanthi NC'	NL/M
N. tabacum 'Bright yellow'	NL/M
Datura stramonium	CL/M
Physalis floridana	–/SM,FL
Capsicum annuum 'Hanbyul'	–/M
Cucumis sativus	–/M
Cucurbita pepo	–/CL,M
Vigna sesquipedalis	–/–
V. unguiculata	–/–
Phaseolus vulgaris	–/–
Vicia faba	–/–
Tetragonia expansa	CRL/–
Brassica camperstris pekinensis 'Chunhawang'	–/–
Raphanus sativus	–/–
Lycopersicon esculentum 'Minicarol'	–/BN
Lycopersicon esculentum 'Seokwang'	–/BN
Lycopersicon esculentum 'Pungsaeng'	–/BN
Lycopersicon esculentum 'Kwangmyung'	–/BN

[a]Symptoms were investigated at 1-2 weeks after mechanical inoculation. BN: Bud necrosis, CL: Chlorotic local, CRL: Chlorotic ring local, FL: Fern leaf, M: Mosaic, NL: Necrotic local, SM: Severe mosaic, –: non-reaction. Inoculated leaves/Upper leaves.

angular crystals in cytosols and vacuoles. The virus particles were arranged linearly with circle and also presented abundantly in xylem vessels (Fig. 5).

Discussion

Cucumber mosaic virus (CMV) having RNA 5 segment was a causal agent for the expression of bud necrosis followed by plant death in tomatoes. CMV having satellite RNA isolated from tobacco plants showing a brilliant yellow symptom expressed necrosis disease in tomato plants (Takanami, 1981). RNA 5 might not be involved in host range but symptom severity of necrosis or mosaic depending upon the host species (Kaper and Tousignant, 1977). CMV typically induced local lesions on *Vigna* species and Chenopodiaceae. However, CMV necrosis strain (CMV-N) isolated from tomato showing bud necrosis in this study had narrow host range by non-infection on *Vigna* species and Cruciferae (Cho et al., 1997).

Tomato necrosis disease was reduced gradually and almost completely disappeared by genetic recombination of satellite RNAs population in eastern Spain (Escriu et al., 2000). The mixed symptoms of necrosis and mosaic on tomato in the field may be influenced by virulence variation between CMV-N and CMV-mosaic (CMV-M) strains. The mixed infection of CMV-N and CMV-M may be a possible reason for strain variation in field-grown tomatoes by the genetic variability of RNA 5 compared with those of other RNA genomes (Aranda et al., 1993).

The cells of tomato infected with CMV-N had virus particles more abundantly in cytosols, vacuoles and especially in xylem vessels than those of CMV-mosaic strain. The long distance movement of virions through xylem or phloem is an important mechanism in symptom expression with cell to cell movement. The movement of virus particles of CMV-N through xylem vessel may be an important

Fig. 3. Symptoms of bud and stem necrosis (left), and necrotic spots (right) in table tomato 'Seokwang' induced by mechanical inoculation of *Cucumber mosaic virus* having RNA 5.

Fig. 4. dsRNA analysis of *Cucumber mosaic virus* (CMV) producing bud necrosis in table tomato. CMV-N: CMV-necrosis strain isolated from table tomato showing bud necrosis. CMV-M: CMV-mosaic strain isolated from table tomato showing mosaic symptom.

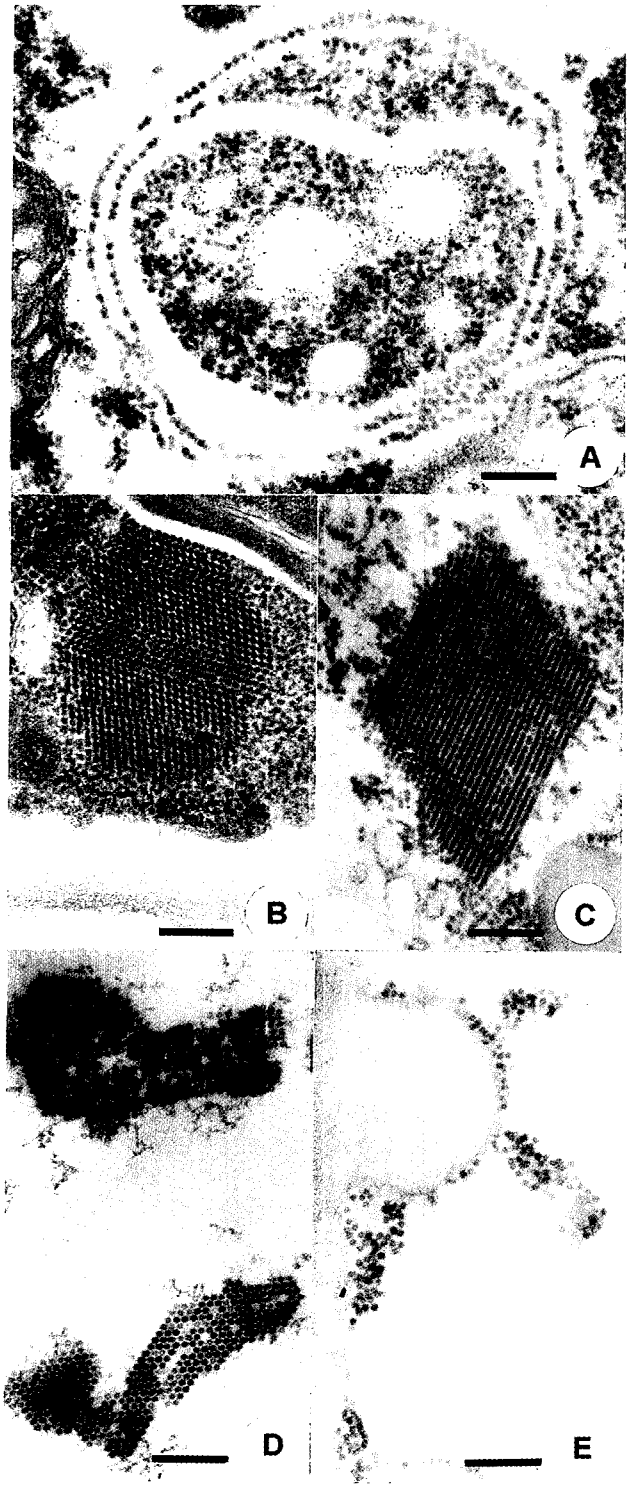

Fig. 5. Virus particles (VP) of *Cucumber mosaic virus* RNA 5 observed frequently in both parenchyma cells and vessels. VPs were arranged lineally (A), and a round crystal (B) or an angled crystal (C) found in cytoplasm. The amorphous crystals of VP were observed mostly in vacuoles (D). VPs adhered abundantly on the surface of secondary cell walls and located in the middle of vessels (E). Bar=250 nm.

factor for the expression of severe symptoms or plant death.

Cross-protection could protect effectively necrosis disease in tomato plants using crude sap of CMV mild strain having non-necrogenic satellite RNA (Gallitelli et al., 1991; Montasser et al., 1991). Because two viruses of CMV-N and CMV-M occurred in combination in the same greenhouse, the vaccination to control virus disease using CMV-RNA 5 in tomato plants may be risk in the field conditions in Korea.

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