INFECTION OF TOMATO AND BELL PEPPER BY ToMV AND TMV AT DIFFERENT GROWTH STAGES AND ESTABLISHMENT OF VIRUS IN SEEDS

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SUMMARY

Tomato and bell pepper were inoculated with tomato mosaic Virus (ToMV) or tobacco mosaic virus (TMV) when they were 35, 50, 90, and 110 days old. Both viruses infected both tomato and bell pepper irrespective of growth stages. The virus in the leaf was quantified by local lesion assay and that in seed was determined by local lesion assay and ELISA. The viruses became established in the seeds, irrespective of growth stage at time of inoculation. However, the concentration of virus was high in seeds when the plants were inoculated early. Bell pepper and tomato are the preferred hosts for TMV and ToMV respectively.

RIASSUNTO

INFEZIONE DI ToMV E TMV SU POMODORO E PEPERONE A DIVERSI STADI DI CRESCITA E INSEDIAMENTO DEI VIRUS NEI SEMI. Piante di pomodoro e peperone di 35, 50, 90 e 110 giorni sono state inoculate col virus del mosaico del pomodoro (ToMV) o col virus del mosaico del tabacco (TMV).

Entrambi i virus hanno infettato sia pomodoro che peperone a prescindere dallo stadio di crescita. Il virus nelle foglie è stato quantificato mediante saggio delle lesioni localizzate e quello nei semi è stato determinato tramite saggio delle lesioni localizzate ed ELISA. I virus si sono stabiliti nei semi indipendentemente dallo stadio di crescita al momento dell'inoculazione, ma, la concentrazione del virus nei semi era alta quando le piante erano inoculate precocemente. Peperone e pomodoro sono gli ospiti preferenziali di TMV e ToMV rispettivamente.

Key words: ELISA, local lesion assay, seed infection, virus concentration.

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INTRODUCTION

Tomato mosaic virus (ToMV) and tobacco mosaic virus (TMV) are economically important viruses infecting tomato and bell pepper, causing moderate to heavy annual losses. ToMV and TMV are highly stable and can survive in soil and in seed. The viruses contaminate the seed coat of tomato and bell pepper. Seed acts as a source of primary infection for ToMV/TMV in tomato (Broadbent, 1965) and TMV in bell pepper (Tosic *et al*., 1980). The rate of seed transmission depends on the concentration of virus in seeds (Chitra *et al*., 1998) and the concentration in seeds in turn depends on the stage at which the plant is infected. Such a study is useful in indexing of seed production plots for viral infection and predicting the concentration, which in turn has epidemiological significance.

Early infection of plants with virus results in higher seed infection and greater yield loss (Broadbent, 1976). Tomato and bell pepper may be infected by ToMV and TMV, but in nature ToMV exclusively infects tomato, and TMV infects bell pepper. The relative susceptibility of these hosts to ToMV and TMV has not been studied. Further, the possible relationship between concentration of virus in leaf to that of seed is not established. Hence, this work was taken up.

MATERIALS AND METHODS

Raising of seedlings. Seeds of tomato and bell pepper were sown in clay pots containing soil, sand and farmyard manure (2:1:1 w/w/w). Four seedlings were maintained per pot. Four replicates of 16 plants each were used per growth stage.

The virus inocula were prepared by triturating ToMV- or TMV-infected dried leaf material (1 g) in 5 ml of 0.05 M PBS (pH 7.2). Tomato and bell pepper plants were inoculated with ToMV or TMV when they were 35, 50, 90 and 110 days old. At each growth stage, the terminal leaf was dusted with carborundum and inoculated by rubbing with a cotton swab soaked in inoculum.

Detection of ToMV/TMV in the inoculated plants. The newly formed leaves subsequent to the inoculated leaves were collected 10 days after inoculation and tested for ToMV/TMV by inoculating the leaf extract (0.2%, w/v in PBS, pH 7.2) onto the indicator plant *Nicotiana glutinosa*. Two leaves of the indicator plant were inoculated with extract of one treatment. The number of necrotic local lesions (NLL) per 100 cm^2 leaf area was calculated.

Detection of ToMV/TMV in seeds. Ripe fruits were collected from all plants. The tomato pulp containing seeds was allowed to ferment at room temperature for 24 h. The seeds were then washed and air-dried. Bell pepper fruits were cut into two. The seeds were removed and air-dried. The virus particles from seed samples (0.3 g) were extracted in phosphate buffer (3 ml) by gently grinding with a mortar and pestle. This inoculum was applied to *N. glutinosa*. The virus concentration was also determined by the indirect (DAC) ELISA procedure described by Hobbs *et al*. (1987). The antisera against ToMV and TMV were obtained from the Danish Government Institute of Seed Pathology for Developing Countries. Each microtitre well was loaded with 100 ml of seed extract in PBS. The virus was detected by alkaline phosphatase-conjugated swine anti-rabbit IgG and p-nitrophenyl phosphate as substrate. Absorbance greater than twice that of negative control was considered positive. The effect of growth stage, virus, replications, and interaction between virus and growth stage on virus concentration was tested based on four replicate samples using analysis of variance and least significant difference values. The local lesion data was subjected to log transformation and the absorbance data were transformed to arcsine values for statistical analysis.

RESULTS

ToMV or TMV produced symptoms in both the crops. In tomato plants the first symptoms of mosaic were seen in the newly formed leaves 12 days after inoculation with ToMV/TMV. All the inoculated plants developed mosaic. In addition leaf narrowing was also observed, when 35 day-old plants were inoculated with ToMV. The first cluster of flowers that were already formed dried and failed to set fruits when 90 day-old plants were inoculated with ToMV. In bell pepper, the inoculated leaves senesced faster and later, mosaic symptoms developed in the subsequent leaves. Thirty five day-old plants showed stem necrosis upon inoculation with TMV. Fruits showed uneven ripening and ringspots when the plants were inoculated at fruit set stage.

Concentration of virus in the leaves. All the leaf and seed extracts of tomato and bell pepper inoculated with ToMV or TMV produced local lesions on *N. glutinosa*. The relative concentration of ToMV in tomato leaves was significantly higher when compared to that of TMV, as evident from local lesion assay. More local lesions were obtained when plants were inoculated at early growth stages (35 and 50 day-old plants) (Table 1). In leaf samples of bell pepper, the concentration of TMV was greater than that of ToMV. Plants up to 90 days old were more susceptible than older plants (Table 2).

Table 1. Concentration of ToMV and TMV in leaves of tomato inoculated at different growth stages based on local lesion assay.

Age of plant (days) at time of inoculation	No. of local lesions 100 cm^{-2} leaf area		
	ToMV	TMV	Mean[a]
35	230	194	212[a]
50	192	165	178[a]
90	140	110	125[b]
110	100	77	88[c]
Mean[b]	166*	136	

[a] Based on eight combined replicate samples for ToMV and TMV.
[b] Based on 16 combined replicate samples for four growth stages.
* Significant at 5 % level.
The values followed by same letter(s) are not significantly different at 5 % level when compared by least significant difference test.

Table 2. Concentration of ToMV and TMV in leaves of bell pepper inoculated at different growth stages based on local lesion assay.

Age of plant (days) at time of inoculation	No. of local lesions 100 cm^{-2} leaf area		
	ToMV	TMV	Mean[a]
35	106	127	116[a]
50	94	115	94[a]
90	35	91	63[b]
110	22	34	28[b]
Mean[b]	60	92*	

[a] Based on eight combined replicate samples for ToMV and TMV.
[b] Based on 16 combined replicate samples for four growth stages.
* Significant at 5 % level.
The values followed by same letter(s) are not significantly different at 5 % level when compared by least significant difference test.

Concentration of virus in seeds. In tomato seeds, the concentration of ToMV was greater than that of TMV (Table 3). Further, the concentration of virus established in seeds was greater in plants inoculated at the first two growth stages. The relative concentration of TMV in seeds collected from inoculated bell pepper plants was higher compared to that of ToMV (Table 4). No significant F-value was obtained for differences among replications and for virus X growth stage interaction.

Table 3. Concentration of ToMV and TMV in tomato seeds collected from plants inoculated at different growth stages.

Age of the plant (days) at time of inoculation	No. of local lesions			ELISA (OD 410 nm)		
	ToMV	TMV	Mean[a]	ToMV	TMV	Mean[a]
35	399	276	338[a]	0.512	0.424	0.468[a]
50	316	250	283[ab]	0.346	0.375	0.360[b]
90	312	235	263[b]	0.306	0.345	0.326[b]
110	285	163	224[b]	0.261	0.322	0.291[c]
Mean[b]	328*	231		0.363*	0.346	

ToMV Threshold value 0.120 TMV Threshold value 0.150
Positive control 0.681 Positive control 0.695

[a] Based on eight combined replicate samples for ToMV and TMV.
[b] Based on 16 combined replicate samples for four growth stages.
* Significant at 5 % level.
The values followed by same letter(s) are not significantly different at 5% level when compared by least significant difference test.

Table 4. Concentration of ToMV and TMV in bell pepper seeds collected from plants inoculated at different growth stages

Age of the plant (days) at time of inoculation	No. of local lesions			ELISA (OD 410 nm)		
	ToMV	TMV	Mean[a]	ToMV	TMV	Mean[a]
35	203	532	367[a]	0.226	0.541	0.383[a]
50	124	413	268[b]	0.159	0.466	0.312[b]
90	104	358	231[bc]	0.123	0.408	0.266[c]
110	98	277	187[c]	0.112	0.374	0.242[d]
Mean[b]	132	395*		0.155	0.447*	

ToMV Threshold value 0.10 TMV Threshold value 0.18
Positive control 0.421 Positive control 0.62

[a] Based on eight combined replicate samples for ToMV and TMV.
[b] Based on 16 combined replicate samples for four growth stages.
* Significant at 5 % level.
The values followed by same letter(s) are not significantly different at 5% level when compared by least significant difference test.

DISCUSSION

ToMV or TMV can infect both tomato and bell pepper. Although ToMV is the most commonly reported virus on tomato, TMV is also known to infect this host (Rast, 1975). ToMV can infect bell pepper (Green and Wu, 1991). Komuro *et al.* (1966) have reported that tomato strain of TMV infected tomato more easily and also multiplied faster after invasion, than TMV. Our study also supported that observation. Further, we have shown that TMV concentration is higher in bell pepper than in tomato.

Early infection leads to seed infection in many virus/host combinations. The present study has shown that ToMV/TMV can enter in the seeds of tomato and bell pepper, irrespective of growth stage at the time of inoculation. Inoculation even at flowering and fruit-set stage can lead to establishment of virus in seeds. However, the concentration of virus in seeds will be higher, if the plants are infected at an early growth stage. Since ToMV/TMV occur in the seed coat, even late infection of the host leads to establishment of virus particles in the seed. Shepherd (1972) reported that ToMV/TMV, probably infect the outermost tissues of the ovule through cytoplasmic connections. These viruses, being stable, can survive in the seed coat derived from the outer wall of the ovule.

Occurrence of ToMV/TMV in tomato (Gooding and Suggs, 1976) and TMV in bell pepper seeds has been reported (Tosic *et al.*, 1980). The present study has shown that even ToMV can invade bell pepper seeds. However, the concentration of TMV in bell pepper was higher than that of ToMV, which indicates that bell pepper is more susceptible to TMV. Likewise ToMV shows a preference for tomato.

As ToMV/TMV infection of tomato/bell pepper at any growth stage can lead to establishment of virus in the seed, proper care has to be taken to raise a virus-free crop for seed production. Further, seed health testing and use of virus-free seed will help in alleviating the virus problem in tomato and bell pepper.

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