J. Turk. Phytopath., Vol. 32, No. 1, 1-7, 2003 ISSN 0378 - 8024

Detection of Tomato Mosaic Tobamovirus (ToMV) on the Local and Imported Tomato Seed by DAS-ELISA, Molecular Techniques and Electron Microscopy#

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ABSTRACT

The applicability of ELISA in combination with dsRNA analysis and RT-PCR were used to determine ToMV in the seeds and cotyledone leaves of seedlings. The virus was not found on the seed coats by ELISA, but detected on the cotyledone leaves of seedlings by ELISA, dsRNA analysis and RT-PCR.

Key words: Tomato Mosaic Virus, ELISA, Double-Stranded RNA (dsRNA), Reverse Trascriptase Polimerase Chain Reaction (RT-PCR)

INTRODUCTION

Because of it's suitable ecological condition and irrigation facilities, the Eastern Mediterranean region of Turkey is known as the most productive area for the early and late vegetable crops production in which tomato production takes an important place. The tomato production however is not correlated with the cultivated area, since the plants are affected by several mechanically, vector and seed transmitted viruses.

Rate of seed transmission is related to the time of infection, growth stage of plant, infected plant species and environmental condition. It was observed that plant grown from infected seeds produce higher percent of infection.

The most common virus diseases infecting tomato crops are Tomato Mosaic Tobamovirus (ToMV), Tomato Yellow Leaf Curl Geminivirus (TYLCV), Cucumber Mosaic Cucumovirus (CMV) and Potato Y Potyvirus (PVY)'es in Turkey (Güldür and Yılmaz, 1994).

The Grants were provided by The Turkish Scientific and Technical Research Council (TÜBİTAK, TARP-2431) and The Research Funds of Çukurova University.

DETECTION OF TOMATO MOSAIC TOBAMOVIRUS (TOMV) ON THE LOCAL AND IMPORTED TOMATO SEED BY DAS-ELISA, MOLECULAR TECHNIQUES AND ELECTRON MICROSCOPY

The seeds used in plantation are mostly imported by firms, but quarantine regulations are not sufficient for detection of viral pathogen in the seeds which results in dissemination of the diseses in the field. Therefore the seeds should be tested before the plantation in a given area by serological and the moleculer techniques.

The objective of this resarch is to detect viruses on the tomato seeds by utilizing of DAS-ELISA test, RNA extraction, dsRNA analysis and Polimerase Chain Reaction (PCR).

MATERIALS and METHODS

The tomato seeds from different farmers and firms (Razan (Petoseed); Roquetero FA 180 (Hazera); Fantastik (Hazera); Tomato F1 Belmonty (Enza Zaden); Falkon (MAY); Domato Mandur (Agromar); Tomato M 74 F1 (Agrotek); 73-33 RZ (RIJK ZWAAN)) were obtained. After germination of seeds in the greenhouse, young cotyledon leaves of seedlings were harvested; 1/3 of the leaf samples were assessed serological based on ELISA against to the antisera of Cucumber Mosaic Cucumovirus (CMV), Tomato Spotted Wilt Tospovirus (TSWV), Pepper Mild Mottle Tobamovirus (PMMV), the other 1/3 of the seedlings were proceeded for the total RNA extraction of ToMV and the RT-PCR tests.

The other part of the samples was mechanically inoculated to *Chenopodium quinoa* Wild, *C. Amaranthicolor* Costa & Reyn, *Datura stromarium* L., *Nicotiana glutinosa* L., *N. tabacum* L. "Samsun", *Nicotiana tabacum* L. "Samsun nn", *N. tabacum* L. "Rustica", *Nicotiana tabacum* L. "Xanthi", *Nicotiana tabacum* L. "Xanthi-nc" and *Gomphrena globosa* L.

The dsRNA analysis was made from the infected *N. tabacum* "Samsun" plants. The procedure was applied one by one to each of seed lots.

Virus Purification, SDS-PAGE Electrophoresis, Electron Microscopy and Serology

The young leaves of infected *N. tabacum* "Samsun" were used in purification of Tomato Mosaic Virüs. The purification was made by Gooding and Hebert, (1967). Molecular weight of ToMV protein was determined with the method described by Laemelli, (1970). The purified material was examined by Elektron Microscopy (Milne, 1993). The infectivity rate of seed transmission was determined by DAS-ELISA on the seeds after germination.

In each lot 25 seeds were used for the testing. DAS-ELISA was perfermed according to the method of Clarck and Adams (1977).

Total RNA Extraction, Double-Stranded RNA (dsRNA) Analysis and Electrophoresis

The young leaves of ToMV infected *N. tabacum* "Samsun" plants and young cotyledon leaves of germinated seeds were used in total RNA extraction (Astruc et al., 1996). In the dsRNA analysis ToMV infected Samsun leaves were used (Valverde et al., 1990). Electrophoresis was performed in 1.0% agarose gel (Galitelli and Minafra, 1994). The size of amplified fragments was determined by DNA molecular weigth markers (SIGMA P9577, PCR Marker, 50-2.000 bp).

Reverse Trascriptase Polimerase Chain Reaction (RT-PCR)

The purified nucleic acide of ToMV was amplified for detection of causal organizm in the tomato seedlings (Castello et al., 1995). RT-PCR was accomplished in a Techne-Genius thermal cycler (model FGEN02TD).

The sequence of complete genome was used for designing the spesific primers (HPLC purify Iyontec) to ToMV (Genbank in www.ncbi.nlm.nih.gov). To detect ToMV, the primers (PrForward 5'-GAAGAGCTAATGCGTCGGGC identical to bases 2671-2690, PrReverse 5'-ATCTGCGTAGCCCCTTGAGA complementary to bases 3157-3176) of ToMV complete genome from Genbank AF155507 were employed in RT-PCR.

Amplification of complementary DNA (cDNA) from the viral RNA, a 25 µl reaction mix containing AMV-Reverse Transcriptase (RT) and RT-buffer (Promega), RNasin (Promega), dATP, dGTP, dCTP, dTTP (Promega) and primer reverse (PrR) in the RT step, for the amplification of cDNA in PCR step the 25 µl of mixture containing $MgCl_2$ and $MgCl_2$-Buffer (Promega), dATP, dGTP, dCTP, dTTP (Promega), Taq polymerase (Promega) and primers reverse and primers forward were used.

RT reaction at 37 C° for 1 hour, PCR reaction at 94 C° for 30 second, 60 C° for 1 minute and 72 C° for 1 minute, for 35 cycle with a final extension at 72 C° for 10 minute for one cycle were carried out in the termocycle.

RESULTS and DISCUSSION

The molecular techniques were employed to detect ToMV on the tomato seeds and seedlings that were provided by different firms and farmers.

After germination of ToMV infected tomato seeds the leaf deformation, and mosaic on the leaves were observed. At the time of harvesting, uneven ripening and discoloration, and necrosis type of symptoms developed on the fruits. The fruits were also reduced in size comparing to that of control (Figure 1). The similar type of symptoms were reported on the fruits and leaves by Jones et al., (1991); Mayhew et al., (1984); Lanter et. al., (1982).

The typical symptoms of ToMV were expressed on the indicator plants (Yorgancı, 1975; Andrade ve ark., 1981; Güldür and Yılmaz, 1994).

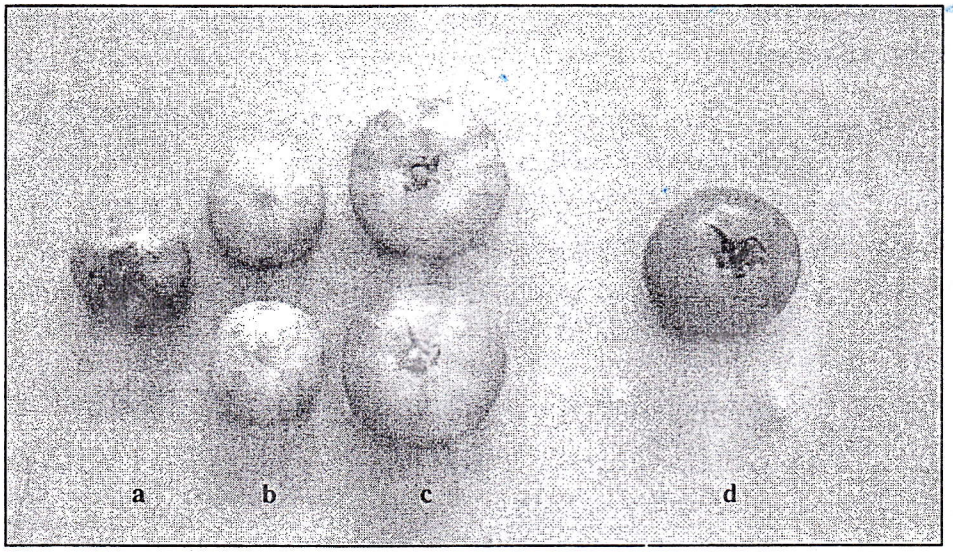


Figure 1. Infected (a,b,c); healthy (d) Tomato Fruits.

ToMV was not directly found on the tomato seed, but easily found on the cotyledone leaves of seedling by ELISA. CMV, TSWV and PMMV were not found out on the same samples.

The straight ToMV particules (300x18 nm) were observed on the purified material under electron microscopy (Figure 2). The moleculer weight of the coat protein was determined as 17.6 kDa by SDS-PAGE. The other workers on the morphology of ToMV particles and the molecular weight of coat protein have reported similar findings (Mayhew et. all., 1984; Fraenkel- Conrat, 1957).

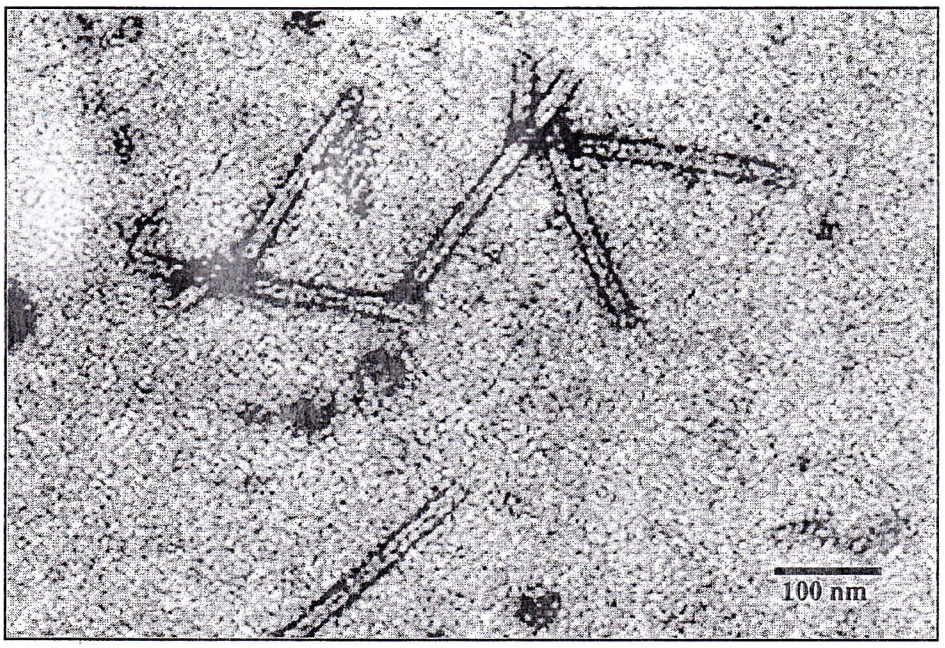


Figure 2. Tomato Mosaic Tobamovirus (ToMV) Particles.

ToMV was diagnosed by dsRNA analysis in the young leaves of tomato seedling (Figure 3). The extract of RNA of ToMV from infected samples were amplified using primers spesific to ToMV. The fragment with size expected (505 bp between Pr forward and Pr reverse of ToMV) for amplification on ToMV RNA was observed by agarose gel electrophoresis analysis (Figure 4).

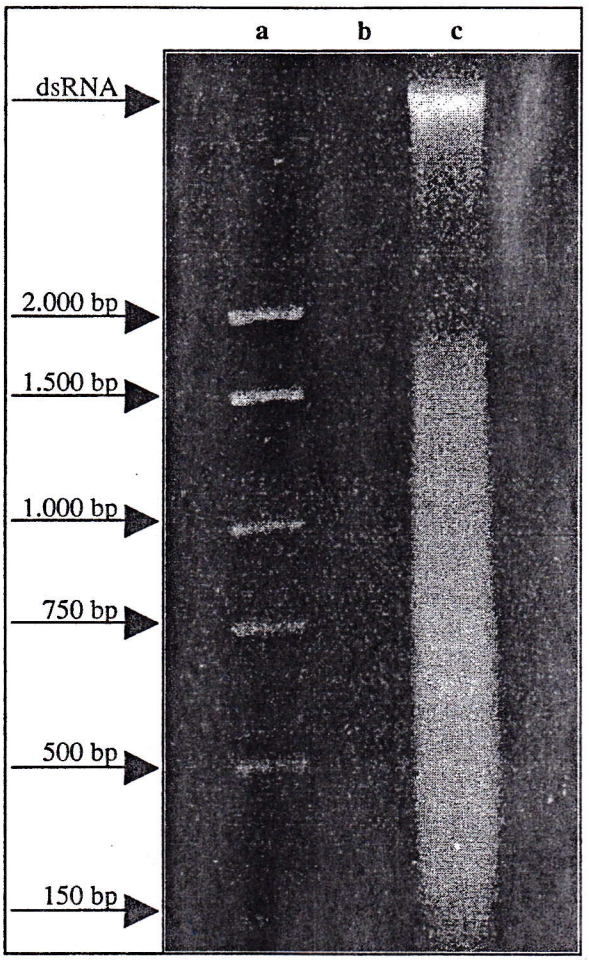


Figure 3. dsRNA analysis; marker (a), healthy (b), and infected tomato (c).

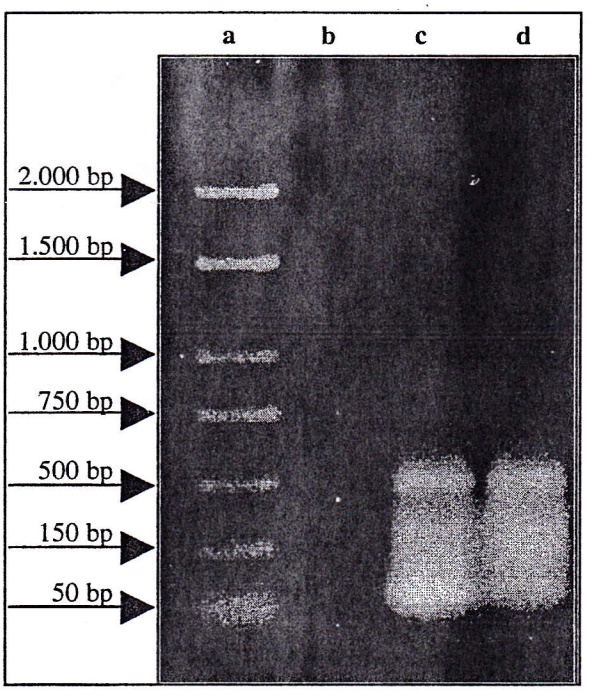


Figure 4. Agarose gel electrophoresis of RT-PCR products (500bp) by spesific primers to ToMV. Marker (a), healthy (b), infected (b,c) tomato.

ÖZET

MOLEKÜLER TEKNIKLERLE YERLI VE YURT DIŞINDAN GETIRILEN DOMATES TOHUMLARINDA DOMATES MOZAYIK VIRÜSÜ (TOMV)' NÜN SAPTANMASI

Domates tohumlarında ve domates fidelerinin kotiledon yapraklarında ToMV nün varlığını saptamak için ELISA, dsRNA analizi ve RT-PCR yöntemleri kullanılmıştır. ELISA testi ile yapılan çalışmalarda domates tohumlarından hazırlanan ekstraklarda ToMV saptanmamıştır. Buna karşılık çimlendirilen domates fidelerinin kotiledon yapraklarında

ELISA testi dahil olmak üzere kullanılan dsRNA analiz ve RT-PCR teknikleri ile ToMV kolaylıkla saptanmış bulunmaktadır.

Anahtar Kelimeler: Domates Mozayik Virüsü, ELISA, Çift İplikli RNA RNA (dsRNA), Reverse Trascriptase Polimeraz Zincir Reaksiyonu (RT-PCR)

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