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Biological, Physico-chemical and Serological Characteristics of TMV Strains Isolated from Tobacco, Tomato and Pepper Plants

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담배, 토마토 및 고추에서 분리된 TMV 계통의 생물학적, 물리화학적 및 혈청학적 특성

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ABSTRACT : Three strains of TMV isolated from tobacco, tomato and pepper plants in Korea were characterized based on biological response, serological relationship, and peptide mapping of the capsid proteins. The strains designated as TMV-common, TMV-pepper, and TMV-tomato could be distinguishable by different visual symptoms on 3 varieties of tobacco, one variety of tomato and pepper for each among 27 plant specieces. Serological relationships were examined by agar gel double diffusion test. Only traceable or weak reaction was observed in the incompatible antigen-antibody combinations. The pepper strain, however, showed trace in reaction with other two antisera. Peptide maps of the capsid proteins digested by V8 protease or by trypsin were also distinguishable, suggesting differences in composition and/or sequence of the amino acids among the strains.

Key words : TMV, strains, tomato, pepper, tobacco

Tobacco mosaic virus (TMV) is distributed worldwide, and is economically important virus which has a wide host range of about 119 plant species including tobacco (Zaitlin, 1975). TMV causes severe economic losses by reducing yield up to 50% and tobacco quality (Lucas, 1975). The virus is also transmitted through seed in pepper and tomato (Neergaard, 1977) which are typical plants damaged severely by the virus due to ease of transmission during the plant cultivation (Fig. 1-a,b). The three crops are cultivated in the similar periods of season and in the same areas. Therefore, TMV strains of the crops may be interrelated, and characterization of the TMV strains is very important to develop control measures for the virus.

In recent years, studies on development of virus-resistant plants by using foreign genes including viral coat protein gene have been greatly

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extended with the help of rapid progresses in molecular biology (Beach, 1993; Fitchen and Beach, 1993). To obtain successful outcomes from the studies, biological and physico-chemical characteristics of viruses are to be understood to provide substantial knowledges for the studies. In this study, the natural TMV isolates from tobacco, pepper and tomato were isolated and identified based on their biological and physico-chemical and serological characteristics, attempting to classify the strains.

MATERIALS AND METHODS

Virus isolate. Fresh leaves with mosaic symptoms of tobacco, pepper and tomato plants were collected from fields. The leaves were squeezed, and the plant sap was inoculated to tobacco (Xanthi-nc). The local lesions produced on the inoculated leaves were individually detached and used for reinoculation of the virus on the plant. Single local lesions were ground with a mortar and pestle and inoculated on the systemic host plants, tobacco (NC 82), pepper (California Wonder) and tomato (Seokwang) for the virus multiplication. Three weeks after inoculation, the leaves with symptoms were dried at 4℃ in a bottle with $CaCl_2$, and stored at -70℃, which were used as inoculum sources.

Host range test. Twenty-seven test plants including tobacco (NC 2326) were inoculated with the virus inocula after dusting 500-mesh carborundum on the leaf surfaces. The inoculated plants were placed in a green house at 22-27℃. Five plants were used for each test plant - virus inoculum combination. Symptom appearance was visually examined up to 4 weeks after inoculation.

Virus Purification. Each virus inoculum was inoculated on the respective susceptible host plants, tobacco (NC 82), pepper (California Wonder) and tomato (Seokwang) for the virus multiplication. Three weeks after inoculation, infected leaves were homogenized in 0.5 M phosphate buffer (pH 7.2) containing 1% 2-mercaptoethanol (100ml/100g leaves). The plant sap was homogenized again with n-butanol to make 8% (v/v), and centrifuged at low speed. The pellet containing chlorophyll and plant debris was discarded, and PEG (MW 8,000) was added to the supernatant, making 4% PEG solution, and was centrifuged two cycles at low speed to precipitate virus. The virus in pellet was resuspended, and is finally purified by density gradient centrifugation by using 38% CsCl solution. Purified virus was tested in spectrophotometry to measure optical density and virus concentration ($E^{0.1\%}_{1cm,\ 260nm} = 3.0$).

Production of polyclonal antibody. One ml of purified virus (2 mg virus) resuspended in phosphate buffered saline (PBS) was emulsified with 1ml of the complete Freund's adjuvant, and 1ml emulsion was injected into the thigh muscle of a hind leg of a New Zealand White rabbit. Boost immunization was performed intramuscularly 2 weeks later with 1mg antigen emulsified in the incomplete Freund's adjuvant (1:1) and the third booster was intravenous injection of 0.5mg antigen in PBS to the marginal vein of the ear. For collection of antiserum, the rabbit was bled from the marginal vein of the ear 10 days after the final booster immunization.

Serological relationships between strains. Serological relationships among three virus isolates from tobacco, pepper and tomato were examined in Ouchterlony agar-gel double diffusion test. For the test, 0.5% agarose (Sigma, Type IV) gel containing 0.1% NaN_3 and 0.75% NaCl was prepared. Wells were made by punching in the gel, and purified virus solutions and TMV antiserum were placed in the wells. One day later, the antigen-antibody reaction was examined.

Partial digestion analysis of TMV coat proteins. Purified TMV coat proteins were partially digested with proteases for the peptide analysis by the modified method of Cleveland, *et al.* (1977). Reaction mixtures contained 20μg of purified coat proteins, 4$\mu\ell$ of 0.1M $NaHCO_3$, 2μg of V8 protease or 200ng of trypsin, making the final volume 40$\mu\ell$ with H_2O. After incubation for 40 minutes at 37℃, the reaction was stopped by adding 10$\mu\ell$ of 5X dissociation buffer (60mM Tris-HCl, pH 6.8, 25% glycerol, 2.5% sodium dodecylsulfate, 14.4mM β-mercaptoethanol, 0.2mg bromophenol blue) and was boiled for 30 minutes.

Partially digested peptides were separated by 15% discontinuous SDS-PAGE and the band patterns were analyzed after staining with Coomassie brilliant blue.

RESULTS AND DISCUSSION

Biological Reaction on differential hosts. Three virus isolates, TMV-common from tobacco, TMV-pepper from pepper, and TMV-tomato from tomato plants were inoculated to 27 test plants including tobacco (NC 2326). Out of the plants tested, 5 differential plant were selected, which can differentiate three virus isolates (Table 1). In NC 2326 tobacco, necrotic local lesions were produced on the inoculated leaves to isolates from pepper and tomato plant, but no systemic symptoms were appeared on the plants (Fig. 1-f). Xanthi-nc tobacco had only necrotic local lesions on the inoculated leaves for all of the virus isolates, and the local lesions formed by the TMV-pepper isolate were smaller and appeared 2 days later than those formed by the other isolates (Fig. 1-e). Samsun tobacco was infected systematically by the inoculation of all the isolates. The tomato plants showed systemic mosaic symptom by inoculation with tobacco and tomato isolate, but no symptom by pepper isolate. In pepper plants, vein necrosis followed by leaf-dropping symptoms in inoculated leaves was observed when the plants were inoculated with tobacco or tomato isolate (Fig. 1-d). Based on the above results, these three TMV isolates were characterized by different symptoms on the differential hosts, and they seem to be different pathotypes or strains.

In this experiment, Samsun was an appropriate host for the multiplication of the TMV pepper isolate, for TMV is seed-borne in pepper (Neergaard, 1977) and the seeds should be certified to virus-free before planting for virus multiplication. Also the plant growth is slow, requiring long time for the virus multiplication.

Morphology of the virus particles. Purified virus particles of each isolate were negatively stained with 2% phosphotungstic acid (PTA) and examined under the transmission electron microscope (Zeiss, EM900). They had the shape of rigid rods with measurements about 300nm in length. There were no differences in shape and size of the virus particles among the isolates.

Purification and antiserum production. In this experiment, purified viruses of three isolates from the respective hosts (tobacco, pepper and tomato) were obtained as colorless pellets in test tubes. Yields of the viruses per 100g leaf tissues were 172mg for the tobacco isolate, 32mg for the pepper isolate, and 189mg for the tomato isolate. The yield of the TMV-pepper was very low, compared to the other isolates, suggesting that other host plants such as Samsun tobacco may be needed for the good viral multiplication.

In the microprecipitin test, the purified viruses reacted with respective antisera of the virus isolates,

Table 1. Symptoms of 3 isolates of TMV on selected differential hosts

Host	Inoculated leaf			Systemic symptom		
	Common	Pepper	Tomato	Common	Pepper	Tomato
Nicotiana tabacum						
cv. NC2326	-	LL	LL	M	-	-
cv. Xanthi-nc	LL	sLL	LL	-	-	-
cv. Samsun	-	-	-	M	mM	M
Lycopericon esculentum						
cv. Seokwang	-	-	-	mM	-	M
Capsicum annum						
cv. California wonder	N, LD	-	N, LD	-	M	-

- : No visual symptom, LL : Local lesions on inoculated leaves
M : Systemic mosaic, mM : Systemic mild mosaic
N : Necrosis, LD : Leaf drop
sLL : Small local lesions on inoculated leaves.

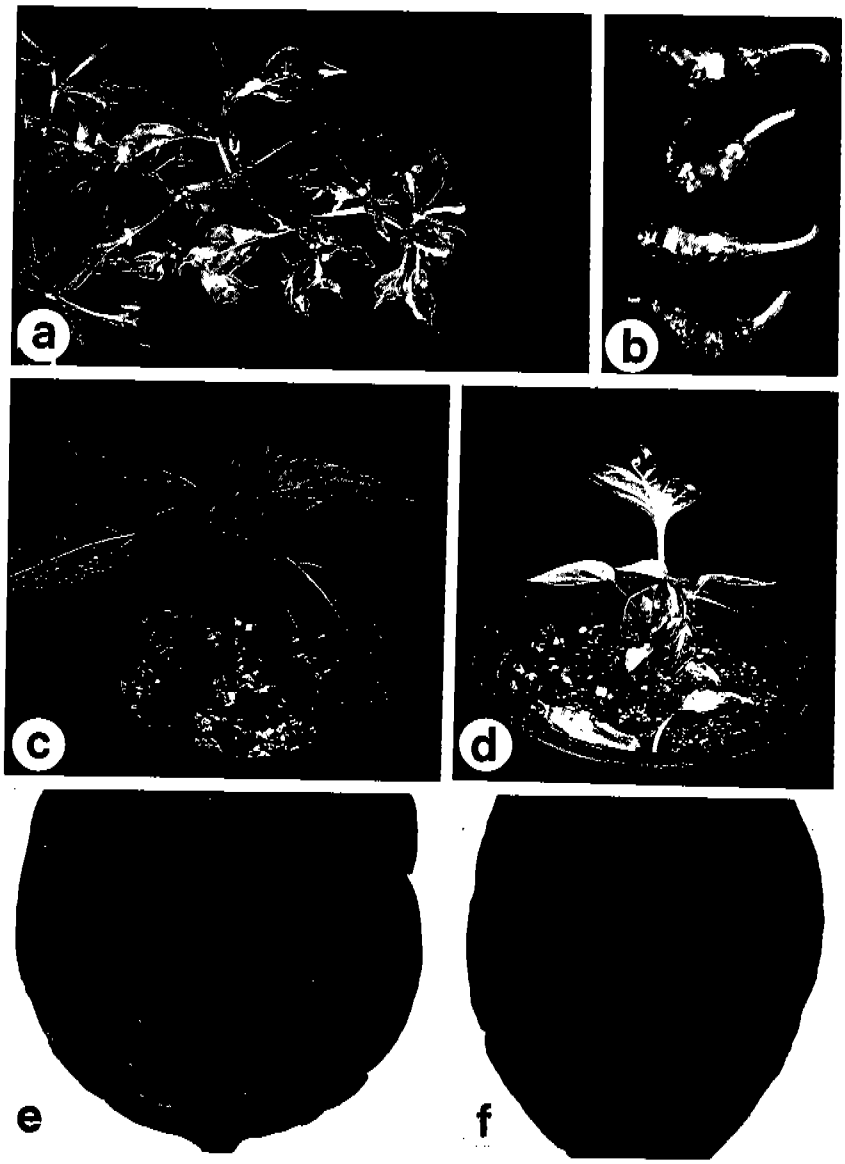

Fig. 1. Symptom of differential hosts caused by inoculation with TMV strains.
a, b; mosaic and deformation of pepper leaves and fruits infected with TMV-peppe strain under the natural field conditions, c; mild mosaic symptom on pepper cv. California Wonder 2 weeks after inoculation with TMV-pepper strain, d; necrosis and leaf dropping of pepper cv. California Wonder 2 weeks after inoculation with TMV-common strain, e; local necrotic symptom of tobacco cv. Xanthi-nc inoculated with TMV-pepper strain (left half) and TMV-common strain (right half), f; local necrotic symptom of tobacco leaf cv. NC 2326 inoculated with TMV-tomato strain (left half), and no symptom of the leaf inoculated with TMV-common (right half).

while the reactions of plant sap of healthy tobacco, pepper and tomato were negative. The antiserum titre was at the dilution of 1/1,024 for the three TMV isolates tested.

Serological relationship. Serological relationships among the TMV isolates were examined by agar gel double diffusion test. The reaction of TMV-tomato with TMV-common antiserum was weak, and that of TMV-pepper was only traceable (Fig. 2-A). Tobacco and tomato isolates had weak reactions with TMV-pepper antiserum (Fig. 2-B), and TMV-pepper strain had very weak (only traceable) reaction with TMV-tomato antiserum. The above results were consistent to those of serologically specific electron microscopy(data not presented here), indicating that serological relationships of the isolates were in order of TMV-common, TMV-tomato and TMV-pepper. The difference in serological reactions suggests that the three isolates may be composed of different capsid protein components.

Partial digestion analysis of TMV coat proteins. About 17 kDa of non-digested coat protein bands of TMV common, pepper and tomato strains (Fig. 3, Lane C, P, and T) were respectively detected. Peptide maps were obtained after partial digestion with V8 protease (Fig. 3, Lane C/V, P/V, and T/V), the 15 kDa protein band was observed in common strain only and 10 kDa protein band in pepper strain only. Protein band patterns between 10 kDa and 14 kDa were different among those strains. Lane C/T, P/T, and T/T on Fig. 3. showed the peptide maps obtained after partial digestion with trypsin. Protein band patterns between 15 kDa and 17 kDa of each strain were not similar and their band patterns were different from V8 digestion. These results showed that amino acid sequences of coat proteins were slightly different among strains and these differences should be one of the factors that influenced to virus-host interactions.

TMV pepper strain and tomato strain have many differences in sequences against TMV common strain. TMV pepper strain showed 69% of DNA sequence maximum homology against TMV Korean common strain (Koh et al., 1992) and TMV tomato strain showed 70% (Lee et al., 1996). An alignment of the capsid protein amino acid sequences of the pepper and tomato strain showed 81% and 86% identity compared to that of TMV-common strain (Lee et al., 1996). The variability observed in coat protein should be involved in virus-specific functions and could affect host range and symptom induction.

Like other living organisms, plant viruses remain substantially like the parent during their replication, but can change to give rise to new types or "strain". Because RNA viruses lack an error-correcting mechanisms during genome replication, they give rise to many mutants involving one or a few nucleotide changes even though slight or

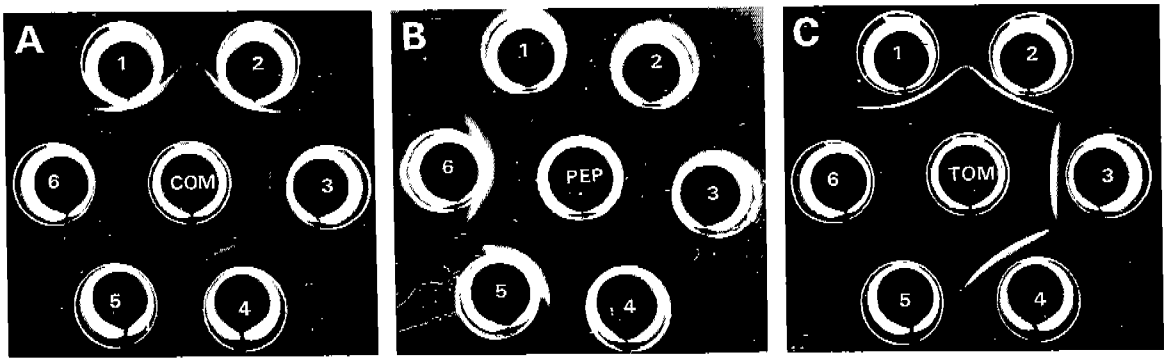

Fig. 2. Serological reactions of 3 isolates of TMV with their antibody by agar gel double diffusion test. Center wells were charged with antisera to TMV-common (COM), pepper (PEP), and tomato (TOM), respectively. Peripheral wells were charged with purified viruses: TMV-common (1,2), TMV-tomato (3,4), and TMV-pepper (5,6).

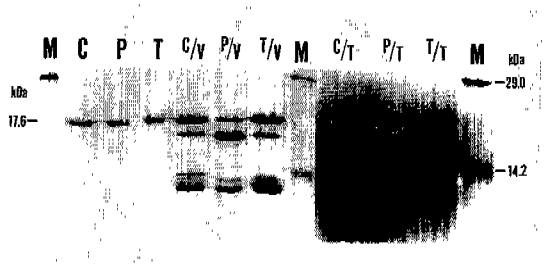

Fig. 3. Peptide mapping analysis of three TMV coat proteins. Samples were run on a 15% polyacrylamide gel with sodium dodecylsulfate and stained with Coomassie brilliant blue. Lane M, Molecular weight standards (α-lactalbumin, 14.2 kDa; carbonic anhydrase, 29.0 kDa); Lane C, coat protein of TMV-common strain; Lane P, coat protein of TMV pepper-strain; Lane T, coat protein of TMV tomato strain; Lane C/V, P/V, T/V indicates the partially digested protein band patterns of TMV coat proteins with V8 protease; Lane C/T, P/T, T/T indicates the partially digested protein band patterns of TMV coat proteins with trypsin.

no change of symptoms on a certain host. From the plant pathologist point of view, the existence of virus strains in the field that cause different kinds of disease is often a matter of considerable practical importance. Reliable criteria for the recognition of strains can be considered in the field of nucleic acids, structural and non-structural proteins, serological properties and biological responses (Matthews, 1991). But these criteria should also be considered for their usabilities. From our results, however, three isolates of the virus might be classified into different strains, and the results could be used for TMV control scheme.

요 약

한국의 담배, 토마토 및 고추에서 분리된 TMV의 3계통을 판별식물에서의 병징반응, 혈청학적 관련성 및 외피단백질의 peptide mapping 조사에 의해 분류하였다. TMV-common, TMV-tomato, TMV-pepper로 명명된 이 계통들은 조사된 27종의 식물중 담배 3품종, 토마토와 고추 각 1품종에 접종하여 서로 다른 병징에 따라 구분할 수 있었다. 한천 gel 확산법에 의해 혈청학적 관련성을 조사한 결과 서로 다른 항원/항체간에는 미약한 양성반응을 나타냈다. 특히 TMV-pepper 계통은 다른 두 계통에 대한 항체와 반응시킨 결과 매우 미약한 반응을 나타냈다. 각 virus 계통의 외피단백질을 V8 protease 또는 trypsin으로 digestion한 결과 각 계통간에 서로 다른 단백질 특성을 나타내 구분이 가능하였다. 이같은 결과로 미루어 각 계통은 단백질 조성 또는 아미노산 서열의 차이가 있을 것으로 사료된다.

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