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Additional Biological Characteristics of TMV Strains Isolated from Tobacco, Tomato and Pepper Plants

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담배, 토마토 및 고추에서 분리한 TMV 계통의 추가적인 생물학적 특성

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ABSTRACTb: Tobacco (*Nicotiana tabacum*) cultivars including NC 82 and KF 114, and *Datura stramonium, Physalis floridana, Gomphrena globosa*, and *Chenopodium* spp. were added to the previous host plants tested for the further examination on the biological characteristics of tobacco mosaic virus (TMV) strains isolated from tobacco (TMV-Common), tomato (TMV-Tomato), and pepper (TMV-Pepper). In TMV-Tomato and TMV-Pepper, different symptoms were noted in *P. floridana* (no symptom development), and NC 82 (local lesion production on the inoculated leaves) from TMV-Common with which systemic mosaic symptoms were developed. Local lesions were developed in KF 114, *D. stramonium, G. globosa*, and *Chenopodium* spp by TMV-Common and TMV-Tomato, while no symptom was observed in KF114 and *G. globosa*. Also the number and size of local lesions were smaller in KF 114 than Xanthi-nc tobacco (local lesion host) infected with TMV-Tomato. Systemic necrosis was induced in Xanthi-nc and KF 114 when infected with TMV-C at high temperature, but not with the other strains.

Key words: **TMV strains, tobacco cultivars, *Datura stramonium, Gomphrena globosa, Physalis floridana.***

Tobacco mosaic virus (TMV) has a wide host range of more than 150 genera in herbaceous, dicotyledonous plants (1). TMV is a very contagious pathogen that can be transmitted mechanically with ease, and causes severe economic losses by reducing yield and tobacco quality (8). In Korea TMV occurs widely in tobacco, tomato, and pepper plants. In tobacco fields, TMV incidences reach more than 10% in some years, and in some fields tobacco plants are damaged to near-devastation by the virus. Also in pepper and tomato, economic losses caused by TMV are severe, as the virus is seed-borne (9) and easily transmitted through mechanical contact.

It is controlled mainly by sanitation; removing inoculum sources and avoiding contamination during cultural practices for which urea treatment (10), and milk treatment (2) are recommended. Recently a resistant tobacco variety KF 114, which has N gene for resistance has been developed (3) to be released in limited areas. In other crops such as tomato and pepper, control of TMV seems not so intensive as in tobacco. TMV is still one of the most damaging problems in cultivating those crops.

Each of the 3 TMV strains, TMV-Common (TMV-C), TMV-Tomato (TMV-T), and TMV-Pepper (TMV-P) has been known to be primary pathogen of the disease in tobacco, tomato, and pepper, respectively. Park *et al.* (11) reported symptoms of the virus strains in the three plant species, and the virus strains could be distinguished by means of symptomatology. Each virus strain develops typical systemic mosaic symptom in the respective plant, but various symptoms in other than their primary hosts.

In this study we examined some more biological characteristics of the three TMV strains, and discussed possible interrelations among the three strains in field conditions.

MATERIALS AND METHODS

Virus isolates and strains. The TMV-C, TMV-T, and TMV-P are those used in the previous study (11), which were isolated from each crop showing mosaic symptoms.

Virus inoculation and symptom examination. Four tobacco cultivars, NC 82 and NC 2326 (TMV-common

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systemic infection plants), and Xanthi-nc and KF 114 (local lesion-forming plants), *Chenopodium amaranticolor, C. quinoa, Datura stramonium, Gomphrena globosa*, and *Physalis floridana* were used for virus inoculation experiments. Leaves with mosaic symptoms were homogenized and the plant sap was inoculated to the plants at about 5~10 leaf stages. Inoculated plants were placed in a greenhouse at 22~27°C. Three plants were tested for each host-strain combination. Symptom appearance was examined visually up to 4 weeks after inoculation. This inoculation method was also used throughout this study, unless noted otherwise.

Comparison of hypersensitive reactions of tobacco plants to TMV strains. The local lesion-forming tobacco plants, Xanthi-nc (at 8 leaf stage) and KF 114 (at 4 leaf stage) were used, and half leaves were inoculated with the inoculum of each TMV strain that was prepared from the dilution of infected leaf sap. The dilution of leaf sap for each strain was optimized in preinoculation tests so that plants formed adequate but not too many lesions on the inoculated leaves. Four days after inoculation, numbers and sizes of local lesions formed on half leaves were examined, and compared with those formed by the TMV-C on the other half leaves. Three leaves were replicated.

Double infection of tobacco plants with TMV strains. Tobacco plants at 8 leaf stage used and inoculated as above with virus strains singly or in combination. Four plants were used for each plant-virus combination.

Symptom development at high temperature. Tobacco plants at 8 true leaf stage were inoculated by the three strains and incubated at 32°C for 3 days and moved into the greenhouse at 22~27°C. Four plants were used for each plant-virus combination to examine symptom deveopment.

Examination of induced resistance in resistant (local lesion-forming) hosts. Xanthi-nc tobacco was inoculated with the TMV-common strain, and 5 and/or 10 days after inoculated the upper leaves of the inoculated plants and the same-stage leaves of fresh plants were inoculated with the virus. The lesion size formed 3 days later by the 2nd or 3rd inoculations was compared with that of the 1st inoculation. Also local lesion forming plants such as Xanthi-nc, *D. stramonium*, and NC 82 and NC 2326 (forming local lesion to tomato and pepper strains) were tested for TMV-T and TMV-P as above (in this time the plants were reinoculated 10 days after the 1st inoculation).

RESULTS

Responses of test plants to TMV infection. Table 1 shows the responses of the host plants tested to the infection of the three TMV strains. In addition to the plants, Xanthi-nc, NC 2326, tested in the previous experiment (11), symtpoms of new host plants such as NC 82 and KF 114 (major tobacco cultivars cultivation in Korea), and wild hosts, *P. floridana C. quinoa, C. amaranticolor, G. globosa*, and *D. stramonium* were examined in this experiment. TMV-T and TMV-P produced local lesions on the inoculated leaves of NC 82 as in NC 2326, and no symptom in *P. floridana*, all of which are systemic hosts of TMV-C. For the three strains, local lesions were formed on the inoculated leaves of all the other host plants tested, except in *G. globosa* and KF 114 which showed little or no symptoms on any leaves by TMV-P.

Comparison of local lesions formed by TMV strains. The number and size of local lesions formed by TMV common strains were not significantly difference between Xanthi-nc and KF 114. On the other hand, TMV-T produced relatively fewer and smaller lesions in KF 114 than in Xanthi-nc, and especially, TMV-P produced no visible lesions in KF 114 (Table 2, Fig. 1).

Symptoms of tobacco plants by double infections. The tobacco plants, NC 82, NC 2326, Xanthi-nc, and KF 114 were inoculated with the virus strains singly or dually (common+tomato strains, common+pepper strains, and tomato+pepper strains). In local lesion-forming tobacco hosts, local lesions were always formed on the inoculated leaves by dual infections. In NC 2326 and NC 82 infected with the common and the other strains, local lesions and

Table 1. Responses of various host plant to the infection of various TMV isolates and strains

TMV strain	*Nicotiana tabacum*				*Chenopodium* spp.[b]	*Datura stramonium*	*Gomphrena globosa*	*Physalis floridana*
	NC82	NC2326	Xanthi-nc	KF114				
Common	-/SM[a]	-/SM	LL/-	LL/-	LL/-	LL/-	LL/-	-/SM
Tomato	LL/-	LL/-	LL/-	LL/-	LL/-	LL/-	LL/-	-/-
Pepper	LL/-	LL/-	LL/-	-/-	LL/-	LL/-	-/-	-/-

[a]Symptoms on inoculated/upper leaves: SM: systemic mosaic symptoms, LL: local lesions, and -: no symptom
[b]*Chenopodium amaranticolor* and *C. quinoa* were used.

Table 2. Comparison of local lesion formation in tobacco plants resistant to TMV

Strain[a]	Lesion number[b]			Lesion size[b]			Significance[c]
	KF 114	Xanthi-nc	X/KF (%)	KF 114	Xanthi-nc	X/KF (%)	
Common	100	100	100	100	100	100	ns
C6	90	58	155	101	110	92	ns
#13	210	251	84	105	71	148	ns
#20	125	136	92	91	95	96	ns
S-2	310	228	136	71	76	93	ns
Tomato	83	238	35	49	82	60	*
Pepper	0	11	0	0	43	0	*

[a]C6, #13, #20, and S-2 are TMV common strains isolated from different areas.

[b]The numbers and sizes of local lesions were examined by half-leaf inoculation method 4 days after inoculation. Figures are relative numbers and sizes of local lesions relative to those formed by the TMV common strain on the other half leaves.

[c]Tested by pooled standard deviation: ns; not significant, *significant at P=0.05% level.

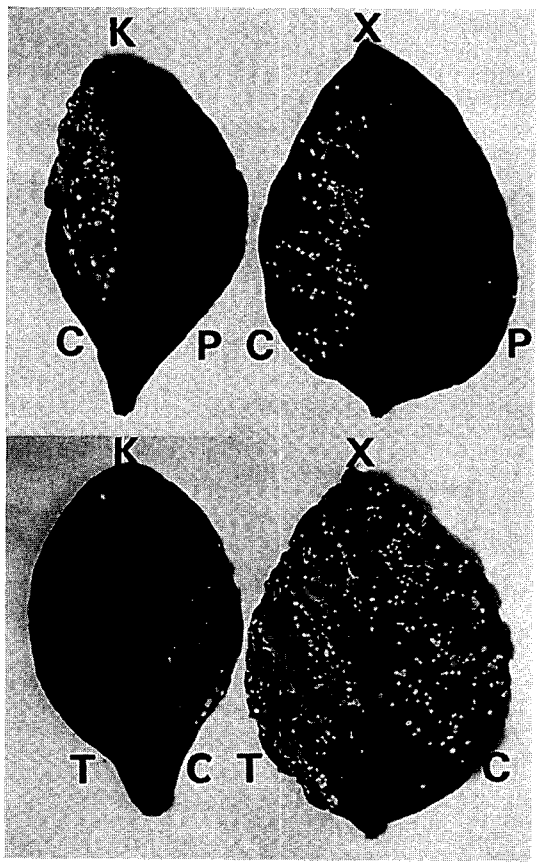

Fig. 1. Symptoms on the inoculated leaves of Xanthi-nc (X) and KF 114 (K) produced by TMV-Common (C), TMV-Pepper (P) and TMV-Tomato (T).

mosaic symptoms were produced on the inoculated leaves and upper leaves, respectively (data not shown). No

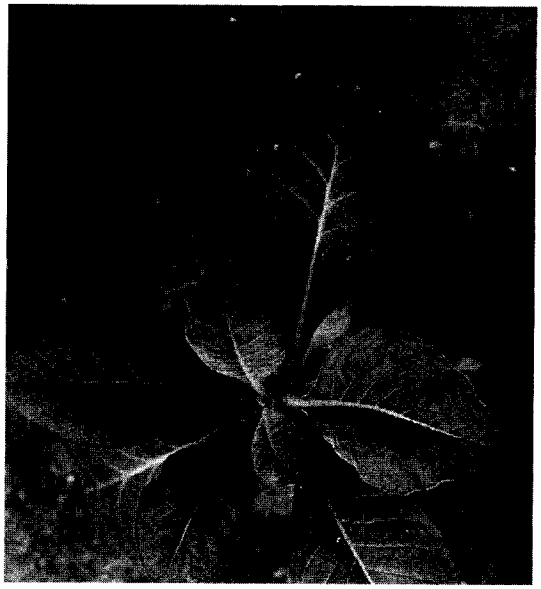

Fig. 2. Symptoms produced in NC 2326 by the dual infection of TMV-Common and TMV-Tomato. Note mosaic symptoms on the upper leaves and necrotic spots (arrows) on the uninoculated leaf.

variations in the type of symptoms were observed in any plants with dual infections of the strains except NC 2326 infected with the common and tomato strains which sometimes showed mosaic symptoms and local lesions on the upper leaves (Fig. 2).

Symptom development at high temperature. Exposure of test plants infected with the TMV-common strain to high temperature (32°C) for 3 days induced systemic necrosis in Xanthi-nc and KF 114. However, no systemic necrosis was observed in the tested plants (NC 82, NC 2326, Xanthi-nc, and KF 114), but a little enlargement of lesion size was noted in those plants infected with TMV-T, and Xanthi-nc with TMV-P (data not shown).

Systemic acquired resistance (SAR) in local lesion-forming hosts. The lesion size formed by the reinoculation of TMV-C and TMV-T was smaller than that by the 1st inoculation on the upper leaves of Xanthi-nc (Fig. 3). This phenomenon, which is called systemic acquired resistance (SAR), was also noted in Xanthi-nc for TMV-P and in *D. stramonium* for all the strains (data not shown). For TMV-C, lesion size decreased by about 60 % in the 2nd inoculation and about 20% in the 3rd inoculation. Lesion size decreased by 43% in the 2nd inoculation for TMV-T. In NC 82 and NC 2326 for TMV-T and TMV-P, no obvious SAR was noted in this experiment.

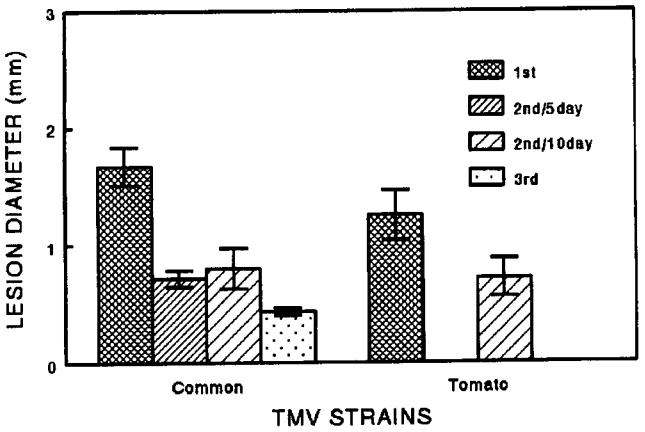

Fig. 3. Comparison of lesion size formed on inoculated leaves of Xanthi-nc by TMV-Common and TMV-Tomato for the 1st inoculation and the consecutive 2nd (5 or 10 days after the 1st inoculation) and 3rd (5 days after the 10-day-later 2nd inoculation) inoculations. The lesion diameter was examined 3 days after inoculation. Each bar and vertical line represent the average and standard deviation of 3 replications.

DISCUSSION

Park *et al.* (11) selected 5 plant hosts to differentiate the three TMV strains, of which systemic tobacco plant of TMV-C, NC 2326, is a local lesion-forming tobacco host to the TMV-T and TMV-P. In our study, NC 82 had the same type of symptoms as NC 2325, and furthermore, local lesions were formed more slowly in NC 82 than NC 2326 by TMV-T and TMV-P. Also different symptoms were developed in the wild hosts *P. floridana* and *G. globosa* by the TMV strains, suggesting that that the proper use of the plants (not involving tomato and pepper plants) can practically differentiate the three TMV strains.

NC 82 is the major flue-cured tobacco cultivar in Korea. It is not systemically infected with the other strains than TMV-C, and disease severity was not increased in NC 82 by the dual infection of TMV-C and the other strains. In field conditions only TMV-C occurred so far as a variety of isolates from tobacco growing areas in Korea were biologically and serologically identical and identified as TMV-C (unpublished data). Thus, TMV strains other than the common one probably can neither infest tobacco plantation nor increase their populations through tobacco plants in field conditions.

KF 114, an F_1 hybrid between cytoplasmic male sterile TMV-resistant cultivar MSNC567 (genotype: NN) and NC 82 (genotype: nn), is currently used as a TMV-resistant cultivar in Korea. It has genotype of Nn and is also cytoplasmic male sterile (3). In KF 114, lesion size was decreased further or no lesion was formed by TMV-T and TMV-P, respectively, compared in Xanthi-nc tobacco. The increase of resistance in KF 114 may be derived from the combination of resistances from both MSNC567 and NC 82 to TMV-T and TMV-P. Thus, there may be no possibility that KF 114 is infected by TMV-T or TMV-P in field conditions. However, TMV-C formed local lesions in KF 114 as well as in Xanthi-nc, and was systemically infected by necrosis at high temperature. Systemic necrosis is often found in Xanthi-nc and KF 114 during the summer in the greenhouse, and in case of KF 114 in fields.

It is well known that tobacco plants with N gene are systemically necrotized upon exposure to high temperature (over 28°C) (4, 5). Kim *et al.* (7) showed the lesion expansion in Xanthi-nc was proportional to the duration of exposure to high temperature. However, no systemic necrosis occurred in the plants infected with the TMV-T and TMV-P. In NC 82 tobacco fields, such type of symptoms has not been found so far. This indicates that resistance gene(s) to the tomato and pepper strains may be less temperature-sensitive than the N gene. Some lesion expansion by the two strains may be related to relatively rapid viral multiplication at the high temperature before the onset of the resistant gene expression.

SAR was induced in resistant plants by the infection of all of the strains in our experiment. SAR is induced by the infection of various pathogens including viruses, fungi and bacteria, and by chemicals such as salicylic acid (6, 12). SAR induced by one pathogen may be effective to other pathogens, and in our study resistant plants infected by a TMV strain became more resistant to the other strains as well as the initial inoculum. Biochemical changes induced by the infection of the three strains may not differ one another, and for this more study is needed in the future.

요 약

담배 품종 NC 82, 및 KF 114와 *Datura stramonium, Physalis floridana, Gomphrena globosa, Chenopodium* spp.를 이전에 조사한 검정식물에 추가하여 TMV Common, Tomato 및 Pepper 계통으로 감염시켜 기주 반응을 조사하였다. TMV Common 계통에 전신감염되는 NC 82와 *P. floridana*는 Tomato와 Pepper 계통에 의해서는 다른 증상 즉 NC 82는 국부병반, *P. floridana*는 무병징을 보였으며, Common과 Tomato 계통에 의해 국부병반이 나타나는 KF 114와 G. globosa는 Pepper 계통

이 감염되었을 때 아무런 증상도 나타나지 않았다. Tomato와 Pepper 계통에 의한 국부병반의 수와 크기는 Xanthi-nc 보다 KF 114에서 상대적으로 작았으나 Common 계통은 차이가 없었다. Common 계통에 감염된 Xanthi-nc와 KF 114는 고온에서 전신괴저 증상을 나타냈으나 다른 계통에 의해서는 이러한 증상이 발생하지 않았다.

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