感染葉組織のフェノール処理液におけるキュウリモザイクウイルスの系統およびトマトアスパーミーウイルスの感染力

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者名	都丸,敬一 宇田川,晃
発行元	日本植物病理學會
巻/号	37巻2号
掲載ページ	p. 73-76
発行年月	1971年3月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

日植病報 37 : 73–76 (1971)
Ann. Phytopath. Soc. Japan 37 : 73–76 (1971)

Infectivity of Phenol Extracts from Leaf Tissues Infected with Strains of Cucumber Mosaic Virus and Tomato Aspermy Virus

Keiichi TOMARU* and Akira UDAGAWA*

都丸敬一*・宇田川　晃*：感染葉組織のフェノール処理液におけるキュウリモザイクウイルスの系統およびトマトアスパーミーウイルスの感染力

Abstract

Phenol extracts from leaf tissues systemically infected with 6 strains of cucumber mosaic virus (CMV) and two isolates of tomato aspermy virus (TAV) showed higher infectivity than that of control phosphate buffer-homogenate, respectively. Difference in kind of assay host has no influence on the higher infectivity of phenol extracts. Phenol extracts from tobacco top leaves infected with CMV-O made at different dates after inoculation indicated always higher infectivity than that of control.

The property that phenol extracts show higher infectivity than usual homogenate seems to be available for one of the criteria in diagnosis or identification of CMV. The higher infectivity of phenol extracts from infected tissues of TAV and chrysanthemum mild mottle virus may be regarded as an additional property which suggests close relationship between CMV and TAV. (Received August 14, 1970)

Introduction

A very high infectivity of phenol extracts from leaf tissues infected with cucumber mosaic virus (CMV), tobacco necrosis virus or pea enation mosaic virus has been reported by Schlegel[5,6] and other workers[1,10].

We examined the infectivity of phenol extracts from tobacco leaf tissues infected with strains of CMV in order to know whether this property can be used for the diagnosis and identification of the virus. In addition, tomato aspermy virus (TAV) was tested for the property described above in comparison with CMV strains. There were many discussions of the relationships between CMV and TAV[4], but as far as we know, no report is found about the infectivity of viral RNA of TAV directly extracted from infected leaf tissues.

Materials and methods

Source of the viruses used in this experiment was as follows; for cucumber mosaic virus (CMV), ordinary strain (CMV-O), mild strain (CMV-C), yellow strain (CMV-Y), yellow mild mottle strain (CMV-YM)[11], legume strain (CMV-LE) and necrotic strain (CMV-N) were used. All of them except CMV-N were isolated from tobacco plants in Japan. CMV-N was originally obtained from

* Hatano Tobacco Experiment Station, Hatano, Kanagawa, Japan. 秦野たばこ試験場

Dr. R. W. Fulton and maintained in our laboratory. TAV[3] from chrysanthemum and chrysanthemum mild mottle virus (CMMV)[8] were obtained from Drs. Inouye and Tochihara, respectively. Alfalfa mosaic virus (AMV-B), an isolate from tobacco plant, was used as standard.

The method described by Schlegel[5,6] was used for extraction by phenol from infected leaf tissues. Systemically infected leaves were separated into half-leaves, the main vein being eliminated, and the one half-leaf was macerated in a mixture of water-saturated phenol and Na-pyrophosphate, while the other half-leaf was macerated in phosphate buffer (0.1 M, pH 7.0) at the same dilution to serve as control. The maceration was carried out with mortar and pestle or with homogenizer. After low speed centrifugation of macerated juice, the supernatant (water phase) was pipetted out and diluted with phosphate buffer 5–10 times and used as inoculum. These procedures were carried out in the cold room. Infectivity of phenol extracts and of control was determined by local lesion assay on cowpea (*Vigna sinensis* var. *sesquipedalis*, cv. Kurodanesanjaku), *Chenopodium* spp. and others. Opposite primary leaves of cowpea[9] and half leaves of the other assay hosts were inoculated for comparison. More than 8 leaves of assay host were used in each trial.

Results and discussion

Representative results among more than two trials in each virus and assay host are shown in Table 1. Phenol extracts in each strain of CMV except CMV-N showed higher infectivity than that of control buffer homogenate when tobacco plant (*Nicotiana tabacum* cv. Bright Yellow or Ky-57) was used as donor host. Phenol extracts of CMV-N from inoculated leaves of tobacco showed less infectivity than

Table 1. Infectivity of phenol extracts from tobacco leaf tissues infected with strains of CMV and two isolates of TAV

Virus	Assay host	Number of local lesions[a]		Phenol extracts
		Phenol extracts	Control	Control
CMV-O	Cowpea	214	102	2.1
CMV-O	*C. quinoa*	40	6	6.7
CMV-O	*C. album*	27	1	27.0
CMV-Y	Cowpea	24	4	6.0
CMV-C	Cowpea	99	40	2.5
CMV-LE	*C. amaranticolor*	75	24	3.1
CMV-YM	Cowpea	24	4	6.0
CMV-N[b]	Cowpea	54	25	2.2
CMV-N	Cowpea	20	130	0.2
CMV-N	Tobacco	16	124	0.1
TAV	Cowpea	38	1	38.0
TAV	*C. quinoa*	51	5	10.2
TAV	*C. album*	44	15	2.9
CMMV	Cowpea	5	1	5.0
CMMV	*C. quinoa*	66	19	3.5
CMMV	*C. album*	37	15	2.5
AMV-B	Cowpea	1	87	0.01
AMV-B	French bean	7	223	0.03

a) Number of local lesions per leaf of cowpea or per half-leaf of the other assay hosts.
b) Systemically infected leaves of *Vinca rosea* was used for source material.

that of control inoculum in the same way as phenol extracts of alfalfa mosaic virus. CMV-N causes only necrotic local lesions on inoculated leaves of tobacco, and it seemed that such material as leaves showing only necrotic local lesions was not suitable for the source. When systemically infected leaves of *Vinca rosea* were used as source material, CMV-N indicated higher infectivity than that of control. On *Chenopodium amaranticolor*, *C. quinoa* and *C. album*, all tested strains of CMV showed the same results as on cowpea leaves.

Phenol extracts of systemically infected tobacco leaves with TAV and CMMV indicated higher infectivity than that of control, respectively, similar to that of strains of CMV. CMMV has no serological relationship with CMV[8], but it showed the same higher infectivity of viral RNA obtained directly from infected leaves by phenol treatment.

An isolate of CMV obtained from soybean (soybean stunt virus) indicated the same property in infectivity of phenol extracts[7] as other strains reported here, in the other separate experiment which will be reported elsewhere.

Then, the infectivity of phenol extracts from top leaves of tobacco plant systemically infected with CMV-O was tested at different dates after inoculation at 25°C in the air-conditioned glasshouse. The result is shown in Fig. 1. The infectivity of phenol extracts was always higher than that of control buffer-homogenate, at any time after inoculation. In Fig. 1, relatively higher infectivity of phenol

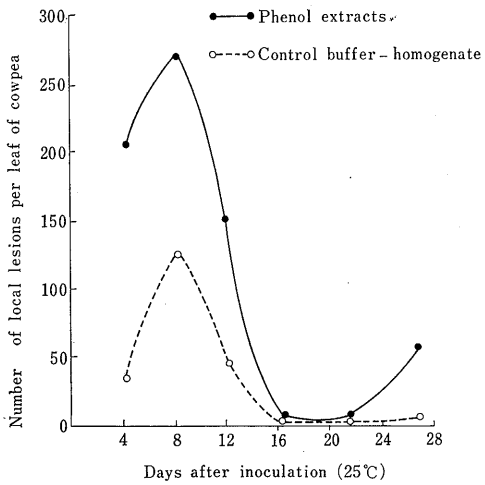

Fig. 1. Infectivity of phenol extracts from systemically infected tobacco top-leaves tested at different dates after inoculation with CMV-O.

extracts from early stage of infection (the 4th day) and from the time of reappearance of symptoms after symptom-masking stage in top leaves (the 27th day) was observed. This phenomenon seems to indicate that the viral RNA which is inactivated in the buffer-homogenate partly plays a role for relatively higher infectivity at these stages of infection, because in these stages multiplication of virus is in exponential stage.

The reason why the phenol extracts of CMV infected leaf tissues indicate equal or higher infectivity to or than buffer-homogenate was suggested by Diener *et al.* (1964)[2]. However, it is not clear yet, what intrinsic properties of viral RNA is related to this unusual higher infectivity of viral RNA.

Nevertheless, from the results reported here, the property that phenol extracts show higher infectivity than usual homogenate seems to be an useful mean to diagnose or identify CMV, especially

to differenciate it from viruses which have similar reactions on several differential hosts of CMV such as alfalfa mosaic virus and tobacco ring spot virus. The higher infectivity of phenol extracts from infected tobacco leaves with TAV and CMMV shows that this property is shared by both virus groups of TAV and CMV. It may be an additional evidence which suggests close relationship between TAV and CMV.

Acknowledgement

We are indebted to Drs. T. Inouye and H. Tochihara for the supply of TAV and CMMV.

Literature cited

1. Babos, P. and Kassanis, B. (1962). Virology 18 : 206–211.
2. Diener, T.O., Scott, H. A. and Kaper, J. M. (1964). *Ibid.* 22 : 131–141.
3. Inouye, T., Asatani, M. and Mitsuhata, K. (1968). Nogaku Kenkyu 52 : 55–64.
4. Lawson, R. H. (1967). Virology 32:357–362.
5. Schlegel, D. E. (1960). Phytopathology 50 : 156–158.
6. Schlegel, D. E. (1960). Virology 11 : 329–338.
7. Takahashi, K., Udagawa, A., Tomaru, K. and Saito, Y. (1970). Ann. Phytopath. Soc. Japan 36 : 374.
8. Tochihara, H. (1970). *Ibid.* 36 : 1–10.
9. Tomaru, K. (1967). Bull. Hatano Tobacco Exp. Sta. 58 : 1–18.
10. Tomaru, K. (1967). *Ibid.* 58 : 27–32.
11. Tomaru, K. and Udagawa, A. (1970). Ann. Phytopath. Soc. Japan 36 : 87–93.

和文摘要

感染葉組織のフェノール処理液におけるキュウリモザイクウイルスの系統およびトマトアスパーミーウイルスの感染力

都丸敬一・宇田川 晃

キュウリモザイクウイルス (CMV) の普通系，軽症系，黄斑系，黄色微斑系，マメ科系およびえそ系の 6 系統およびトマトアスパーミーウイルス (TAV)（井上ら，1968）およびキク微斑ウイルス (CMMV)（栃原，1970）を用い，感染葉のフェノール処理液の感染力を対照のりん酸緩衝液磨砕汁と比較した。その結果，いずれもフェノール処理液では対照より高い感染力を示し，定量用の検定植物をかえてもこの性質は変わらなかった。タバコに CMV 普通系を接種したのち，4 日から27日まで経時的に検定した頂葉のフェノール処理液の感染力はつねに対照より高かった。以上の結果から，このフェノール処理液が高い感染力を示す性質は，CMV 群ウイルスの診断，同定の一手段として用いうるものと考えられる。また TAV および CMMV が CMV と同様にフェノール処理液で高い感染力を示すことは，TAV 群ウイルスと CMV 群ウイルスとの類縁関係を示す一つの新しい性質といえるであろう。