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植物 바이러스病의 感染機構 및 增殖抑制에 關한 硏究 (第1報)

—抗生物質 및 抗바이러스性 化合物의 TMV增殖에 미치는 影響—

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Studies on the Infection Mechanism and Inhibition of Multiplication in Plant Virus Diseases (I)

—A Screening Test for the Effect of Antibiotics and Organic Compounds on the Multiplication of Tobacco Mosaic Virus—

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ABSTRACT

A number of antibiotics and organic compounds were tested for their effect on the multiplication of tobacco mosaic virus(TMV) in floating leaf discs.

1) Among six antibiotics tested, kanamycin sulfate showed 24% or more inhibition to TMV multiplication under these experimental conditions. Aureomycin hydrochloride and chloramphenicol showed 19% and 14% inhibition respectively.

2) For screening of organic compounds two environments were used, a diffuse daylight environment(25 foot-candles and laboratory temperature) and an artificial light environment for 12 hours per day(300 foot-candles at 4 to 5°C above laboratory temperature). A wide range of organic compounds increased virus multiplication in the diffuse daylight environment but had less effect, or inhibited virus multiplication, in the artificial light environment.

3) The following compounds were among the most effective in increasing TMV multiplication: glucose-1-phosphate, L-aspartic acid, glucose, and 5-bromouracil. The following compounds were most effective in inhibiting TMV multiplication: thiouracil, uracil, DL-isoleucine, L-leucine, and zinc chloride.

INTRODUCTION

The mechanism of virus multiplication and the chemotherapy of plant virus disease are subjects that are now receiving much attention.

The literature in these subjects is too voluminous to be covered here(Nichols, 1953; Gray, 1957; Hirai and Shimomura, 1960; Taniguchi, 1962). In general the evidence indicates that, although a number of plant viruses may have similar requirements, others may behave differently toward antibiotics and chemical treatments(Gray, 1955, 1957; Lindner *et al.*, 1959). This paper gives the results of tests to determine the effect of a number of antiviral organic compounds on the multiplication of tobacco mosaic virus

(TMV).

MATERIALS AND METHODS

The method used involved the inoculation of tobacco leaves with TMV, the cutting out of discs from the infected leaves, and floating these discs on water and on solutions of the antibiotics and organic compounds during the period of TMV multiplication. After the floating period the TMV in the leaf discs were purified by heating a suspension of the homogenized discs to precipitate certain impurities and then running the virus suspension through a cycle of slow and high speed centrifugations. The concentration of the purified TMV suspension was determined by half-leaf method of local-lesion bioassays on *Nicotiana glutinosa*. The details of the method are given below.

The lateral mature leaves from Turkish tobacco(Var. N.T. Samsun and Xanthi) about 60cm tall were used in all tests. About 1 week prior to inoculation the plants were topped, and all except the lateral mature leaves were removed. These remaining leaves were inoculated by brushing with carborundum(600mesh) and TMV-Vulgare and washing out immediately. The following day, the leaves were harvested, discs were cut with a sharp cork borer, and were floated immediately on distilled water in Petri dishes.

Ten 15mm discs were used in each treatment when approximately 300 mg samples were desired. Three or more samples were floated on distilled water, each in a different Petri dish, as controls, and two samples were floated on 10 m*l* of water solution of each antibiotic and chemical to be tested. Samples for antibiotic test were placed in the constant temperature chamber which provides 300 foot-candles of fluorescent light incident on the surface of Petri dish lids for 12 hours each day. Some samples were held in the laboratory under incandescent light (300 foot-candles) for 10 hours each day.

Others were kept in diffuse daylight of about 25 foot-candles. In this diffuse daylight environment the temperature was 4 to 5 degrees lower than under artificial lights.

In general,if an antibiotic or a compound were found to be toxic at the concentration at which it was tested, the sample was discarded and the compound was tested again in a later experiment at a more dilute concentration. The solutions were removed every 48 to 56 hours and leaf discs rinsed with distilled water. Fresh solution then was added. This procedure was followed in order to remove unattached bacteria and their metabolites and to maintain the concentration of the chemical. After TMV multiplication had continued for 7 days, the solution was removed and the discs rinsed with distilled water. The samples were then frozen at -10°C and held at this temperature until virus assays were made. All the virus assays were made through the bioassay on the leaves of *Nicotiana glutinosa* by half-leaf method.

RESULTS AND DISCUSSION

Various antibiotics have been tested for their effects on the multiplication of plant virus diseases by many workers and they found that some of antibiotics were capable of reducing the rate of virus production in the plant tissues (Gray, 1955, 1957; Shimomura and Hirai, 1959). Results obtained from the test showed that kanamycin sulfate was the most

effective among antibiotics tested in the inhibition of TMV multiplication (24% inhibition) under these experimental conditions. Aureomycin hydrochloride and chloramphenicol showed 19% and 14% inhibition of TMV respectively (Table 1). Effect of antibiotics on leaves of tobacco plant, aureomycin hydrochloride produced slight yellowing and kanamycin sulfate produced slight water-soaking area on the leaves. The rest of antibiotics produced no chemical injury at all.

The detailed data of virus assay are not given, however they showed that approximately twice as much TMV was produced in the artificial light environment as in the diffuse daylight environment. Schlegel and Rawlins (1954) also found light to increase virus production. It is possible that some of the increased virus production in the artificial light environment may have been due to the higher temperature in this environment. Yarwood (1952) reported 31°C to be optimum for the multiplication of TMV.

Diffuse daylight environment(Table 2): In this environment L(+)-glutamic acid and colchicine were the most effective inhibitors of those tested. A great variety of compounds caused an increase in virus multiplication in the diffuse daylight environment. The most effective in decreasing order of effectiveness were glucose-1-phosphate, glucose, and L-aspartic acid.

Artificial light environment(Table 3): In this environment the most effective compounds produced inhibition of TMV multiplication in decreasing order as below, thiouracil, zinc, DL-isoleucine, and L-isoleucine. Earlier workers have reported some of these compounds to show activity in plant virus chemotheraphy. Thompson *et al.*(1953) reported on the effectiveness of benzaldehyde, thiosemicarbazone, and results with zinc chloride were reported by Weintraub *et al* (1952). Inhibition of tobacco mosaic virus multiplication by thiouracil was reported by Commoner and his co-workers(1951, 1952). The results indicated that in mature leaf discs in a low intensity daylight environment a number of organic compounds caused an increase in virus multiplication. On the contrary, in the artificial environment where the light intensity was much higher and greater the photosynthesis in plant tissues probably contained a greater amount of metabolites. In this environment treated organic compounds usually produced an inhibition in TMV multiplication. It may be possible that some of the results may be related to different amounts of chemicals taken up in the two environments.

Table 4 showed the effect of 8-azaguanine with different concentration on the biosynthesis of TMV in tobacco leaf tissue. Increasing the concentration of 8-azaguanine resulted in decreasing amounts of biosynthesis of TMV until a concentration of 30.0×10^{-4} M was reached. At that concentration, the amount of biosynthesis of TMV increased as compared to the amount at 30.0×10^{-4} M concentration (Table 4).

When concentrations of $10\times, 20\times, 30\times$ and 40×10^{-4}M of 8-azaguanine were tested against each of three TMV strains(Vulgare, Dahlemense, and Common), percentage of virus survival decreased directly with increasing concentrations for all three TMV strains until the 30.0×10^{-4}M concentration. At that concentration, the percentage survival of TMV-

Table 1. Effect of antibiotics on the multiplication of tobacco mosaic virus

Antibiotics	Solvent	Conc. of chemicals (μg/ml)	%Increase(+) or decrease(−) in TMV multiplication & average % of deviation from mean	Effect of antibiotics on leaves
Aureomycin hydrochloride	MtOH	100	−19±6.4	Slight yellowing
Blasticidin S	H_2O	100	+12±3.2	None
Chloramphenicol	Acetone	200	−14±0.7	None
Kanamycin sulfate	H_2O	50	−24±8.2	Slight water-soaking
Penicillin K salt	H_2O	300	−6±4.6	None
Streptomycin hydrochloride	H_2O	200	−2±1.7	None

Table 2. The effect of certain compounds on multiplication of TMV in the diffuse daylight environment

Compounds tested	Conc. of chemicals	Changes in virus yield	Average deviation from mean	Toxicity to host*
Amino acids	per cent	per cent	per cent	—
L-Aspartic acid	0.10	+21	±4.6	—
L(+)-Glutamic acid	0.10	−27	±3.7	—
DL-Benzoylalanine	0.10	+11	±4.9	—
Pyrimidines and related compounds				
5-Bromouracil	0.01	+14	± 4.8	—
5-Methyluracil	0.01	−15	±11.2	S_2
6-Methyluracil	0.05	+ 7	± 1.4	S_1
6-Methylthiouracil	0.05	+ 9	± 1.4	S_2
Uracil	0.01	−21	± 2.3	—
Miscellaneous compounds				
Aureomycin	0.10	−23	± 2.4	C_1
Colchicine	0.10	−27	± 3.2	—
Glucose	0.10	+31	± 4.7	—
Glucose-1-phosphate	0.10	+73	±11.9	—
Maleic acid	0.50	+ 7	± 2.3	—
Nicotinic acid	0.10	+13	± 4.1	S_1B_2
Riboflavin	0.10	−19	± 5.4	C_1
Thiamin	0.25	+ 3	± 2.4	—

* Toxicity of a compound was recorded on a basis of 1—3 for increasing toxicity. Following is the code for types of injuries produced.

B(1—3): Browning of edges or brown spots
C(1—3): Chlorosis
S(1—3): Water-soaked spots

—: No visible injury
+: per cent increase in virus production
−: per cent decrease in virus production

Vulgare increased greatly (29%), the survival of TMV- Dahlemense increased slightly(4%), and that of the Common strain decreased by 10% (Fig. 1). It seems to appear probable that the concentration of chemicals cause the critical point of the virus multiplication in the plant tissue.

Acknowledgements

Table 3. The effect of certain compounds on multiplication of TMV in the artificial light environment

Compound tested	Conc. of chemical	Changes in virus yield	Average deviation from mean	Toxicity to host
Amino acids and related compounds	per cent	per cent	per cent	
Cystine	Saturated	− 4	± 2.3	—
Glycine	0.005	+ 3	± 2.9	—
Hydroxyphenylglycine	0.01	−11	± 1.4	B_1
DL-Isoleucine	0.01	−57	± 4.2	—
D-Isoleucine	0.01	− 6	± 1.9	—
L-Isoleucine	0.01	−46	± 3.1	—
DL-Leucine	0.05	−12	± 4.7	—
DL-Methionine	0.01	−24	± 3.2	—
DL-Serine	0.05	−11	± 5.7	C_1
DL-Tryptophan	0.01	− 3	± 5.5	—
Pyrimidines and related compounds				
Cytosine	0.05	− 5	± 5.3	C_1
5-Hydroxyuracil	0.05	+17	± 4.0	—
5-Methyluracil	0.10	+ 6	± 3.8	—
6-Methylthiouracil	0.05	−17	± 5.9	C_2
Thiouracil	0.10	−86	± 3.7	—
Uracil	0.05	−27	± 5.4	S_2
Miscellaneous compounds				
Ascorbic acid	0.10	−17	± 8.7	S_1
Glucose	0.10	−25	± 5.7	—
Glucose-1-phospate	0.50	+21	±11.4	—
Phenylacetate	0.01	− 9	± 1.0	—
Tryptophan blue	0.01	−39	± 4.8	—
Zinc chloride	0.01	−67	± 5.8	B_3

Table 4. Effect of 8-azaguanine on the biosynthesis of TMV in tobacco leaf tissue

8-Azaguanine conc. (10^{-4}M)	Local lesion formed		Survival (%)
	treated	untreated	
0	104*	104	100
1.0	109	111	98
5.0	106	109	97
10.0	87	98	89
20.0	81	99	82
30.0	76	96	79
40.0	82	97	85

* Lesion counts are average for 8 half-leaves

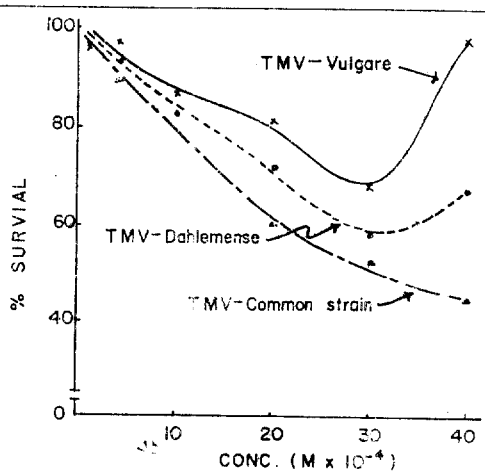

Fig 1. Relationships between concentration of 8-azaguanine and the percentage survival of 3 strains of TMV

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摘 要

抗生物質 및 抗바이러스性 化合物의 담배 모자이크 바이러스(TMV) 病 增殖에 미치는 影響을 實驗한 바 그 結果는 다음과 같다.

1. 여섯種類의 抗生物質中에서 kanamycin sulfate는 TMV增殖을 生體組織안에서 24% 減少시켰으며, aureomycin hydrochloride 및 chloramphenicol은 각각 19%, 14% 減少시켰는데 稻熱病菌에 有效한 blasticidin S는 오히려 TMV 增殖을 12% 增加시킨 結果를 얻었다.

2. 抗바이러스性 化合物의 TMV 增殖抑制를 보기 위하여 두가지의 環境條件을 使用하였다. 日光條件(25燭光, 實驗室温度)下에서 많은 化合物이 TMV增殖을 增加시킨데 반하여 人工 環境條件(300燭光, 實驗室温度보다 4—5°C높다)에서는 TMV增殖을 減少시켰다.

3. TMV增殖을 뚜렷하게 增加시킨 化合物은 glucose-1-phosphate, L-aspartic acid, glucose, 및 5-bromouracil등이었고, TMV增殖을 抑制한 化合物은 thiouracil, uracil, DL-isoleucine, L-leucine 및 zinc chloride등이었다.

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