STUDIES ON THE INFECTION OF CUCUMBER MOSAIC VIRUS IV. VIRUS INFECTIBILITY OF EPIDERMAL CELL AND MESOPHYLL CELL

STUDIES ON THE INFECTION OF CUCUMBER MOSAIC VIRUS

IV. VIRUS INFECTIBILITY OF EPIDERMAL CELL AND MESOPHYLL CELL

By

Yoshio EHARA and Tadao MISAWA

Department of Agronomy, Faculty of Agriculture,*
Tohoku University, Sendai, Japan
(Received, July 7, 1967)

Introduction

Many studies on the mechanical infection of plant virus and the cytological observations of the infected cell have been examined usually with the epidermal cell, and especially with hair cell of the tobacco leaf (3, 11, 13, 15). These experiments were done by the combination of systemic host and viruses. Generally virus seems to replicate in the epidermal cell of the systemic host. But here to fore there has been Dijkstra's report (4), as to whether or not the virus replciates in the epidermal cell of the local lesion host. While it is thought regardless of species of the host that the epidermal cell differs physiologically from the mesophyll cell, therefore, the epidermal cell may differ from the mesophyll cell in regard to the virus infectibility. And moreover the virus infectibility of the epidermal cell and the mesophyll cell, may differ between the local lesion host and the systemic host.

We have investigated the process of the virus infection by the combination of cucumber mosaic virus (CMV) and its local lesion host (cowpea). In a previous paper (5), we reported that the release of nucleic acid from the virus particle would be performed in the primary infected epidermal cell. But it was obscure, whether the CMV particle was produced in the primary infected epidermal cell or not.

Therefore, we have investigated the difference of the virus infectibility in the epidermal cell and the mesophyll cell by the combinations of cucumber mosaic virus and its local lesion host (cowpea) or systemic host (tobacco plant). Our results will be reported in this paper.

* Laboratory of Phytopathology

Materials and Methods

The virus used was the ordinary strain of cucumber mosaic virus (CMV). The plants used were cowpea (*Vigna sinensis* Endle var. Kurodane Sanjaku) which forms the necrotic local lesion and tobacco (Bright yellow) which infects systemically by CMV. This virus was multiplied in tobacco plants and purified by the method of Scott (10). Infectious nucleic acid was extracted by the phenol method from the purified virus (6). Cowpea and tobacco plants were grown in a growth cabinet (9). After the inoculation, inoculated leaf tissues were floated on 1 % sucrose solution in the covered petri-dish, and incubated under continuous fluorescent light of 3000 lux at 28°C. After a given hours, the incubated tissues were homogenized with the phosphate buffer (1/10 M, pH 7.0) and the squeezed sap was inoculated to cowpea and tobacco by the carborundum method for the bioassay. Namely, the degree of virus replication in the inoculated leaf tissue was measured by the number of local lesion formed on the cowpea primary leaf, and by the number of infected tobacco plants. Details of the method used will be described in each experiments.

Results

I) Virus replication in epidermal cell

In this experiment, we investigated the virus infectibility of the epidermal cell of cowpea and tobacco leaf. The lower epidermis of tobacco or cowpea leaf were inoculated by rubbing with virus suspension containing the carborundum as an abrasive. Three hours after inoculation, the epidermal tissue of both plants was stripped out and incubated for 72 hours under the above mentioned conditions. Leaf disks of both plants from which the epidermis was not stripped were used as the control, and they were treated under the same conditions. After incubation, the equal weight of these epidermal tissues and leaf disks of the control were homogenized with phosphate buffer respectively and the infectivity of these saps were bioassayed by using cowpea and tobacco as follows. Bioassay with cowpea was done by the opposite leaf method of the primary leaves. One leaf was inoculated with the sap of some treated epidermal tissue and opposite leaf with sap of the control, and then numbers of local lesion formed by both inocula were compared. Bioassay with tobacco plants were done by comparing the ratio of numbers of diseased plants with numbers of inoculated plants.

In the case of the tobacco epidermal tissue, its homogenized sap has the infectivity as shown in Table 1. By the inoculation with sap of the epidermal tissue, local lesions of 10–20 % of the control were formed on the cowpea leaf, and every inoculated tobacco plant appeared the symptom. Thus, as a matter of course, it was recognized atht CMV can replicate in the primary infected epidermal cell of tobacco.

Table 1. Infectivity of the inoculated tobacco epidermal cell.
(Four replications)

Test plant	Infection			
Cowpea	12/53	10/41	16/73	11/103 *
Tobacco	2/2	—/—	3/3	2/2 **

* Number of local lesion by infection with sap of infected epidermis / Number of local lesion by infection with sap of infected leaf disk as control
Each value is an average of 5 plants

** Number of plants appeared symptom / Number of plants inoculated

Table 2. Infectivity of the inoculated cowpea epidermal cell
(Four replications)

Test plant	Infectivity			
Cowpea	0	0	0	0 *
Tobacco	0/3	0/3	0/3	0/3 **

* Formed local lesion number

** Number of plants appeared symptom / Number of plants inoculated

On the contrary, when the sap of inoculated cowpea epidermal tissue was used as the inoculum, the cowpea and tobacco plant did not appeared the symptom together as shown in Table 2. Therefore, the replication of virus in the cowpea epidermal tissue was not recognized. It was also interest to note that local lesion-like symptom was never formed on the stripped epidermal tissue which was inoculated.

It seems therefore that infectibility of the epidermal cell of tobacco and cowpea leaf for cucumber mosaic virus differ greatly from each other. Therefore it is presumed that CMV does not multiply in the epidermal tissue of cowpea, or even if CMV multiplies, its quantity is so small as it can not be estimated by bioassay.

II) virus infectibility of mesophyll cell

a) Tobacco leaf

After stripping the epidermal tissue from the tobacco leaf, the bared mesophyll tissue was inoculated immediately with CMV. Inoculation was done by gently rubbing with a paint brush soaked with CMV solution. The fragments of the tissue were then immediately cut out from the inoculated portion, and incubated as

Table 3. Infection of tobacco mesophyll cell

Experiment	Addition of a carborundum in inoculum	Infection by sap of mesophyll cell*				
I	+	5/83	5/111	3/32	4/28	4/31
	−	2/153	0/126	0/136	0/126	3/123
II	+	3/79	4/126	3/98	4/72	5/113
	−	0/99	1/172	2/160	1/159	1/122
III	+	8/158	4/168	2/91	2/121	8/231
	−	2/238	3/180	3/188	2/206	1/167

+ addition, − no addition

* $\frac{\text{Number of local lesion by infection with sap of inoculated mesophyll tissue}}{\text{Number of local lesion by infection with sap of infected leaf disk as control}}$

5 seedlings were used in each experiment

described above. As the control, unstripped leaf fragments were inoculated by the carborundum method and incubated under the same condition. After incubation for 96 hours, the disks were homogenized, and the squeezed sap was used for the bioassay by the opposite leaf method of cowpea. Results are shown in Table 3. Several local lesions were formed by the sap of fragment which was inoculated directly to mesophyll tissue, although it was less as compared with the control. There is a question as to whether remained CMV on the tissue infected the assay-plant. As CMV is very sensitive, it is destroyed on the surface of tissue during 96 hours, so such virus particle is not related with the infection of assay plant. Whatever have shown is that CMV can infect directly the mesophyll cell without through the epidermis and replicate there.

When the mesophyll tissue from which the epidermis was stripped, was immersed in virus suspension for a time, no infection occured. Therefore this fact may suggest that CMV can not infect through both the injured mesophyll cell formed by the treatment of epidermis stripping or the uninjured mesophyll cell. As the stripped mesophyll cell is delicate and damaged cells seems to die quickly, the virus would not infect through such injured cells, but injured cells formed by rubbing with brush. This assumption is clear from that the infection by sap of leaf tissue which was inoculated by the inoculum containing a carborundum was superior to which inoculated without a carborundum.

b) Cowpea leaf

As soon as the epidermal tissue of cowpea leaf is stripped, CMV or its viral

nucleic acid was inoculated in the bared mesophyll tissue by the above mentioned method. Then leaf disks were cut out from these inoculated portion, and leaf disks from which the epidermis was not stripped were used as the control, and these disks were incubated during 24 hours as cited above, and the number of the local lesions formed per a given area (1 cm square) were observed. Results are shown in Table 4 and 5. In the case of the inoculation with whole virus, local lesion was not formed in these mesophyll whether the inoculum included carborundum or not. On the contrary, when viral nucleic acid was used, local lesions were formed only where the abrasive was used. But the number of local lesion were few. Therefore, it was demonstrated that the mesophyll cell of cowpea was able to be infected by CMV nucleic acid, although it was not infected by CMV particle.

Table 4. Local lesion formation in mesophyll cell of stripped cowpea epidermal tissue by inoculation with CMV particle. (Five replciations)

Treatment	Carborundum	Local lesion formation				
Stripping of epidermis	+	0	0	0	0	0
Stripping of epidermis	−	0	0	0	0	0
Non stripping of epidermis (control)	+	15	14	17	19	20

Each value is lesion numbers per 1 cm^2
+ addition, − no addition

Table 5. Infection of cowpea mesophyll cell by inoculation with viral nucleic acid. (Five replciations)

Treatment	Carborundum	Local lesion formation				
Stripping of epidermis	+	2	0	1	1	2
Stripping of epidermis	−	0	0	0	0	0
Non stripping of epidermis (control)	+	28	35	25	26	9

Each value is lesion numbers per 1 cm^2
+ addition, − no addition

Discussion

Viral inclusion bodies are recognized frequently in the epidermal cells of plants infected systemically by viruses (1, 2, 7), and in these epidermal cells virus seems to replicate (12). Generally, it has been reported that in the systemic host plant virus is able to replicate not only in the mesophyll cell, but also in the epidermal cell. Also in the experiment of the combination of CMV and its systemic host (N. *tobacum*), virus replication in the epidermal cell was observed. And as appearance of its infectivity was comparatively greater, it seemed that virus spread from the primary infected cell to many surrounding cells where it replicated. More-

over, when mesophyll cells from which their epidermis were stripped were directly inoculated with virus, infection occurred. Therefore, it is assumed that epidermal cells and mesophyll cells of *N. tobacum* are able to be infected directly by CMV particle, and that the every processes of infection including the decoating were performed in the cells of both tissues.

However in the case of the local lesion host the infection process seems to differ from the systemic host. In the previous paper, we reported that in the combination of CMV and its local lesion host (cowpea leaf), the decoating of the inoculated virus would be performed in the primary infected cell of the epidermis. Moreover, in these experiments, CMV replication in the cowpea epidermal cell was not observed through a bioassay. Therefore it is assumed that CMV particle is not produced in the copwea epidermal cell. From the above two points, we presume that in the combination of CMV and cowpea the virus replication starts in the mesophyll cell by the transmission of the viral nucelic acid released by decoating or any infectible precursor occured in the primary infected epidermal cell.

When cowpea mesophyll cells without epidermis were inoculated with CMV particle, local lesion did not form. However local lesion did form with the inoculation of CMV-nucleic acid, and thus it is evident that cowpea mesophyll cell is infected only by CMV-nucleic acid. The phenomenon seems to due to whether cowpea mesophyll cells immediately loss the function that supports the decoating process of the virus, owing to the injury by the occasion of inoculation, or that these cells naturally have not such a function. Kontaxis (8), and Yamaguchi and Shimomura (14) experimented on the virus infectivity of the mesophyll cell by the combinations of tobacco mosaic virus (TMV) and its systemic host (*N. tobacum*) and its local lesion host (*N. glutinosa*). They found that the mesophyll cell of *N. glutinosa* was not infected by TMV particle although it was infected by TMV-nucleic acid, but the mesophyll cell of *N. tobacum* was infected by TMV particle. These findings are similar to our experimental results. But Dijkstra (4) reported that TMV was able to replicate exceedingly in the epidermal tissue of its local lesion host – *N. glutinosa*. Therefore, it may be that the results of this experiment are not a general phenomenon of the local lesion host and the systemic host. Both their findings and ours leads us to the conclusion that the inoculated virus particle decoats in the epidermal cell and the mesophyll cell does not have the function that supports the decoating in the CMV-cowpea. If this hypothesis is true, it means that cowpea mesophyll cells can be infected only by any infective precursor, for example, viral nucleic acid transmitted from the primary infected epidermal cell. As this hypothesis was obtained by bioassay, confirmation of these phenomena should become more precisely with the development of serological and other techniques for dealing with intracellular virus.

Summary

Virus infectibility of epidermal cell and mesophyll cell were investigated by the combination of cucumber mosaic virus and its local lesion host (copwea) or its systemic host (tobacco plant). Virus replication in these tissues was bioassayed by the cowpea or tobacco.

In the case of the tobacco plant which infects systemically by CMV, the virus is able to replciate in the epidermal cell. Also, when the virus was inoculated directly to the bared mesophyll cell, infection occured. Therefore, CMV can infect directly the epidermal cell and the bared mesophyll cell of tobacco plant and replicate there.

In the case of cowpea which shows necrotic local lesion by the infection with CMV, virus replication was not recognized in the epidermal cell so far asb ioassay. Also, when virus was inoculated directly to the bared mesophyll cell, local lesion was not formed. But when viral nucleic acid was inoculated, a few lesion formed. Therefore, the mesophyll cell of cowpea leaf can not be infected with whole virus, but with viral nucleic acid. So that, in the combination of CMV and cowpea, virus replication would start in the mesophyll cell by the transmission of the viral nuclic acid released by decoating or by any infective precursor produced in the primary infected epidermal cell.

References

1) Bawden, F.C. and F.M.L. Scheffield (1939). Ann. Appl. Biol., **26**, 102.
2) Bawden, F.C. (1950). Plant viruses and virus diseases. Chronica Botanica Company, p. 37–55.
3) Benda, G.T.A., (1958). Virolog. 6, 718.
4) Dijkstra, J. (1966). Virus of plants (A.B.R. Beemster and J. Dijkstra. eds), North-Holland Publishing Company, Amsterdam, p 19.
5) Ehara, Y and T. Misawa (1967). Tohoku J. Agr. Res., **17**, 193.
6) Gierer, A. and G. Schramm (1956). Nature, **177**, 702.
7) Kassanis, B. and F.M.L. Scheffield (1941). Ann. Appl. Biol., **28**, 360.
8) Kontaxis, D.G., (1961). Virolog., **16**, 244.
9) Misawa, T. and Y. Ehara (1964). Tohoku J. Agr. Res., **14**, 143.
10) Scott, H. (1963). Virolog., **29**, 103.
11) Scheffield, F.M.L. (1963). Ann. Appl. Biol., **23**, 498.
12) Schramm, G. und B. Rottger (1959). Zeits. für Naturforsch., **14**, 510.
13) Wildman, S.G. (1960). The Virus (F.M. Burnet and W.M. Stanley. eds) Vol. 2, p. 1–31, Academic Press, New York.
14) Yamaguchi. A, and T. Shimomura (1964). Host-parasite relations in plant pathology, Symposium held at the Hungarian Academy of Sciences (Z. Kiraly and G. Ubrizsy. eds). p. 59.
15) Zech, H. (1954). Exptl. Cell. Res., **5**, 560.