Necrotic Lesion Formation Induced by Citrinin on Leaves Systemically Infected with Tobacco Mosaic Virus

Yuko OHASHI*, and Toru SHIMOMURA*

大橋祐子*・下村 徹*: タバコ・モザイク・ウイルスに全身感染させた
葉のシトリニン処理による壊死斑の誘導

It was previously reported¹⁾ that leaves of systemic hosts, *Nicotiana tabacum* Xanthi or Samsun, inoculated with tobacco mosaic virus (TMV) or leaves of local lesion hosts, *N. glutinosa* or *N. tabacum* Xanthi-nc, inoculated with TMV and incubated at 30C, developed necrotic lesions if they were treated with actinomycin D. This paper describes similar necrotic lesion formation induced by citrinin^{2,3,4)} on both systemic and local lesion hosts. Citrinin was supplied from Nippon Soda Co., Ltd., and Wako Pure Chem. Ind. Ltd., Japan. It was produced by several species of genera *Penicillium* and *Aspergillus* and is well known as one of the yellowish toxic substances found in *Penicillium citrinum*-infected rice grains.

Leaves of *N. glutinosa* were inoculated with TMV (Common strain OM, 10 μ g/ml) and the plants were incubated at 30C, a temperature at which no lesions appear and in which TMV spreads systemically, and were illuminated continuously by fluorescent lamps (about 6,000 lux). One day after inoculation, the inoculated leaves were detached and their petioles were put into vials containing 0.5 ml of a solution of 100-200 μ g/ml citrinin. After the complete absorption of citrinin through the petioles, the leaves were placed on wet filter paper in covered plastic vessels and incubated at 30C under illumination. About 6-8 hr after citrinin treatment, necrotic lesions appeared on the treated leaves as shown in Fig. 1a. These lesions appeared as necrotic rings and turned distinct dark brown. Lesion number was decreased proportionally with the dilution of the inoculum and lesion size became larger gradually as the interval between inoculation and chemical treatment prolonged. These results show that the necrotic areas corresponds with the initial sites of virus infection. No lesions formed on uninoculated control leaves of *N. glutinosa*, but were treated with citrinin, nor on untreated control leaves incubated constantly at 30C after the inoculation.

Necrotic lesions also formed on *N. glutinosa* leaves inoculated with TMV 1 day before the incubation at 30C if they were injected intercellularly with 50 μ g/ml citrinin or were floated on a solution of 30-80 μ g/ml citrinin in Petri dishes. These leaves were then incubated at 30C under continuous illumination. As is shown in Fig. 2, water-soaked rings appeared on *N. glutinosa* leaves floated on a solution of

* Institute for Plant Virus Research, Tsukuba Science City, Yatabe, Ibaraki 300-21, Japan
植物ウイルス研究所

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Fig. 1. Citrinin induced lesions formation on leaves systemically infected with TMV. a: On *N. glutinosa* leaf treated with 75 $\mu\text{g}/\text{leaf}$ of citrinin 1 day after the inoculation. b: On Samsun tobacco leaf treated with 100 $\mu\text{g}/\text{leaf}$ of citrinin 1 day after the inoculation.

citrinin 3-4 hr after the treatment.

Further experiments were conducted to determine whether lesions would be formed or not if infected leaves of systemic host were treated with citrinin. Leaves of *N. tabacum* Samsun were inoculated with 2 $\mu\text{g}/\text{ml}$ of TMV and the plants were incubated at 30C under illumination. One day after the inoculation, the inoculated leaves were detached, treated with citrinin by absorbing it through the petioles as described above, and then incubated at 30C. Necrotic rings appeared occasionally on the treated leaves as shown in Fig. 1b.

According to the results of our previous experiment⁵⁾, treatments of actinomycin D, ultraviolet light irradiation, and dark incubation, all of which were able to induce necrotic lesions on the leaves systemically infected with TMV, inhibited nucleic acid synthesis in the cytoplasmic ribosomal fraction more effectively than that in TMV fraction. We now examined the effect of citrinin on nucleic acid synthesis in the infected leaves.

Leaves of *N. glutinosa* were inoculated with 50 $\mu\text{g}/\text{ml}$ of TMV and the plants were incubated at 30C under illumination. One day after inoculation, the inoculated leaves were detached and the petioles were put into vials containing a solution of 5 μCi ^3H -uridine and of 75 μg citrinin. After the solution was completely absorbed through the petioles, the leaves were placed on wet filter paper in vessels incubating at 30C under illumination. After 18 hr's incubation, nucleoproteins were isolated from the treated leaves and then virus and ribosomal fractions were separated by the sucrose linear gradient centrifugation. These methods were the same as in our

Fig. 2. Citrinin induced lesions formation on *N. glutinosa* leaf systemically infected with TMV. Leaves of *N. glutinosa* were inoculated with TMV, incubating the plants at 30C. One day after the inoculation, the inoculated leaves were detached, floated on a solution of 50 $\mu\text{g}/\text{ml}$ of citrinin, incubating at 30C.

previous paper⁵⁾. The results of the experiment are shown in Table 1. In citrinin treated leaves, the rate of the incorporation of ³H was more effectively inhibited in

Table 1. Effect of citrinin on ³H-uridine incorporation into nucleoproteins of *N. glutinosa* leaves systemically infected with TMV at 30C

Treatments	³ H cpm/OD ₂₆₀		
	TMV	Ribosome	TMV/Ribosome
Citrinin(75 µg/leaf)	24,200	7,150	3.38
Untreated	37,200	24,700	1.51

ribosomal fraction than that in TMV fraction, although the both incorporation rates were below those of the untreated leaves. In this respect, the radioactivity pattern of citrinin treated leaves showed a similar trend to that of actinomycin D treated leaves⁵⁾. However, further studies are needed to determine whether or not necrotic lesion formation by both these chemicals is based from a common physiological activity. Another possibility that necrotic lesions are induced by the degradation of cell membranes in citrinin treated leaves can not be excluded, since this chemical is known to exhibit its antifungal activity by damaging cell membranes and by changing the permeability in fungi³⁾. The previous paper⁶⁾ suggested that the degradation of cell membranes is one of the early host responses for lesion formation in the hypersensitive hosts.

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