Comparison of Virus Production in Infected Plants between an Attenuated Tomato Strain (L₁₁A) and Its Wild Strain (L) of Tobacco Mosaic Virus

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An attenuated tomato strain (L₁₁A) of tobacco mosaic virus (TMV) has been used in Japan to protect greenhouse tomato plants from mosaic disease by TMV¹⁾. The complete nucleotide sequence of L₁₁A was compared with that of the ancestral wild strain (L)²⁾. It was shown that ten nucleotide substitutions were found in L₁₁A, of which only three were found to be responsible for amino acid changes. These three nucleotide substitutions were all located in 130 K and 180 K protein genes. Since the 130 K and 180 K proteins were thought to take part in viral RNA replication³⁾, it was presumed that the level of virus production of L₁₁A was lower than that of L. In this paper, we demonstrated that the number of accumulated virus particles is lower in L₁₁A-infected cells than L-infected ones, suggesting that the mutations found in the 130 K and 180 K protein genes caused the limited level of multiplication of the virus in infected cells. Part of this paper was presented at the annual meeting of the Phytopathological Society of Japan in 1981⁴⁾.

In the first experiment, leaves of tomato (*Lycopersicon esculentum* Mill. cv. Fukuju No. 2) and tobacco (*Nicotiana tabacum* L. cv. Samsun) plants were inoculated with 10-fold diluted sap of infected leaves or purified virus (0.5 mg/ml) and about two weeks later they were harvested for virus purification. Virus was purified from the leaves by the method described previously⁵⁾. The virus yields of L₁₁A and L were shown in Table 1. The yield of L₁₁A was clearly less by 4-6 folds than that of L in tomato as well as tobacco plants although the virus yield was different between the two hosts.

This result provided two possibilities: one is that the total number of L₁₁A-infected cells in an inoculated leaf was less than that of L-infected ones. The other is that the virus yield per infected cell was different between these two strains. To ascertain which possibility is correct, protoplasts were isolated from infected tomato leaves and the virus yields in them were examined.

Virus was inoculated at 0.5 mg/ml onto either cotyledon of tomato plants at 1-2 true leaf

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Table 1. Comparison of virus yield from infected plants between L₁₁A and L^{a)}

Expt.	Plant	L ₁₁ A	L	L/L ₁₁ A
1	Tomato	270 ^{b)}		5.2
	Tomato		1,408	
2	Tomato	255		6.0
	Tomato		1,537	
3	Tomato	226		—
4	Tobacco	612		—
5	Tobacco	460		—
6	Tobacco		3,236	—

a) Plants were inoculated with virus and incubated for about two weeks. The infected tomato plants and the inoculated tobacco leaves were used for virus purification.

b) mg virus per kg infected leaves.

Table 2. Comparison of number of virus particles in infected protoplast between L₁₁A and L^{a)}

Expt.	Virus	Leaf position ^{b)}	No. of harvested protoplasts	Amount of virus (mg)	Infection ^{c)} (%)	Estimated no. of virus particles per infected protoplast
1	L ₁₁ A	4	4.0×10^6	44.7	60	2.9×10^5
	L	4	1.9×10^6	163.7	92	1.4×10^6
2	L ₁₁ A	8	1.5×10^6	23.5	97	2.5×10^5
	L	8	1.3×10^6	72.5	50	1.7×10^6
	L ₁₁ A	5	5.2×10^5	38.5	93	1.2×10^6
	L	5	1.3×10^6	501.2	99	6.0×10^6

a) Virus was inoculated at 0.5 mg/ml onto either cotyledon of tomato plants at 1–2 true leaf stage (Expt. 1) or 1–2 true leaves at 4–5 true leaf stage (Expt. 2). After incubation for two weeks, protoplasts were isolated from the upper infected leaves.

b) Number of true leaves from the base.

c) The level of infection was determined by staining the protoplasts with TMV specific fluorescent antibody.

stage (Expt. 1) or 1–2 true leaves at 4–5 true leaf stage (Expt. 2). Protoplasts were isolated from infected upper leaves 2 weeks after inoculation as described previously⁶⁾ and the proportion of infected protoplasts was determined by the method of Otsuki and Takebe⁷⁾. The virus amount accumulated in the protoplasts was estimated by measuring the virus amount precipitated with polyethylene glycol as described by Otsuki⁸⁾. As shown in Table 2, the percentage of infected cells varied from leaf to leaf. However, the estimated number of virus particles of L per infected protoplast was always higher by 4–6 folds than that of L₁₁A. This result coincided with the result of Koiwa *et al.* by electronmicroscopic observation⁹⁾. They showed that virus particles in L₁₁A-infected tissues were less in number than that in L-infected ones. When protoplasts were inoculated *in vitro* with L₁₁A or L, the virus yield⁶⁾ and the viral RNA production¹⁰⁾ of L₁₁A were almost the same level as that in L-inoculated protoplasts kept up to three days. It may need more prolonged period if any remarkable difference appears between the two strains. Experiments using protoplasts are, however, usually conducted with a short period such as 1–3 days since thereafter protoplasts gradually lose their viability.

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In the next experiment, the virus multiplication in leaf discs was examined to see whether there was any difference in multiplication curve between $L_{11}A$ and L . As described previously⁵⁾, leaf discs were prepared from leaflets of tomato by means of a cork-borer (14 mm in diameter) immediately after inoculation with L or $L_{11}A$ at the concentration of 0.5 mg/ml floated in the medium, and cultured for 6 days at 32 or 22 C under the continuous illumination of fluorescent light (approximately 2,000 lux). At any given days after the culture, the homogenate of the ten discs was assayed for infectivity using Xanthi nc tobacco leaves. As shown in Fig. 1, the pattern of virus multiplication of $L_{11}A$ was similar to that of L for at least 3 days although the efficiency was lower. However, at the later stage (4–6 days postinfection), $L_{11}A$ production was significantly reduced while L continued to increase. The difference in virus production at the later stage was more pronounced when the intact leaves on plants were used for an infection experiment¹¹⁾. Such a phenomenon has been termed autoregulation¹¹⁾. Since the number of local lesions per unit virus (virus specific infectivity) was not significantly different between $L_{11}A$ and L ¹²⁾, the amount of $L_{11}A$ was also lower in this experiment than that of L .

Our data show that virus yield of $L_{11}A$ was one fourth to one sixth of that of L . Watanabe *et al.*¹⁰⁾ presumed that $L_{11}A$ moves more slowly from cell to cell than L does, because of remarkably reduced production of 30 K movement protein in $L_{11}A$ when compared with L .

Fig. 1. Virus growth patterns of $L_{11}A$ and L in tomato leaf discs.

$L_{11}A$ at 22 C: ○, $L_{11}A$ at 32 C: ●, L at 22 C: □, L at 32 C: ■.

Tomato leaflets were inoculated with virus at 0.5 mg/ml and rinsed with tap water. The leaf discs were cut out from them and incubated either 22 C or 32 C. Relative no. of local lesions defined as:

$$\frac{(\text{Total number of local lesions by homogenate of discs}) \times (\text{dilution factor})}{(\text{Total number of local lesions with standard suspension with } L \text{ at } 0.1 \mu\text{g/ml})}$$

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The present result shown in Table 2, however, revealed that even if cell-to-cell movement of $L_{11}A$ was slower than that of L , the number of accumulated virus particles per infected cell were lower in $L_{11}A$ than in L .

The $L_{11}A$ -encoded 30 K and coat proteins are identical to those of L . However, 130 K and 180 K proteins of $L_{11}A$ differ from those of L by amino acid substitution. The 130 K and 180 K proteins are responsible for virus multiplication. In conclusion, our present results suggest that the mutations in 130 K/180 K proteins of $L_{11}A$ are responsible for the limited production of virus in each of the infected cells (autoregulation). Further study is needed to elucidate the molecular mechanism of the autoregulation which the altered 130 K/180 K proteins of $L_{11}A$ involves.

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和 文 摘 要

西口正通・本吉総男・大島信行：タバコモザイクウイルスの強弱両系統， L および $L_{11}A$ ，の感染植物におけるウイルス産生量の比較

タバコモザイクウイルスの強弱両系統， L および $L_{11}A$ ，のウイルス産生量の比較を行った。ウイルスに感染したトマトおよびタバコからのウイルス収量は $L_{11}A$ のほうが少なく L の約 $1/5 \sim 1/6$ であった。感染トマト葉からプロトプラストを分離し感染率およびウイルス量を調べ、感染プロトプラスト当りのウイルス量を比較すると、 $L_{11}A$ 感染プロトプラスト当りのウイルス量は L の場合より少なく $1/4 \sim 1/6$ であった。 $L_{11}A$ のウイルスの増殖曲線は接種4日後プラトーに到達しその後減少するのに対し、 L は4日以後も増加を続けた。このように細胞における $L_{11}A$ のウイルス量が少ないのは、増殖能の低下とともに感染4日以降におけるウイルス増殖の減少によることが示唆された。

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