Control of Tomato Mosaic Disease by Attenuated Virus

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The mosaic disease of tomato which is caused by tobacco mosaic virus(TMV) is the most serious disease of tomato in Japan, especially in plastic greenhouses, though cucumber mosaic virus and others are also the cause of mosaic disease of tomato. More than 90% of viruses isolated from mosaic-diseased tomato is TMV among which tomato strain of the virus is most prevalent, over 90%.[4] In order to prevent the mosaic disease (TMV), seed disinfection, soil fumigation, and some cautions against the mechanical transmission of TMV are carried out, but these methods are not so effective. Consequently many tomato growers began to use attenuated virus in their tomato fields. The attenuated virus is a kind of preventive against the tomato mosaic disease by using the phenomenon of interference which works between the strains of a plant virus.

History of attenuated virus in Japan

The first attenuated virus of TMV named as L_{11} was selected from avirulent strains which had been produced in tomato stems inoculated with a tomato strain of TMV,TMV-L, and incubated for 14 days at 35°C, through local lesions of *Nicotiana glutinosa*.[6] Later this attenuated virus was improved by several passages through local lesions of *N. glutinosa* and tomato plants in the field. The reformed virus was named as $L_{11}A$[2] and almost symptomless while L_{11} caused very mild symptoms in young tomato seedlings inoculated(Plate 1).

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Another attenuated strain, $L_{11}A237$[7], was developed in 1977 for the prevention of the mosaic disease of TMV-resistant tomato cultivars which were being raised by tomato growers in the last ten years. This attenuated virus was produced by successive passages of $L_{11}A$ through GCR237 tomato bearing homozygous TMV-resistant gene,Tm-1. $L_{11}A$ multiplies hardly in such resistant cultivars and consequently even the tomato plants previously inoculated with $L_{11}A$ are readily infected with wild TMV which is pathogenic to Tm-1-type resistant tomatoes.

Effects of attenuated virus inoculation

The effects of attenuated virus for the prevention of tomato mosaic disease were investigated by challenging the parent virus L to tomato plants which were pre-inoculated with L_{11}, in the open fields of Hokkaido National Agricultural Experiment Station mainly in 1963-1965.[3,6] The main effects of inoculation of attenuated virus were suppression of disease symptoms and prevention from reduction of fruit setting caused by the infection of wild TMV. These good effects were reflected in fruit yields.

Early growth of tomato plants infected with L_{11} decreased slightly, but soon after recovered. In the experiment of 1965 the blooming of the first truss of the plants inoculated with L_{11} was delayed 1-2 days compared with untreated plants, but such a delay of blooming did not always occur.

Leaf symptoms of L_{11}-inoculated tomato plants were suppressed to mild mottling or no

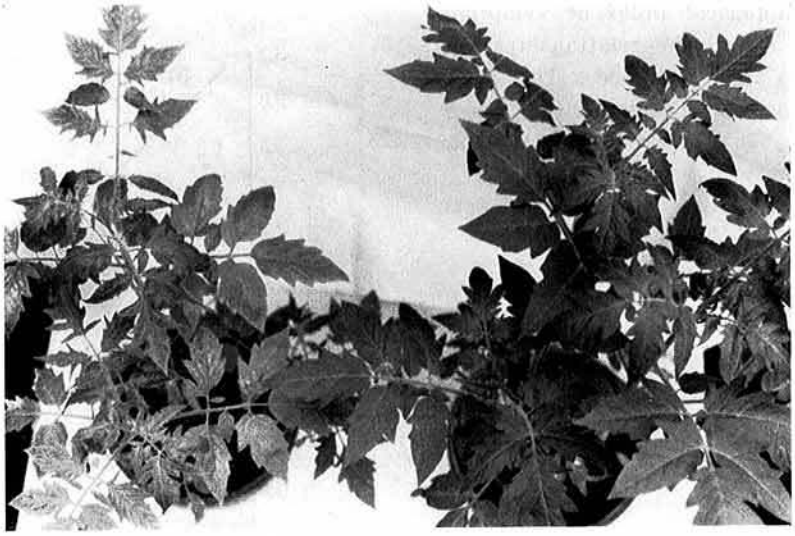

Plate 1. Tomato Plants infected with parent virulent virus L (left) and attenuated virus L_{II} A (right). The symptoms of the right plant is invisible to the naked eye.

symptoms during about the first one and a half months, but thereafter severe mottling appeared gradually. The severity of the mottling was most influenced by the time of inoculation of the virulent virus challenged. The tomato plants infected with the virulent virus alone showed systemically developed severe leaf symptoms from two to three weeks after inoculation. The cause of the symptoms which appeared on the plants pre-inoculated with L_{II} might be a decline of the attenuated virus multiplication in the late stage of tomato growth followed by the multiplication of the virulent virus inoculated or transferred from the other parts of the field. The degree of these leaf symptoms has a close relationship to fruit yields. Fig. 1 shows the change of leaf symptom of tomato plants in each plot through growing season in the experiment of 1963. Plots of this experiment are shown in Table 1. There are three main plots inoculated at different two dates or not inoculated with L and each of which has three subplots inoculated or not inoculated with L_{II}. To know the relationship between degree of symptom and fruit yields, the area enclosed by each curved line showing the change of symptoms was measured by a planimeter and used as

Table 1. Dates of L- and L_{II}-inoculation[6)]

Main plot		Subplot	
Plot name	Date of L-inoculation	Plot name	Date of L_{II}-inoculation
A	May 25	1	May 10
		2	June 9
		3	not inoculated
B	June 24	1	May 10
		2	June 9
		3	not inoculated
C	not inoculated	1	May 10
		2	June 9
		3	not inoculated

Table 2. Numerical index showing the degree of leaf symptoms[6)]

Treatment	Index of symptoms
A1	1.98
A2	2.76
A3	2.89
B1	1.69
B2	2.09
B3	2.49
C1	1.71
C2	1.59
C3	1.95

expressing the numerical index of symptom (Table 2). Fig. 2 shows the relationships between fruit yields and the indices. The index of the symptom of each plot shows a high correlation with fruit yield of each plot, especially with the total yield.[6]

Table 3 shows number of flowers and percentage of fruit setting of tomato plants in the first to the fifth truss in the experiment of 1965. Percentages of fruit setting in the plants of L1, L2, and L3 plots were different between trusses, because the failure of fruit setting was restricted to the trusses which had been blooming or going to bloom at the time when plants became systemically infected with the virulent virus and mosaic symptoms appeared. The time of L-inoculation and appearance of symptom and the progress of flower formation are shown in Fig. 3. The time of blooming of each truss is represented by the time of its first flower blooming. The tomato plants pre-inoculated with L_{11} were saved from this damage (Table 3). In plants of L2 plot inoculated with the virulent L alone on May 27, the percentage of fruit setting was

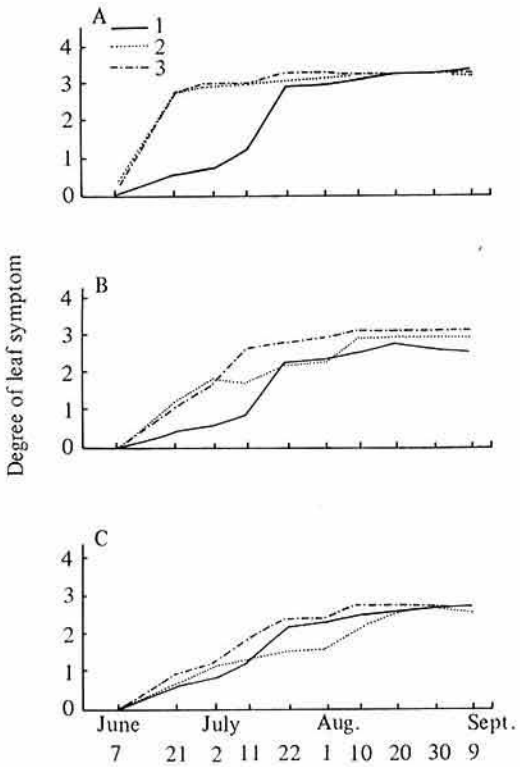

Fig. 1. Changes of leaf symptom through growing season in the experiment of 1963[6]

The degree of symptoms (S) of each plot is expressed as follows.

$$S=\frac{1a+2b+3c+4d}{a+b+c+d+e}$$

where a, b, c, d and e express the numbers of plants showing mild mottling, medium mottling, severe mottling, symptoms consist of mottling, mulformation of leaves, stunting, and necrosis, and no sympoms respectively. The numbers, 1, 2, 3, and 4 represent the grades of severity for respective symptoms of a, b, c, and d. (Some plants of B2 and B3 plots were possibly infected with wild TMV before L_{11}- or L-inoculation, so it was supposed that these plots showed high degree of symptom in the survey of June, 21 and July, 2.)

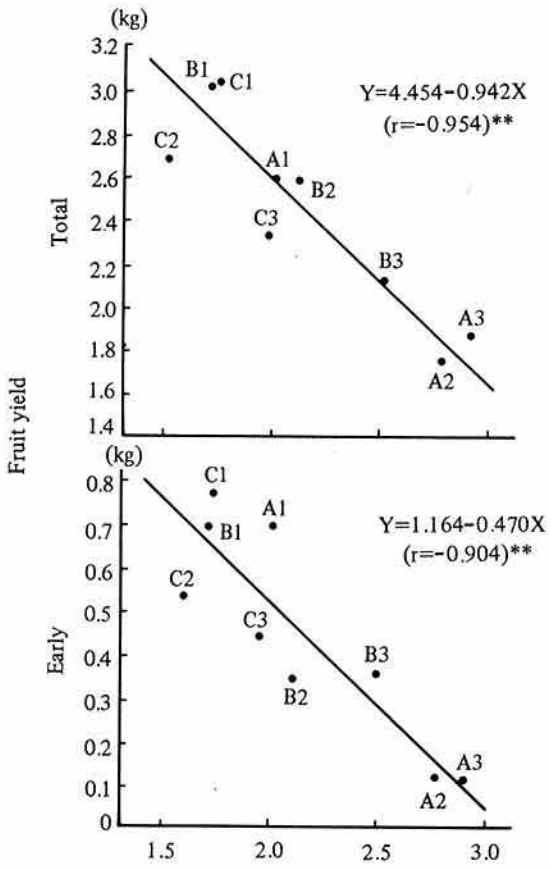

Fig. 2. Relationships between indices of symptom and fruit yields[6]

Table 3. Number of flowers and percentage of fruit setting in successive trusses from the first to the fifth truss[3)]

Truss number	Treatment								L.S.D. (5% level)
	L1	L2	L3	L_{11}+L1	L_{11}+L2	L_{11}+L3	L_{11}	Control	
	Number of flowers								
1st	9.6	9.6	11.4	10.0	9.9	9.2	8.7	10.2	1.4
2nd	8.1	10.5	10.0	11.2	10.7	9.8	10.0	10.0	1.4
3rd	9.0	10.1	10.5	10.8	10.3	9.7	10.2	10.5	n.s.
4th	8.6	10.5	9.3	10.5	10.1	8.8	10.6	9.4	n.s.
5th	7.8	8.0	8.1	7.3	7.3	7.5	7.6	7.3	n.s.
Total	43.1	48.7	49.3	49.8	48.3	45.0	47.1	47.4	n.s.
	Percentage of fruit setting								
1st	44.4	25.4	51.2	48.3	57.9	63.2	63.3	51.1	
2nd	55.3	47.7	54.6	51.1	63.6	66.5	65.1	63.9	
3rd	63.5	64.3	39.9	58.5	60.4	65.1	63.6	63.8	
4th	61.0	62.6	35.4	53.9	58.0	68.6	62.1	65.5	
5th	35.6	31.6	37.3	34.6	28.7	45.2	31.4	41.6	
Total	52.0	46.3	43.7	49.3	53.7	61.7	57.1	57.2	

L1, L2 and L3: Inoculated with L on April 30, May 27, and June 23, respectively.
L_{11}: Pre-inoculated with L_{11} on April 23.

markedly decreased in the first and second trusses. In the plants of L3 plot inoculated on June 23, it was in the third and fourth trusses. The ability of flowers to set fruit appeared to be recovered about a month after inoculation of the virulent virus. This phenomenon was interpreted as a shock reaction resulting from the infection according to Boyle and Wharton (1957). In the plants of L1+L_{11} plot inoculated with L seven days after L_{11}-inoculation severe symptoms appeared in early June, about nine days before the first flower blooming. Accordingly the marked decrease in the fruit setting owing to the shock reaction was expected in the first truss, but no more than the slight decrease in fruit setting was observed. This seemed to mean that the shock reaction was inhibited by the pre-inoculation of the attenuated virus regardless of the appearance of the virus symptoms. The number of flowers is not so different between the plots except L1. In L1 plot the numbtr of flowers is fewer than those of other plots, but its cause is not clear although it might be caused by severe leaf symptoms. L1, L2, and L3 plots inoculated with virulent L alone yielded 27, 16, and 24% less weight of fruit

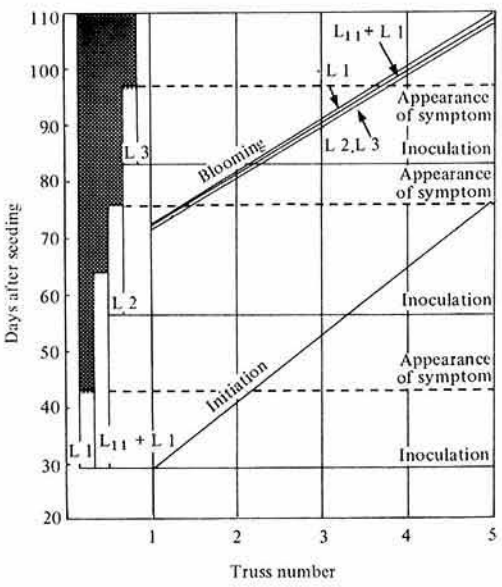

Fig. 3. Progress of flower formation in relation to time of L inoculation and of appearance of symptom.[3)]

In this figure the interval between blooming times and that between intiation times of trusses are drawn regularly as 9 and 12 days respectively.[3)]

Table 4 Control of tomato mosaic disease by inoculation with $L_{11}A$

(Shizuoka Agr. Exp. Sta., 1974)

Plot	Outbreak of mosaic disease				Grade of mosaic (Mar. 15)		Plant TMV-detected (Mar. 14)	Yield index
	Jan. 4	Jan. 28	Feb. 20	Mar. 15	M	m		
L_{11} A-inoculated at cotyledonary stage	0%	0%	14%	29%	7%	21%	100%	165
do + virulent TMV-ino. at planting date	11	18	68	100	75	25	100	144
virulent TMV-ino. at planting date	100	100	100	100	100	0	100	87
Customary cultivation	50	86	100	100	100	0	100	100
Careful cultivation against wild TMV	0	0	29	39	21	18	74	123

Planting date of tomato seedlings, cv. Tōkō-K: Nov. 9. Inoculation of virulent TMV: Nov. 9.
Yield index: Index of weight of good quality fruit.

respectively than the plot inoculated with L_{11} alone(L_{11} plot) which yielded as much as the non-inoculated control plants infected naturally with wild TMV to some extent. The plants inoculated with L more than one month after pre-inoculation of L_{11} yielded just as well as those inoculated with L_{11} alone. Even L1+L_{11} plot inoculated with L one week after pre-inoculation of L_{11} yielded 19% higher than L1 plot inoculated with L alone.[3]

Table 4 shows the results of $L_{11}A$ inoculation to tomato plants in the plastic greenhouse which was carried out by Shizuoka Agricultural Experiment Station. The inoculation of the attenuated virus was done in cotyledonary stage of tomato plant. In this table the fourth plot was cultivated customarily without virus inoculation and the fifth plot was not inoculated with $L_{11}A$ but cultivated with special attention against contamination of wild TMV. In he first and fifth plots the percentages of outbreak of mosaic disease were lower than those of other plots. On Feb. 20 the outbreak of mosaic disease was also fewer in the second plot, but on Mar. 15 it became 100%. These three plots show higher yield index than other two plots. Especially the $L_{11}A$-inoculated plots yielded higher.[8]

Properties of attenuated viruses

Some properties of $L_{11}A$ were compared with the parent virulent strain, L. Dilution end point of L_{11} A was $10^{-7}-10^{-8}$, while L was still infective at 10^{-8}. $L_{11}A$ lost its infectivity after heat treatment at 90°C for 10 min., while L did not. The shape of virus particles and the length distribution pattern of $L_{11}A$ after purification was much the same as those of L. The sap of tomato leaves infected with L_{11} A retained about 50% of original infectivity after a week-storage at 25°C and less than 10% after a year-storage, whereas that of leaves infected with L retained 80 and 50% of infectivity after respective periods. $L_{11}A$ was suspended at 3.3 mg/ml either in 0.01 M phosphate buffer(pH 7.0) or in the same buffer containing 2% sucrose, then lyophilized and the resultant virus powder was stored at 22–25°C. The samples without sucrose completely lost their infectivities after 27 months storage, whereas the samples with sucrose were highly infectious still at the same stage of storage. The effect of temperature on the multiplication of $L_{11}A$ and L in tomato leaf discs was compared. The optimal temperature for $L_{11}A$ was 25–28°C., while that for L was 28–30°C. At 35°C, L multiplied considerably but $L_{11}A$ did not.[5]

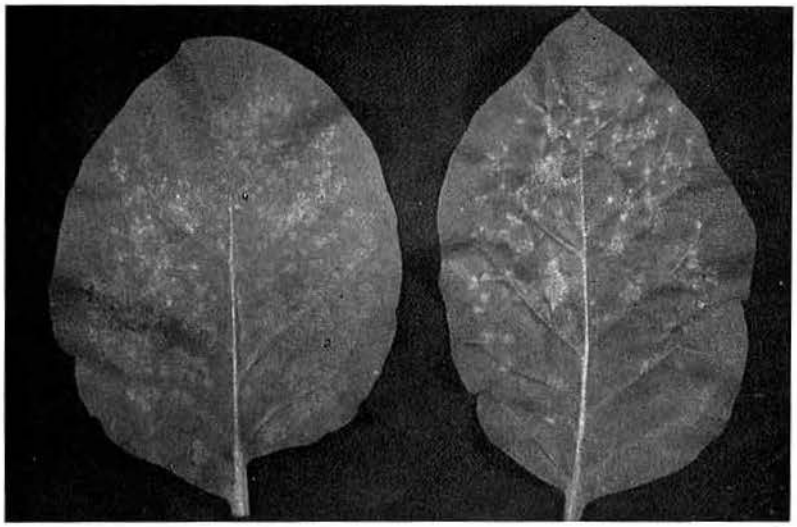

Plate 2. Leaves of *Nicotiana tabacum* cv. Xanthi nc inoculated with $L_{m}A237$ (left) and $L_{m}A$ (right).

Fifty-four species from 15 families among 94 species belonging to 20 families were found to be susceptible to $L_{m}A$, and 66 species from 16 families were found to be susceptible to L. Samsun tobacco showed very mild mottling due to the infection of L_{m} A, but other tobacco varieties, Ambalema, Bright Yellow, and White Burley showed local lesions followed by mild mosaic with the exception of Ambalema. Other solanaceous plants systemically infected didn't show symptoms or showed very mild symptoms with the exception of *Petunia hybrida* and *Physalis floridana* which showed severe symptoms similar to plants infected with virulent L. Most of plants infected with $L_{m}A$ seemed to show only mild symptoms.

$L_{m}A237$ causes almost no symptoms on tomato and Samsun tobacco, but differs in that it multiplies much faster than $L_{m}A$ in TMV-resistant Tm-1-type tomato cultivars. This attenuated virus causes sometimes necrotic rings on the inoculated leaves of Xanthi nc tobacco in the environment in which $L_{m}A$ causes nectrotic spots.[7] (Plate 2)

Plate 3. Inoculation of attenuated virus to tomato seedlings with a power sprayer.

Practical procedure of inoculation of attenuated virus for tomato seedlings

Tomato seeds are heat-treated at 70°C for 3–4 days and sown in rows on carbonized rice hulls or sterilized soil in seedbed. Virus inoculum is sap of tomato leaves infected with attenuated virus which is diluted 20–120 times by water and added with 10–20 g caborundum, 600–800 mesh, per *l*. Tomato seedlings of 1–2 true leaf stage are inoculated by spraying at a distance of 2–10 cm from them under working pressure of 5 kg/cm^2 with a manual or a power sprayer. (Plate 3)

References

1) Boyle, J. .S. & Wharton, D. C. : The experimental reproduction of tomato internal browning by inoculation with strains of tobacco mosaic virus. *Phytopathology*, **47**(4), 199–207 (1957).
2) Goto, T. & Nemoto, M. : Studies on control of plant virus diseases by interference of attenuated virus—Selection of TMV attenuated strain and influence on various plants inoculated with the attenuated strain. *Hokkaido Nat. Agr. Exp. Sta. Bull.*, **99**, 67–76 (1971).
3) Komochi, S., Goto, T. & Oshima, N. : Studies on the control of plant virus diseases by vaccination of attenuated virus. III. Relation in fruit setting as a shock reaction resulting from infection of tomato plants with TMV. *J. Jap. Soc. Hort. Sci.*, **35**(3), 67–74 (1966).
4) Komuro, Y., Iwaki, M. & Nakahara, M. : Viruses isolated from tomato plants showing mosaic and/or streak in Japan, with special reference to tomato strain of tobacco mosaic virus. *Ann. Phytopath. Soc. Japan*, **32**, 130–137 (1966).
5) Nishiguchi, M. & Oshima, N. : Some properties of an attenuated tomtao strain ($L_{11}A$) of tobacco mosaic virus. *Ann. Phytopath. Soc. Japan*, **45**, (5), 654–659 (1979).
6) Oshima, N., Komochi, S. & Goto, T. : Study on control of plant virus diseases by vaccination of attenuated virus. (1) Control of tomato mosaic disease. *Hokkaido Nat. Agr. Exp. Sta. Bull.*, **85**, 23–33 (1965).
7) Oshima, N. et al. : A new attenuated virus L_{11} A237. *Ann. Phytopath. Soc. Japan*, **44**(4), 504–508 (1978).
8) Shizuoka Agricultural Experiment Station : Protection of tomato against mosaic disease by attenuated virus. *New Technique of Agriculture*, No. 24. (1977).