



# HOKKAIDO UNIVERSITY

Title	The Relative Amount of the Ribonucleoprotein Components of Alfalfa Mosaic Virus
Author(s)	MATSUMOTO, Tsutomu; MURAYAMA, Daiki
Citation	Journal of the Faculty of Agriculture, Hokkaido University, 56(3), 248-255
Issue Date	1971-01
Doc URL	<a href="https://hdl.handle.net/2115/12850">https://hdl.handle.net/2115/12850</a>
Type	departmental bulletin paper
File Information	56(3)_p248-255.pdf



# THE RELATIVE AMOUNT OF THE RIBONUCLEOPROTEIN COMPONENTS OF ALFALFA MOSAIC VIRUS

Tsutomu MATSUMOTO and Daiki MURAYAMA

(Laboratory of Plant Pathology, Faculty of Agriculture,  
Hokkaido University, Sapporo, Japan)

Received November 17, 1969

## Introduction

Several reports concerning the relative amount of ribonucleoprotein components of multicomponent viruses have been published (BANCROFT and KAESBERG, 1960; PAUL, 1963; AGRAWAL, 1964; SCHNEIDER and DIENER, 1966; JASPARS and MOED, 1966; SEMANCIK and KAJIYAMA, 1967; VAN KAMMEN, 1967).

BANCROFT and KAESBERG (1960), and KELLEY and KAESBERG (1962) reported that alfalfa mosaic virus, one of the multicomponent viruses, consisted of top-a (68S), top-b (73S), middle (89S) and bottom component (99S). Variation of the relative amount of ribonucleoprotein components was not found between different strains and the ages of the plants following inoculation.

JASPARS and MOED (1966) reported that the amount of top component-a in strain II (AMV isolate 425) and alfalfa yellow mosaic virus was larger than in those of alfalfa mosaic virus strain ATCC-106. And they also reported that the ratio of the top component-a increased as the infected plants grew older.

The present investigation was undertaken to study whether the relative amount of the virus components varied according to different strains of the virus, the host plants and their ages on the basis of virus translocation, concentration of the virus nucleoprotein and the specific infectivity in inoculated tobacco leaves at different periods of time after inoculation. Such an investigation is important in order to know the biological roles particularly of the multiplication of multicomponent viruses. A part of the results of the present studies was presented at the annual meeting of the Hokkaido Branch of the Phytopathological Society of Japan held in 1968.

### Materials and Methods

Four strains of alfalfa mosaic virus; HN-4 and HN-6 isolated by MATSUMOTO et al., (1968), PC-Y (potato calico strain) isolated from diseased potato tubers obtained in Yatsugatake Seed Potato Foundation Farm, Nagano Prefecture, Japan, by MATSUMOTO and MURAYAMA, and ATCC-106 (kindly provided by Dr. J. B. BANCROFT), were used in the experiments. The strains were inoculated on tobacco plants (White Burley) and pea (Beikoku Ōsaya) grown in pots in a greenhouse. When they grew about 15 cm high, the inoculation was made. The inoculated plants were maintained in a controlled room (25~27°C, 2,000 lux for 15 hours/day) and a greenhouse (in May and June). The crude extracts (in 0.01 M phosphate buffer, pH 7.0 and at a dilution of 1 : 10) used as the inoculum were prepared by grinding the systemically infected tobacco leaves 6~8 days after inoculation.

The purification of the virus was made mainly with the procedure reported by BANCROFT et al., (1960). About 100 g of leaves were homogenized in 200 ml of 0.1 M phosphate buffer (pH 7.0) containing 5 g of ascorbic acid per liter. The extracts expressed through two layers of gauze were clarified adding one-third volume of chloroform-n-butanol (1 : 1, v/v) and then 2 cycles of differential centrifugation (10,000 rpm for 15 minutes and 30,000 rpm for 90 minutes, by using a RP 30 rotor) were done with a Hitachi-40 P ultracentrifuge. The resultant pellets were suspended in 0.01 M phosphate buffer (pH 7.0). The supernatant thus prepared followed by the centrifugation at 10,000 rpm for 15 minutes was used as the purified virus solution. The concentration of the virus in purified preparations was determined spectrophotometrically based on  $E_{260}^{1\%1\text{cm}} = 4.87$  as approximately 1 mg/ml virus concentration mentioned by FRISH-NIGGEMEYER and STEERE (1961).

The ratio of the components was estimated from the areas under the schlieren peaks in the sedimentation diagrams obtained by SPINCO model E and Hitachi UCA-1A analytical ultracentrifuges. The areas of the schlieren diagrams were magnified to ten-fold by means of Nikon profile projector (model 6C) and were measured with a planimeter.

### Results

#### (1). Translocation of alfalfa mosaic virus in tobacco plants.

To know the effect of the different period of the infected plants on the relative amount of the virus components, translocation of the virus in each phyllotaxis of infected tobacco plant was investigated. The detection of translocation of the virus in the plant was made by assaying the existence of the

virus in each phyllotaxis of the inoculated plant, on the local lesion host. The virus strain HN-6 was used in the studies. The inoculated plants were kept in a controlled room as mentioned above. Observation of symptom appearance was made every day. Bean (var. Kairyo Ōtebo) was used as the assay plant. When the plants had grown about 15 cm in height (primary leaf stage), inoculations were made.

TABLE 1. Translocation of alfalfa mosaic virus in tobacco plant (White Burley)

Days after inoculation Phyllotaxis	3		4		5		6		7	
	Local <sup>b)</sup> lesion	Symp- <sup>c)</sup> tom	Local lesion	Symp- tom	Local lesion	Symp- tom	Local lesion	Symp- tom	Local lesion	Symp- tom
1 a)	425	SC	503	MY	1289	Y	937	Y	607	Y,N
2	0	—	0	—	0	—	0	—	0	—
3	0	—	0	—	0	—	0	—	0	—
4	0	—	1102	Mo	0	—	1531	Mo	1366	Mo,N
5	0	—	0	—	1374	Mo	1754	Mo	1219	Mo,N

a). No. 1 means the first lower leaf inoculated.

b). Average number of local lesions produced on 8 half leaves of beans.

c). Mo: Mosaic, N: Necrosis, Y: Yellowing, MY: Mild yellowing,

SC: Slight chlorosis, —: No symptom

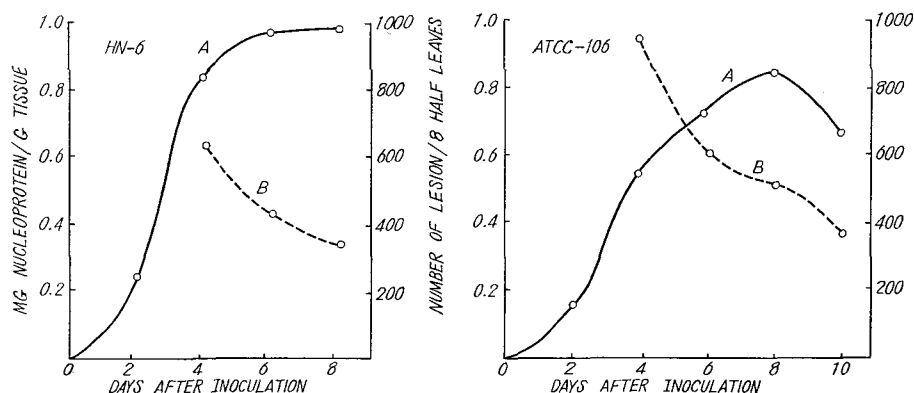
As the results in Table 1 show, a very high concentration of the virus was found only in inoculated leaves in the case of 3 days after inoculation. The yellow mosaic symptom followed by necrosis, in general, appeared on inoculated leaves 7 days after inoculation. Some of the inoculated leaves died about 10 days after inoculation. The highest concentration of the virus was detected in the fourth or fifth leaf, 4-5 days after inoculation. Severe mosaic and necrosis appeared on the fourth and fifth expanded leaves after 6 or 7 days. According to the results obtained from this experiment, it appears that the existence of the virus in inoculated plants varied according to the position of the leaves and the virus was found only in young expanded ones. Thus, there was close relationship between the age of plants after infection and the relative amount of components of AMV was recognized.

(2). The amount of virus nucleoprotein and the specific infectivity during different periods after inoculation.

Detection of AMV in inoculated leaves, determination of the amount of purified virus and their specific infectivity were examined at different periods following infection. Some strains of AMV used in previous experiment were also used in this experiment. The infected leaves used for purification

were harvested every other day and they were stored in a refrigerator ( $-30\sim-35^{\circ}\text{C}$ ). The virus purification and the bioassay were carried out at the same time. The assay of specific infectivity of the virus in each sample was made from the number of local lesions on the primary leaves of bean (Kairyō Ōtebo), produced by the virus at the concentration of  $10\ \mu\text{g}/\text{ml}$ .

Four days after infection  $0.848\ \text{mg}$  of purified virus nucleoprotein (HN-6) was isolated from one gram of the inoculated leaves (Fig. 1). The maximum



**Fig. 1.** The concentration of nucleoprotein and the specific infectivity of AMV (HN-6 and ATCC-106 strain) harvested from inoculated tobacco leaves at different times after inoculation. Curve A shows the amount of purified virus nucleoprotein (mg/g inoculated leaves of wet weight). Curve B shows the specific infectivity of the nucleoprotein (0.01 mg/ml) of AMV purified from inoculated tobacco leaves (total number of local lesions produced on half leaves of bean).

concentration ( $0.996\ \text{mg}/\text{g}$ ) of the virus nucleoprotein occurred 8 days after infection. From one gram of leaves infected with ATCC-106 4 days after inoculation  $0.561\ \text{mg}$  of nucleoprotein was obtained. The maximum concentration of the virus ( $0.867\ \text{mg}/\text{g}$ ) was recognized 8 days after inoculation and the concentration decreased to  $0.694\ \text{mg}/\text{g}$  10 days after inoculation. The specific infectivity of the strains, HN-6 and ATCC-106, decreased about 50% from 4 to 8 days and 60% from 4 to 10 days after inoculation, respectively. The difference of the specific infectivity between the two strains was impossible to compare because the preparations were obtained at the different times. The results obtained in this study agree closely with those reported by KUHN and BANCROFT (1961).

(3). The relative amount of nucleoprotein components of alfalfa mosaic virus. As described above, it was clear that the best material for the determi-

nation of the relative amount of nucleoprotein components at the different periods of the infected plants was found to be the inoculated leaves. In most cases, no virus was detected in the leaves except the inoculated and new expanded ones. As to the upper leaves of infected plant, it was necessary to know the time during which the translocation of the virus in the phylotaxis has occurred. The amount of virus nucleoprotein and the specific infectivity were compared in two purified virus preparations obtained from inoculated leaves, 4 and 8 or 10 days after inoculation. The specific infectivity determined by 4-days' preparation was higher than those by 8 or 10 days' one. So far as the systemically infected leaves, the relative amount of virus components was determined by using the leaves 6 and 10 days after inoculation.

The experimental results on the relative amount of the components of AMV are given in detail in Table 2. The figure of the top component

TABLE 2. The relative amounts of the components of alfalfa mosaic virus

Virus strains	Test plants	Test <sup>(1)</sup> leaves	Days after inoculation	Conditions of virus propagation	Components (%) <sup>(2)</sup>		
					Top	Middle	Bottom
HN-6	Tobacco (White Burley)	L	6	Greenhouse	48	21	31
do.	Pea (Beikoku Osaya)	S	6	do.	48	23	29
do.	Tobacco (White Burley)	L	4	Controlled room	47	25	28
do.	do.	L	8	do.	42	30	28
do.	do.	S	6	do.	46	20	34
do.	do.	S	10	do.	42	26	32
ATCC-106	do.	L	4	do.	44	24	32
do.	do.	L	10	do.	41	31	28
HN-4	do.	L	5	do.	60	14	26
do.	do.	L	10	do.	58	21	21
PC-Y	do.	S	6	do.	59	16	25

(1). L and S represent inoculated and systemically infected leaves, respectively.

(2). The percentage of the components was estimated from the areas under the schlieren peaks in the sedimentation diagrams.

shown in Table 2 represents a mixture of top-o, top-a and top-b, as reported by KELLEY et al., 1962 and JASPARS et al., 1966.

The relative amount of the top component of HN-4 and PC-Y was

higher than that of HN-6 and ATCC-106. Significant differences of the virus multiplication were not found between different conditions, in controlled room and greenhouse, and in inoculated and systemically infected leaves. Nevertheless, the obvious differences of the relative amount of virus components between strains were recognized. The relative amount of middle component of HN-6 and ATCC-106 strain in the inoculated tobacco leaves appeared to increase with the age of the plants after inoculation. The same tendency was observed in the preparations, both from HN-4 strain in the inoculated tobacco leaves and HN-6 strain in the systemically infected tobacco leaves. On the contrary, the top and bottom components decreased with age. However, preparations of HN-6 strain in tobacco plants and pea, respectively, showed almost the same ratio of the components.

### Discussion

Differences in the relative amount of ribonucleoprotein components were found among different strains of AMV. The ratio of the top-a of AMV isolate 425 and alfalfa yellow mosaic virus was higher than those of ATCC-106 strain (JASPARS and MOED, 1966). In this study, we also found that the strains used could be characterized by the ratio between the top and bottom component. Strain HN-4 and PC-Y contain more top component than that of HN-6 and ATCC-106. Top component represents the three top components (o, a, b) as designated by KELLEY, et al., (1962) and JASPARS, et al., (1966). The relative amount of virus components in pea has not greatly altered the characterization of the strain. Therefore, the relative amount of components of AMV may be closely correlated with the genetic factor of strains (JASPARS and MOED, 1966., MATSUMOTO, 1966).

In response to the variation of age of the plant after inoculation, the relative amount of top-a increased as the plant grew older (JASPARS and MOED, 1966). In the case of tobacco ringspot viurs (SCHNEIDER and DIENER, 1966) and tobacco rattle virus (SEMANCIK and KAJIYAMA, 1967), the ratio of middle component decreased with age of inoculated plants. In the present study, the ratio of middle component of 4 days' preparation after inoculation was higher than those of 8 to 10 days' preparation. The reason why the relative amount of the middle component increased with age after inoculation needs to be studied further.

On the other hand, the relative amount of the virus components of broad bean true mosaic virus (PAUL, 1963) and cowpea mosaic virus (AGRAWAL, 1964., VAN KAMMEN, 1967) was varied by the growth conditions of

the inoculated plants and different host plants. Therefore, experiments on other host plants and the different growth conditions responsible for the existence of virus component of AMV is made in advance.

### Summary

The purpose of this study was to determine whether the relative amount of ribonucleoprotein components of AMV varied according to the virus strains, the host plants and the age of plants after inoculation, or not. The ratio of the components was estimated from the areas under the schlieren peaks in the sedimentation diagrams. In comparison with different virus strains multiplied in tobacco plants (White Burley), HN-4 and PC-Y (potato calico strain) contained much more top component than HN-6 and ATCC-106. However, preparations of HN-6 strain grown in both tobacco plants (White Burley) and pea (Beikoku Ōsaya) showed almost the same ratio of the components. Attempts were made to determine whether the ratio of the components changed during the different periods after inoculation under the same environmental conditions (25–27°C, 2,000 lux for 15 hours/day). The best material for the determination of the relative amount of nucleoprotein components at the different periods after the inoculation was found to be the inoculated leaves. Because, in most cases, no virus was detected in the leaves except the inoculated and new expanded ones. It was found that virus nucleoproteins increased rapidly after inoculation. The specific infectivity of the strains, HN-6 and ATCC-106, decreased about 50% from 4 to 8 days and 60% from 4 to 10 days after inoculation, respectively, in the inoculated tobacco leaves. The ratio of the middle component of 4 days' preparation was higher than that of 8 to 10 days' one.

### Acknowledgment

The authors wish to express their thanks to Dr. T. KODAMA, Institute for Plant Virus Research, for his continuous, important, and helpful advice and Dr. J. B. BANCROFT, Purdue University, U.S.A., for generous donation of the ATCC-106 strain of alfalfa mosaic virus. The authors are indebted Mr. D. SAITO, Medical School, Hokkaido University, for making schlieren diagrams of the analytical ultracentrifugation.

Thanks are also due to members of this Laboratory of Plant Pathology for their ready help and apt comments. We also express our sincere gratitude to Mr. You Hsin HAN, Associate Professor of Taiwan Provincial Chung Hsing

University, Taiwan for his kind assistance.

### References

- 1). AGRAWAL, H. O. (1964). Identification of cowpea mosaic virus isolates. Mededel. Landbouwhogeschool Wageningen 64 (5): 1-53.
- 2). BANCROFT, J. B. and KAESBERG, P. (1960). Macromolecular particles associated with alfalfa mosaic virus. Biochem. Biophys. Acta. 39: 519-527.
- 3). FRISCH-NIGGEMEYER, W. and STEERE, R. L. (1961). Chemical composition of partially purified alfalfa mosaic virus. Virology 14: 83-87.
- 4). JASPARS, E. M. J. and MOED, J. R. (1966). The complexity of alfalfa mosaic virus. In "Viruses of plants" (Beemster, A. B. R. and Dijkstra, J. eds): 188-195. North-Holland Publ., Amsterdam.
- 5). KELLEY, J. J. and KAESBERG, P. (1962). Biophysical and biochemical properties of top component a and bottom component of alfalfa mosaic virus. Biochim. Biophys. Acta. 61: 865-871.
- 6). KUHN, C. W. and BANCROFT, J. B. (1961). Concentration and specific infectivity changes of alfalfa mosaic virus during systemic infection. Virology 15: 281-288.
- 7). MATSUMOTO, T. (1966). Studies on the alfalfa mosaic virus strains. (Master thesis).
- 8). MATSUMOTO, T., KODAMA, T. and MURAYAMA, D. (1968). On the alfalfa mosaic virus strains occurring on forage crops in Hokkaido. Journ. Fac. Agr., Hokkaido Univ. 56: 1-16.
- 9). MURAYAMA, D. and MATSUMOTO, T. (1968). On the relative amounts of the ribonucleoprotein components of alfalfa mosaic virus. Ann. Phytopath. Soc. Japan 34: 368.
- 10). PAUL, H. L. (1963). Untersuchungen über das Echte Ackerbohnenmosaik-Virus. Phytopath. Z. 49: 161-175.
- 11). SCHNEIDER, I. R. and DIENER, T. O. (1966). The correlation between the proportions of virus-related products and the infectious component during the synthesis of tobacco ringspot virus. Virology 29: 92-99.
- 12). SEMANCIK, J. S. and KAJIYAMA, M. R. (1967). Comparative studies on two strains of tobacco rattle virus. J. Gen. Virology 1: 153-162.
- 13). VAN KAMMEN, A. (1967). Purification and properties of the components of cowpea mosaic virus. Virology 31: 633-642.

## PLATE I

Photographs were taken 10 minutes after centrifugation (35,600 rpm); sedimentation was shown from left to right. The components from right to left are bottom, middle and top component. The sedimentation diagrams of HN-6 strain prepared from infected tobacco leaves 4 and 8 days after inoculations were shown in Fig. A-1 and A-2. The sedimentation diagrams of HN-6 strain prepared from systemically infected tobacco leaves 6 and 10 days after inoculation were shown in Fig. B-1 and B-2, respectively. The sedimentation diagrams of ATCC-106 strain prepared from inoculated tobacco leaves 4 and 10 days after inoculation were shown in Fig. C-1 and C-2. The sedimentation diagrams of PC-Y strain prepared from systemically infected tobacco leaves 6 days after inoculation and HN-4 prepared from inoculated tobacco leaves 5 days after inoculation were shown in Fig. D and Fig. E, respectively.

