Some Properties of Hop Latent and Apple Mosaic Viruses Isolated from Hop Plants and Their Distributions in Japan

Yoshiaki KANNO*, Nobuyuki YOSHIKAWA* and Tsuyoshi TAKAHASHI*

Abstract

This paper reports some properties of hop latent virus (HLV) and apple mosaic virus (ApMV) isolated from hop plants and the distributions of the viruses in hop gardens in Japan. HLV isolated in this study was 650 nm in length and 13 nm in width and had a single coat protein species with $M_e$ 2.98 $\times$ 10^6 Da. In immunosorbent electron microscopy, the virus was trapped and decorated with antiserum against HLV from England, but not with antisera against hop mosaic virus or American hop latent virus. The spherical virus isolated in this study formed precipitation lines with antiserum against ApMVs, but not with antiserum against prunus necrotic ringspot virus and Humulus japonicus virus in double diffusion tests and was identified as ApMV. Purified ApMV contained isometric particles of 22 and 24 nm in diameter and some quasi-isometric particles of 24×26–28 nm, and comprised a single coat protein species with $M_e$ 27.500 Da and four nucleic acid species with $M_r$ 1.39, 1.11, 0.72 and 0.34×10^6 Da. Distributions of HLV and ApMV in commercial hop plants were investigated by DIBA and ELISA, respectively. HLV was detected in the hops collected from all of 21 hop gardens located in 5 prefectures and the incidence of infection was 75.2% (158/210). ApMV was detected in 37.2% (67/180) of hop plants in 14 out of 18 hop gardens.

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Key words: hop (Humulus lupulus L.), hop latent virus, apple mosaic virus.

INTRODUCTION

Virus diseases are common in hop gardens throughout the world. At least six viruses have been known to infect commercial hop plants naturally. They are hop mosaic virus (HMV)$^1$, hop latent virus (HLV)$^{15,16,17,20,21,29}$, American hop latent virus (AHLV)$^{24,25}$, prunus necrotic ringspot virus (PNRSV)$^{6,8,20}$, apple mosaic virus (ApMV)$^{24,26,27}$ and arabis mosaic virus$^{28}$. The first three viruses are carlaviruses and with the exception of HMV they infect hop plants latently. The other three viruses have isometric particles and induce several diseases in hop plants$^{8,9,24,26}$.

In Japan, two viruses have been isolated from commercial hop plants$^{25}$. One was serologically identified as ApMV$^{24}$ and induced ring and band pattern mosaic disease in hop plants$^{24}$. The other was the filamentous virus reported by Inoue and Murayama$^{12,13}$, which seemed to be HLV because it infected hop plants latently and induced necrotic local lesions in the inoculated leaves of Phaseolus vulgaris$^{23}$. These two viruses, however, were not characterized in detail, especially their nucleic acids and proteins, and their distributions in hop gardens in Japan were not thoroughly investigated.

In this paper, we report some properties of HLV and ApMV isolated from hop plants and the distribution of these viruses in hop gardens located in five prefectures in Japan.

* Faculty of Agriculture, Iwate University, Ueda 3-chome, Morioka 020, Japan
MATERIALS AND METHODS

Viruses and indicator plants. A symptomless hop plant (Humulus lupulus L. cv. Shinshuwase; plant No. Y-7) infected with filamentous virus-like particles that had been recognized by electron microscopy was propagated vegetatively and used as an inoculum source and for purification of HLV. Leaf extracts were prepared by grinding hop leaves with 0.05 M phosphate buffer (pH 8.0) containing 0.5% 2-mercaptoethanol (2-Me) and 2% polyvinylpyrrolidone (PVP) and they were inoculated to P. vulgaris (cv. Toiku D-4 and Top Crop), Nicotiana clevelandii, N. debneyi, Chenopodium quinoa, C. murale and Datura stramonium.

ApMV was transferred from hop leaves (cv. Saaz; plant No. M-1) with mosaic symptoms to Cucumis sativus (cv. Suyo) and C. quinoa by mechanical inoculations. Infected C. quinoa leaves were used for purification of the virus.

Purification. HLV was purified from the hop leaves by the procedure of Tavantzis27) except that we used equilibrium density gradient centrifugation in CsCl instead of Cs2SO4 as a final step. Purification of ApMV from infected C. quinoa leaves was carried out essentially as described by de Sequeira10).

Electron microscopy. Leaf extracts and purified preparations were negatively stained with 2% uranyl acetate and examined in a Hitachi H-800 electron microscope.

Proteins and nucleic acids. The coat proteins were electrophoresed in SDS-10% polyacrylamide gels (SDS-PAGE) using the buffer system of Laemmli17). The standard proteins used were: phosphorylase b (97,400 ; 97.4K), bovine serum albumin (66.3K), aldolase (42.4K), carbonic anhydrase (30.0K), trypsin inhibitor (20.1K) and lysozyme (14.4K). The protein bands were stained with 0.1% Coomassie Brilliant Blue.

Nucleic acids were extracted from purified viruses by the SDS-phenol method7), denatured with formaldehyde and electrophoresed in 1% agarose gels containing formaldehyde22). The gels were stained with 0.01% toluidine blue O. Tobacco mosaic virus-RNA (2.19 × 106) and cucumber mosaic virus-RNAs (1.27, 1.13, 0.82 and 0.35 × 106) were used as size standards.

Serology. Antisera against HLV and ApMV were prepared in rabbits by intramuscular injections of purified virus preparations (0.15-0.2 mg for HLV and 0.7-1.0 mg for ApMV) emulsified with an equal volume of Freund's complete adjuvant on four successive weeks.

Immunosorbent electron microscopy (ISEM) was conducted as described by Milne and Luisoni16) using antisera against HLV, HMV and AHLV which were kindly supplied by Dr. A.N. Adams.

Double diffusion tests12) were conducted to clarify the serological relationships of ApMV isolated in this study with other ilarviruses. Antisera against the following viruses were used; ApMV isolated from hop, PNRSV isolated from peach (these two antisera were kindly supplied by Dr. T. Sano), ApMV and PNRSV isolated from hops (kindly supplied by Dr. R.E. Klein) and Humulus japonicus virus (HJV)3) (kindly supplied by Dr. A.N. Adams).

Detection. For the detection of HLV from hop plants, dot-immunobinding assay (DIBA) was carried out as described by Yoshikawa et al.28) Leaf samples (0.05 g) were ground with 0.165 M disodium phosphate, 0.018 M trisodium citrate (pH 9.0) containing 0.1% diethylthiocarbamate (DIECA), 0.5% 2-Me, 0.5 mM ethylenediaminetetraacetic acid and 2% PVP and the resulting saps were applied to the nitrocellulose sheets. Antiserum against HLV isolate Y-7, which had been cross-absorbed with an extract of healthy hop leaves, was used at a dilution of 1/2,000 as a first antibody.

ApMV was assayed by indirect ELISA described by Koenig16). Leaf saps were prepared by grinding tissues (0.2 g) with 1 ml of 0.02 M phosphate buffer (pH 8.0) containing 0.01 M DIECA and 0.02 M thioglycolic acid, followed by centrifugation for 5 min at 10,000 rpm and dilution in 0.05 M carbonate buffer (pH 9.6) at 1/10 to give a final dilution of 1/50. Anti-ApMV IgG (1 mg/ml) which had been cross-absorbed against healthy C. quinoa proteins was used at a dilution of 1/1,000.

Anti-rabbit IgG goat IgG conjugated with alkaline phosphatase (Tago Inc.) was used as a second antibody at a dilution of 1/2,000 in both DIBA and indirect ELISA.
RESULTS

Reactions of indicator plants
Leaf extracts of a Y-7 hop plant were inoculated to several indicator plants. In *P. vulgaris*, pinpoint brown spots appeared in the inoculated primary leaves within 6 days. The virus also infected the inoculated leaves of *C. murale* symptomlessly, but it did not infect *N. clevelandii* and *N. debneyi* which are known to be susceptible hosts for HMV, or *C. quinoa* and *D. stramonium* which are diagnostic hosts for AHLV. These reactions indicated that the Y-7 plant had been infected with HLV, but not with HMV and AHLV.

*C. quinoa* plants inoculated with leaf extracts of hop plant M-1 showed chlorotic spots in the inoculated leaves and chlorotic spots, ring pattern mosaic and distortion in the upper leaves. In cucumber, the virus induced chlorotic spots in inoculated cotyledons, followed by either systemic mosaic or top necrosis in the first true leaves. The virus also induced necrotic spots in the inoculated leaves of *C. amaranticolor*. From these results, the virus isolated from hop plant M-1 is thought to be ApMV which is similar to that reported by Sano et al.24)

Properties of the purified viruses
After CsCl equilibrium density gradient centrifugation, HLV formed a band separated from host components (Fig. 1a) and the purified preparation eluted from the gradient had an UV-absorption spectrum typical of filamentous virus particles with maximum and minimum absorptions at 259 nm and 248 nm, respectively. The $A_{260/280}$ ratio was 1.20 (corrected for light scattering), indicating 5-6% RNA content. Purified preparation contained slightly flexous particles with a modal length of 650 nm (196 particles) and a width of 13 nm (Fig. 1b). Yields of the purified HLV were approximately 70-200 μg/100 g of fresh hop leaves.

ApMV was purified from infected *C. quinoa* leaves by the method of de Sequeira10). After sucrose density gradient centrifugation, two bands were formed at the positions of about 14 mm and 18 mm below the meniscus. The upper band contained isometric particles of 22 nm in diameter (127 particles) (Fig. 2a), and the lower band contained isometric particles of 24 nm in diameter (162 particles) and some quasi-isometric particles of 24×26-28 nm (Fig. 2b). When these bands were inoculated to cucumber plants, only the lower band had high infectivity. Hop plants derived from meristem tip culture were inoculated with purified virus and they showed ring and band pattern mosaic symptoms. The purified preparation had an UV-absorption profile typical of nucleoprotein with maximum and minimum

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Fig. 1. A band (arrowhead) formed after CsCl equilibrium gradient centrifugation (a) and electron micrograph of purified HLV particles (b). Bar represents 200 nm.
absorptions at 259 nm and 245 nm, respectively. The $A_{260/280}$ ratio was 1.32 (corrected for light scattering). Yields of the purified ApMV were approximately 150-400 μg/100 g of fresh C. quinoa leaves.

### Proteins and nucleic acids

HLV coat protein migrated as a single species in SDS-PAGE and its $M_r$ was estimated as 34,700 daltons (Da) (average of three determinations) (Fig. 3a). A second polypeptide with smaller $M_r$ 28,000 Da was detected in some preparations, in agreement with the report by Adams and Barbara\(^2\). HLV nucleic acid denatured with formaldehyde migrated as a single band with a $M_r$ $2.98 \times 10^6$ Da (Fig. 3b) in a 1% agarose gel.

ApMV had single coat protein species with $M_r$ 27,500 Da (average of three determinations) (Fig. 4a) and four nucleic acids species with $M_r$ 1.39, 1.11, 0.72 and $0.34 \times 10^6$ Da (Fig. 4b).

### Serology

Antisera produced against purified HLV and ApMV had homologous titers of 1/256 and 1/512 in microprecipitin tests, respectively. ISEM showed that our HLV isolate was trapped and heavily decorated with homologous antiserum and with antiserum against HLV from England, but not with
Fig. 4. Electrophoretic analysis of viral coat protein and nucleic acids of ApMV. (a) Electrophoresis of viral coat protein in a SDS-10% polyacrylamide gel. Lane 1: marker proteins, lane 2: viral coat protein. (b) Electrophoresis of viral nucleic acids denatured with formaldehyde in a 1% agarose tubular gel. Lane 1: marker nucleic acids, lane 2: viral nucleic acids.

Table 1. The number of the filamentous virus particles from leaf sap trapped by antisera against three carlaviruses

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>No. particles ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homologous</td>
<td>46.2 ± 5.0</td>
</tr>
<tr>
<td>HLV</td>
<td>33.8 ± 3.3</td>
</tr>
<tr>
<td>HMV</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>AHLV</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>PBS</td>
<td>0.8 ± 0.7</td>
</tr>
</tbody>
</table>

a) Infected hop leaf was ground in 0.165 M disodium phosphate, 0.018 M trisodium citrate buffer (pH 9.0) containing 0.1% DIECA, 0.5% 2-Me and 0.5 mM EDTA and centrifuged briefly. The supernatant was used for tests.
b) Antisera were diluted at 1/128 in PBS (0.02 M phosphate buffer pH 7.0, 0.15 M NaCl).
c) The number of particles counted on 75×58 mm at a magnification of ×15,000. (Average of 5 replicants ± standard deviation.)

antisera against HMV and AHLV (Table 1). Thus, the filamentous virus isolated in this study was serologically identified as HLV.

In a double diffusion test, our isolate of ApMV formed precipitation lines with homologous antiserum and antisera against ApMVs isolated in America and Japan, but not with antisera against HJV and PNRSVs isolated from hop and peach (Fig. 5).

**Distribution**

The distributions of HLV and ApMV were investigated in 21 hop gardens located in 5 prefectures in Japan. HLV was detected in 75.2% of samples (158/210 samples), the incidence ranging from 40-100% within the gardens (Table 2).

ApMV was detected in 37.2% samples (67/180 samples). Some infection occurred in 14 of the 18 gardens tested (Table 2).

**DISCUSSION**

The filamentous virus isolated from a symptomless hop plant (Y-7) was identified as HLV from the reactions of indicator plants and from serological tests using antisera against three carlaviruses from hop plants.
Fig. 5. Double diffusion test with ApMV isolated in this study and antisera against some ilarviruses. Center well (v) contained purified ApMV preparation. Each outer well contained homologous antiserum (a) and antisera against (b) ApMV isolate from hop, (c) PNRSV isolate from peach in Japan, (d) HJV, (e) PNRSV isolate from hop and (f) ApMV isolate from hop in America.

Table 2. Distributions of HLV and ApMV in hop gardens in Japan

<table>
<thead>
<tr>
<th>Location</th>
<th>Cultivar</th>
<th>HLV</th>
<th>ApMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iwate Prefecture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Kirin II</td>
<td>9/10(b)</td>
<td>2/10</td>
</tr>
<tr>
<td>B</td>
<td>Kirin II</td>
<td>10/10</td>
<td>1/10</td>
</tr>
<tr>
<td>C</td>
<td>Kirin II</td>
<td>10/10</td>
<td>0/10</td>
</tr>
<tr>
<td>D</td>
<td>Shinshuwase</td>
<td>8/10</td>
<td>0/10</td>
</tr>
<tr>
<td>E</td>
<td>Shinshuwase</td>
<td>7/10</td>
<td>6/10</td>
</tr>
<tr>
<td>F</td>
<td>Shinshuwase</td>
<td>7/10</td>
<td>6/10</td>
</tr>
<tr>
<td>G</td>
<td>Shinshuwase</td>
<td>7/10</td>
<td>6/10</td>
</tr>
<tr>
<td>H</td>
<td>Shinshuwase</td>
<td>7/10</td>
<td>6/10</td>
</tr>
<tr>
<td>I</td>
<td>Shinshuwase</td>
<td>5/10</td>
<td>8/10</td>
</tr>
<tr>
<td>J</td>
<td>Shinshuwase</td>
<td>5/10</td>
<td>NT(a)</td>
</tr>
<tr>
<td>K</td>
<td>Golden Star</td>
<td>5/10</td>
<td>NT</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>158/210</td>
<td>67/180</td>
</tr>
</tbody>
</table>

a) Leaf samples were collected in June-August 1991 and stored at -20°C until used.
b) No. of infected plants/no. of tested plants.
c) Not tested.

Purified HLV was 650 nm, slightly shorter than the 675 nm reported for a British isolate of HLV. An American isolate and a Chinese isolate were reported to be 610 nm and 670 nm, respectively. The variations in particle size may reflect the differences of isolate or experimental conditions.

The molecular weight of nucleic acid (2.98 × 10^6 Da) of HLV isolated in this study is similar to that of British isolate (2.9 × 10^6 Da). Both isolates had a major polypeptide (our isolate 34,700 Da, British
isolate 33,000 Da) and a second polypeptide (our isolate 28,000 Da, British isolate 28,500 Dal. It is not
known whether the second polypeptide is a breakdown product of the major polypeptide or not.

ApMV isolated in this study reacted with antisera against ApMVs reported by Sano et al.24) and
isolated in America. Meanwhile, there were differences in symptoms in inoculated cucumber plants
between our ApMV isolate and the ApMV reported by Sano et al.24) Our isolate could infect cucumber
systemically and could be transferred from cucumber to cucumber. In contrast, the isolate of Sano et
al.24) could not infect cucumber systemically and could not be transferred from cucumber to cucumber.

The purified ApMV preparation contained three types of particle (22, 24 and 24×26-28 nm) which
were consistent with those reported previously, although the particle sizes of our isolate were smaller
than those of ApMV isolated by Sano et al.24) (25, 32 and 30×30-36 nm).

The physical properties of ApMV isolated from hop have not been previously described. Our isolate
of ApMV comprised a single coat protein species (estimated M, 27,500 Da) and four nucleic acid species
(estimated M, 1.39, 1.11, 0.72 and 0.34×10^8 Da), in common with other ilarviruses11).

HLV was detected in 75.2% samples from hop gardens located 5 prefectures. The virus occurred in
all gardens although the incidence of HLV infection varied between gardens. The incidence of ApMV
(37.2%) was lower than that of HLV. However, more than 50% of samples were infected with ApMV
in 9 hop gardens out of the 18 tested. In these gardens the reduction of yield by ApMV infection could
be considerable, because ApMV can induce severe symptoms in hop19,20). The difference between
the incidences of HLV and ApMV in hop gardens might be due to the different modes of transmission of
these two viruses. HLV infection might occur at a high level because it can be transmitted by the
damson-hop aphid5). In contrast, ApMV apparently does not have an insect vector and may not be
transmitted through pollen because male hops are not cultivated in commercial hop gardens in Japan.

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agricultural co-operative associations for supplying hop leaf samples.

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   59 : 437-446.

和 文 摘 要

菅野善明・吉川信幸・高橋 士：日本のホップから分離されたホップ潜在ウイルスおよびリンゴモザイクウイルスの性状と栽培圃場における発生分布

日本のホップから分離されたホップ潜在ウイルス (hop latent virus：HLV) およびリンゴモザイクウイルス (apple mosaic virus：ApMV) の性状を栽培圃場における発生分布を調査した。本研究で分離した HLV は長さ 650 nm、幅 13 nm で、一種類の外被タンパク質（分子量 34,700 グルトン）と一種類の核酸（分子量 2.98×10^6 グルトン）からなっていた。免疫電顕法において、このウイルスは英国で分離された HLV に対する抗体を用いて陽性と判定されたが、hop mosaic virus および American hop latent virus に対する抗体とは反応しなかった。ホップから分離された球状ウイルスは対数減滅法で、ApMV に対する抗体を用いて沈降線を形成したが、プラズマクロティックリングスボットウイルスおよび Humulus japonicus virus に対する抗体との反応は認められず、ApMV と同定された。精製した ApMV は直径 22 nm、24 nm の球形粒子および 24×26-28 nm の桿状粒子からなり、外被タンパク質は分子量 27,500 グルトンの 1 種類で、核酸は分子量がそれぞれ 1.39、1.11、0.72 および 0.34×10^6 グルトンの 4 種類からなっていた。ホップ栽培圃場における HLV および ApMV の発生分布を DIBA および ELISA によってそれぞれ調査したところ、HLV は 5 県 21 園場の全ての圃場のホップから検出され、その感染率は 75.2% (158/210) であった。ApMV は 18 園場中 14 園場において発生が認められ、その感染率は 37.2% (67/180) であった。