Some Properties of Hop Mosaic Virus Isolated in Japan

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Abstract

Hop mosaic virus (HMV) was isolated from a mosaic-diseased hop plant (*Humulus lupulus* L. cv. Sunshine). The virus systemically infected two plant species, hop (cv. Shinshuwase) and *Nicotiana occidentalis* and only the inoculated leaves of *N. clevelandii*, *Chenopodium quinoa*, *Cucurbita maxima* among 24 species of 11 families. The virus was purified from infected leaves of *N. occidentalis*, a new host suitable for propagation of HMV. Purified virus particles were 625 nm in length, 13 nm in width and composed of a single coat protein species with Mr 32,800 daltons (Da) and a nucleic acid with Mr 2.91×10^6 Da. In immunosorbent electron microscopy using antisera against three carlaviruses (HMV, hop latent virus: HLV and American hop mosaic virus) the virus isolated in this study reacted with an antiserum against HMV isolated in England and also weakly with antisera against HLVs isolated in Japan and England. The survey of HMV infection in commercial hop plants indicated that HMV did not occur in hop gardens in Japan though some cultivars introduced for breeding are infected with the virus.

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Key words : hop (*Humulus lupulus* L.), hop mosaic virus, *Nicotiana occidentalis*.

INTRODUCTION

Three carlaviruses are known to infect commercial hop plants (*Humulus lupulus* L.), *i.e.* hop latent virus (HLV)^{2,4,14}, hop mosaic virus (HMV)^{1,3} and American hop latent virus (AHLV)^{2,5}. These viruses can be distinguished from each other by host reactions and serology. HMV causes hop mosaic disease in sensitive hop cultivars, in contrast to HLV and AHLV which infect hop plants latently^{1,3,10}.

In Japan, a filamentous virus was isolated from hop plants and identified as HLV^{8,9,16}. Recently, Kanno *et al.*⁹ reported that HLV is prevalent in hop plants throughout 21 hop gardens located in 5 prefectures. Purification and characterization of HMV has not been conducted in Japan and the incidence of HMV was not been investigated extensively although Sano and Shikata¹⁷ reported the occurrence of HMV in one limited cultivar (Brewers Gold) by serology.

In this paper, we report a new host suitable for propagation of HMV, the purification and characterization of HMV isolated in Japan, and a survey of its occurrence in commercial hop plants.

MATERIALS AND METHODS

Virus. The HMV isolated used in this study was obtained from a mosaic-diseased hop plant (cv. Sunshine : plant No. S-1) maintained in Iwate Experimental Farm, Asahi Breweries Ltd. S-1 hop leaves were ground in 0.02 M phosphate buffer (pH 8.0) containing 0.5% 2-mercaptoethanol and 1% polyvinylpyrrolidone (PVP) and the sap was inoculated to *Nicotiana clevelandii*^{1,3,13}. The inoculated leaves of *N. clevelandii* were used as inoculum sources in further experiments.

Host range. In preliminary tests, it was found that the virus could infect *N. occidentalis*

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systemically and inoculum for host range tests was therefore prepared by grinding infected leaves of *N. clevelandii* or *N. occidentalis* in 0.05 M borate buffer (pH 8.0) containing 1% sodium sulfate. Inoculated plants were grown in a greenhouse maintained at 20–30°C. After 3–4 weeks, inoculated and uninoculated upper leaves were ground in 10 volumes of PBS (0.02 M phosphate buffer, 0.15 M NaCl, pH 7.4) containing 1% PVP and 0.1% thioglycolic acid, followed by centrifugation at 10,000 rpm for 5 min. The supernatants were assayed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)⁷⁾. Both Anti-HMV IgG for coating and IgG-alkaline phosphatase conjugate were used at a dilution of 1 µg/ml.

Virus purification. The virus was purified from infected leaves of *N. occidentalis* by the procedure described by Adams and Barbara¹⁾ except that the crude extract was clarified by a half volume of carbon tetrachloride.

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

Coat protein and nucleic acid. The molecular weight (Mr) of coat protein was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemmli system¹¹⁾. The standard proteins used were: phosphorylase b (97,400: 97.4 K), bovine serum albumin (66.3 K), aldolase (42.4 K), carbonic anhydrase (30.0 K), trypsin inhibitor (20.1 K) and lysozyme (14.4 K). The protein bands were stained with 0.1% Coomassie Brilliant Blue.

Nucleic acid was extracted from purified virus preparation by the SDS-phenol method⁶⁾. The Mr of nucleic acid denatured with formaldehyde was estimated by electrophoresis in 1% agarose tubular gels containing formaldehyde¹⁵⁾. The gels were stained with 0.01% toluidine blue O. Tobacco mosaic virus-RNA (2.19×10^6) and cucumber mosaic virus-RNAs (1.27 , 1.13 , 0.82 and 0.35×10^6) were used as size standards.

Serology. Antiserum against HMV was prepared in a rabbit by intramuscular injections of purified virus preparations (0.38–0.5 mg) 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 Luisoni¹²⁾ using antisera against HLV from Japan (HLV-J)⁹⁾, HLV from England (HLV-E)²⁾, HMV from England (HMV-E)¹⁾ and AHLV²⁾. The latter three antisera were kindly supplied by Dr. A.N. Adams.

RESULTS

Host range of HMV isolated in Japan

Among 24 species of 11 families, the virus systemically infected two species, hop (cv. Shinshuwase) and *N. occidentalis*, without causing obvious symptoms; it also infected the inoculated leaves only of *N. clevelandii*, *Chenopodium quinoa* and *Cucurbita maxima*. No virus was recovered from the following species: *Amaranthus caudatus*, *Gomphrena globosa* (Amaranthaceae); *Basella rubra* (Basellaceae); *Beta vulgaris*, *C. murale* (Chenopodiaceae); *Brassica campestris* var *pekinensis*, *Raphanus sativus* (Cruciferae); *Cucumis sativus* (Cucurbitaceae); *Zea mays* (Gramineae); *Phaseolus vulgaris*, *Vicia faba*, *Vigna sesquipedalis* (Leguminosae); *Sesamum indicum* (Pedaliaceae); *Datura stramonium*, *Lycopersicon esculentum*, *N. debneyi*, *N. glauca*, *N. tabacum* cv. Xanthi nc (Solanaceae); *Petroselinum crispum* (Umbelliferae).

N. occidentalis as a propagation host of HMV

Adams and Barbara¹⁾ purified HMV from systemically infected *N. clevelandii*. Our virus isolate (HMV-J), however, was recovered from only the inoculated leaves of *N. clevelandii* without systemic infection. To search for a suitable propagation host for purification of HMV, we compared the virus concentrations in leaves of systemically infected *N. occidentalis* and hop plants by DAS-ELISA. A_{405} values of ELISA were 1.27 for infected *N. occidentalis* (4 weeks after inoculation), 0.63 for naturally infected S-1 hop, 0.41 for inoculated hop (cv. Shinshuwase, 8 weeks after inoculation) and 0.04 to 0.08 for healthy *N. occidentalis* and hop (cv. Shinshuwase) plants. We also compared the amounts of virus particles in systemically infected leaves of *N. occidentalis* and in the inoculated leaves of *N. clevelandii*

by ISEM tests. The number of virus particles trapped by HMV antiserum from leaves of infected *N. occidentalis* was about 20 times more than that from the inoculated leaves of *N. clevelandii*. These results indicate that *N. occidentalis* is thought to be a most suitable propagation host for HMV purification.

Properties of purified virus

The UV-absorption spectrum of purified preparations showed a maximum at 259 nm and a

Fig. 1. Electron micrograph of purified HMV particles. Bar represents 200 nm.

Fig. 2. Electrophoretic analyses of the coat protein and the nucleic acid of HMV. (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 acid denatured with formaldehyde in a 1% agarose gel. Lane 1: marker RNAs, Lane 2: viral nucleic acid.

Table 1. The numbers of virus particles trapped by antisera against three carlaviruses^{a)}

Antisera ^{b)}	The numbers of particles $\pm$ S.D. ^{c)}
Homologous	48.4 $\pm$ 9.2
HMV-E	55.8 $\pm$ 8.0
HLV-J	17.6 $\pm$ 2.4
HLV-E	14.2 $\pm$ 4.7
AHLV	0.8 $\pm$ 0.7
PBS buffer	0.8 $\pm$ 0.4

- a) Infected leaves of *N. occidentalis* were ground in 10 volumes of 0.5 M borate buffer (pH 8.0) containing 1% sodium sulfate and then centrifuged at 10,000 rpm for 5 min. The supernatants was used for tests.
 b) Antisera were diluted at 1/20 in PBS. HMV-E and HLV-E were against English isolates and HLV-J was against Japanese isolate.
 c) The number of particles counted on 75 $\times$ 58 mm at a magnification of $\times$ 15,000 (Average of 5 replicants $\pm$ standard deviation).

Fig. 3. Immunosorbent electron microscopy of HMV isolated in this study using antisera against (a) HMV-J, (b) HLV-J and (c) AHLV. Each antiserum was diluted at 1/20 in PBS and used for decorations.

minimum at 248 nm, and the A_{260}/A_{280} ratio was 1.18 (corrected for light scattering), indicating that virus particle contained 5–6% nucleic acid. The yields of purified HMV were 3–8 mg/kg of fresh leaves of *N. occidentalis*, assuming an extinction coefficient $A_{260}=2.1$ for carnation latent virus¹⁸⁾.

Purified virus preparations contained filamentous virus particles with a modal length of 625 nm (208 measurements) and a width of 13 nm (Fig. 1).

Coat protein of HMV migrated as a single species in 10% and 15% polyacrylamide gels containing SDS and its Mr was estimated as 32,800 daltons (Da) (average of three determinations) (Fig. 2a). Nucleic acid from purified virus preparation migrated as a single band with Mr 2.91×10^6 Da under denatured conditions (average of three determinations) (Fig. 2b).

Serological relationships

An antiserum produced against purified HMV preparation had a homologous titer of 1/512 in microprecipitin tests. Serological relationships of the virus isolated in this study with carlaviruses from hop plants were investigated by ISEM tests. As shown in Table 1, the numbers of virus particles trapped by homologous antiserum are almost the same as those trapped by an antiserum against HMV from England (HMV-E) and more than were trapped by antisera against HLVs from Japan (HLV-J) and from England (HLV-E). The virus was not trapped by an antiserum against AHLV. The virus particles were also decorated heavily with antiserum against HMV-E and weakly with those against HLV-J or HLV-E (Fig. 3). From these results, the virus isolated in this study was serologically identified as HMV.

Detection of HMV in hop gardens in Japan

The occurrence of HMV in commercial hop plants was investigated in 22 hop gardens located in 7 prefectures by DAS-ELISA. HMV was not detected in any samples in 218 hop plants. However, the virus

was found in 5 cultivars (Sunshine, Northern Breweres, Becka, Early Zugg and Spalt) out of 19 cultivars which had been introduced for breeding.

DISCUSSION

This is the first report on the biological, physical and serological properties of HMV isolated in Japan.

Three filamentous viruses (HLV, HMV and AHLV) were reported to infect hop plants^{1-5,15)}. These viruses can be distinguished from each other by host reactions. The virus isolated in this study infected the inoculated leaves of *N. cleveandii* reported to be a host plant for HMV, but not hosts for HLV and AHLV. The virus did not infect *P. vulgaris*, *C. murale* (diagnostic hosts for HLV) and *D. stramonium* (a diagnostic host for AHLV). From these results, the virus isolated in this study is thought to be HMV.

Our HMV isolate (HMV-J) was different from the isolate in England (HMV-E) in some host reactions. In contrast with HMV-E which infected systemically both *N. cleveandii* and *N. debneyi*, HMV-J infected only the inoculated leaves of *N. cleveandii* and not *N. debneyi*. A Chinese isolate of HMV is also reported to infect *N. cleveandii* systemically¹⁹⁾. Hop plants (cv. Shinshuwase) inoculated with purified HMV did not show any obvious symptoms. This may be because the cultivar Shinshuwase is tolerant to HMV infection.

N. occidentalis was a satisfactory propagation host for virus purification. The virus concentration in leaves of infected *N. occidentalis* is higher than in the infected leaves of hop plants and in the inoculated leaves of *N. cleveandii* and we obtained sufficient purified virus to analyze its physical properties and to prepare antiserum.

Purified HMV was 625 nm in length, slightly shorter than 650 nm reported for HMV-E¹⁾ and 655 nm for a Chinese isolate¹⁹⁾. The molecular weight of HMV-J nucleic acid (2.91×10^6 Da) was essentially similar to that of HMV-E (3.0×10^6 Da)¹⁾ and of a Chinese isolate (2.95×10^6 Da)¹⁹⁾. However, coat protein of HMV-J (32,800 Da) was slightly smaller than that of HMV-E (34,000 Da)¹⁾ and of a Chinese isolate (34,200 Da)¹⁹⁾. These variations may reflect differences in experimental conditions and/or virus isolate.

In ISEM tests using antisera against three carlaviruses from hop plants, HMV-J was trapped and decorated heavily with an antiserum against HMV-E. HMV-J also shared a distant serological relationship with HLV, in agreement with the results of Adams and Barbara⁹⁾.

The survey of HMV infection in commercial hop plants indicated that HMV did not occur in hop gardens in Japan although some cultivars introduced for breeding are infected with the virus. The result contrasts with the high incidence of HLV (75.2% of hop plants tested)⁹⁾.

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和 文 摘 要

菅野善明・飯田 久・吉川信幸・高橋 壯：わが国のホップから分離されたホップモザイクウイルスの2, 3の性質

モザイク症状を呈したホップ (*Humulus lupulus* L. 品種：Sunshine) からホップモザイクウイルス (hop mosaic virus : HMV) を分離した。本ウイルスの宿主範囲を 11 科 24 種の植物について調査した結果、ホップ (品種：信州早生) および *Nicotiana occidentalis* に全身感染し、*N. clelandii*, *Chenopodium quinoa* およびカボチャでは接種葉のみに感染した。HMV の増殖宿主として *N. occidentalis* が適しており、本植物の感染葉から HMV を精製した。精製ウイルス粒子は長さ 625 nm, 幅 13 nm のひも状粒子で、分子量 32,800 ダルトンの 1 種類の外被タンパク質と分子量 2.91×10^6 ダルトンの 1 種類の核酸からなっていた。本ウイルスとこれまでにホップから分離された 3 種 carlavirus (HMV, ホップ潜在ウイルス ; HLV, American hop latent virus) との血清関連を免疫電顕法により調査した結果、本ウイルスは英国で分離された HMV に対する抗血清と強く反応し、日本および英国で分離された HLV に対する抗血清と弱く反応した。わが国のホップ栽培圃場における HMV の発生を調査したところ、栽培ホップにおける発生は認められなかったが、育種材料として用いられている数種導入品種から HMV が検出された。