# 土壌伝染性ひも状ウイルスの核酸および外被蛋白質の諸性質


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# Some Properties of Nucleic Acids and Coat Proteins of Soil-Borne Filamentous Viruses

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**Abstract**

The properties of nucleic acids and coat proteins of barley yellow mosaic virus (BaYMV), wheat yellow mosaic virus (WYMV), wheat spindle streak mosaic virus (WSSMV) and oat mosaic virus (OMV) were studied. They have two species of single-stranded RNAs, designated as RNA-1 and RNA-2. The molecular weights (MWs) of RNA-1 and RNA-2 of each virus were: $2.6\times10^6$ and $1.5\times10^6$ for BaYMV and WYMV, $2.6\times10^6$ and $1.4\times10^6$ for WSSMV, and $2.8\times10^6$ and $1.8\times10^6$ for OMV. All the viruses had single coat protein with MWs of $33\times10^3$ (K) for BaYMV, WYMV and WSSMV, and 30 K for OMV. The association of two modal lengths of virus particles and two species of RNAs, and the transmissibility by the fungus *Polymyxa graminis* demonstrate that these viruses are distinct from potyviruses, and a new virus group should be established for them. We propose that BaYMV is a type member of the new group to be designated as the barley yellow mosaic virus group, bymovirus.

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**Key words:** barley yellow mosaic virus, wheat yellow mosaic virus, wheat spindle streak mosaic virus, oat mosaic virus, classification, bymovirus.

## INTRODUCTION

Soil-borne filamentous viruses in cereal plants, barley yellow mosaic (BaYMV), wheat yellow mosaic (WYMV), wheat spindle streak mosaic (WSSMV), rice necrosis mosaic (RNMV) and oat mosaic (OMV) viruses, have been reported to have several common properties. They are flexuous filaments 100～1,000 nm long; BaYMV, WYMV, WSSMV and RNMV have two modal lengths of 200～300 nm and 500～600 nm[11,12,25,28,29] while OMV has one modal length of 600～750 nm[7,30]. These viruses are transmitted by the fungus *Polymyxa graminis*[4,7,17,24], they infect only cereal plants[7,14–16,24], their infectivities in sap are considerably unstable[14–16,24,26], they are serologically related[2,11,28–30], and pinwheel and cylindrical cytoplasmic inclusions are observed in infected cells[8,11].

These common properties suggest that the viruses belong to one group, and they have been grouped as possible members of subgroup 2 of potyviruses[20]. However, Hollings and Brunt[9] suggested that they should be excluded from the potyvirus group. Among the classification criteria, the properties of their nucleic acids and coat proteins are still unknown except for the German isolates of BaYMV. Therefore, we investigated the properties of these viruses, except for RNMV, in order to compare them in one laboratory, and discuss here the classification of these soil-borne filamentous viruses.

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## MATERIALS AND METHODS

***Viruses.*** BaYMV and WYMV were originally collected from Tochigi and Ibaraki, Japan, respectively[28]. WSSMV[29] and OMV[30] were kindly supplied by Dr. J.T. Slykhuis, Cell Biology Research Institute, Ottawa, Canada, and Dr. T.T. Hebert, North Carolina State University, North Carolina, U.S.A., respectively[29]. Purified rice dwarf virus (RDV), tobacco mosaic virus (TMV) and bean common mosaic virus (BCMV) were kindly supplied by Dr. I. Kimura and Dr. F. Sakai, National Institute of Agrobiological Resources, Tsukuba, Ibaraki, Japan, and Dr. F. Fukumoto, National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan, respectively.

***Propagation and purification of viruses.*** The viruses were propagated in the following hosts by sap-inoculation: BaYMV in barley (cvs. New Golden or Gose Yonkoku), WYMV in wheat (cv. Hatakedakomugi), WSSMV in wheat (cv. Norin No. 64), and OMV in oat (cv. Taiho). Inocula were prepared by grinding infected leaves in 0.1 M phosphate buffer (pH 7.0) containing 0.001 M potassium cyanide, and adding carborundum. Seedlings at the one- to two-leaf stage were inoculated by rubbing with fingers, and were grown in a growth chamber controlled at 15 C during day and 13 C at night or in a screen house in winter. Each virus was purified from the infected leaves according to the method of Usugi and Saito[30], and the final pellets were resuspended in 0.1 M citrate buffer (pH 7.0) at the concentration of about $A_{260}=2.0$.

Healthy component of oat was prepared according to the same method for purification of the virus, except that CsCl isopycnic gradient centrifugation was omitted.

***Electrophoresis of nucleic acids.*** Nucleic acids of BaYMV, WYMV, WSSMV, OMV and BCMV were released by adding 1 $\mu$l of 20% SDS to 20 $\mu$l of purified virus. Nucleic acids of TMV and RDV were prepared according to the methods of Fraenkel-Conrat *et al.*[3] and Miura *et al.*[21], respectively.

Electrophoresis of nucleic acids were performed principally according to the method of Peacock and Dingman[23]. Slab gels ($14\times11\times0.2$ cm) containing 2% acrylamide, 0.11% bisacrylamide and 0.5% agarose were prepared, and the nucleic acids were electrophoresed at 40 mA/gel for 5 hr in the electrophoresis buffer (30 mM tris, 36 mM $NaH_2PO_4$, pH 7.8, 1 mM EDTA and 0.1% SDS). Molecular weight (MW) markers were 23 S (MW: $1.009\times10^6$) and 16 S (MW: $0.534\times10^6$) ribosomal RNAs of *Escherichia coli*[1], TMV-RNA (MW: $2.19\times10^6$)[10] and BCMV-RNA (MW: $3.33\times10^6$)[5]. After electrophoresis, the gels were washed in sterile dist. water and stained with ethidium bromide. RNA bands were made visible under U.V. light.

***Treatment of nucleic acids with nucleases.*** The gels after electrophoresis were soaked in RNase A (5 $\mu$g/ml, Sigma) either in sterile dist. water or in $2\times$SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0) or in DNase (5 $\mu$g/ml, P-L Biochemicals) in 0.05 M magnesium sulfate, pH 7.0, for 3 hr at 37 C. Nucleic acids were treated with $S_1$ nuclease (350 units/ml, Bethesda Research Laboratories) by soaking the gels in 0.03 M sodium acetate, 0.05 M sodium chloride, 0.01 M zinc sulfate and 5% glycerol, pH 4.6, for 6 hr at 37 C.

***Electrophoretic analysis of coat proteins.*** The properties of the coat proteins were examined by SDS polyacrylamide gel electrophoresis (SDS-PAGE), limited proteolysis and immunoblotting techniques. Purified viruses were incubated in 2% SDS, 2% 2-mercaptoethanol for 2 min at 100 C. The 5~10 $\mu$l samples were electrophoresed at 18 mA/gel for 5 hr on 5 and 15% discontinuous polyacrylamide slab gels ($14\times11\times0.1$ cm) containing 0.1% SDS according to Laemmli[18]. After electrophoresis, the gels were stained with Coomassie brilliant blue R (CBB). Limited proteolysis in SDS polyacrylamide gels was performed as described by Matsuoka *et al.*[19] Immunoblotting was performed as described by Omura *et al.*[22]

Antisera against BaYMV, WYMV, WSSMV and OMV, which had been prepared by Usugi and Saito[28–30], were used without absorption with healthy plant component.

## RESULTS

### *Nucleic acids*

Nucleic acids of all the four viruses were separated into two bands by electrophoresis (Fig. 1). All the bands disappeared after treatment with RNase A both in dist. water and in 2×SSC. No significant effect on the bands was observed in treatment with DNase. The bands almost disappeared after treatment with $S_1$ nuclease, but RDV-RNA remained under the same condition (data not shown). These results indicate that all the viruses examined have two species of single-stranded RNA. The slower and faster migrating bands were designated as RNA-1 and RNA-2, respectively.

The MWs of RNA-1 of BaYMV, WYMV and WSSMV were similar ($2.6\times10^6$), but that of OMV ($2.8\times10^6$) was much larger than those of the other viruses (Fig. 1). The MWs of RNA-2 of BaYMV and WYMV were similar ($1.5\times10^6$), and that of WSSMV ($1.4\times10^6$) was slightly smaller than those of BaYMV and WYMV. The MW of RNA-2 of OMV ($1.8\times10^6$) was apparently larger than those of the rest of the viruses.

### *Coat proteins*

Coat proteins released from purified preparations of BaYMV, WSSMV and OMV migrated as two major bands in SDS-PAGE. With BaYMV and WSSMV, several minor bands were also observed (Fig. 2). The MWs of two bands were $33\times10^3$ (K) and 26.5 K for BaYMV and

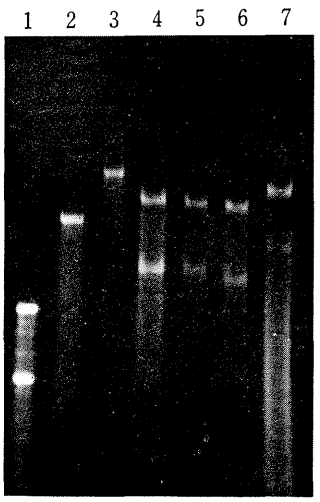


Fig. 1. Electrophoresis of nucleic acids of soil-borne filamentous viruses in a 2% polyacrylamide-0.5% agarose composite gel. Samples were 23 S and 16 S ribosomal RNAs of *Escherichia coli* (lane 1); tobacco mosaic virus (TMV) RNA (lane 2); bean common mosaic virus (BCMV) RNA (lane 3); barley yellow mosaic virus (BaYMV) RNA (lane 4); wheat yellow mosaic virus (WYMV) RNA (lane 5); wheat spindle streak mosaic virus (WSSMV) RNA (lane 6); oat mosaic virus (OMV) RNA (lane 7).

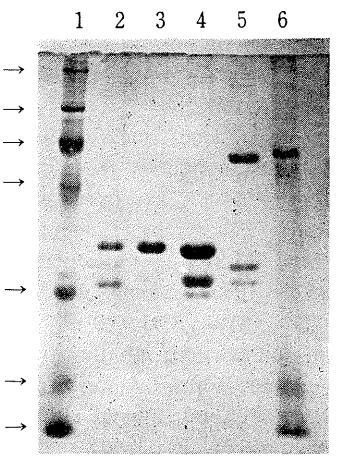


Fig. 2. Electrophoresis of coat proteins of soil-borne filamentous viruses in a 5 and 15% discontinuous SDS polyacrylamide gel. Samples were molecular weight standards (from top to bottom), myosin H chain (MW: $200\times10^3$ (K)), phosphorylase b (MW: 97.4 K), bovine serum albumin (MW: 68 K), ovalbumin (MW: 43 K), α-chymotrypsinogen (MW: 25.7 K), β-lactoglobulin (MW: 18.4 K) and cytochrome C (MW: 12.3 K). Arrows indicate the positions of standards (lane 1); BaYMV (lane 2); WYMV (lane 3); WSSMV (lane 4); OMV (lane 5); healthy component of oat (lane 6).

WSSMV, and 30 K and 26.5 K for OMV. Only one major band with a MW of 33 K and several minor bands were observed in WYMV. The 68 K band observed in the OMV sample was found to be a healthy component.

Limited proteolysis of major protein bands of each virus in SDS polyacrylamide gel was examined (Fig. 3). No significant difference was observed between peptide maps of two major bands of BaYMV, WSSMV and OMV. Peptide maps of the slower migrating bands were different among four viruses.

Immunoblotting showed that antisera against BaYMV, WYMV and WSSMV strongly reacted with all the protein bands including the minor ones of these viruses (Fig. 4a). On the other hand, antiserum against BaYMV weakly reacted with protein bands of OMV and *vice versa* (Fig. 4b).

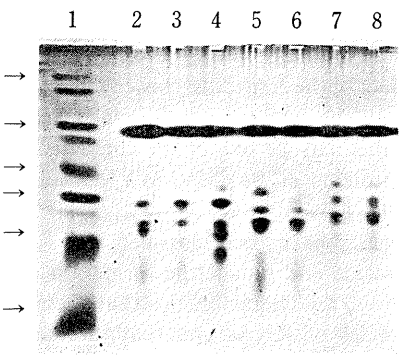


Fig. 3. Patterns of peptide bands produced after electrophoresis of partial *Staphylococcus* V8 protease (0.4 $\mu$g/well) digests of major protein bands of soil-borne filamentous viruses. Samples were molecular weight standards (from top to bottom), ovalbumin (MW: 43 K), $\alpha$-chymotrypsinogen (MW: 25.7 K), $\beta$-lactoglobulin (MW: 18.4 K), lysozyme (MW: 14.3 K), bovine trypsin inhibitor (MW: 0.62 K), and insulin B chain (MW: 0.34 K). Arrows indicate the positions of standards (lane 1); 33 K protein of BaYMV (lane 2); 26.5 K protein of BaYMV (lane 3); 33 K protein of WYMV (lane 4); 33 K protein of WSSMV (lane 5); 26.5 K protein of WSSMV (lane 6); 30 K protein of OMV (lane 7); and 26.5 K protein of OMV (lane 8).

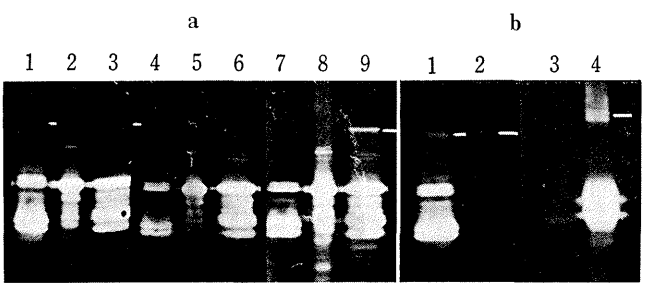


Fig. 4. a, Immunoblotting of coat proteins of BaYMV, WYMV and OMV. Samples were BaYMV (lanes 1, 4 and 7); WYMV (lanes 2, 5 and 8); WSSMV (lanes 3, 6 and 9). Lanes 1~3 were treated with antiserum against BaYMV, lanes 4~6 with antiserum against WSSMV and lanes 7~9 with antiserum against WYMV. Spears indicate the position of healthy plant component. b, Immunoblotting of coat proteins of BaYMV and OMV. Samples were BaYMV (lanes 1 and 3); OMV (lanes 2 and 4). Lanes 1 and 2 were treated with antiserum against BaYMV, and lanes 3 and 4 with antiserum against OMV.

## DISCUSSION

The present study shows that nucleic acids of BaYMV, WYMV, WSSMV and OMV were composed of two species of single-stranded RNAs as in the case of German isolates of BaYMV[11]. Association of two species of RNAs with BaYMV, WYMV and WSSMV seems to correspond to two modal lengths of particles of these viruses[11,25,28,29]. OMV was reported to have one modal length of particles[7,30], however, the existence of two peaks in particle-length distribution (250～300 nm and 650～700 nm) of OMV in some experiments (unpublished data) was supported by the finding of two species of RNAs in this study as in the case of BaYMV, WYMV and WSSMV. The larger size of the RNA-1 of OMV seems to be attributed to larger sizes of virus particles as compared to the other viruses. These results demonstrate that the soil-borne filamentous viruses have two species of RNAs corresponding to two modal lengths of virus particles.

Coat proteins of BaYMV, WSSMV and OMV were composed of two major bands in SDS-PAGE. Limited proteolysis and immunoblotting revealed that the faster migrating band of each virus was the degradative product of the respective slower migrating band as described for German isolates of BaYMV[3]. Thus, all the four viruses examined in the present study were found to have single coat protein, and this may be one of the characteristics of these viruses, as in cases of potyviruses[27]. The coat proteins of BaYMV, WYMV and WSSMV have MWs of 33 K, while that of OMV has a smaller MW of 30 K.

Soil-borne filamentous viruses have been classified into subgroup 2 of potyvirus group[20], because they are flexuous filaments and induce the formation of pinwheel and cylindrical cytoplasmic inclusions[8,11]. However, the characteristics that these viruses have two modal lengths of virus particles and two species of RNAs and that they are transmitted by the fungus *P. graminis* but not by aphids (unpublished data), demonstrate that these viruses are distinct from potyviruses. The evidence that none of the viruses is serologically related to potato virus Y, bean common mosaic virus, bean yellow mosaic virus, beet mosaic virus, blackeye cowpea mosaic virus and turnip mosaic virus, all of which belong to the potyvirus group (unpublished data), support the idea that the viruses studied here should be excluded from the potyvirus group[9]. None of the eight monoclonal antibodies to potato virus V, which reacted with one or more aphid-borne potyviruses, reacted with BaYMV and WYMV (B.D. Harrison, personal communication). Thus, a new group should be established for these viruses.

Among BaYMV, WYMV, WSSMV, RNMV and OMV, the former three are closely related with respect to the sizes of their virus particles, RNAs and coat proteins, and also with respect to their serological relationships (Fig. 4a). RNMV seems to be more closely related to BaYMV, WYMV and WSSMV than to OMV on the basis of particle-length distribution[12] and serological relationship[28,30]. OMV is not as closely related because it has larger particles and RNAs, has coat protein of smaller size, and is serologically distant from the others[30] (Fig. 4b).

RNMV has been proposed to be a type member of this virus group[13], but information on its properties is scarce and its occurrence is limited to Japan[15] and India[6]. On the other hand, BaYMV is distributed widely in several Asian and European countries, and is economically the most important in this virus group. Furthermore, BaYMV was the first to be found as a filamentous virus[16] and is the most well characterized in this virus group. Therefore, we propose BaYMV to be a type member of a new virus group designated as the barley yellow mosaic virus group, bymovirus.

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## 和 文 摘 要


宇杉富雄・柏崎　哲・大村敏博・土崎常男：土壌伝染性ひも状ウイルスの核酸および外被蛋白質の諸性質

オオムギ縞萎縮ウイルス (BaYMV), コムギ縞萎縮ウイルス (WYMV), wheat spindle streak mosaic virus (WSSMV) および oat mosaic virus (OMV) の核酸および外被蛋白質の諸性質を調べた。これらのウイルスはいずれも大小2種類 (RNA-1, RNA-2) の一本鎖 RNA を有していた。BaYMV および WYMV の RNA-1 および RNA-2 はそれぞれ $2.6\times10^6$, $1.5\times10^6$ であり, WSSMV では $2.6\times10^6$, $1.4\times10^6$, OMV では $2.8\times10^6$, $1.8\times10^6$ であった。BaYMV, WYMV および WSSMV は分子量 33,000 の, また OMV は分子量 30,000 の単一成分の外被蛋白質を有していた。土壌伝染性ひも状ウイルスが2成分の粒子より成り立ち, 2種類の核酸を有していること, また, *Polymyxa graminis* によって伝搬されることはこれらのウイルスが potyvirus とは明らかに異なっていることを示している。よってわれわれは土壌伝染性ひも状ウイルスを分類するために BaYMV を代表とする barley yellow mosaic virus group, すなわち bymovirus group の新設を提案する。