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PHYSICAL AND CHEMICAL PROPERTIES OF JAPANESE ISOLATE OF BARLEY YELLOW DWARF VIRUS

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Introduction

Barley yellow dwarf virus (BYDV), the type member of luteovirus, is one of the most widespread and economically important plant viruses in the world, and has several isolates distinguished on the bases of vector specificity,[11,19] serological relationships[20,21,23] and cytopathological alterations[7]. As the virus is restricted to phloem tissues of the infected plants, small amounts of virus yield makes the physico-chemical studies difficult. In 1983, KOJIMA *et al.*[12] reported the occurence of BYDV in barley in Japan. In our previous papers on aphid transmission,[12,14] and cytopathology,[13] they were concluded that this Japanese isolate has close affinity to GILL and CHONG's subgroup-2[7]. The replicative forms of luteovirus-genome are known to consist of genomic and subgenomic dsRNAs and their profiles on polyacrylamide gel electrophoresis are known to be characteristic to each subgroup[4,6]. In this paper, dsRNA associated with BYDV-805 in the infected leaf tissues and the properties of virus components such as coat protein and nucleic acid of this isolate are reported.

Materials and Methods

Virus isolate and vector

The BYDV-805 isolate was originally isolated from barley (*Hordeum vulgare* cv. Minori-mugi) in the field and maintained on barley plants. Non-viruliferous aphids, *Rhopalosiphum padi*, were reared on barley in a growth chamber with 16 hr. light at 25°C.

Purification

Whole plant of the infected barley or oat were harvested, chopped into

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piece about 1 cm long, and stored at −30°C. Frozen leaf tissue (100 g) were ground with liquid nitrogen in a motar, and macerated in 300 ml of 0.1 M phosphate buffer (pH 7.0) with sea sand for 6–7 hrs. at room temperature[17]. The homogenate was clarified by adding 1/3 volume of chloroform, and blending for 3 min. After low speed centrifugation at 6,000 g for 15 min. polyethylene glycol (PEG 6,000) and sodium chloride were added to the aqueous phase to make concentration of 8% (w/v) and 0.2 M, respectively. The mixture was incubated at 4°C overnight and the virus was pelleted by centrifugation at 10,000 g for 15 min. The pellet was resuspended in 0.1 M phosphate buffer containing 1% Triton X-100. After low speed centrifugation at 10,000 g for 15 min, the supernatant was layered onto 20% sucrose cushion and centrifuged at 27,000 rpm for 180 min. in a Hitachi RP-30 rotor. The pellets were resuspended in 2 ml of 0.1 M phosphate buffer, layered onto sucrose density gradient columns (10–40%), and centrifuged at 22,500 rpm for 180 min. at 20°C in Hitachi RPS-25 rotor. A virus zone was collected by ISCO UA-2 fractionator. After the virus zone was diluted in buffer and centrifugated at 27,000 rpm for 180 min. in Hitachi RP-30 rotor, the pellet was resuspended in a small volume of double distilled water and stored at −30°C.

SDS-PAGE

Viral coat protein prepared from the purified virus preparation was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system of LAEMMLI[15]. Molecular weight was estimated by using SDS-PAGE Standard of Low Molecular Weight (Bio Rad Lab.).

SsRNA extraction and PAGE

Nucleic acid was extracted from the purified virus by SDS and phenol method[18]. The purified virus preparation was treated by 1% SDS in TAE (40 mM tris, 20 mM sodium acetate, 2 mM EDTA, pH 7.8). After heating at 60°C for 5 min. the preparation was emulsified with a equal volume of TAE-saturated phenol containing 0.1% 8-hydroxyquinoline. The mixture was separated by centrifugation for 10 min. at 10,000 g. An equal volume of chloroform : isoamyl alcohol (24 : 1) mixture was added to the aqueous phase to remove phenol, and the nucleic acid preparation was concentrated by ethanol precipitation, dried under vacuum and resuspended in TAE. The nucleic acid was analyzed under denaturing condition on 2.5% PAGE containing 7 M urea[16]. After electrophoresis the gel was stained with ethidium bromide (1 μg/ml), visualized under uv-transilluminator (Model TM-15, UVP. Inc.).

DsRNA extraction and PAGE

For extraction of dsRNAs from infected plants, a procedure described previously was used[6]. Frozen leaf tissue (100 g) were ground in liquid nitrogen in a mortar and stirred for 30 min. in a extraction medium containing 100 ml of 2×STE (100 mM tris, 2 mM EDTA, 200 mM sodium chloride, pH 8.0), 100 ml of STE-saturated phenol, 5 ml of bentonite (40 g/l) and 50 ml of chloroform : isoamyl alcohol (24 : 1) mixture. After centrifugation at 6,000 g for 30 min., the aqueous phase was collected. The residues and phenol phase were re-extracted in 50–100 ml of 2×STE with sea sand in a mortar. After the second centrifugation, absolute ethanol was added to the combined aqueous phase to 15%. Cellulose powder (Whatman CF-11 cellulose powder) was added to the ethanol solution (5.5–6.0 g/100 g tissues) and then stirred for 2 hrs. at room temperature. The CF-11 cellulose bound with dsRNA was then collected by centrifugation, and washed in 500 ml of a 15% ethanol-STE solution. After washing, the cellulose powder was packed in a 1×30 cm column, and washed in 200 ml of 15% ethanol-STE. The retained dsRNAs were eluted in STE without ethanol and fractionated. The retained dsRNAs precipitated in ethanol, resuspended in 50 mM tris-HCl (pH 7.5) containing 20 mM $MgCl_2$ and 2 mM $CaCl_2$, and incubated at 37°C for 30 min. with 1 μg/ml of DNase I (Sigma). The reaction was stopped by phenol treatment. The aqueous phase was made up to 2 M LiCl, and incubated at 4°C overnight. The sample was then centrifuged at 10,000 g for 5 min., and dsRNA in the LiCl supernatant fraction was subjected to ethanol precipitation 3 times. The precipitate was resuspended in TAE. PAGE under non-denaturing condition was performed in a 5% PAGE in 2×TAE, at 35 mA for 20 hr. at room temperature. After electrophoresis, the gel was stained with ethidium bromide. The RNA samples were tested for RNase-A sensitivity by the method described previously[26]. The RNA samples were made up to high (2×SSC) or low (0.1×SSC) salt conditions, treated with 50 μg/ml of RNase-A (Boehringer-Mannheim) and incubated at 25°C for 15 min. After treatment, the samples were incubated at 25°C for 15 min. with 5 μg/ml of Proteinase-K (Boehringer-Mannheim) and 0.05% SDS to remove RNase-A.

ELISA

The procedure for ELISA was essentially the same as those described previously[2,17]. In this experiment, we used antisera to BYDV-805 and -Ns (aphid non-specific isolate) which has kindly supplied by Dr. Y. C. PALIWAL (Chemistry and Biology Research Institute, Agriculture Canada).

Results

SDS-PAGE of protein

In SDS-PAGE analysis, a single band was observed (Fig. 1). In those experiments, migration rates were expressed as the ratio of distance of each protein migration to that of cytochrome-C (Rm). The migration rates plotted are shown to be logarithmic Fergason plots[8] (Fig. 2-A). HEDRICK and SMITH plots[9] which were plotted by each protein slope on Fig. 2-A was shown in Fig. 2-B. From the parabolic curve, a molecular weight of the coat protein was determined to be about 22,500 (Fig. 2-B).

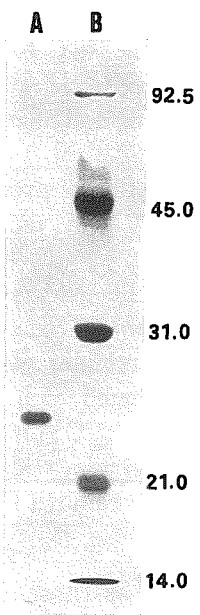

Fig. 1. SDS-PAGE of BYDV-805 coat protein. Lane A: BYDV-805 coat protein, lane B: standard proteins as makers.

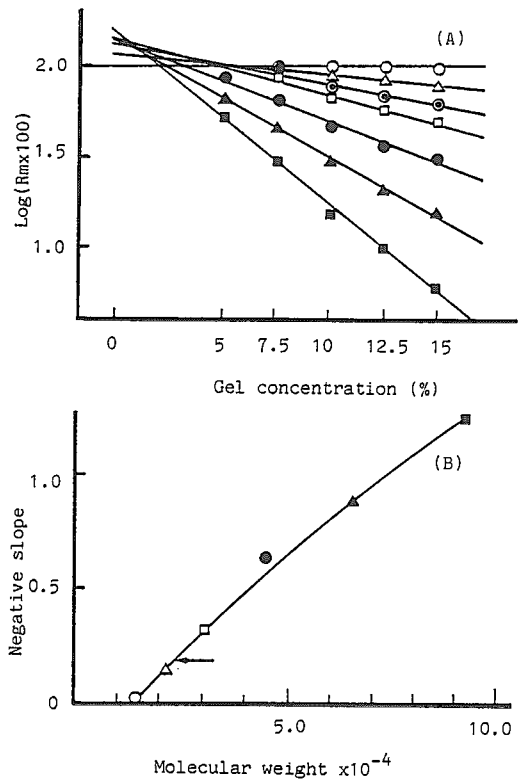

Fig. 2. Fergason plots of migration of BYDV-805 and standard proteins in SDS-PAGE (A), and Hedrick and Smith plots of standard proteins used for estimation of molecular weight (B).

BYDV-805 coat protein (⊙), lysozyme (○, Mr; 14,400), soybean trypsin inhibitor (△, Mr: 21,500), carbonic anhydrase (□, Mr: 31,000), ovalbumin (●, Mr: 45,000), BSA (▲, Mr: 66,200) and phosphorylase B (■, Mr:92,500). An arrow indicates the estimation for the molecular weight of BYDV-805 coat protein to be 22,500.

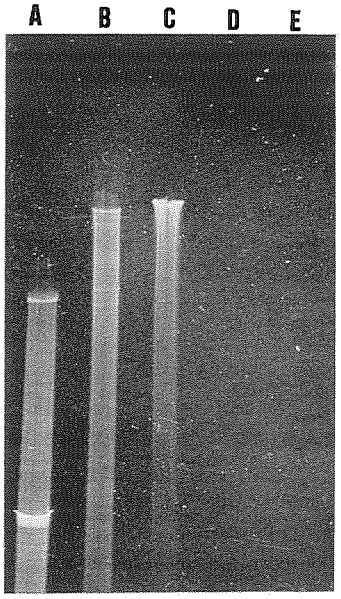

Fig. 3. Electrophoresis of BYDV-805 genome RNA (lane B) in 2.5% PAGE under 7M urea denaturing condition. *E. coli* rRNA (lane A), TMV-RNA (lane C) were used as standards. Lane D and E showed TMV-RNA (E) or BYDV-805 (D) treated with RNase-A under a high salt condition.

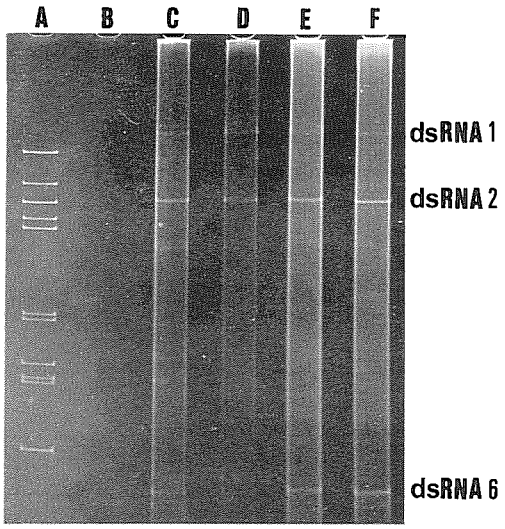

Fig. 4. Profiles of dsRNAs on 5% PAGE. Lane B to F show profiles of dsRNAs purified from healthy oats (lane B) and BYDV-805 infected plants grown in the spring (C), summer (D), autum (E) and winter (F). Lane A shows RDV genome as markers.

SsRNA

The nucleic acid extracted from purified virus preparation was susceptible to digestion by RNase-A in 2×SSC (Fig. 3), indicating that the BYDV-805 nucleic acid is ssRNA. The absorbance ratios of 240/260 nm and 280/260 nm were 0.48 and 0.54, respectively. The result suggests that the BYDV-805 has one component of ssRNA with a molecular weight of 1.95×10^6.

DsRNA

The yield of dsRNA associated with BYDV-805 from oat infected in the winter was about 50 μg/100 g. In contrast, about 1/8 to 1/16 yield was obtained from the infected oat in the summer. Three major bands of the virus specific nucleic acids extracted from the BYDV-805 infected oat grown in four seasons were detected (Fig. 4). In addition, some minor bands were

TABLE 1. A comparison of molecular weights of major dsRNA species extracted from plants infected with BYDV-805, BYDV New York isolates and beet western yellows virus isolates

Band	Molecular weight ($\times 10^6$)@				
		BYDV		BWYV$	
	BYDV-805#	Group 1*	Group 2**	STFL	ST-9
1	3.5	3.6	3.8	3.6	3.6
2	2.1	2.0	1.6		2.2
3		1.2	1.2	1.4	1.4
4		0.55	0.55		
5		0.50			
6	<0.46				0.46

@: Molecular weights were determined from the mobilities of dsRNAs on 5% PAGE relative to dsRNA makers of tobacco mosaic virus and rice dwarf virus.
#: Present study.
*: GILDOW *et al.* (1983). Including MAV, PAV, and SGV.
**: GILDOW *et al.* (1983). Including RPV and RMV.
$: FALK and DUFFUS (1984).

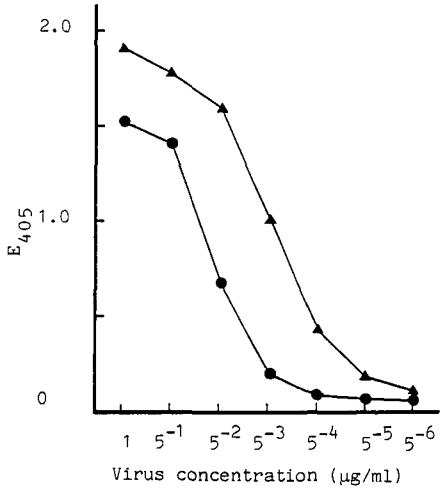

Fig. 5. Reactions of purified BYDV-805 preparation on enzyme linked immunosorbent assay with antisera (IgG) to BYDV-805 (●) and BYDV-Ns (▲).

also detected from the infected oat. No dsRNA bands were detected from healthy ones. The nature of these RNAs was verified by RNase-A treatment under high and low salt conditions. Both dsRNAs from RDV and BYDV-805 infected oat were degraded by RNase-A treatment in $0.1\times$SSC, but not in $2\times$SSC, suggesting that these bands were dsRNA. Each molecular weight was estimated from the relative mobilities of these dsRNA to dsRNA markers of RDV genome segments and replicative form of TMV genome on 5% PAGE. The estimated molecular weight were 3.5×10^6 for dsRNA-1 and 2.1×10^6 for dsRNA-2 (Table 1). The third dsRNA was smaller than RDV genome segment 12 (Mr : 0.48×10^6). In this experiment, no other bands corresponding to dsRNA-3, -4 and -5 described by GILDOW *et al.*[6] were detected.

ELISA

Serological relationship between BYDV-805 and -Ns was examined by ELISA. The optimum reaction was obtained when 1 μg/ml IgG for coating the plates and 300-fold dilution enzyme conjugate were used. As shown in Fig. 5, purified BYDV-805 reacted not only homologous IgG but also with heterologous ones.

Discussion

Previous investigations of chemical and physical properties of BYDV isolates have shown that they consisted of a single coat protein species[3,8,24] and a single ssRNA species[1,22]. The molecular weights of the proteins in the BYDV isolates MAV, PAV and P-PAV were estimated to be 23,500, 24,450[24] and 24,400,[8] respectively. In this experiment, result of Hedrick and Smith plots showed the molecular weight of the protein of BYDV-805 was smaller than those of other isolates.

On the other hand, BYDV-805 has a single species of ssRNA with a molecular weight of 1.95×10^6. This value is similar to that of the BYDV isolates and those of other luteoviruses[1,4,10,25].

Recent studies on cytopathology and serological analysis of the BYDV isolates suggested that they could be classified into two distinct subgroups. In addition, GILDOW *et al.*[6] suggested that these isolates were also categorized into two subgroups by comparing the dsRNAs profiles extracted from the infected plants. By using five New York isolates, they reported the differences in the number of dsRNA associated with the isolates in their electrophoretic mobilities, i. e. 5 for subgroup 1 and 4 species for subgroup 2. We obtained three major bands of dsRNA from BYDV-805 infected oat.

The first and second bands almost corresponded to those of subgroup 1 (Table 1), but the third band which migrated faster than RDV genome segment 12, was not found in the other isolates. Furthermore, some minor bands which did not correspond to those from New York isolates were also detected. These isolates which belong to the same subgroup, reacted with each other in serological blocking tests for aphid transmission[20]. In ELISA, however, they had low reactivity even within the same subgroup[21]. BYDV-805 reacted with BYDV-Ns IgG in ELISA at the same level as homologous reaction. The results reveal that BYDV-805 has similar propertis to those of subgroup 1. But BYVD-805 has high degree of transmission efficiency by *R. padi* and moderates degree of that by *Sitobion avenae*, but is not transmitted by *R. maidis* and *Schizaphis graminum*[12,14]. The transmission characteristics of BYDV-805 were similar to those of PAV which belong to subgroup 1. Nevertheless, cytopathological alteration was similar to those of subgroup 2[13]. Thus, based on vector specificity and serology, BYDV-805 is categorized into subgroup 1. But cytopathological feature suggests that it belongs to subgroup 2. Futhermore, the dsRNA patterns indicate that it differs from the both subgroups. These ambiguous results suggest that BYDV-805 seems to be a novel variant of BYDV. Further comparative studies with other BYDV isolates are required to resolve such problems.

Summary

Protein and nucleic acid compositions of a barley yellow dwarf virus isolate were determined. BYDV-805 consists of a single coat protein species with a molecular weight of 22,500 and a single ssRNA species with a molecular weight of 1.95×10^6. Analysis of virus specific dsRNA isolated from the infected oat showed two major species (molecular weight 3.5×10^6, 2.1×10^6). In addition, an extra species which migrated faster than that of rice dwarf virus (RDV) gemome segment 12 (0.48×10^6) was detected. The smallest species has not been detected in plants infected with any other known isolates. In double antibody sandwich ELISA, the virus isolate reacted not only with homologous antibody but also with antibody to BYDV-Ns (Canada Isolate). Relationship of BYDV-805 and previously described isolates of BYDV was discussed.

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