

オオムギ黄萎病(新称)の発生

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著者名	小島,誠 松原,旭 築瀬,誠司 鳥山,重光
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The Occurrence of Barley Yellow Dwarf Disease in Japan

Makoto KOJIMA*, Akira MATSUBARA*, Seiji YANASE*
and Shigemitsu TORIYAMA**

小島 誠*・松原 旭*・築瀬誠司*・鳥山重光**:
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Abstract

Barley plants showing yellow dwarf symptoms were found in the barley fields at Nagaoka, Niigata Prefecture in May, 1980. The causal agent was transmitted in a persistent manner by *Rhopalosiphum padi* to gramineous plants but neither *R. maidis* nor *Schizaphis graminum*. Cereals such as barley, wheat, rye and oat exhibited typical yellow dwarf symptoms of stunting and either yellowing or red leaf discoloration. Other plants such as rice, maize, sorghum, Japanese millet, Job's tears and Italian ryegrass showed no symptoms but proved to be symptomless carriers of the agent. Partially purified preparations containing small spherical virus particles about 25nm in diameter were infectious when they were fed by aphids through membranes and the inoculated plants showed typical symptoms caused by barley yellow dwarf virus. Thermal inactivation point of the agent was between 65 and 70°C. In the ultrathin sections, electron dense virus-like particles about 22nm in diameter were found only in phloem tissues of affected barley. Based on these results, the causal agent of the disease was identified as barley yellow dwarf virus.

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Key Words: BYDV, transmission, electron microscopy.

Introduction

In May, 1980, barley plants showing symptoms characteristic of barley yellow dwarf virus (BYDV) were found in the fields at Nagaoka, Niigata Prefecture. Cereals showing yellow dwarf symptoms have been described at different districts in Japan¹¹⁾, and one of this syndrome was presumed to be caused by BYDV. Although BYDV has been known to distribute throughout the world⁹⁾, only few experimental tests have been reported so far in Japan to identify the causal agent. Toriyama and Yora,¹¹⁾ successively isolated BYDV from *Agropyron tsukusiense* var. *transiens* showing yellows and oats showing red leaf symptoms. They confirmed that their isolate was transmitted by *Rhopalosiphum padi* and *Macrosiphum avenae-akebiae* but not by *R. maidis****. However, the virus has not been widely distributed in Japan to cause serious damages on cereal yields. As Rochow pointed out,⁹⁾ symptoms of barley

* Faculty of Agriculture, Niigata University, Niigata 950-21, Japan 新潟大学農学部

** Faculty of Agriculture, The University of Tokyo, Tokyo 113, Japan 東京大学農学部

*** In a report of Toriyama and Yora (1972)¹¹⁾ it was incorrectly stated that BYDV was transmitted by *R. maidis* but not by *R. padi*. In that report the species name should be interchanged.

yellow dwarf in the field can easily be confused with those caused by other factors, and thus transmission by aphids is considered to be indispensable for identification. We report here the results of aphid transmission tests and electron microscopic investigations to determine if the agent responsible for yellow dwarf symptoms on barley is BYDV.

Materials and Methods

Virus. The virus isolate used was originally isolated from infected barley plants (*Hordeum vulgare* cv. Minori-mugi) at Nagaoka, Niigata Prefecture in May, 1980. This isolate (805) which is transmitted selectively by *Rhopalosiphum padi* was maintained by serial transmission to barley (cv. Minori-mugi) seedlings at intervals of 5–6 weeks.

Plants and aphids. Stock colonies of virus-free aphids, *Rhopalosiphum padi*, were maintained on caged barley plants and were grown under continuous fluorescent illumination at 20 C. Other aphid species, *Rhopalosiphum maidis* and *Schizaphis graminum* were also maintained in the same manner. Barley plants were used for infectivity tests, and to provide the sources of infected materials for purification.

Transmission and infectivity assay. Infectivity of the concentrated preparations was estimated by a membrane-feeding method⁷⁾. Inocula usually were suspended in 20 % sucrose dissolved in neutral 0.01 M phosphate buffer. Following an acquisition access period of about 20 hours at 15 C, 5 aphids per plant were transferred to barley seedlings for a 5-day inoculation access period. Aphids were then killed by fumigation with a nicotine sulfate solution, and the plants were placed in a greenhouse for at least 4 weeks. In some transmission experiments, symptom observations were also done in a controlled environment chamber at 20 C with 10,000 lux of mercuric light.

Virus purification. Entire shoots of infected barley plants were harvested, chopped into pieces about 5 cm long, and stored at –20 C in plastic bags until use for purification. Virus extraction from plants was done by means of Driselase (Kyowa Hakko Kogyo Co., Ltd., Japan) assisted procedure¹⁰⁾. Clarification and concentration procedures were followed by the modified method described previously⁵⁾.

Electron microscopy. Sap from affected barley plants was examined by dip method using 2 % PTA. Each fraction from virus extraction procedures was also checked in the same manner. Fixation and embedding of leaf tissues for ultrathin sectioning were made according to a method described previously⁶⁾. Ultrathin sections were cut in a Reichert Om 4 ultra-microtome and were examined in a JEM 100 B electron microscope at 80 KV.

Results

Assay for virus particles by dip method

No rods, filamentous or baciliform particles were observed in the dip preparations made from naturally infected barley leaves.

Table 1. Daily transmission of the virus isolate by *Rhopalosiphum padi*

Aphid No. ^{a)}	Daily transfer									
	1	2	3	4	5	6	7	8	9	10
I	● ^{b)}	●	●	●	●	●	●	●	○	D ^{c)}
II	●	× ^{d)}	×	×	●	●	D	●	●	●
III	●	●	●	●	●	●	●	●	●	●
IV	●	●	●	●	●	●	●	D	●	●
V	×	×	×	●	●	●	●	○	●	○
VI	×	×	×	●	●	●	●	●	●	○
VII	●	×	●	×	×	●	●	×	●	●
VIII	●	×	●	●	●	●	●	●	●	●
IX	●	×	●	×	×	●	○	●	○	D
X	×	×	●	×	×	×	●	●	●	○

a) Nymphs given the acquisition access periods for 5 days were used.

b) ●: infected; ○: not infected.

c) Aphids died.

d) Test seedlings died.

Observation of inclusion bodies

Strips of epidermis from naturally infected plants and those from the affected plants after inoculation, were stained with 0.5% phloxine solution in water and examined under the light microscope. No inclusion bodies were visible.

Table 2. Transmissibility of the virus isolate by three aphid species

Aphid species	Transmission (%)
<i>Rhopalosiphum padi</i>	38/42 (90)
<i>Rhopalosiphum maidis</i>	0/17 (0)
<i>Schizaphis graminum</i>	0/43 (0)

Mechanical inoculation

To determine whether the virus is sap transmissible, naturally-infected barley plants were ground in 0.1 M phosphate buffer, pH 7.4, and then resultant juice was rubbed on Carborundum-dusted seedlings of barley. These experiments were repeated twice using 10 seedlings of barley in 1980. All attempts failed to transmit the virus by sap inoculations.

Aphid transmission

The virus isolate originally was transmitted from naturally-infected barley grown at Nagoka to barley seedlings by aphids. Cherry oat aphids (*R. padi*) were collected from a yellow-dwarfed barley and transferred three times (in groups of 5) to seedlings of barley at 3-days intervals. In the preliminary transmission experiments, all test plants developed chlorosis, yellowing of leaves, erect growth, and stunting of plants similar to those observed in the field (Plate I-A). In addition, batches of 10 non-viruliferous *R. padi* that acquired the virus from infected plants for 5 days were daily transferred singly to seedlings of barley. As shown in Table 1, the virus was persistently transmitted by *R. padi*.

Virus-vector relationships

Nymphs of *R. padi*, *R. maidis*, and *S. graminum* that were allowed a 5-day acquisition access period on infected barley were transferred to healthy barley seedlings for a 5-day inoculation access period using 10 insects per test plant. *R. padi* transmitted the virus to 90 % of the test plants, but both *R. maidis* and *S. graminum* failed to transmit the virus (Table 2).

Host range and symptomatology

Host range and symptomatology of the virus isolate was tested using various cereal plants and grasses. All twelve gramineous plants tested were susceptible (Table 3).

Table 3. Host range and symptomatology of the virus isolate

Plant species	Symptoms
<i>Hordeum vulgare</i>	
Minori-mugi	chlorosis, yellowing, stunting (severe), erectness
Kōgen-mugi	do.
Asama-mugi	do.
<i>H. disticum</i>	
Hoshi-masari	chlorosis, yellowing, stunting (moderate)
<i>Triticum aestivum</i>	
Muka-komugi	chlorosis, yellowing, stunting (moderate)
Yuki-chabo	do.
Nōrin 16	do.
<i>Avena sativa</i>	
Zen-shin	chlorosis, red-leaf, stunting (moderate)
Hōyō	do.
Moiwa	do.
<i>A. byzantina</i>	
Coast Black	do.
<i>Secale cereale</i>	
Petkus	chlorosis, stunting (moderate)
<i>Oryza sativa</i>	
Koshi-hikari	symptomless (+) ^{a)}
Blue Bonnet	do.
Balilla	do.
<i>Zea mays</i>	
Heigen-wase	do.
Golden Cross Honey Bantam	do.
<i>Sorghum vulgare</i>	
Janome	do.
<i>Panicum crus-galli</i> var. <i>trumentaceum</i>	
Futago-mochi	do.
<i>Coix lachryma-jobi</i> var. <i>frumentacea</i>	
Hato-mugi	do.
<i>Lolium multiflorum</i>	
Mammoth B	do.

a) Proved to be carrying the virus by back inoculation.

No symptoms, however, were developed about half of the plants tested such as rice, maize, sorghum, Japanese-millet, Job's tears and Italian ryegrass. Symptoms were usually less severe in oat than in barley plants, and mild in wheat, two-rowed barley and rye plants, (Table 3). In barley, leaf chlorosis appeared first on young leaves and then yellowing progressed downwards. Leaves of infected barley plants were erect and some old leaves were often serrated. In barley the infected plants were remarkably stunted. Leaf chlorosis was also common in oat and wheat and accompanied by reddening in oat and by yellowing at the tips in wheat. They were moderately stunted.

Purification of the virus

In preliminary experiments, the concentrated preparations from infected barley and viruliferous aphids after chloroform clarification and differential centrifugation were found to be infectious by means of aphid-feeding through membranes, but they contained only a few virus-like particles.

To investigate the presence of virus particles and infectivity, samples obtained from each step of the extraction procedure were assayed (Table 4). Extracts from infected barley after grinding in 0.1 M citrate buffer (pH 6.0), were centrifuged at 3,000 rpm and the supernatants (S₁) were discarded. The sediments containing fibrous materials were suspended in 0.1 M citrate buffer, pH 6.0 containing Driselase (1 %, w/v) and shaken for 1-2 hr at room temperature (or 28 C). Then the mixtures were reground in the mortar and pestle with carborundum and centrifuged at 3,000 rpm. The resulting supernatants (S₂) were decanted and saved for further purification. The sediments were re-ground in the same manner adding small volume of 0.1 M phosphate buffer (pH 7.4) for further extraction, and centrifuged. Such extraction process was repeated several times (S₃-S₇). Each resulting supernatant (S₁-S₇) was processed by chloroform clarification, PEG concentration and differential centrifugation. The final clarified concentrated preparations were assayed for infectivity and examined by an electron

Table 4. Relationships between presence of virus-like particles and infectivity in each fraction

Experiment	Assay	Fraction						
		S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇
I	EM ^{a)}	—	+	‡‡ ^{c)}		‡‡	•	•
	Infectivity ^{c)}	0/6	6/6	7/10		5/5	•	•
II	EM	•	+	‡‡		‡‡	•	•
	Infectivity	•	4/4	5/5		3/8	•	•
III	EM	•	+	‡‡			‡‡	
	Infectivity	•	5/5	5/6			3/5	

a) Observed by an electron microscope at the 20,000 magnification.

—: no particles; +: aver. number ~5; ‡‡: ~10; ‡‡‡: 10~per EM negative film (5.9×8.2cm).

b) Numerator: number of plants infected;

Denominator: number of plants infested with fed aphids.

c) Combined S₃ and S₄.

Table 5. Thermal inactivation point of the virus isolate

Experiment ^{a)}	Temperature (C)					Control ^{b)}
	55	60	65	70	75	
I	5/6	•	5/7	0/7	•	5/5
II	•	•	3/6	•	0/2	4/8
III	•	•	5/6	0/7	0/15	5/7
IV	•	2/9	1/8	0/8	•	6/9
V	•	4/12	2/10	•	•	3/9

a) Concentrated ($\times 25$) preparations from plants were diluted to three times (Exp. I) and to ten times (Exp. II and III), respectively, and extracts from aphids (800 aphids/4.0 ml) were used in Exp. IV and V.

b) Non-heated preparations.

microscope. As shown in Table 4, the preparations from S_2 to S_7 were infectious but preparations from S_2 contained less virus-like particles than that combined S_3 and S_4 . Electron micrographs showed that these infectious preparations contained small spherical particles about 25 nm in diameter (Plate I-B).

Thermal inactivation point

The concentrated (25-fold) preparations from barley used above and extracts from viruliferous aphids (*R. padi*) were heated at 55–75 C for 10 min and used for inocula. As shown in Table 5, the inactivation point (10 min) for the virus isolate in both partially purified preparations from plants and in crude insect extracts was between 65 and 70 C.

Virus-like particles in infected plant tissues

Because of similarities of the virus isolate to BYDV in aphid transmissibility and symptomatology, attempts were made to detect the virus particles in phloem tissues of the infected plants by ultrathin sections. Some degenerated phloem cells were observed in the infected plants and electron-dense spherical particles were found in those cells (Plate I C). These particles were restricted to sieve tubes, companion and phloem parenchymatous cells. None of such particles was seen in mesophyll and epidermal cells of the infected plants and in healthy leaves. The particles were scattered singly or in masses within the lumen of affected cells or associated with vesicle structure. The particles were uniform in shape and size and measured about 22 nm in diameter.

Discussion

So far in Japan, wheat yellow leaf virus (WYLV)³⁾ is the only aphid-borne virus known to affect cereals. The virus, however, is transmitted in a semi-persistent manner by *R. maidis*. The virus investigated here appears to be similar in many respects to BYDV^{1,2,4,8)}, a type member of luteovirus group, but differs from previously reported viruses of barley in Japan¹²⁾ in a number of ways, i.e., manner of transmission, symptomatology, morphology, and localization of the virus in host plant cells.

BYDV occurs as several vector specific and nonspecific variants^{1,8)}. The present isolate of BYDV is similar to the isolate from *Agropyron tsukusiense* var *transiens*¹¹⁾ in vector specificity, but mild in the symptoms on wheat. Even though we must await the results of further chemical and serological studies to determine relationships of this virus isolate, we tentatively identify the isolate from barley at Nagaoka as similar to *R. padi*-specific variant of BYDV based on its vector specificity.

The cropping areas of barley are increasing in Japan, due to a re-orientation program for paddy field utilization. Thus, occurrence and spread of BYDV must be closely monitored because of wide host range of BYDV among cereals, and wild grasses and widespread occurrence of aphid vectors.

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和文摘要

オオムギ黄萎病（新称）の発生

小島 誠・松原 旭・築瀬誠司・鳥山重光

1980年5月、新潟県長岡市のオオムギ（ミノリムギ）に黄化萎縮症の発生をみた。その病因を探索した結果、ウイルスに起因することが判明した。その病原ウイルスはムギクビレアブラムシ（*Rhopalosiphum padi*）により永続的にムギ類、禾本科牧草、イネ等に伝染した。ムギミドリアブラムシ（*Schizaphis graminum*）、トウモロコシアブラムシ（*R. maidis*）では伝染しなかった。接種したオオムギ、コムギ、ライムギ、カラスムギでは葉身の黄化あるいは赤化を伴った萎縮症状が現われた。他の禾本科植物では病徴は認められなかった（不顕性感染）。病植物から部分純化した標品には直径約 25 nm の球状粒子が認められ、膜吸汁法により病原性が確認された。また、本ウイルスの不活化温度は65～70℃であった。一方、感染オオムギの超薄切片法による観察の結果、直径約 22 nm の球状ウイルス様粒子が篩部組織にのみ認められた。これらのことから、本ウイルスは barley yellow dwarf virus の一系統と考えられ、本病をオオムギ黄萎病と呼ぶことにしたい。

Explanation of Plate

Plate I.

- A. A barley plant (cv. Minori-mugi) naturally-infected with an isolate of BYDV: note the chlorosis and yellowing on the leaves and stunting.
- B. An electron micrograph of BYDV from partially purified preparation obtained from the affected barley, stained with 2% PTA. Bar represents 100 nm.
- C. An ultrathin section of a companion cell of an infected barley plant 12 days after inoculation. Note a mass of the virus-like particles and small vesicles containing fibrous materials. Bar represents 100 nm.

Plate I

