

# 低温下におけるコムギ縞萎縮ウイルス(WYMV)の冬コムギ(*Triticum aestivum*)体内での増殖

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## Propagation of Wheat Yellow Mosaic Virus in Winter Wheat under Low Temperature Conditions

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### Abstract

The seasonal process of infection and propagation of wheat yellow mosaic virus (WYMV) in winter wheat (*Triticum aestivum* L.) was studied in a field in Morioka, Japan. In the susceptible winter wheat cultivar Nanbukomugi, WYMV was first detected by ELISA only in roots on November 30, about two months after seeding in a field naturally and seriously infested with the virus. The incidence and concentration of the virus in roots and shoots of Nanbukomugi then increased when temperatures dropped below 5°C and with snow cover, and reached a high level before snow melt. Disease incidence in the cultivar in the same field was 96% when wheats were sown at the usual time. In contrast, in the resistant cultivar Hachimankomugi was asymptomatic and virus incidence was low during the same period. In a cross-transplanting test between infested and non-infested fields, virus infection occurring from mid-October through early November in Nanbukomugi resulted in symptom expression in spring. These results suggest that WYMV, infecting during the conducive period, propagated under low temperature conditions which induced a high virus incidence and concentration in the susceptible cultivar before symptoms were expressed, thus resulting in a high disease incidence.

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**Key words :** winter wheat, wheat yellow mosaic bymovirus, virus propagation, low temperature condition.

### INTRODUCTION

In recent years, wheat yellow mosaic disease has caused economic losses in winter wheat (*Triticum aestivum* L.) production in the Tohoku district of northern Japan. The pathogen of this disease is wheat yellow mosaic virus (WYMV), which belongs to the genus *Bymovirus*. Wheat spindle streak mosaic virus (WSSMV), which is involved in a similar disease of winter wheat in North America, has a close serological relationship to WYMV<sup>5)</sup>.

Circumstantial evidence suggests that, like barley yellow mosaic virus and WSSMV, WYMV is transmitted by *Polymyxa graminis* Ledingham, a soil-borne fungus<sup>7)</sup>. The use of resistant cultivars would be the most effective method for avoiding damage by the disease, but resistant cultivars have not been widely accepted by growers.

The disease incidence and severity caused by WYMV depend on weather conditions, especially during early growth ; a warm, wet autumn followed by a cold winter results in serious damage in the spring<sup>3)</sup>. A few studies have examined the process of infection and propagation of the virus in winter wheat crops under field conditions. Carroll *et al.* reported the detection of WSSMV from

wheat roots in autumn and from shoots in spring<sup>1)</sup>. In this study, we report on the conducive period for infection of WYMV during autumn by cross-transplanting test, and the temporal variation of the WYMV incidence in winter wheat plants. The results obtained provide evidence for the propagation of WYMV in susceptible winter wheat under low temperature conditions in winter before symptom expression in spring.

### MATERIALS AND METHODS

**Field conditions** All experiments were carried out at the Tohoku National Agricultural Experiment Station, Morioka, Iwate Prefecture from September 1994 to April 1995. The non-infested field had previously grown white clover followed by winter wheat in 1993-1994. No WYMV was found in this wheat crop. The infested field, which had a climate and soil type similar to the non-infested field, had been continuously cropped with winter wheat for the previous 10 years. WYMV occurred seriously and uniformly in this field in 1992-1993. Soil temperature (5 cm depth, three locations) and air temperature (30 cm height, one location) were monitored daily in the non-infested field from October 1994 to April 1995 with Pt-sensors and a Model M-166 recorder (Yokogawa Weathac Inc.). Daily mean soil and

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air temperatures were calculated from maximum and minimum temperatures of each point. Snow continuously covered both fields from December 15 to March 11.

**Monitoring incidence and concentration of WYMV in wheat plants from autumn through spring** For monitoring the incidence and concentration of WYMV from autumn through spring, two wheat cultivars Nanbukomugi and Hachimankomugi, susceptible and resistant to WYMV, respectively, were sown in the infested field. The two cultivars were sown at one-week intervals for three weeks from September 20, 1994 onwards to observe the effect of sowing dates on virus incidence and concentration. Each plot was a 2 m long row of 25 plants at 8 cm intervals. There were five replicates. At each sampling, four plants per replicate (including roots to about a 15 cm soil depth) were collected randomly, so that 20 plants per sowing time per cultivar were sampled. Plants were sampled from the field five times from October 27, 1994 to March 25, 1995. Additional samples were taken before and after the snow melted (March 6 and 15, 1995, respectively). The plants were carefully washed by ultrasonication in tap water until soil particles were thoroughly removed. After removing excess water with paper towels, plant shoots and roots were separated with a razor. Each sample was sealed in a plastic bag and stored at  $-80^{\circ}\text{C}$  until further processing. All sampling was done in one day. Virus incidence in the shoots and roots was evaluated by enzyme-linked immunosorbent assay (ELISA).

To examine disease incidence and severity, the two cultivars were established in other plots in the same infested field as mentioned. The number of tillers of these plants was counted on December 12, 1994, just before being snowed, to compare the effect of sowing dates on wheat growth before wintering. Twenty plants in the middle of each plot were scored for disease severity on March 28, 1995, when symptoms were most distinctive. Disease incidence was expressed as the percentage of plants with characteristic symptoms. Disease index was rated on a scale of 0–4, where 0, no symptom; 1, slight yellow mosaic on the youngest leaf; 2, distinctive yellow mosaic on two or three younger leaves; 3, yellow mosaic and dwarf of whole plant and 4, severe dwarf and serious yellow mosaic often associated with necrosis of the youngest leaf. Disease severity in each plot was calculated using the following equation:  $\text{disease severity} = 100 \times \sum(\text{scale value (0 to 4)} \times \text{number of plants with each scale value}) / (4 \times \text{total number of plants observed})$ .

**Cross-transplanting test to determine the conducive period for viral infection** The cultivar Nanbukomugi was established in infested and non-infested fields five times at one-week intervals starting on September 20. A total of 125 plants in five replicates was established for each of the five sowing dates. At the second leaf stage, 20 uniform-sized plants were dug up to a 15 cm depth. Their roots were gently washed free of soil using tap water. Plants were then carefully trans-

planted from the infested field into the non-infested field and vice versa (Table 2). As controls, twenty plants were transplanted in the same fields in both the infested and non-infested field on the same dates. Disease severity of these plants was estimated on April 10, 1995 as described before. Virus incidence in the shoots was evaluated by ELISA.

**Serological assays** Double antibody sandwich ELISA was performed at each sampling time using polyclonal antiserum to WYMV, supplied by Dr. Kashiwazaki (National Agricultural Research Center). Purification of  $\gamma$ -globulin and preparation of  $\gamma$ -globulin-alkaline phosphatase conjugate were performed as per Clark and Adams<sup>2)</sup>. Both  $\gamma$ -globulin and  $\gamma$ -globulin-alkaline phosphatase conjugate were used at a dilution ratio of 1 : 800. Whole-shoot samples were ground in 1 : 20 (w/v) of 0.1 M phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20 (v/v) with a pestle and mortar. Whole-root samples were ground in 1 : 10 (w/v) of 0.1 M citrate buffer (pH 7.0) containing 0.05% Tween 20 (v/v)<sup>6)</sup> with a trace of quartz sand to ease grinding. Root and shoot sap from healthy greenhouse-grown plants were added to 10 wells in each ELISA test as a control. All samples were stored in micro-test tubes at  $-80^{\circ}\text{C}$  until serological assay. The absorbance value at 405 nm ( $A_{405}$ ) of each well was measured by a plate reader (Bio Rad model 3550) after substrate incubation for 60 min at  $20^{\circ}\text{C}$ . The ELISA value of each sample was calculated as follows:  $\text{ELISA value} = A_{405} \text{ of a sample} - \text{mean } A_{405} \text{ of controls}$ . A sample was taken as WYMV-positive when the ELISA value was higher than 0.1.

## RESULTS

### *Changes of WYMV incidence and concentration in susceptible and resistant cultivars from autumn through winter*

For all three sowing dates, the increase in virus incidences in the susceptible cultivar Nanbukomugi increased in early winter following the same trends (Fig. 1A). In this cultivar, WYMV was detected in the roots at every sampling for each sowing date, but was not detected in the shoots of any samples until December 26. On January 31, 1995, the virus incidence in both roots and shoots exceeded 90% in all plots. The incidence in shoots remained over 90% until March 25 for all sowing dates. In Hachimankomugi, WYMV was detected at low levels from plants sampled on January 31, March 6, 15 and 25 (Fig. 1B).

The temporal variation of virus concentration in WYMV-positive Nanbukomugi showed the same trend as virus incidence. Small ELISA values were first recorded in some root samples on November 30, 1994. The ELISA values in both root and shoot samples of WYMV-positive plants, then increased to a high level ( $\approx 2.0$ ) by January 31, 1995. The virus concentration in WYMV-positive Hachimankomugi was high in some January 31 samples, but was generally low in spring

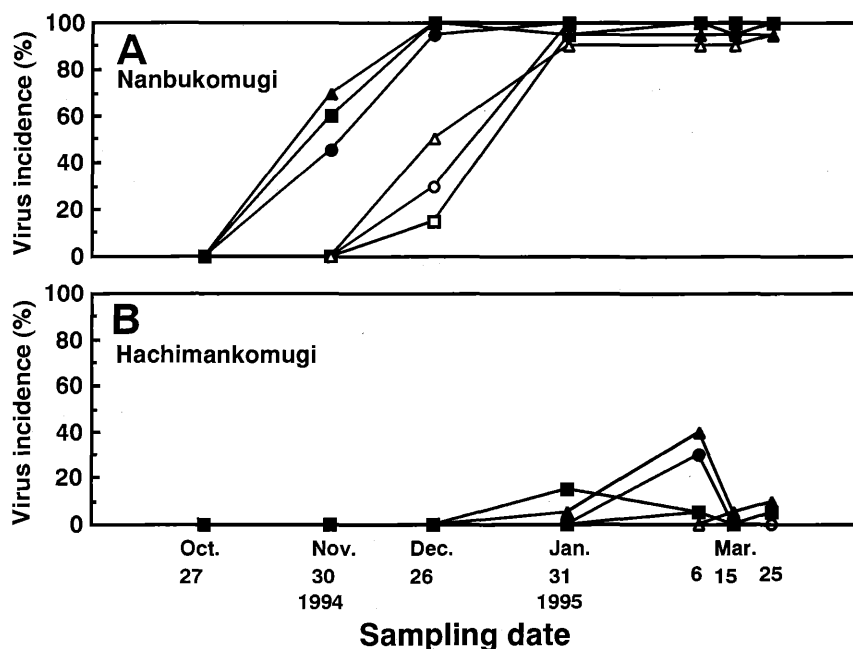


Fig. 1. Wheat yellow mosaic virus incidence<sup>a)</sup> from autumn through spring in roots and shoots of winter wheat cultivars Nanbukomugi (susceptible) and Hachimankomugi (resistant) sown at different times. Symbols indicate virus incidence determined by ELISA of shoot samples sown on September 20 (□), 28 (○) and October 4 (△), and of root samples sown on September 20 (■), 28 (●) and October 4 (▲).

a) The virus incidence indicates percentage of plants in 20 samples in which virus was detected by ELISA.

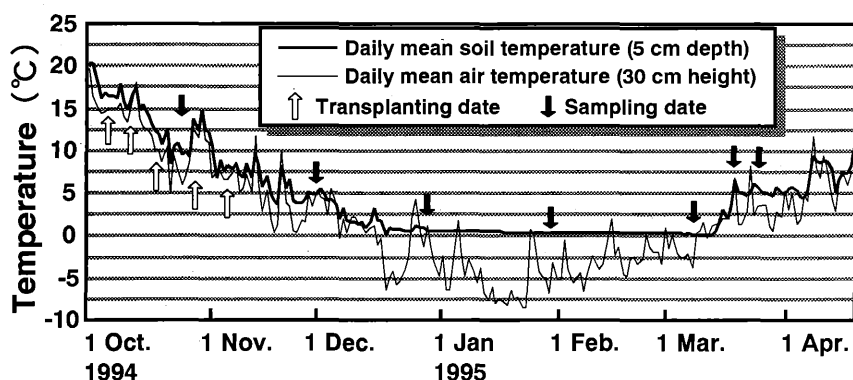


Fig. 2. The daily mean soil (5 cm depth) and air (30 cm height) temperatures from October 1 in 1994 to April 15 in 1995.

(data not shown). The mean soil temperatures under the snow in the field, when the virus incidence and virus concentration in wheat plants increased, was almost always between 0°C and 5°C (Fig. 2).

In both cultivars, the number of tillers before wintering decreased with late sowing dates. Yellow mosaic was first detected on Nanbukomugi in the September 20-sown plots on March 16, 1995, one week after the snow disappeared. The yellow mosaic progressed by the end of March while air temperatures remained between 0°C and 5°C. The disease incidence and severity in Nanbukomugi decreased with the later of sowing dates (Table 1). No disease symptoms developed in Hachimankomugi regardless of sowing date.

#### Conducive period for WYMV infection

In transplanting from the infested to the non-infested

fields, the virus incidence and disease severity increased with the lateness of transplanting date (Table 2). The virus incidence and disease severity were low when wheat was transplanted from the non-infested to the infested field after October 28. Plants grown in the infested field for 17 and 20 days from mid-October to early November before transplanting showed high virus incidences (60%). During this period, the mean soil and air temperatures in the field were almost 7°C to 15°C and 5°C to 15°C, respectively (Fig. 2).

#### DISCUSSION

Carroll *et al.*<sup>1)</sup> reported that WSSMV incidence increased from autumn to early spring probably by replication of the virus or by the secondary infection by

Table 1. The number of tillers (NT) before wintering, disease incidence (DI) and disease severity (DS) in spring in susceptible and resistant cultivars of winter wheat

| Cultivar                          | Sowing date  | NT                 | DI(%) <sup>a)</sup> | DS <sup>b)</sup>           |
|-----------------------------------|--------------|--------------------|---------------------|----------------------------|
| Nanbukomugi<br>(susceptible cv.)  | 1994 Sep. 20 | 10.1 <sup>c)</sup> | 98                  | 44.8±4.06a <sup>d,e)</sup> |
|                                   | Sep. 28      | 5.7                | 96                  | 34.3±3.84 b                |
|                                   | Oct. 4       | 3.4                | 66                  | 20.8±6.27 c                |
| Hachimankomugi<br>(resistant cv.) | 1994 Sep. 20 | 11.2               | 0                   | 0.0±0.00 d                 |
|                                   | Sep. 28      | 8.2                | 0                   | 0.0±0.00 d                 |
|                                   | Oct. 4       | 5.4                | 0                   | 0.0±0.00 d                 |

NT was counted on December 12, 1994.

DS and DI were estimated on March 28, 1995.

a) Percentage of plants with wheat yellow mosaic disease symptoms.

b)  $100 \times \sum(\text{scale value (0 to 4)} \times \text{number of plants of each scale value}) / (4 \times \text{total number of observed plants})$ .

c) Average of 100 plants.

d) Average of 100 plants±confidence interval (95%).

e) Numbers followed by the same letter did not differ significantly from each other according to Tukey's multiple range test,  $P=0.05$ .

Table 2. Effect of transplanting on the WYMV-infested and non-infested fields on virus incidence (VI) and disease severity (DS) in Nanbukomugi in 1994-1995 growing season

| Sowing date | Transplanting date | Fields                      | VI <sup>a)</sup>   | DS <sup>c)</sup> |
|-------------|--------------------|-----------------------------|--------------------|------------------|
| Sep. 20     | Oct. 5             | infested → non-infested     | 3/20               | 1.0              |
|             |                    | infested → infested         | 20/20              | 39.6             |
|             |                    | non-infested → infested     | 19/20              | 50.0             |
|             |                    | non-infested → non-infested | 0/20               | 0.0              |
| Sep. 28     | Oct. 12            | infested → non-infested     | 5/20               | 2.0              |
|             |                    | infested → infested         | 20/20              | 42.6             |
|             |                    | non-infested → infested     | 20/20              | 48.1             |
|             |                    | non-infested → non-infested | 0/20               | 0.0              |
| Oct. 4      | Oct. 21            | infested → non-infested     | 4/19 <sup>b)</sup> | 2.3              |
|             |                    | infested → infested         | 19/20              | 31.9             |
|             |                    | non-infested → infested     | 18/20              | 42.9             |
|             |                    | non-infested → non-infested | 0/20               | 0.0              |
| Oct. 11     | Oct. 28            | infested → non-infested     | 12/20              | 14.3             |
|             |                    | infested → infested         | 19/20              | 21.0             |
|             |                    | non-infested → infested     | 8/20               | 26.7             |
|             |                    | non-infested → non-infested | 0/20               | 0.0              |
| Oct. 18     | Nov. 8             | infested → non-infested     | 12/20              | 21.3             |
|             |                    | infested → infested         | 18/20              | 8.0              |
|             |                    | non-infested → infested     | 7/20               | 1.3              |
|             |                    | non-infested → non-infested | 0/20               | 0.0              |

a) Number of WYMV-positive plants/number of tested plants.

b) One plant was lost by cold damage.

c)  $100 \times \sum(\text{scale value (0 to 4)} \times \text{number of plants of each scale value}) / (4 \times \text{total number of observed plants})$ . ELISA samples were collected and DS were estimated on April 10, 1995.

viruliferous *P. graminis* under low temperature conditions. Our results showed that the virus incidence and concentration increased both in roots and shoots of the susceptible cultivar under low temperatures (below 5°C) before the symptom development in spring. Our transplanting experiments showed that the most effective virus infection for symptom expression occurred between mid-October and early November when the daily mean soil temperature was between 7°C and 15°C. Plants transplanted from the infested field to the non-

infested field before this period show little virus incidence, concentration or symptoms, probably because soil temperatures were too high for the vector or the virus to infect the plants. Viral infections after this conducive period also had little or no effect on disease incidence in spring. These results suggest that WYMV infection during the conducive period, followed by replication under low temperature conditions, induced the high virus incidence and concentration before symptom expression.

In this study, WYMV was detected by ELISA in a few plants of the resistant cultivar Hachimankomugi both in roots and shoots, but no symptoms were observed in shoots of any plants. As slight WYMV symptoms have occasionally been observed in Hachimankomugi in Morioka (Ohto, unpublished), this cultivar is not completely resistant (immune) to WYMV.

It has been noted that earlier seeding can result in serious damage by WYMV<sup>3)</sup> and barley yellow mosaic virus<sup>4)</sup>. The usual time for wheat seeding in Morioka is the end of September. In our experiment, the disease severity of plots that had been sown on September 20 was more serious than that of plots sown on October 4 (Table 1), although plants in these two plots grew during the same conducive period for viral infection in the infested field and showed similar virus incidences. Growth in the two plots before snow, when no symptoms were detected, was much different (Table 1). Thus the growth stage of wheat plants during the conducive period for infection seems to affect disease development in spring.

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#### Literature cited

1. Carroll, J.E., Bergstrom, G.C. and Gray, S.M. (1993). Temporal aspects of winter wheat infection by wheat spindle streak mosaic virus (WSSMV) in New York, USA. In Proceedings of the Second Symposium of the International Working Group on Plant Viruses with Fungal Vectors (Hiruki C. ed.), American Society of Sugar Beet Technologists, Denver, Colorado, pp. 79-82.
2. Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.
3. Ikata, S. and Kawai, I. (1940). Studies on the wheat yellow mosaic disease. Noji-kairyō-siryō, Ministry of Agriculture and Forestry 154: 1-123 (in Japanese).
4. Kusaba, T., Toyama, A., Yumoto, A. and Tatebe, Y. (1971). Studies on the ecology of soil-borne barley yellow mosaic and its control in two-rowed barley. Spec. Bull. Tottori Agric. Exp. Stn. 2: 1-208 (in Japanese).
5. Usugi, T. and Saito, Y. (1979). Relationship between wheat yellow mosaic virus and wheat spindle streak mosaic virus. Ann. Phytopathol. Soc. Jpn. 45: 397-400 (in Japanese).
6. Usugi, T., Kuwabara, T. and Tsuchizaki, T. (1984). Detection of barley yellow mosaic virus, wheat yellow mosaic virus and soil-borne wheat mosaic virus by ELISA. Ann. Phytopathol. Soc. Jpn. 50: 63-68 (in Japanese).
7. Usugi, T., Kashiwazaki, S., Omura, T. and Tsuchizaki, T. (1989). Some properties of nucleic acids and coat proteins of soil-borne filamentous viruses. Ann. Phytopathol. Soc. Jpn. 55: 26-31.

#### 和 文 摘 要

大藤泰雄・内藤繁男：低温下におけるコムギ縮萎縮ウイルス (WYMV) の冬コムギ (*Triticum aestivum*) 体内での増殖

盛岡における、コムギ縮萎縮ウイルスの冬コムギへの感染とコムギ体内での増殖の季節的過程について調査するために、圃場試験を行った。コムギ縮萎縮病感受性品種ナンブコムギでは、汚染圃場に播種およそ2ヵ月後の11月30日に初めてウイルスが根より検出された。その後、ナンブコムギの根・茎葉部におけるウイルス検出率およびウイルス濃度は、積雪下の5°C以下の温度条件で増加し、融雪前に高い水準に達した。同一圃場で通常の時期に播種したナンブコムギでは、発病率は96%であった。これに対し、抵抗性品種ハチマンコムギでは、ウイルス検出率は低く推移し、病徴も認められなかった。汚染圃場と未汚染圃場との間での相互移植試験では、春の病徴発現に有効なウイルスの感染は、10月中旬から11月上旬に起こっていた。これらの結果から、感染適期に感染し冬期間低温下で増殖したウイルスにより、病徴出現前の高いウイルス検出率・濃度と引き続く高率の発病が引き起こされることが、示唆された。