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Yukio Shirako
Tohoku University

Myron K. Brakke
University of Nebraska-Lincoln

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Spontaneous Deletion Mutation of Soil-borne Wheat Mosaic Virus RNA II

By YUKIO SHIRAKO*† AND MYRON K. BRAKKE

Agricultural Research Service, U.S. Department of Agriculture and Department of Plant Pathology, Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska 68583, U.S.A.

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SUMMARY

The wild-type (WT) isolate of soil-borne wheat mosaic virus has two species of rod-shaped virions 281 nm and 138 nm in length, which are designated as virions 1·0L and 0·5L, respectively. We reported previously that virions shorter than 0·5L arose in assay plants inoculated with separated and recombined 1·0L RNA and 0·5L RNA and suggested that these short virions were caused by deletion mutation of 0·5L RNA. We now report that these short virions arose after a period of several months in wheat plants that had been inoculated manually with unpurified WT isolate, and also when plants infected naturally in fields infested by the fungal vector, *Polymyxa graminis*, were grown at 17 °C. The sizes and relative proportions of virions shorter than 0·5L varied both from plant to plant and in the same plant sampled at different times. This indicates that the virions shorter than 0·5L arose by continued and spontaneous deletion mutation of 0·5L RNA.

Soil-borne wheat mosaic virus (SBWMV) (Brakke, 1971) has a bipartite RNA genome separately encapsidated in stiff, hollow rods 20 nm in diameter. Component I has virions 281 nm long and component II has virions 138 nm or less in length (Brakke, 1977; Tsuchizaki *et al.*, 1973; Shirako & Brakke, 1984). Because of the variety of lengths found in component II, it is convenient to designate the components by the relative lengths of the virions [1·0L for 281 nm, 0·5L for 138 nm, etc. (Shirako & Brakke, 1981, 1984)]. These designations apply to virions and to the corresponding RNA species whose mol. wt. is proportional to the length of the virion. In practice it is often more convenient to estimate the molecular weight of the RNA than to measure the lengths of the virions.

Virus isolated from field-grown plants had two components (1·0L and 0·5L), whereas that from plants in other fields had three components (1·0L, 0·5L and 0·35L) (Brakke, 1977). Repeated manual transfer of virus with three components gave rise to an isolate with only 1·0L and 0·35L (Tsuchizaki *et al.*, 1973; Brakke, 1977), which was designated as Lab 1 (Shirako & Brakke, 1984). We previously reported that SBWMV requires 1·0L RNA (RNA I) and either 0·5L RNA or 0·35L RNA (RNA II) for infection (Shirako & Brakke, 1984). Inoculation with 1·0L RNA and 0·35L RNA gave progeny of 1·0L and 0·35L only. However, inoculation with 1·0L RNA and 0·5L RNA gave progeny of 1·0L, 0·5L and also virions shorter than 0·5L. The sizes and the relative proportions of the virions shorter than 0·5L varied from one assay plant to another. Furthermore, a new isolate having 1·0L and 0·4L RNAs (Lab 2 isolate) was recovered from one of the assay plants. Lab 2 isolate produced 0·35L virions in addition to 1·0L and 0·4L in one of six infected plants after several months. From these results, we postulated that the virions shorter than 0·5L, including 0·35L of Lab 1 isolate, arose by deletion mutation of 0·5L RNA. However, it was unclear whether the deletion occurred spontaneously in the infected plants or *in vitro* either during RNA preparation, inoculation or both. In this paper, we report that the deletion mutation of 0·5L RNA occurs continually and spontaneously in infected wheat plants

† Present address: Faculty of Agriculture, Tohoku University, 1-1 Tsutsumidori–Amamiyamachi, Sendai 980, Japan.

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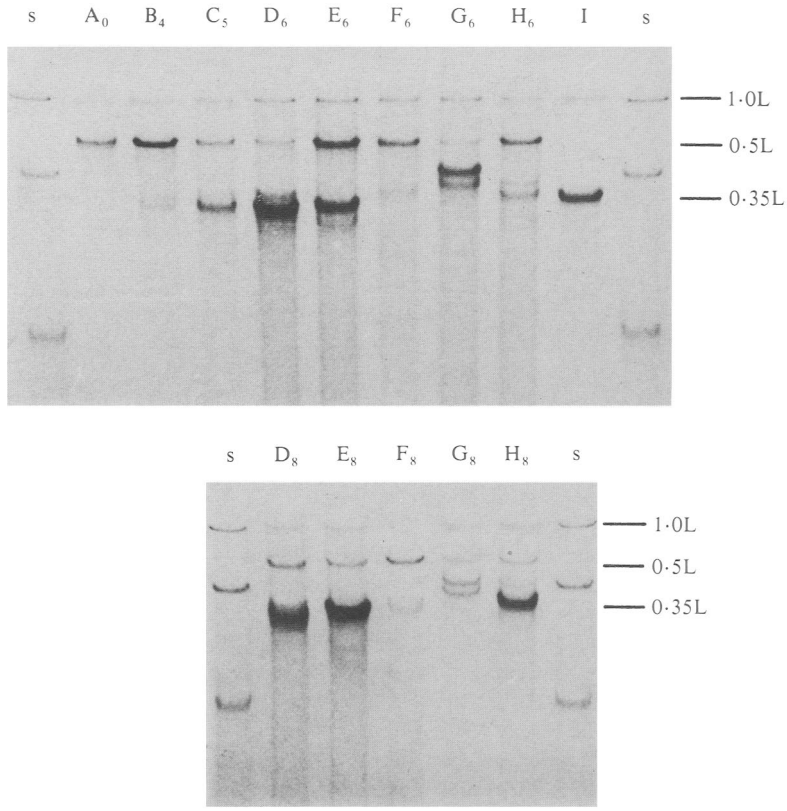

Fig. 1. Polyacrylamide–agarose composite gel pattern of progeny viral RNA produced after the manual inoculation of wheat plants with WT isolate. (A_0) WT RNA (1·0L RNA and 0·5L RNA) in the inoculum. (B_4) Progeny virus RNA 4 months after inoculation. (C_5) Progeny virus RNA 5 months after inoculation. In (B_4) and (C_5), virus was purified from bulked, infected plants. (D_6) to (H_6) Progeny virus RNA 6 months after inoculation. Virus was purified separately from each of five infected plants. (D_8) to (H_8) Progeny virus RNA 8 months after inoculation. Virus was purified separately from the same plants as at 6 months. (I) Lab 1 RNA (1·0L RNA and 0·35L RNA). (s) Molecular weight standards: tobacco mosaic virus RNA (mol. wt. $2{\cdot}19 \times 10^6$) and *Escherichia coli* ribosomal RNA (mol. wt. $1{\cdot}01 \times 10^6$ and $0{\cdot}53 \times 10^6$).

inoculated manually with WT isolate (1·0L and 0·5L) and also in plants infected in fields by the fungal vector, *Polymyxa graminis* Led. (Estes & Brakke, 1966).

Wheat plants (*Triticum aestivum* L. cv. 'Michigan Amber') at the two-leaf stage were inoculated by rubbing leaves with an extract of infected leaves in 0·1 M-K_2HPO_4 using Celite as an abrasive. The source leaves were collected from a field in early spring and were selected because gel electrophoretic analysis of RNA from virus purified from these leaves showed that only the 1·0L and 0·5L components were present (WT isolate) (Fig. 1, A_0). Inoculated plants were kept at 17 °C in a growth chamber with 20 klx of cool white fluorescent light. Four, 5, 6 or 8 months after inoculation, virus was purified from bulked or individual infected plants and the sizes and the relative proportions of viral RNA species were analysed in 2% acrylamide, 0·5% agarose composite gels after formaldehyde denaturation as previously described (Shirako & Brakke, 1984). Virus purified from bulked, infected plants 4 months after inoculation consisted of 1·0L and 0·5L virions with trace amounts of shorter virions (Fig. 1, B_4). The relative amounts of virions shorter than 0·5L increased during the succeeding month, after which more than half of component II virions were shorter than 0·5L (Fig. 1, C_5). Six months after inoculation, virus was purified separately from each of five infected plants. The sizes and relative proportions of

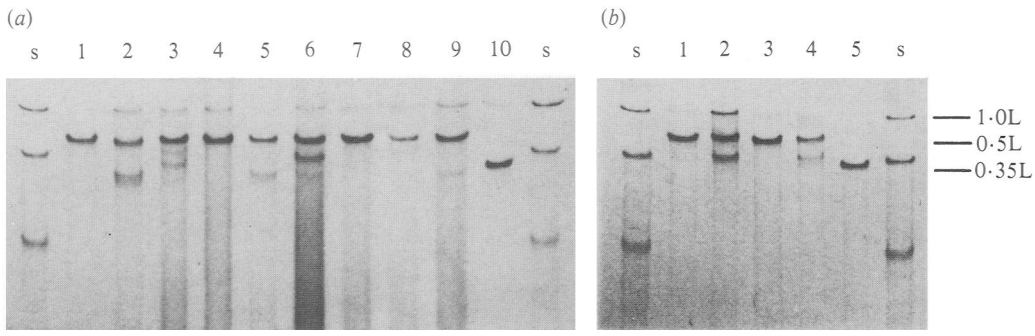

Fig. 2. Polyacrylamide–agarose composite gel pattern of virus RNA from naturally infected wheat plants. (*a*) (1) Virus RNA from the infested field in Nebraska in March. (2) to (9) Virus RNA from eight individual infected wheat plants which were collected in November from the same field as in (1) but grown in a greenhouse at 17 °C until the following March. (10) Lab 1 RNA. (s) Molecular weight standards (see Fig. 1). (*b*) (1) Virus RNA from the infested field 1 in Kansas in March. (2) Virus RNA from bulked, infected wheat plants which were collected in November from the same field as in (1) but grown in a greenhouse at 17 °C until the following March. (3) and (4) are the same combination as (1) and (2) but the plants were collected from field 2 in Kansas. (5) Lab 1 RNA. (s) Molecular weight standards (see Fig. 1).

the virions shorter than 0·5L varied from plant to plant (Fig. 1, D_6 to H_6). Virus from the five individual plants 8 months after inoculation gave results similar to those at 6 months, although there was an increase in the ratio of virions shorter than 0·5L to those of 0·5L in some plants (Fig. 1, E_8 and H_8).

In order to examine the occurrence of virions shorter than 0·5L in plants infected naturally in infested fields, wheat plants were collected in November from one field in Nebraska and from two in Kansas and then transplanted to pots and grown in a greenhouse at about 17 °C until spring. Field plants show no mosaic in November, the virus being confined to roots (Brakke *et al.*, 1965). Ten of 49 plants collected in Nebraska, 17 of 52 from field 1 in Kansas, and 5 of 32 plants from field 2 in Kansas had developed mosaic disease by March. Virus purified from bulked, infected plants collected in March from the Nebraska field had 1·0L and 0·5L virions only (Fig. 2*a*, 1). In contrast, virus purified separately from eight infected plants grown from November to March in the greenhouse at 17 °C contained additional virions shorter than 0·5L and, furthermore, the sizes and the relative proportions of these short virions varied from plant to plant (Fig. 2*a*, 2 to 9). Similar results were obtained with infected plants from the two fields in Kansas (Fig. 2*b*). Therefore, the virions shorter than 0·5L appeared in plants infected with vector-transmitted virus from field soil, provided the host plants were grown at 17 °C for several months. Furthermore, virus purified from bulked plants collected from two different fields in Nebraska in early April (average temperature, 9·3 °C) had 1·0L and 0·5L virions and no detectable shorter ones. However, when plants were collected from these two fields in early May (average temperature, 16·9 °C), virions shorter than 0·5L were present in addition to 1·0L and 0·5L. This suggests that the shorter virions also may form during continued growth of plants in the fields at the higher temperature.

Thus the virions shorter than 0·5L appeared in plants regardless of the mode of infection, whether manually with isolated 1·0L RNA and 0·5L RNA (Shirako & Brakke, 1984), manually with WT virions (1·0L and 0·5L), or naturally by the vector. The data are most easily explained by repeated deletion mutation of component II RNA in infected wheat plants. All virions of 0·5L and shorter are functionally component II, coding for capsid protein and probably other proteins (Tsuchizaki *et al.*, 1975; Hsu & Brakke, 1983). The alternative to continued formation of deletion mutants would be that several types of component II virions of RNA were present in all isolates of the virus. It is a question then of why one or another becomes dominant in a plant. We have never observed a shift from a shorter to a longer dominant virion. Virions of 0·5L may

be replaced by 0·4L, and 0·4L by 0·35L, but not the converse. This suggests that shorter RNAs have a competitive advantage over longer ones, as happens in other situations (Huang & Baltimore, 1977; Kacian *et al.*, 1972). If this is true and if the 0·35L RNA is always present, why do those of intermediate size between 0·35L and 0·5L become dominant in many plants? Having no good answer to this question, we conclude that deletion mutants occur continually with newly formed, highly competitive mutants eventually dominating the population. Hybridization with cDNA copies of each RNA II species or determinations of whole nucleotide sequences will be needed to prove the deletion hypothesis.

If deletion mutation of 0·5L RNA is occurring continually and spontaneously in the infected wheat plants, it would be expected that 0·35L would replace 0·5L in the fields. However, undeleted 0·5L still predominates over short virions in infected wheat plants in early spring. There might be several explanations for this apparent contradiction, such as (i) deletion mutants may not be transmitted by the vector, (ii) deletion mutants are produced and/or multiply only at higher temperatures, e.g. 17 °C in growth chambers, but not at low winter temperature, or (iii) plants infected with deletion mutants may not survive the winter because such mutants cause a more severe disease than does WT (Shirako & Brakke, 1984). In any case, the RNA sequence that is lost during mutation from 0·5L RNA to 0·35L RNA, approximately 1050 bases, is not required for infection and multiplication if the hosts are kept at warmer (15 to 17 °C) temperatures, but still must be essential for survival in fields.

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