Rapid Antigenic Modification of Wheat Streak Mosaic Virus in vitro Is Prevented in Glutaraldehyde-fixed Tissue

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SUMMARY

Reactions of virions of the potyvirus wheat streak mosaic virus (WSMV) with homologous antiserum in leaf-dips depended on the age of infection of the leaf. Virions from young and recently expanded leaves were completely or partially covered with immunoglobulin whereas virions from leaves infected earlier were not decorated. However, in ultrathin sections virions could be immunolabelled in leaves of all ages. These results suggest that the virions can be partially degraded upon extraction, particularly those from older leaves. Thus, a negative reaction with leaf-dip serology of a suspected WSMV infection does not in itself constitute evidence that the plant is not infected with WSMV. The same principle has implications for the routine serological identification of potyviruses in leaf-dips.

Several long flexuous viruses that have been reported to infect wheat (Triticum aestivum L.) in North America are agropyron mosaic virus (Slykhuis, 1973), hordeum mosaic virus (Slykhuis & Bell, 1966), wheat streak mosaic virus (WSMV) (Brakke, 1971) and wheat spindle streak mosaic virus (WSSMV) (Slykhuis, 1976). Only the latter two cause disease epidemics and are economically important in North American wheat production. In Nebraska and Kansas, WSMV and WSSMV epidemics can occur simultaneously in many geographical areas. Wheat samples which are suspected of being infected with either virus are routinely tested by the leaf-dip procedure of Ball & Brakke (1968) (as modified by Lin, 1984), hereafter referred to as leaf-dip. Flexuous virus particles that do not decorate in rabbit anti-WSMV antiserum are assumed to be WSSMV since it is the only other known flexuous virus infecting wheat naturally in this region. In 1987, a sample of wheat with symptoms similar to those resulting from WSSMV infection, i.e. chlorotic spindle-shaped lesions, was tested by leaf-dip serology with rabbit anti-WSSMV serum. The reaction was negative; no IgG decoration was observed. Hence, the virus was tentatively presumed to be WSSMV. However, the concentration of virus was moderate, more like that of WSMV than of WSSMV, which typically shows only very few particles in leaf-dips. This prompted further investigation. The results showed that WSMV is unusually susceptible to antigenic alteration in vitro even for a potyvirus. Leaf age appeared crucial in the routine serological identification of this virus.

Field-infected wheat leaves (T. aestivum L.) with symptoms of WSSMV infection were ground with water in a mortar and pestle. The sap was mechanically inoculated onto leaves of young wheat plants (second-leaf stage) of the cultivar 'Michigan Amber'. Other wheat plants were mechanically inoculated with WSMV type strain PV57 (American Type Culture Collection, 1980). The plants were rinsed with water after inoculation and maintained in a greenhouse at 28 to 30 °C without additional lighting.

Leaf tissue of the original field-infected sample plants and leaves of various ages from non-vernalized greenhouse-grown plants (mechanically inoculated 9 months earlier with PV57), were fixed and embedded in LR White resin as described (Langenberg, 1986). Ultrathin...
sections were labelled with the two-step method described by Lin & Langenberg (1983). Rabbit antiserum to WSMV was prepared as described (Brakke & Ball, 1968). Antiserum to the cylindrical inclusion body protein of WSMV was prepared as reported (Brakke et al., 1987), while antiserum to WSSMV was received from K. Zagula Haufler (Michigan State University, East Lansing, Mich. U.S.A.). Leaf-dips were made by the leaf-dip method, a rapid procedure by which detection of IgG on virions is greatly enhanced by immunogold-labelled goat anti-rabbit IgG.

Leaves of plants that had been mechanically inoculated with sap from the original field-infected plants showed mosaic symptoms within 5 days, and a leaf-dip made in anti-WSMV serum showed heavy decoration of all virus particles present. A leaf-dip from the same leaf in anti-WSSMV serum did not show decoration of any of the virions. Since the original field-infected source plants could have been doubly infected with WSMV and WSSMV, ultrathin sections from flag leaves of these plants were immunolabelled with rabbit anti-WSSMV. No virion aggregates were immunolabelled (Fig. 1). Virus-infected cells contained neither membranous inclusions, nor pinwheels with large, open arms which are characteristic of WSSMV infection of which the cylindrical inclusions (CI) and pinwheels would be similar to those observed with WSMV infections. Ultrathin sections of the original flag leaves were, therefore, also incubated with either rabbit anti-WSMV or rabbit anti-WSMV CI protein. All virion aggregates in ultrathin sections were labelled positively with anti-WSMV IgG (Fig. 2) as were all pinwheels and cylindrical inclusions with rabbit anti-WSMV CI serum (Fig. 3). Therefore, virus was WSMV and not WSSMV or a mixed infection of the two.

Results similar to those obtained with the flag leaves of field-infected wheat were observed in thin sections of older leaves of WSMV PV57-infected plants 9 months after inoculation (not shown). Moreover, leaf-dips made from tissue of the oldest green leaves of these plants, known to be infected with WSMV only, had virions that were not decorated with anti-WSMV IgG (Fig. 4a). Virions from young unfurling leaves of the same 9-month-old WSMV-infected plants were entirely decorated (Fig. 4b) with the procedure used for Fig. 4(a). In progressively older leaves some virions were decorated partially or not at all.
Fig. 2. Ultrathin section as in Fig. 1 treated with anti-WSMV serum, then with goat anti-rabbit IgG bound to colloidal gold. All virus aggregates (v) labelled. Some gold label is also present over cylindrical inclusions which is attributed to WSMV virion or capsid protein attachment to CI (see Langenberg, 1987). The infecting virus is, therefore, WSMV. Bar marker represents 250 nm.

Fig. 3. Ultrathin section as in Fig. 1 treated with anti-WSMV cylindrical inclusion body protein serum, followed by goat anti-rabbit IgG bound to colloidal gold. All cylindrical inclusions and pinwheels label positively, indicating that tissue is infected with WSMV only. Bar marker represents 250 nm.
Degradation of virions in cell sap and therefore no label with homologous antiserum could not be prevented by the crushing of fresh tissue from older leaves in a phosphate buffer containing 0.05% sodium azide or 0.1 M-sodium fluoride. The highly toxic sodium fluoride was used to stop some enzymic reactions (Sabatini et al., 1963). Sodium azide was routinely present in all phosphate buffers to prevent the growth of microorganisms and the presence of unknown lytic enzymes in the buffers used for leaf dips. Less degradation, shown by fewer particles partially decorated or undecorated (Fig. 4c), was seen when such leaf tissue pieces were crushed in a droplet of buffered 1% glutaraldehyde. Only when older leaf pieces were vacuum-infiltrated with buffered 1% glutaraldehyde just before being crushed in a droplet of the same buffered 1% glutaraldehyde, did all particles label positively and consistently. Glutaraldehyde was neutralized before IgG decoration of virions, by washing the grids with 0.1 M-ammonium acetate in 0.05 M neutral phosphate buffer.

Regardless of the age of the leaves sampled, all virion aggregates in ultrathin sections of WSMV-infected tissue were immunolabelled positively with anti-WSMV IgG. Thus, virions of WSMV were shown to occur in the non-degraded state in intact cells, even in older leaves.

A bias exists towards the utilization of young, newly infected plants or leaves. Because the youngest leaves from field collections survive shipment better and look fresher, they are normally selected for leaf-dip assays. Most research on virion purification or ultrastructural investigations uses plants specifically infected for these methods. It is for these reasons that differences in WSMV virion reactivity with homologous antiserum between older and young leaves went undetected in this laboratory prior to 1987.

The antiserum to WSMV used in the experiments described here was made to viruses which had been extracted from the youngest, fully expanded leaves (Brakke & Ball, 1968). A higher yield of virus can be extracted from these leaves than from others.

The coat protein of WSMV from older leaves often migrates faster in SDS-PAGE than that of virus from young leaves, indicating that virions in older leaves may have partially degraded coat protein (Lane & Skopp, 1983; Brakke et al., 1987). In this respect WSMV is probably not largely
different from other viruses in the potato virus Y (PVY) group. Hiebert & McDonald (1976), Shukla & Ward (1988), Shukla et al. (1988) and Stein et al. (1986) reported capsid protein heterogeneity in viruses of this group.

Antigenic differences in the PVY group result from capsid protein heterogeneity with resultant severe problems in separation and classification (Shukla & Ward, 1988; Shukla et al., 1988). Consequences for the identification of potyvirus virions in leaf-dips may be more serious than for identification of the specific virus by immunolabelling in ultrathin sections.

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REFERENCES


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