Effect of a Non-Localized Infection by Southern Bean Mosaic Virus on a Cell Wall Glycoprotein from Bean Leaves

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We thank D. Wuddah for technical assistance and L. C. Vining for his critical reading of the manuscript. Research supported by grants from the National Research Council of Canada.

Accepted for publication 19 June 1975.

ABSTRACT

A cell-wall-related glycoprotein was extracted from primary leaves of *Phaseolus vulgaris* L. 'Black Valentine'. The amount of glycoprotein in leaves systemically infected with southern bean mosaic virus (SBMV) was compared with that in abraded leaves and in untreated controls. It was recovered in significantly greater amounts at 24 and 72 hours from the abraded plants, and this response is attributed to wounding. Less glycoprotein was obtained from leaves that were similarly injured during inoculation with SBMV. This suggests that intercellular spread of SBMV was associated with suppression of some aspects of cell wall modification.

Phytopathology 65:1350-1351

Glycoproteins have been extracted from Pinto bean plants locally infected with tobacco necrosis virus (TNV) or tobacco mosaic virus (TMV) (1, 6), and from Black Valentine bean plants infected with SBMV. Similar glycoproteins were obtained from plants that had been mechanically injured (2). Their protein and carbohydrate composition suggest that they are associated with cell wall synthesis. A postinfection time study in plants with localized infections, indicated that the glycoproteins were formed in response to the wounding caused by transmitting the virus, and not through virus infection itself (6). We suggested that wound-induced redifferentiation of the cell wall may localize the virus if it occurs in advance of virus spread (6). Wu (8) has investigated the leaves of *Nicotiana glutinosa* infected by the very mild strain of TMV and has also suggested that the events of wound-healing may be responsible for limiting the spread of virus. However, he concluded that the effective wound-healing response was induced by necrobiosis of infected cells and not by the injury caused during inoculation.

The objective of the present study was to determine the effect of a nonlocalized virus infection on the amount of wound-enhanced glycoprotein in the host plant. For this purpose we infected the primary leaves of Black Valentine bean with SBMV.

MATERIALS AND METHODS.—*Plants and viruses.*—Conditions used for germination and growth of *Phaseolus vulgaris* L. 'Black Valentine' (Asgrow) were the same as described previously for the cultivar Prince (5). SBMV (American Collection - 17) was maintained on bean cultivar Bountiful and purified by the method of Grogan and Kimble (4). Bean cultivar Pinto was used as the local lesion test host for Black Valentine plants with systemic infection of SBMV.

For inoculation of Black Valentine plants, preparations of SBMV were diluted to 40 μg/ml with distilled water and 5% (w/v) of diatomaceous earth (Celite) added as an abrasive. This inoculum was applied with a brush to the upper surface of the primary leaves which were then rinsed with water. The relative virus content of the inoculated leaves was determined at 24-hour intervals in the following way. The primary leaves from 10 plants were weighed, macerated, strained through cheesecloth, and diluted proportionately with water. Celite (5%, w/v) was added and the extract assayed by infecting the primary leaves of the local lesion host Pinto. Relative virus contents were determined from the numbers of lesions per gram fresh weight produced in the assay plants.

Extraction of glycoproteins.—The primary leaves of 10-day-old Black Valentine plants were treated at 0900 hours in one of the following ways; (i) inoculated with SBMV, (ii) abraded (virus omitted from the inoculum) (iii) left uninoculated (control). Glycoproteins were extracted from primary leaf samples of 20 plants at 12-hour intervals up to 72 hours, and then at 24-hour intervals up to 120 hours using the procedures previously described (1). After the dialysis step, part of the non-dialyzable insoluble material was used for carbohydrate determination (7) to provide information on insoluble carbohydrate present in the glycoprotein material. The remainder was dried (100 C for 12 hours) and weighed.

RESULTS.—The amount of glycoprotein material measured as dry weight and as total carbohydrate in the recovered product is shown in Table 1. The results for abraded and control plants are similar to those obtained previously with Pinto bean plants (6). In the untreated control plants the amount of glycoprotein extracted was comparatively small and relatively constant during the period sampled. Wounding the plants by abrasion was associated with a marked rise in the quantity obtained at 24 hours. There was also a second period, between 60-96 hours during which levels of glycoprotein were obtained. By 96-120 hours the recovery had fallen and was similar to those found in the other two treatments. In SBMV-infected plants only minor changes in glycoprotein content occured, the amounts recovered being similar to those from untreated controls. Values for the relative virus content of the primary leaves (Table 2) indicate that the SBMV content continued to increase over the period sampled.

DISCUSSION.—The larger amounts of the glycoprotein detected in two peaks at 24 and 72 hours in the abraded plants and its absence from the untreated control plants implies that the substance is produced as a result of the wounding during inoculation. Except for the time at which the peaks occur this result is identical to that

TABLE 1. Amounts of glycoprotein material by weight and as carbohydrate in control, abraded, and southern bean mosaic virus (SBMV)-inoculated Black Valentine bean

Postinoculation time (hours)	Dry weight of glycoprotein (mg/g fresh weight)			Insoluble carbohydrate (mg/g fresh weight)		
	Control	Abraded	SBMV	Control	Abraded	SBMV
0	0.24[a]	0.35	0.26	0.073	0.069	0.061
12	0.19	4.27	0.16	0.062	0.787	0.031
24	0.48	15.69	0.46	0.068	2.689	0.064
36	0.62	10.61	0.29	0.119	1.487	0.092
48	0.55	0.46	0.19	0.107	0.105	0.075
60	0.45	4.37	0.21	0.063	0.640	0.066
72	0.47	7.02	0.12	0.077	1.293	0.045
96	0.48	0.37	0.16	0.061	0.068	0.067
120	0.52	0.22	0.19	0.039	0.063	0.050

[a]Each datum is the arithmetic mean from four tests except those at 12, 36, and 60 hours which are the means from three tests.

TABLE 2. Relative southern bean mosaic virus (SBMV) content in infected leaf samples from Black Valentine bean assayed on cultivar Pinto, a local lesion host

Postinoculation time, (hours)	Lesions on Pinto (no. per gram fresh weight)
0	0.00[a]
24	1.89
48	4.13
72	227.45
96	473.19
120	260.20

[a]Arithmetic mean from three tests with eight plants per treatment. Infected leaf extracts (1 g) were diluted to 5 ml (0, 24) 10 ml (48, 72, 96) and 20 ml (120 hours). An inoculum of SBMV (10 μg/ml) induced a mean of 372 lesions per gram fresh weight in three tests.

obtained with Pinto plants (6).

It has been suggested that wound-healing procedures are a normal part of the symptomology of virus infections (3). The significance of the association of wound healing with localized virus infection has recently been noted by Kimmins and Brown (6) and Wu (8) who have suggested that the process of redifferentiation may produce a zone of tissue which precludes, partially or completely, cell-to-cell spread of the virus. Both groups have postulated that successful localization would depend on the relative rates of virus spread and wound response. Kimmins and Brown (6) attributed the wound response to injury caused in transmitting the virus, whether by natural or artificial means, and it was anticipated that the level of glycoprotein in SBMV-infected Black Valentine leaves would increase for this reason. Localization would not occur only because intercellular spread of the virus would have taken place in advance of the wound response. Our unexpected failure to detect any increase in glycoprotein suggests that cell wall modification was suppressed by the SBMV infection. If wound healing is partly or entirely a postinfection response to necrobiosis of cells at the infection center as suggested by Wu (8), then the cycle of SBMV infection may involve less tissue damage and a slower or smaller wound-induced wall growth. However, our previous data (6) indicate that the wound response, as measured by glycoprotein production, does not depend on the presence of virus and can be attributed to the initial injury made to establish virus infection rather than postinfection wounding.

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