Serological Relationships Among Beet Western Yellows, Barley Yellow Dwarf, and Soybean Dwarf Viruses

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ABSTRACT

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The potential genetic vulnerability of substantial numbers of United States soybean cultivars to beet western yellows virus (BWYV), and the similarity of BWYV to soybean dwarf virus (SDV), a virus capable of inducing severe losses of soybean in Japan, led to serological studies between SDV and BWYV and other closely related viruses. Two antisera (from Japan) prepared against the dwarfing strain (SDV-DS) and the yellowing strain of SDV (SDV-Y) were tested against BWYV, turnip yellows virus (TuYV), beet mild yellowing virus (BMYV), and three isolates of barley yellow dwarf virus (BYDV). The virus-antiserum mixtures were subjected to density-gradient centrifugation and analyzed photometrically and by virus neutralization. Antiserum prepared against the SDV-Y and SDV-DS isolates of the SDV from Japan reacted with BWYV isolates from the United States and Europe, with BMYV from Europe, with TuYV from Europe, and with the RPV isolate of BYDV. The SDV antisera did not react with the MAV and PAV isolates of BYDV in a manner identical to the reactions of BWYV, TuYV, and BMYV antiserum with these two BYDV isolates. Neither saline nor antiserum to the beet yellows virus, or healthy shepherd's purse reacted with any of the virus isolates. Reciprocal tests with the SDV were not made.

Beet western yellows virus (BWYV) causes stunting and chlorosis of a wide range of dicotyledonous species in North America, Europe, and Asia. A number of BWYV strains or variants with distinctive host ranges occur naturally on many wild and cultivated species (11, 12).

The virus induces yellowing, leaf-rolling, and stunting of various legumes including *Cicer arietinum*, *Lathyrus odoratus*, *Pisum sativum*, *Trifolium alexandrinum*, *T. incarnatum*, and *Vicia faba* and has recently been reported to induce similar symptoms on soybean, *Glycine max* (L.) Merr. (3, 4, 8, 10).

Soybean dwarf virus (SDV) first was reported by Tamada et al. from Japan in 1969 (22). The virus, apparently restricted in host range to members of the Leguminosae, is transmitted in a persistent manner by the aphid *Acyrthosiphon* (*Aulacorthum*) *solani*. The disease is one of the most important maladies of soybean in Japan. In 1971 and 1972, the soybean crops in Hokkaido showed a high percentage of infection and severe production losses resulted (21).

The recent studies on the potential genetic vulnerability of substantial numbers of U.S. soybean cultivars to BWYV (8), the demonstrated economic significance of SDV on soybean, and the similarity of SDV to BWYV in regard to symptomology, vector transmission, particle morphology, and virus localization within phloem tissue prompted this study of the serological relationships among these viruses.

MATERIALS AND METHODS

Beet western yellows virus isolates tested in these studies came originally from radish (*Raphanus sativus* L.), sugarbeet (*Beta vulgaris* L.), broccoli (*Brassica oleracea* L. var *Botrytis* L.), and lettuce (*Lactuca sativa* L.), isolates 1, 3, 7, and E, respectively (4, 10). Turnip yellows virus (TuYV) was from turnip (*Brassica rapa* L.) and beet mild yellowing virus (BMYV) was from sugarbeet (11, 12). These virus isolates were maintained in desiccated plant tissue.

For activation of virus strains, desiccated tissue was ground in 0.05 M phosphate buffer (pH 7.0) containing 0.01 M glycine in the proportion of one part plant tissue (fresh wt) to one part diluent. These extracts were placed directly on sucrose density-gradients (20-60%); centrifuged 2 hr at 73,450 *g* in a Beckman SW 50.1 rotor; and, after dilution with buffer to 20% sucrose, the virus zones (18-26 mm from the top of the tubes) were fed to aphids (5).

Barley yellow dwarf virus isolates were obtained from W. F. Rochow (USDA-ARS, Cornell University, Ithaca, NY 14853) and were maintained in oats (*Avena byzantina* K. Koch) (16).

Nonviruliferous green peach aphids [*Myzus persicae* (Sulz.)] were reared on radish, and *Rhopalosiphum padi* (L.) and *Macrosiphum avenae* (Fab.) were maintained on virus-free oats.

The handling of aphids, strains of BWYV, membrane feeding technique, and antigen and antiserum preparation were as previously reported (6, 13). Extracts

for antigen preparations, infectivity neutralization, and antigen scanning pattern analysis were prepared from shepherd's purse [*Capsella bursa-pastoris* (L.) Medic.] infected with BWYV, BMYV, or TuYV. Extracts of BYDV were prepared from oats.

Frozen plant material was ground in a food grinder 1:1 with 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine and then homogenized at 45,000 rpm in a VirTis homogenizer. Crude extracts were heated to 45 C and then were clarified by low-speed centrifugation (20 min, 12,100 *g*). Clarified juice was ultracentrifuged (2 hr, 80,800 *g*). Pellets were resuspended in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine.

Gradient columns for scanning patterns were made by layering 4, 7, 7, and 7 ml, respectively, of 10, 20, 30, and 40% sucrose dissolved in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine. Centrifugation was done in a Beckman SW-25 rotor for 3 hr at 58,600 *g*. The gradient columns were scanned photometrically with an ISCO Model D density-gradient fractionator using the sensitive scale ($A^{254} = 0.5$).

All density-gradient fractions used in feeding extracts were adjusted to 20% sucrose (by dilution with buffer) before they were placed on the membranes. This dilution prepared the samples for membrane feeding by the test aphids. The resulting preparations were concentrated about 40 times greater than the crude juice.

Aphids that had fed through membranes were tested for virus transmission to shepherd's purse plants. Similar aphids from the same colony, but which did not have access to virus, also were tested on this host as controls.

Antisera to two isolates of SDV, ASSDV-DS (dwarfing strain) and ASSDV-Y (yellowing strain) were kindly supplied by T. Tamada (Hokkaido Central Agricultural Experiment Station, Naganuma, Hokkaido, Japan) (14).

RESULTS

Two antisera prepared against the dwarfing strain and yellowing strain of SDV were tested against the BWYV isolates, TuYV, BMYV, and against three isolates of BYDV. Antisera prepared against BWYV, TuYV, BMYV, the beet yellows virus (BYV), and healthy shepherd's purse extracts were tested against the same isolates and served as controls (Table 1). The virus-antiserum mixtures were subjected to density-gradient centrifugation, analyzed photometrically (1), and in the case of BWYV, TuYV, and BMYV, tested for virus neutralization (7) (Fig. 1) (Table 2).

A positive reaction in the case of BWYV, TuYV, and BMYV was based on the reduction or elimination of virus antigen in the scanning patterns of sucrose density-gradient columns and the reduction or elimination of infectivity in the normal virus zone. A positive reaction with isolates BYDV was based on the reduction or elimination of virus antigen in the scanning patterns of sucrose density-gradient columns.

Antiserum prepared against the two strains of SDV from Japan reacted positively with BWYV isolates from the United States and Europe, with BMYV from Europe, with TuYV from Europe, and with the RPV strain of BYDV. The antiserum did not react with the MAV and PAV isolates of BYDV in a manner identical to the reaction of BWYV, BWYV-E, TuYV, and BMYV antiserum to these same isolates. Antiserum to the beet yellows virus, healthy shepherd's purse, and saline did not react with any of the virus isolates. Reciprocal tests with the SDV were not made because the antigen was not available.

DISCUSSION

A number of viruses which cause reddening and cupping of the leaves of affected plants and are transmitted in a persistent manner by aphids form a natural group, the luteoviruses (20). Characterizations of the luteoviruses have described small (25-30 nm diameter) isometric particles which are confined to the phloem of infected plants and are not mechanically transmissible.

Serological studies have established close reciprocal relationships between BWYV as it occurs in the United States and Europe and malva yellows virus (Duffus,

TABLE 1. Serological interactions of antiserum to isolates of the yellowing (Y) and dwarfing strain (DS) of soybean dwarf virus (SDV) with isolates of beet western yellows virus (BWYV), turnip yellows virus (TuYV), and barley yellow dwarf virus (BYDV)

	Antigen[b]								
Antiserum[a]	BWYV-1	BWYV-3	BWYV-7	BWYV-E	TuYV	BMYV	BYD-PAV	BYD-RPV	BYD-MAV
ASSDV-Y	+[c]	+	+	+	+	+	−	+	−
ASSDV-DS	+	+	+	+	+	+	−	+	−
ASBWYV	+	+	+	+	+	+	−	+	−
ASBWYV-E	+	+	+	+	+	+	−	+	−
ASTuYV	+	+	+	+	+	+	−	+	−
ASBMYV	+	+	+	+	+	+	−	+	−
ASBYV	−	−	−	−	−	−	−	−	−
ASHSP	−	−	−	−	−	−	−	−	−
Saline	−	−	−	−	−	−	−	−	−

[a]Antisera to different strains of virus and controls: antiserum to beet yellows virus (ASBYV) (BYV) and healthy shepherd's purse (ASHSP) (HSP).

[b]The virus samples were obtained from frozen plant material, clarified by heating to 45 C and low-speed centrifugation, and pelleted by ultracentrifugation. Pellets were resuspended in buffer to approximately 1/130 of the original volume of sap. The sample was mixed with an equal volume of serum and incubated 0.5 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation (3 hr at 58,600 *g* in a Beckman SW 25 rotor) and analyzed photometrically and by virus neutralization.

[c]A positive reaction with BWYV, TuYV, and BMYV was based on the reduction or elimination of virus antigen in scanning patterns and in infectivity tests. A positive reaction with BYDV was based on the reduction or elimination of virus antigen in scanning patterns.

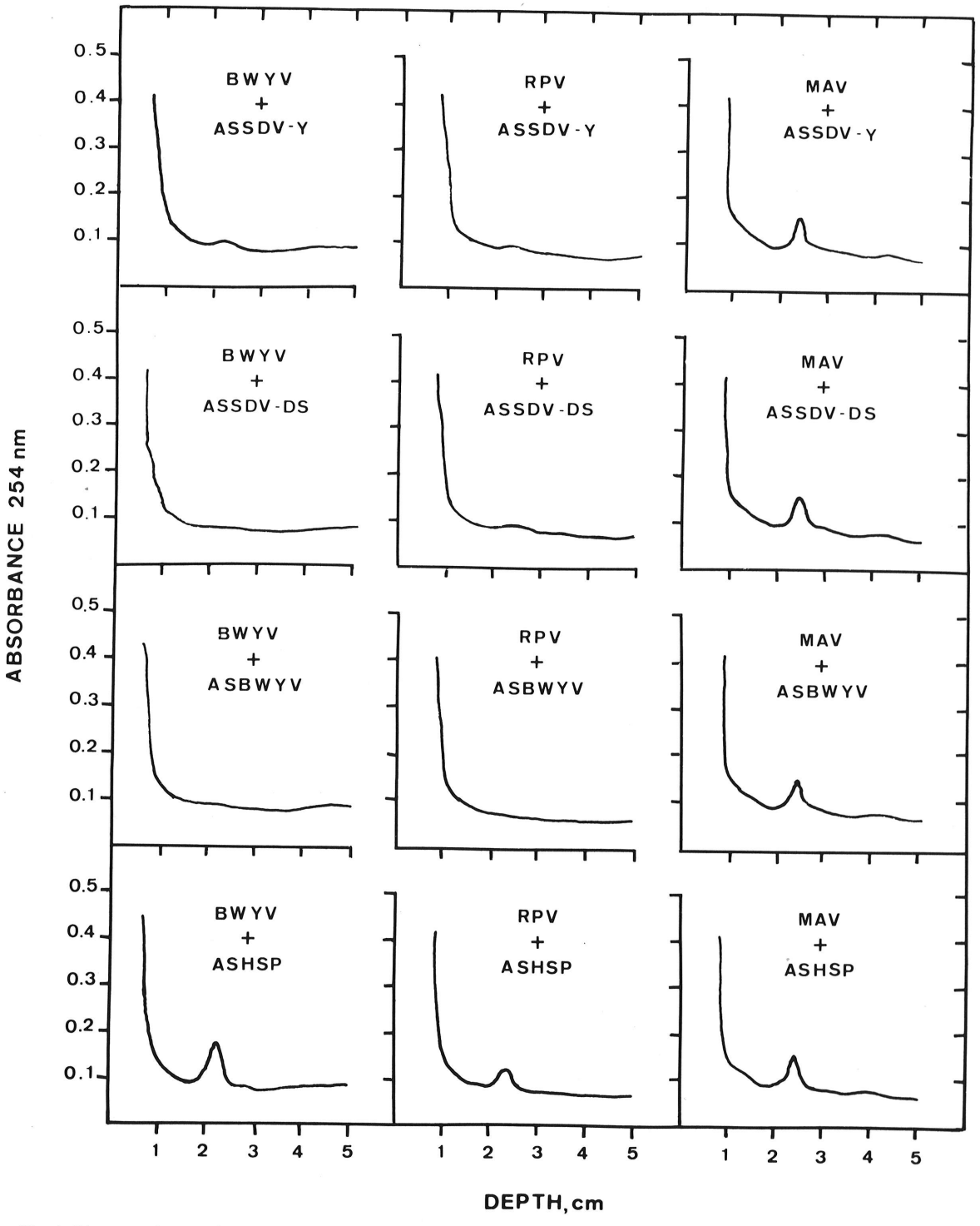

Fig. 1. Photometric scanning patterns of mixtures of beet western yellows virus (BWYV), the RPV and MAV isolates of barley yellow dwarf virus, and antisera against the soybean dwarf virus (SDV), BWYV and controls. The virus samples were obtained from frozen plant material, clarified by heating to 45 C and low-speed centrifugation (20 min at 12,100 *g*), and pelleted by ultracentrifugation (2 hr at 80,800 *g*). The virus sample was mixed with an equal volume of serum (ASSDV-Y, antiserum against the yellowing strain SDV; ASSDV-DS, antiserum against the dwarf strain SDV; ASBWYV, antiserum against BWYV; ASHSP, antiserum against healthy shepherd's purse) and incubated 0.5 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation (3 hr at 58,600 *g*) and analyzed photometrically.

unpublished), TuYV from England and Germany (11), and BMYV from England (12) and Germany (Duffus and Nagi, *unpublished*). A reciprocal relationship between BWYV and the RPV isolate of BYDV also has been established (9, 19). Antiserum prepared against the MAV and PAV isolates of BYDV reacted positively with BWYV (9), but the reciprocal reaction was negative (19). It should be pointed out that other studies showed a close serological relationship between MAV and PAV, but also revealed that RPV was distinct from the other two isolates (18).

Duffus and Gold (7) found no serological relationship between BWYV and potato leaf roll virus; and, in recent studies, Murayama and Kojima (15) showed no close serological relationship between the SDV and potato leaf roll virus.

The results of these experiments demonstrate a serological relationship between SDV from Japan and BWYV from the United States and Europe, BMYV from Europe, TuYV from Europe, and with the RPV strain of BYDV. The reaction of both SDV antisera, although not tested in a reciprocal manner, was identical to the reaction of several BWYV antisera to all the antigens tested, including the strains of BYDV, which probably indicates a close relationship of SDV to BWYV.

Soybean dwarf virus has a reported host range restricted to the legumes and is transmitted only by the foxglove aphid, *Acyrthosiphon* (*Aulacorthum*) *solani* and not by *Myzus persicae*, or *Acyrthosiphon pisum*. Thus, SDV differs significantly from commonly encountered strains of BWYV from United States, Europe, and Japan. It should be remembered, however, that many variants of BWYV have been distinguished on the basis of host range and virulence (4).

Different variants seem to predominate in different plant species. For many years, malva yellows virus was considered to be a distinct virus, based on its characteristic symptoms and host relations (2). Typical variants of this virus have been shown to be closely related serologically to more typical BWYV (Duffus, *unpublished*). This is also true for TuYV, that until recently was known to occur only in Europe and was thought mainly to attack members of the Cruciferae (11). Serological evidence also indicates that BMYV, thought for many years to be distinct on the basis of host range and epidemiology, is closely related to BWYV (12). Thus, in Europe for a number of years, at least three virus strain types have been identified. These appeared to be separate entities based on host and epidemiological differences, but now appear to be closely related serologically.

Soybean dwarf virus is the first member of a group of legume yellowing viruses found in various parts of the world to be characterized. Soybean dwarf virus, as well as other yellowing viruses of legumes, have seemed to be a distinct group based on host range and vector specificity. However, the serological relationship to BWYV may indicate that BWYV plays a role in the yellowing diseases of legumes and perhaps involves strain types transmitted by different aphid species with affinities for this group of plants.

The existence of susceptibility and resistance in soybean cultivars to BWYV, first introduced into the United States over 65 yr ago, could indicate that active selection for resistance to BWYV took place in Asia before the present century (8). Studies on susceptibility and resistance to BWYV and SDV in Japanese and United States soybean cultivars may further elucidate the relationships of these viruses.

Beet western yellows virus presents a potential threat to the soybean industry of the United States. The recently published work on the susceptibility of a broad range of soybean cultivars to BWYV (8), coupled with the results of the studies reported here which show a serological relationship of BWYV to SDV (a virus that causes severe losses in soybean production in Japan) indicate that these viruses may be as closely related as strains, and may occur together in certain legumes. Thus, they could, through a mechanism such as heterologous encapsidation, as suggested by Rochow (17), produce a new pathogen with broad vector and host range base, which would be capable of causing severe soybean losses.

The isolates of BWYV tested in soybean susceptibility studies (8) were collected in California on sugarbeet and cruciferous crops. Attempts should be made to collect

TABLE 2. Effect of antisera of the yellowing (Y) and dwarfing strains (DS) of soybean dwarf virus (SDV) on the aphid transmission of beet western yellows virus (BWYV), turnip yellows virus (TuYV), and beet mild yellowing virus (BMYV)

	Infectivity of virus zone					
Antiserum[a]	BWYV-1[b]	BWYV-3	BWYV-7	BWYV-E	TuYV	BMYV
ASSDV-Y	5[c]	3	1	1	3	0
ASSDV-DS	1	3	0	2	2	0
ASBWYV-E	0	0	0	0	0	0
ASTuYV	1	0	0	0	0	0
ASBMYV	0	0	0	0	0	0
ASBYV	20	20	19	20	20	16
ASHSP	20	20	20	20	20	14

[a] Antisera to different strains of SDV and controls. Antiserum to beet yellows virus (ASBYV) antiserum to healthy shepherd's purse (ASHSP).

[b] The virus samples were obtained from infected shepherd's purse, clarified by heating to 45 C and low speed centrifugation, and pelleted by ultracentrifugation. Pellets were resuspended in buffer to approximately 1/130 of the original volume of sap. The virus sample was mixed with an equal volume of serum and incubated 0.5 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation (3 hr at 58,600 *g* in a Beckman SW 25 rotor) and samples for infectivity assays were removed from the virus zone as determined photometrically.

[c] The number of plants infected of 20 inoculated with groups of 25 green peach aphids accession-fed on each sample through membranes.

yellowing isolates from commercial soybean fields in the USA and to compare the serology and host range and crop damage of these isolates with those of BWYV and SDV.

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