Characterization of Peanut Stunt Virus Strains by Host Reactions, Serology, and RNA Patterns

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ABSTRACT

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Thirteen peanut stunt virus (PSV) isolates were characterized by host and serological reactions. Reactions in gel diffusion tests were used to separate the isolates into four serotypes: I, II, III, and IV. Isolates of serotype III, a new serotype, infected Davis and Bansei soybean but did not infect peanut or Perfected Wales pea. Isolates in serotypes I, II, and III were closely related serologically but distantly related to serotype IV (PSV-W). All PSV isolates had RNA 1–4 of similar molecular weights. An RNA 5 was found in the two PSV isolates tested in serotypes I, II, and III but not in PSV-W. Relative infectivity of six of the PSV isolates on white clover was determined by inoculating plants of 20 different white clover clones. Infectivity of the six isolates varied among clover clones.

Peanut stunt virus (PSV) is economically important and has been reported from the United States (18), Japan (23), France (6), Spain (5), the U.S.S.R. (14), Hungary (2), Poland (24), and Morocco (7). PSV naturally infects peanut (*Arachis hypogaea* L.) (19), bean (*Phaseolus vulgaris* L.) (11,23), soybean (*Glycine max* (L.) Merr.) (12), white clover (*Trifolium repens* L.) (21), red clover (*T. pratense* L.) (2), arrowleaf clover (*T. vesiculosum* L.) (16), crimson clover (*T. incarnatum* L.) (16), subterranean clover (*T. subterraneum* L.) (16), alfalfa (*Medicago sativa* L.) (5), crownvetch (*Coronilla varia* L.) (22), hoary-pea (*Tephrosia* sp.) (25), tobacco (*Nicotiana tabacum* L.) (10), celery (*Apium graveolens* L.) (24), yellow lupine (*Lupinus luteus* L.) (24), and pea (*Pisum sativum* L.) (4). Strains of PSV have been found since the virus was first described in the United States in 1966 (18). In 1969, Mink et al reported PSV-W as a new serotype of PSV different from PSV-E (21). Subsequently, PSV-J (23), PSV-V (3), PSV-H (6), PSV-B (5), and PSV-T (25) were reported; most are closely related serologically to PSV-E (PSV-V is closely related but serologically different from PSV-E), except for PSV-J and PSV-B, which are more closely related to PSV-W. In 1979, Beczner and Devergne (2) reported a new PSV strain, PSV-Tp, from *T. pratense*. PSV-Tp is distinct from the two serotypes PSV-V and PSV-W (2).

Information on the variability of PSV was needed to help determine criteria used to identify and select for PSV resistance in white clover. We collected 13 PSV isolates from several states in the United States and determined their relationship by indicator host reactions and serology. The relative infectivity of selected PSV isolates on different genotypes of white clover also was examined.

MATERIALS AND METHODS

Thirteen PSV isolates were obtained from diverse sources and maintained in *T. repens* and *N. tabacum* 'White Burley' plants. Sources of the PSV isolates were: PSV-W (G. I. Mink, Washington State University, Prosser); PSV-V (PSV-74-23) and PSV-76-69 (S. A. Tolin, Virginia Polytechnic Institute and State University, Blacksburg); PSV-E (T. T. Hebert, North Carolina State University, Raleigh); PSV-1, PSV-3, and PSV-5 (various clovers from Alabama, E. M. Clark, Auburn University); PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, and PSV-101-16 (white clover clones from virus resistant screening program, Clemson, SC); and PSV-2 (Worthmore cowpea from Clemson, SC, M. R. McLaughlin).

Host reactions of the PSV isolates were compared on several selected indicator plants (Table 1) grown in a greenhouse. Four or five plants of each species were sap-inoculated with infected leaf tissue ground in 0.03 M sodium phosphate buffer, pH 8.0. Inoculation of some species was repeated. Inoculated and noninoculated leaves were tested by enzyme-linked immunosorbent assay (ELISA) to determine if plants were infected.

Sources of antisera were: PSV-W (G. I. Mink), PSV-T (PVAS-249, American Type Culture Collection, Rockville, MD), and PSV-B2 homologous serum for PSV-76-69 (S. A. Tolin). Antisera to PSV-2 and PSV-E were prepared in rabbits; an intravenous injection of 1 mg of virus was followed by biweekly subcutaneous injections of 1 mg of virus (emulsified with Freund's complete adjuvant for the first injection and with Freund's incomplete adjuvant for subsequent injections). Serum collections began after the third injection.

Gel double-diffusion serological tests were conducted in 0.8% agarose in 0.03 M phosphate buffer, pH 7.0, plus 0.1% NaN_3, with wells 4 mm apart. Antigens for gel diffusion serology were in sap extracted in 0.03 M sodium phosphate buffer, pH 7, plus 0.005 M DIECA, from PSV-infected leaves of white clover, peanut, or tobacco plants.

PSV isolates were propagated in cowpeas and purified by the Francki et al protocol with modifications (26). PSV-RNA was extracted by incubation in proteinase K overnight at room temperature, heated at 60 C for 5 min and cooled rapidly. Then, 2.5 vol of phenol-hydroxyquinoline (0.1%):*m*-cresol (9:1, v/v) were added, mixed with a Vortex mixer for 2 min, and centrifuged at 3,000 *g* for 15 min. Cold (−20 C) isopropanol (5 vol) was added to the aqueous phase and held overnight at −18 C. Precipitated RNA was collected by centrifugation at 12,000 *g* for 40 min and resuspended in electrophoresis buffer (0.04 M Tris, 0.02 M sodium acetate, 0.001 M Na_2EDTA, pH 7.2). Electrophoresis of RNA was at 5 mA/gel for 3.5 hr in 1.8% acrylamide/0.5% agarose gels made in electrophoresis buffer containing 0.2% SDS. Tobacco mosaic virus RNA (2.05×10^6 d), *Escherichia coli* 23S RNA (1.07×10^6 d), and *E. coli* 16S RNA (0.55×10^6 d) were used as standards. The gels

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were scanned with a GCA-McPherson spectrophotometer equipped with a gel scanning attachment. The quantitative estimation of the RNA component composition was done by the method of Lot and Kaper (15).

The *T. repens* clones were selected from two levels of a screening program designed to identify PSV resistance. All clones were screened by sap- and aphid-inoculation for resistance to PSV-E and assigned to group A if field-infected and to group B if not field-infected (8). Two other clones with known susceptibility to PSV-E were chosen as inoculation controls. Selected *T. repens* clones were propagated by stolon cuttings. Ten plants of each clone were sap-inoculated, four times at 3- to 4-day intervals, with each of six PSV isolates. Ten plants of each clone were in a row, and 10 or 12 rows, corresponding to 10 or 12 clones receiving the same virus isolate, were grouped together on the greenhouse bench. This was done twice so that, for most clones, 20 plants of each clone were inoculated with each isolate and indexed for infection. Infections were determined (by ELISA tests of noninoculated leaves) 4 wk or longer after the last inoculation. ELISA tests were done with immunoglobulin from the PSV-B2 antiserum as previously described (17).

RESULTS

Host reactions. Reactions of indicator plants to the 13 PSV isolates are shown in Table 1. Isolates were divided into three groups, primarily on the basis of their reactions on Bansei and Davis soybean, Perfected Wales pea, and peanut. One group, isolates PSV-W, PSV-1, PSV-3, PSV-5, and PSV-E, infected Perfected Wales pea and peanut but not Davis or Bansei soybean. A second group, isolates PSV-76-69, PSV-2, and PSV-V, infected both soybean varieties, Perfected Wales pea, and peanut. A third group, isolates PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, and PSV-101-16, infected both soybean varieties but either did not infect Perfected Wales pea and peanut or infected them at a low frequency (e.g., PSV-60-4 and PSV-65-24 infected two of 10 inoculated pea plants). On Blackeye cowpea, isolates in the first group usually caused severe symptoms, including leaf malformation, ring spots, and necrotic lesions, whereas isolates of the third group caused relatively mild mosaic symptoms. In the first group, PSV-W caused malformation and systemic necrotic spots on Perfected Wales pea and malformation of pinto bean, whereas the other isolates caused a mild mosaic on these plants. In the second group, PSV-2 caused only a faint mosaic on pinto bean, whereas PSV-V and PSV-76-69 caused dwarfing.

All PSV isolates induced local lesions on *Chenopodium amaranticolor* Coste & Reyn. and both necrotic local lesions and chlorotic systemic spots on *Gomphrena globosa* L.; symptoms on *G. globosa* varied in severity with the virus isolate. None of the PSV isolates infected Rutgers tomato (*Lycopersicon esculentum* Mill.), and some isolates (PSV-5, PSV-60-4, PSV-2, PSV-V, and PSV-76-69) caused systemic mosaic on Chicago Pickling cucumber (*Cucumis sativus* L.).

Serological tests. Sap extracted from infected white clover and peanut plants consistently gave sharp precipitin lines in gel diffusion tests, but sap from infected tobacco did not. Two precipitate bands were formed with some virus-isolate/serum combinations, a straight band near the central well and a curved band near the antigen. The curved band was used to place the 13 isolates into different serotypes by the presence or absence of spurs between bands from adjacent antigens.

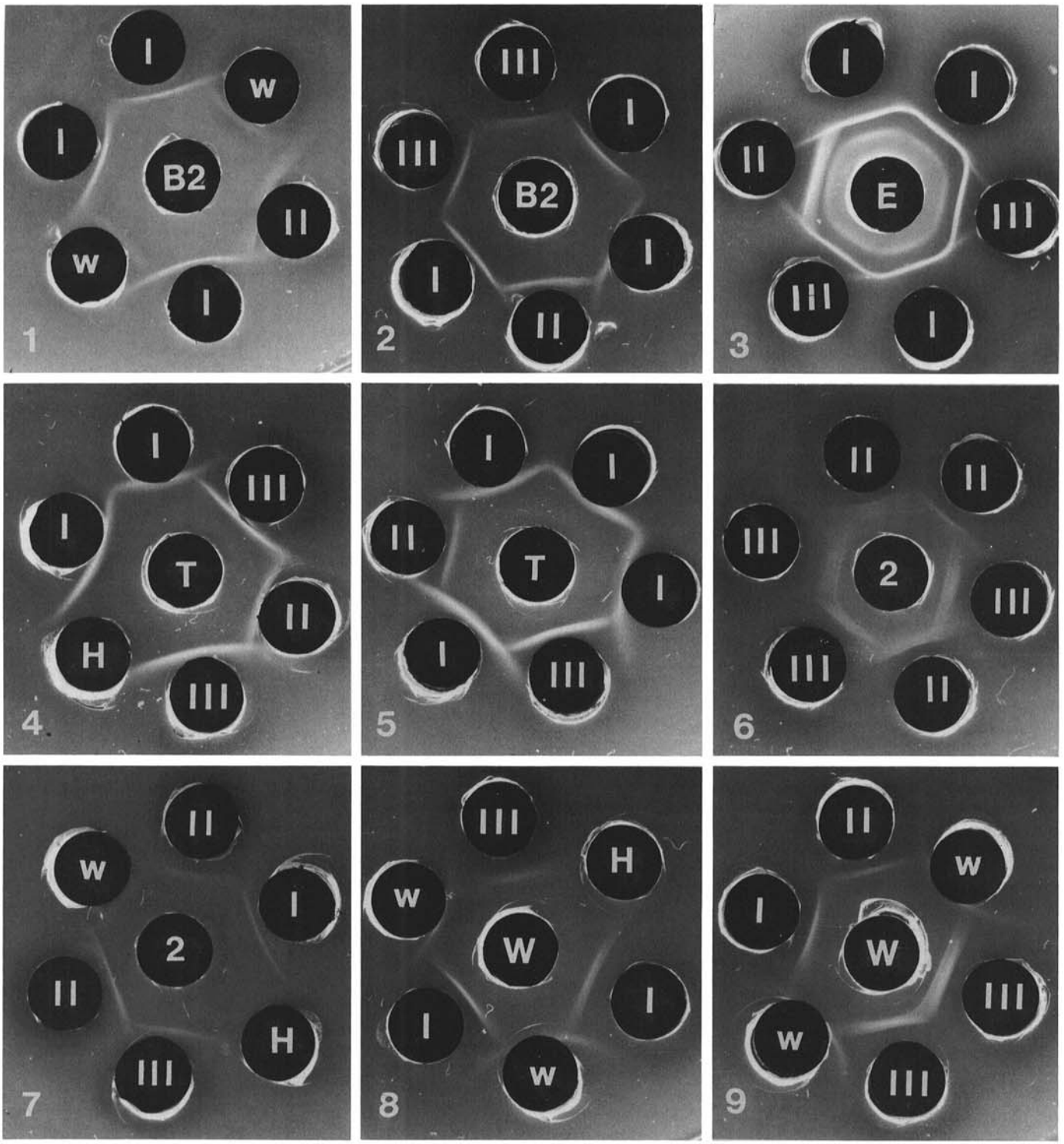
On the basis of serological reactions with five antisera (Table 2), the 13 isolates were grouped into four serotypes. Serotype I included PSV-1, PSV-3, PSV-5, PSV-E, and PSV-76-69; serotype II included PSV-2 and PSV-V; serotype III included PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, and PSV-101-16; and serotype IV comprised only PSV-W. The precipitin lines among isolates within each serotype were confluent in tests with all five antisera. The confluent reaction lines between serotype I and serotype III isolates with PSV-B2 antiserum and between serotype I and serotype II isolates with PSV-2 antiserum indicated that these three serotypes were closely related (Figs. 1, 2, 6, and 7).

TABLE 1. Reactions of indicator plants to 13 PSV isolates

PSV isolates	Soybean cv. Davis		Soybean cv. Bansei		Pea cv. Perfected Wales		Peanut		Pinto bean		Cowpea cv. Blackeye		Cucumber cv. Chicago Pickling		*Chenopodium amaranticolor*		*Gomphrena globosa*		Tomato cv. Rutgers	
	I/P[a]	Symptoms[b]	I/P	Symptoms	I/P	Symptoms	I/P	Symptoms	I/P	Symptoms	I/P	Symptoms	I/P	Symptoms	I/P	Symptoms	I/P	Symptoms	I/P	Symptoms
First group																				
PSV-W	0/4	...	0/1	...	5/5	Mo,Ma,SN	3/4	Stu	5/5	Epi,Mo/Mo,Ma	5/5	LLcn/Mo,Chls	0/0	...	0/0	...	0/0	...	0/6	...
PSV-1	0/7	...	0/2	...	6/9	Mo	8/8	Stu	3/8	Epi/slight Mo	5/5	LLcn/RS,Ma,NL	0/5	...	4/4	LLcn+/–	4/4	LLn+/Chls++	0/6	...
PSV-3	0/6	...	0/3	...	6/7	Mo	5/7	Stu	6/8	Epi/slight Mo	5/5	LLcn/RS,Ma,NL	0/5	...	4/4	LLcn++/–	4/4	LLn+/Chls+	0/6	...
PSV-5	0/7	...	0/2	...	8/8	Mo	6/9	Stu	8/8	Epi/Mo	5/5	LLcn/RS,Ma,NL	4/5	Mo,VN	4/4	LLcn++/–	4/4	LLn+/Chls+++	0/6	...
PSV-E	0/7	...	0/2	...	9/9	Mo	6/8	Stu	7/7	Epi/Mo	5/5	LLcn/Mo,Chls	0/5	...	4/4	LLcn+/–	4/4	LLn+/Chls++	0/6	...
Second group																				
PSV-76-69	2/5	Mo	1/2	Mo	9/9	Mo	4/8	Stu	8/8	Epi/Mo,Dr	0/0	...	3/5	Mo	4/4	LLcn+++/–	4/4	LLn++/Chls+++	0/6	...
PSV-2	4/8	Mo	0/2	...	4/6	Mo	0/7	...	7/9	Epi/slight Mo	5/5	LLcn/RS,Ma,NL	1/5	Mo	4/4	LLcn+/–	4/4	LLn+++/Chls+++	0/6	...
PSV-V	4/6	Mo	2/2	Mo	3/8	Mo	4/6	Stu	8/8	Epi/Mo,Dr	0/0	...	4/5	Mo	4/4	LLcn+/–	4/4	LLn+++/Chls++	0/6	...
Third group																				
PSV-60-4	6/7	Mo	1/2	Mo	1/8	Mo	0/8	...	8/8	Epi/Mo	5/5	LLcn/Mo	1/5	Mo	4/4	LLcn+/–	4/4	LLn+/Chls++	0/6	...
PSV-65-24	2/6	Mo	2/2	Mo	1/8	Mo	0/7	...	8/8	Epi/Mo	5/5	LLcn/Mo	0/5	...	4/4	LLcn+++/–	4/4	LLn+++/Chls++	0/6	...
PSV-80-11	6/7	Mo	2/2	Mo	0/8	...	0/4	...	7/9	Epi/Mo	5/5	LLcn/Mo,Chls	0/4	...	4/4	LLcn+++/–	4/4	LLn+++/Chls++	0/6	...
PSV-100-26	5/6	Mo	1/2	Mo	0/9	...	0/3	...	7/8	Epi/Mo	5/5	LLcn/Mo	0/5	...	4/4	LLcn+/–	4/4	LLn++/Chls+	0/6	...
PSV-101-16	3/7	Mo	0/2	...	0/9	...	0/4	...	7/9	Epi/Mo	5/5	LLcn/Mo,Chls	0/5	...	4/4	LLcn+/–	4/4	LLn++/Chls+	0/6	...

[a]Number of plants infected/number of plants inoculated.
[b]Inoculated leaves/noninoculated leaves. + = Few, ++ = several, +++ = many local lesions; – = no infection. Chls = chlorotic spots, Dr = dwarf, Epi = epinasty, LLcn = local chlorotic and necrotic lesions, LLn = local necrotic lesions, Ma = malformation, Mo = mosaic, NL = necrotic lesions, RS = ring spots, SN = systemic necrosis, Stu = stunt, VN = vein necrosis.

Among these three serotypes, serological differences were observed. With PSV-T and PSV-E antisera, serotype I isolate reaction lines formed spurs over serotype III isolate reaction lines and serotype III spurred over serotype II isolate reaction lines; with PSV-B2 antiserum, serotype I or III spurred over serotype II isolate reaction lines; and with PSV-2 antiserum, serotype I or II spurred over serotype III isolate reaction lines (Figs. 1–7). The serotype IV isolate was different from all other serotypes because PSV-W formed straight reaction lines that usually did not touch reaction lines of other isolates with PSV-B2, PSV-E, PSV-T, or PSV-2



Figs. 1–9. Reactions of 13 PSV isolates with antisera to PSV-B2, PSV-E, PSV-T, PSV-2, and PSV-W in gel double-diffusion serological tests. **1,** With antiserum to PSV-B2. Antigens (clockwise from top well): I = PSV-1, W = PSV-W, II = PSV-2, I = PSV-5, W = PSV-W, I = PSV-E. **2,** With antiserum to PSV-B2. Antigens: III = PSV-65-24, I = PSV-E, I = PSV-76-69, II = PSV-V, I = PSV-E, III = PSV-60-4. **3,** With antiserum to PSV-E. Antigens: I = PSV-E, I = PSV-3, III = PSV-101-16, I = PSV-E, III = PSV-60-4, II = PSV-2. **4,** With antiserum to PSV-T. Antigens: I = PSV-5, III = PSV-60-4, II = PSV-2, III = PSV-101-16, H = healthy tissue, I = PSV-E. **5,** With antiserum to PSV-T. Antigens: I = PSV-3, I = PSV-E, I = PSV-5, III = PSV-60-4, I = PSV-1, II = PSV-2. **6,** With antiserum to PSV-2. Antigens: II = PSV-2, II = PSV-V, III = PSV-100-26, II = PSV-2, III = PSV-100-26, III = PSV-80-11. **7,** With antiserum to PSV-2. Antigens: II = PSV-2, I = PSV-76-69, H = healthy tissue, III = PSV-60-4, II = PSV-2, W = PSV-W. **8,** With antiserum to PSV-W. Antigens: III = PSV-60-4, H = healthy tissue, I = PSV-E, W = PSV-W, I = PSV-76-69, W = PSV-W. **9,** With antiserum to PSV-W. Antigens: II = PSV-V, W = PSV-W, III = PSV-80-11, III = PSV-65-24, W = PSV-W, I = PSV-3.

TABLE 2. Serological reactions of 13 PSV isolates with antisera to PSV-B2, PSV-T, PSV-E, PSV-2, and PSV-W

Antigen reaction type[a]	PSV-B2 antiserum	PSV-T, PSV-E antisera	PSV-2 antiserum	PSV-W antiserum
Homologous	Serotype I: PSV-1, PSV-3, PSV-E, PSV-5, PSV-76-69 Serotype III: PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, PSV-101-16	Serotype I: PSV-1, PSV-3, PSV-E, PSV-5, PSV-76-69	Serotype II: PSV-2, PSV-V Serotype I: PSV-1, PSV-3, PSV-E, PSV-5, PSV-76-69	Serotype IV: PSV-W
Heterologous	Serotype II: PSV-2, PSV-V Serotype IV: PSV-W	Serotype II: PSV-2, PSV-V Serotype III: PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, PSV-101-16 Serotype IV: PSV-W	Serotype III: PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, PSV-101-16 Serotype IV: PSV-W	Serotype I: PSV-1, PSV-3, PSV-76-69, PSV-5, PSV-E Serotype II: PSV-2, PSV-V Serotype III: PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, PSV-101-16

[a] Homologous = antigens formed precipitin bands that fused with those of other antigens of this group but spurred over precipitin bands of heterologous antigens. Heterologous = antigens formed precipitin bands that fused with those of other antigens of this group but were under spurs formed by antigens with homologous reactions.

TABLE 3. Homologous and heterologous antisera titers among three PSV strains

Antiserum	Test	Antigen PSV-E	PSV-2	PSV-W
PSV-E	Microprecipitin	512[a]	128	64
	Gel diffusion	64	32	16
PSV-2	Microprecipitin	64	128	64
	Gel diffusion	32	64	16
PSV-W	Microprecipitin	...[b]	...	...
	Gel diffusion	16	8	64

[a] Reciprocal titer of greatest serum dilution that reacted with virus.
[b] Test not done.

antiserum. Conversely, reaction lines of the other isolates were straight and usually did not touch the reaction line of PSV-W with PSV-W antiserum (Figs. 8 and 9).

The serum titers to three isolates of PSV (E, 2, and W) were measured with homologous and heterologous purified virus by microprecipitin and gel diffusion serology. The serological differentiation indexes (twofold dilution steps between homologous and heterologous antigen reactions with a serum) between PSV-E and PSV-2 varied between 1 and 2 for the different combinations and procedures (Table 3), whereas those for PSV-W with either PSV-E or PSV-2 were between 1 and 3.

RNA components. Five principal RNA components were found in six of the PSV isolates, but only four components were found in PSV-W (Fig. 10). The molecular weights (in the nondenaturing gels) of RNA 1–4 of all PSV isolates were $1.14–1.20 \times 10^6$, $0.96–1.06 \times 10^6$, $0.75–0.84 \times 10^6$, and $0.37–0.44 \times 10^6$, respectively. RNA 5 of PSV isolates E, 3, 2, 101-16, and 80-11 had molecular weights of about 7×10^4, but RNA 5 of PSV-V had a molecular weight of 22×10^4.

The electrophoresis patterns also reflected the RNA component compositions of PSV isolates. There were no apparent differences between PSV-V and PSV-2 in serotype II or between PSV-101-16 and PSV-80-11 in serotype III; both serotypes had a high proportion of RNAs 3 and 4. In serotype I, PSV-E had a lower proportion of RNAs 3 and 4 than did PSV-3. PSV-W had a higher proportion of RNA 1, similar to PSV-E; in another preparation of virus, however, the RNA 3 proportion of PSV-W increased.

Reaction of *T. repens* to PSV isolates. The susceptibility of *T. repens* to six PSV isolates was determined on two groups of clones (Table 4). Even though all the clones had originally been inoculated with PSV-E and found to be uninfected, when 20 plants of each clone were inoculated with PSV-E, one or more plants of all but two clones were infected. In group A clones, all of which were susceptible under field conditions, PSV infection rates varied among clover clones and virus isolates. PSV-101-16, originally isolated from white clover clone SC 101-16, infected 90% of the plants of this clone. In group B clones, none of which were infected by PSV under field conditions, clone BCR-39 was very susceptible to all PSV isolates by sap-inoculation, whereas BCR-36 was infected by only one isolate. The control clones, 2682 and LA-1, were highly susceptible to most of the PSV isolates. Overall, some clones were susceptible to all PSV isolates and others were more susceptible to certain isolates.

TABLE 4. Frequencies of infection of *Trifolium repens* clones with six PSV isolates

Clones	Isolate PSV-E (%)	PSV-1 (%)	PSV-3 (%)	PSV-5 (%)	PSV-2 (%)	PSV-101-16 (%)	All (%)
Group A[a]							
SC 60-4	5	0	10	0	0	0	2
SC 65-24	16	0	30	0	35	30	16
SC 80-11	20	15	40	10	40	20	23
SC 80-28	20	5	40	10	25	20	20
SC 84-3	20	0	60	0	44	20	27
SC 100-26	35	0	42	30	45	20	29
SC 101-16	40	30	40	20	35	90	38
SC 101-30	10	0	0	0	0	10	3
SC 102-20	35	45	63	65	15	20	42
SC 102-36	0	0	10	5	5	10	5
All	20	11	34	15	24	24	21
Group B[b]							
BCR-18	15	0	0	5	25	6	8
BCR-36	0	0	0	0	0	11	2
BCR-39	94	90	55	100	95	95	88
SC 44-21	15	21	0	10	15	21	14
SC 56-2	15	25	10	25	20	5	17
SC 58-22	50	5	15	0	40	35	24
SC 60-32	62	50	5	16	67	19	36
SC 65-13	58	50	0	10	32	16	26
SC 66-31	14	35	0	12	16	0	13
NC 308-7	21	0	0	0	11	0	5
All	34	27	9	18	32	22	23
Susceptible controls[c]							
2682	100	80	80	70	90	30	75
LA-1	60	70	22	40	80	20	49
All	80	75	53	55	85	25	62
All 22 clones	29	22	22	18	31	23	24

[a] Clones screened for resistance to PSV-E that were field-infected. About 20 plants of each clone were inoculated with each isolate but because of plant deaths, 10–20 were scored; the exception was PSV-101-16, where 10 plants were inoculated and 10 were scored.
[b] Clones screened for resistance to PSV-E that were not field-infected. About 20 plants of each clone were inoculated with each isolate but because of plant deaths, 14–20 were scored.
[c] Clones known to be susceptible to PSV-E but not selected for any virus resistance characters; 10 plants of each clone were inoculated with each isolate, and 9 or 10 were scored.

DISCUSSION

Four PSV serotypes could be distinguished by the reactions of 13 PSV isolates with antisera to PSV-E, PSV-T, PSV-B2, PSV-2, and PSV-W in gel diffusion serology. PSV serotypes I, II, and IV, typified by PSV-76-69 (serologically similar to PSV-E), PSV-V, and PSV-W, respectively, have been reported (19). Serotype III, which includes isolates from *T. repens* at Clemson, is a new serotype of PSV serologically closely related to serotypes I and II and distantly related to serotype IV. Spur formations in gel diffusion serology tests among serotypes I, II, and III indicated that at least one epitope was common to all three serotypes and that

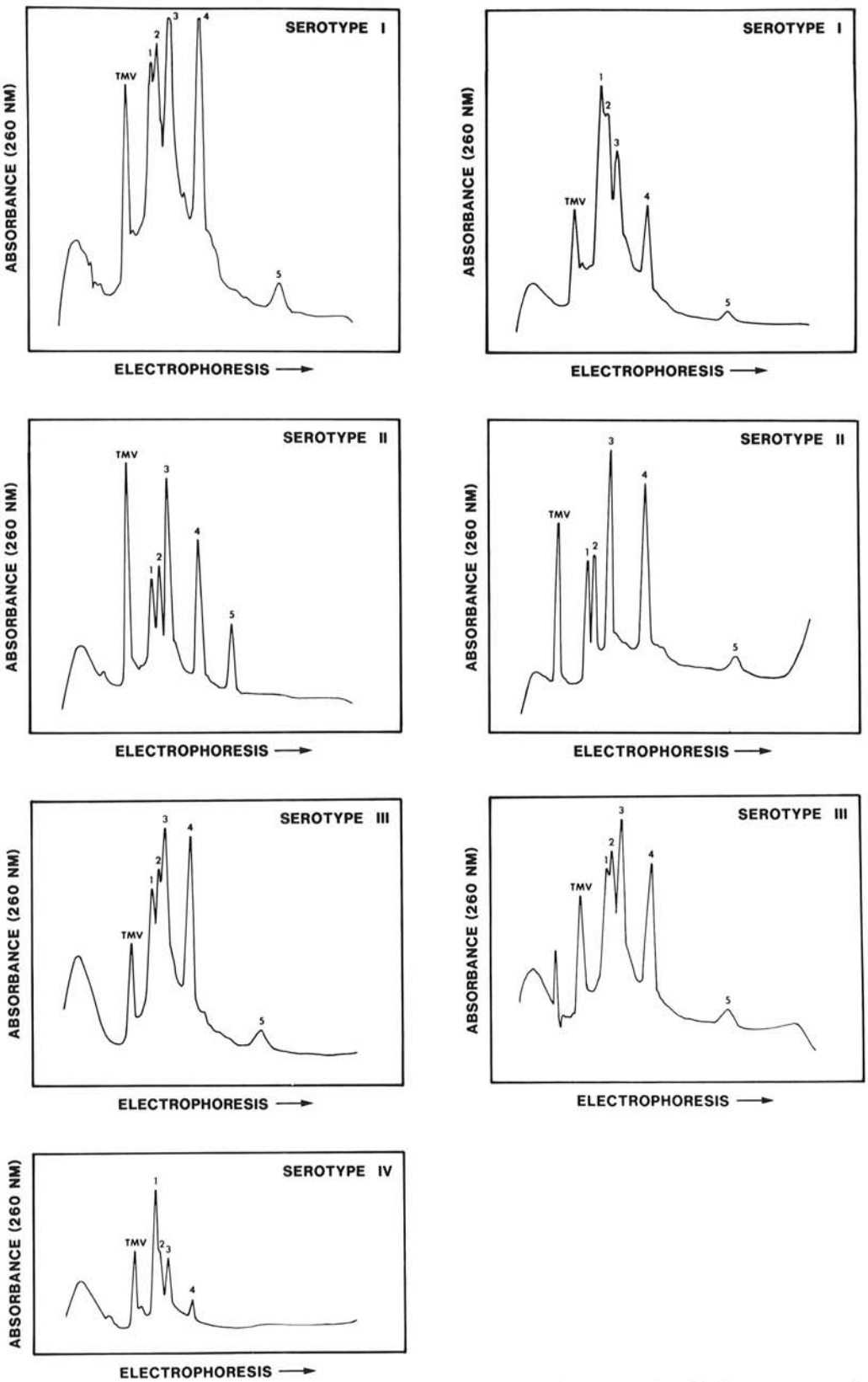


Fig. 10. RNA band patterns obtained by UV absorption scan of polyacrylamide/agarose gels after electrophoresis. The peanut stunt virus RNAs used were: serotype I, PSV-3 (left) and PSV-E (right); serotype II, PSV-V (left) and PSV-2 (right); serotype III, PSV-101-16 (left) and PSV-80-11 (right); serotype IV, PSV-W.

serotypes II and III had at least one epitope not contained by the other serotype but contained by serotype I (i.e., serotype I had epitopes a, b, c, and d; serotype II had epitopes a and d; and serotype III had epitopes a and b). Because serotypes I, II, and III are closely related serologically and distantly related to serotype IV, it would be appropriate to combine serotypes I, II, and III into an eastern group. Until recently, the western serotype isolates were not differentiated by serology (20) but there were differences between PSV-W and Arkansas isolates closely related to PSV-W (1). By competition hybridization, nucleic acids of serotype I and II isolates (i.e., PSV-V and PSV-76-69) are closely related but are very distantly related to serotype IV isolates (i.e., PSV-W) (4).

Serotype III infected Davis and Bansei soybean but not Perfected Wales pea or peanut. PSV-W was grouped with isolates of serotype I in the host tests but was clearly distinct from serotype I isolates by the severe symptoms caused on Perfected Wales pea and pinto bean. Although in most cases the host reactions agreed with the serological reactions, PSV-2 and PSV-V in serotype II had some host reaction differences on peanut and PSV-76-69 differed from other serotype I isolates in several host reactions. The relationship of these serotypes to the European serotype of PSV has not been established (2).

Electrophoresis of PSV-RNAs revealed a low molecular weight RNA in all PSV isolates belonging to the eastern group. We did not determine if this RNA was a breakdown product of the genome RNA or a satellite RNA. The absence of a small RNA in PSV-W might be a characteristic of the western group, since the Spanish isolate closely related to PSV-W also had none (5). Kaper et al (13) demonstrated that RNA 5 is present in the PSV-1976 isolate. PSV-V has a low molecular weight RNA of similar size to that of the RNA 5 of the PSV-1976 isolate (13). The size of the low molecular weight RNA of most PSV isolates was much less. Although the RNA component compositions of PSV isolates in serotypes II and III apparently did not differ, the RNA component composition probably is not characteristic of the serotype, e.g., the RNA component compositions of PSV-E and PSV-3 in serotype I were dissimilar and the RNA component composition of PSV-W varied in different preparations of purified virus.

The infection rates obtained with the *T. repens* control clones are typical of our experience with inoculation of this host with other viruses (9). Even though a clone is susceptible, 100% infection is seldom obtained by mechanical or aphid inoculation of several plants of that clone. Many factors are probably involved in this phenomenon. The six PSV isolates used to inoculate *T. repens* clones represented three of the four serotypes and all three isolate groups based on host reaction. The variation in clone susceptibilities among the four isolates of serotype I was as great as that between any one of the serotype I isolates and either the serotype II or III isolates. Some PSV isolates, however, infected more plants of some *T. repens* clones than did other isolates, and more plants of some clones were infected by more isolates than other clones. This indicates that there are different alleles or genes in *T. repens* for resistance to PSV strains. Because all three serotypes infected *T. repens* clones of both group A and group B, the importance of serotype III—which infected *T. repens* plants with some level of resistance to PSV-E (serotype I)—in resistance to PSV in *T. repens* cannot be predicted.

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