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Host differentiation and serological homology of pea seed-borne mosaic virus isolates*

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Abstract

Seven isolates of pea seed-borne mosaic virus (PSbMV) were compared on selected *Pisum sativum* L. differentials and by microprecipitin and SDS-gel serology and particle length. All isolates were characterized by 750 nm particle-length modes and were closely related serologically, but some were readily distinguished on *P. sativum* differentials. Isolate distinctions were of the magnitude typical for virus strains. Differentials, diverse *Pisum* germplasm from U.S. Plant Introduction accessions, provided a practical means of PSbMV strain differentiation.

Additional keywords: Virus assays, virus strains, strain distinctions, *Pisum sativum,* host reactions, virus-induced plant necrosis, symptom diversity.

Introduction

Viruses reported to be seed-borne in peas *(Pisum sativum* L.) in Czechoslovakia (Musil, 1966), Japan (Inouye, 1967), the USA (Hampton, 1969; Mink et al., 1969; Stevenson and Hagedorn, 1969), and the Netherlands (Bos, 1970) resembled each other in many respects. However, published descriptions suggested that more than one virus might be involved. For example, particle lengths were reported to be 700 nm in Czechoslovakia (Musil, 1970), frequently shorter than 700 nm in the USA (Hampton, 1969; Stevenson and Hagedorn, 1969; Hampton et al., 1974) and 750

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nm in Japan (Inouye, 1967). Furthermore, the virus in Japan (Inouye, 1967) was not transmitted by the pea aphid [*Acyrthosiphon pisum* (Harris)], whereas the virus in the USA was readily transmitted by this aphid (Aapola et al., 1974). Yet when the viruses from Japan and the USA were compared serologically they appeared to be closely related if not identical (Mink et al., 1974), and the priority name pea seed-borne mosaic virus (OSbMV) was adopted.

We have now compared isolates of this virus from Japan and the USA with some apparently related isolates from Czechoslovakia and the Netherlands by (1) inoculation of selected *P. sativum* lines under defined conditions and by (2) subsequently assembling seven isolates at Corvallis, Oregon, USA, and examining these under a single set of conditions. Evidence that these virus isolates comprise a single virus, pea seed-borne mosaic virus, and the extent to which they were distinguishable by *Pisum* differential hosts are presented.

Materials and methods

Pisum sativum differentials, selected from U.S. Department of Agriculture Plant Introduction accessions in preliminary PSbMV-resistance screening studies at Corvallis, were distributed as seed to each of the authors. Each of us, operating within prearranged experimental guidelines, tested one or two representative virus isolates at our respective locations. Isolates were originally obtained from naturally infected pea seed. Symptoms induced by these isolates on *Pisum* differentials were recorded, and the data were exchanged among the authors and summarized (Table 1).

Virus isolate C-4-24, from Oregon, was the twenty-fourth PSbMV isolate derived from infected seedlot C-4 (Hampton et al., 1974). Isolate WA-1, from Washington was purified and characterized by Knesek et al. (1974). Isolate WI-1, from Wisconsin, was reported by Stevenson and Hagedorn (1969). Isolate P202 was described by Inouye (1967). Isolate E210, from the Netherlands, was characterized by Bos (1970), after which a deviant type, E224, was isolated. Isolated PsS_2, from Czechoslovakia, was characterized by Musil (1970).

These seven isolates were gathered at Corvallis for further comparisons. They were supplied to the senior author in infected seed (isolates P202-ST and PsS_2-ST) or in desiccated tissue from infected pea plants (the remaining five isolates). Each isolate was established in plants of pea cultivar 447 or bell bean [*Vicia faba* var. *minor* (Peterm.) Beck] in insect-screened glasshouses and separated from plants with other isolates by plastic-film dividers. These isolates were compared by symptomatology in selected hosts (Table 2), serology, and modal particle length.

Antisera to PSbMV were prepared at three locations by injecting purified infectious virus preparations into rabbits. The rabbits were bled successively, and antibody titer was determined with homologous antigen. Antisera to PSbMV-551 and PSbMV-SL25 were prepared by six injections of virus, ranging from 100 to 660 μg per injection, with Freund's incomplete adjuvant. For microprecipitin serology, each isolate was purified by previously reported methods (Knesek et al., 1974), standardized to 100μg/ml, and tested against two to four PSbMV antisera. Viral antigens used in SDS-gel tests (Purcifull and Batchelor, 1977) consisted of buffer extracts from desiccated tissue supplied by each author, extracts from pea plants infected with each isolate, or both. Although microslide gel tests (McCrum et al., 1971;

Table 1. Symptoms[1] induced in *Pisum sativum* differentials by seven isolates of pea seed-borne mosaic virus[2].

Host group	Plant Introtion no. of differential	Symptoms induced at designated test location by indicated isolate													
		Corvallis C-4-24		Prosser WA-1	Madison WI-1	Sakai P202		Wageningen E210		Wageningen E224		Bratislava PsS_2			
I	142774	N!	8/9	lr	N!	N!	24/24	N!	15/16	lr,VC	12/12	N!			
	174319	N!	3/3	N!	N!	N!	22/22	N!	12/12	lr,VC	12/12	N!			
	269771	N!	8/9	lr	N!	N!	21/24	N!	12/12	lr	12/12	N!			
	272146	N!	7/10	N!	N!	N!	19/23	N!	16/16	lr	10/12	N!			
	272155	N!	7/9	lr	N!	N!	15/15	N!	11/16	lr,VC	8/12	N!			
II	140297	n,St	4/5	N!	VC,M	N!	10/10	LR,n	8/18	lr	10/11	n,St			
	206823	N!(n)	7/10	lr,VC	LR,VC	lr	23/23	lr	3/3	lat(lr)	18/19	lr			
	263033	lr,VC	3/3	N!	lr,VC	n	24/24	LR,n	15/16	lr,VC	12/12	n,N			
III	175877	LR,VC	4/6	LR	LR,VC	LR	15/15	LR(N!)	15/16	lr,VC	10/13	LR			
	204724	LR,VC	1/2	lr,VC	LR,VC	LR	15/15	LR(N!)	4/12	lr,VC	9/12	LR			
	269765	LR,VC	5/5	lr	lr,VC	LR	12/12	lr,VC	9/12	lr,VC	11/12	LR			
	269804	LR,VC	4/4	LR	lr,VC	LR	11/11	lr,VB	16/16	lr,VC	12/12	LR,lr			
	272171	LR,VC	8/9	LR,VC	LR,VC	LR	24/24	lr	13/16	lr,VC	15/16	LR			
IV	206813	lr,VC	1/3	lr,VC	lr,VC	lr,LR	16/16	LR,VC	12/12	lr,VC	10/12	LR,lr			
	261669	lr, VC	3/3	lr	m,VC	LR,lr	18/18	LR,VC	9/12	lr,VC	15/16	LR,lr			
	269777	lr,VC	10/10	LR	LR,VC	LR,lr	23/23	LR	16/16	lr,VC	11/11	LR			
V	193586	Is	8/11	I	Is	Is	13/16	Is	11/12	Is	9/12	I			
	269774	I	9/9	I	Is	I	16/16	Is	15/16	I	20/20	I			
	269818	I	5/5	I	I	I	12/12	I	16/16	I	20/20	I			

[1] Where data were available, symptom symbols are followed by a fraction representing the number of plants developing symptoms per number of plants inoculated. Symptom symbols are: N! = rapid, whole-plant necrosis; n = localized tissue necrosis; St = stunting; lat = symptomless infection; LR,lr = leafroll (severe, mild); VC = pronounced vein clearing; VB = vein banding; M,m = leaf mosaic (severe, mild). Symbols within parentheses represent exceptional symptoms.
Immune response was indicated by absence of symptoms and absence of assayable virus. I = immune; Is = immune, some susceptible plants. Fractions indicate no. of immune plants per no. of plants inoculated.
[2] A summary of disease symptoms obtained by co-authors.

Tabel 1. Symptomen geïnduceerd door zeven isolaten van het erwterolmozaïekvirus in differentiërende cultivars van Pisum sativum.

Table 2. Symptoms[1] induced in selected hosts by pea seed-borne mosaic virus isolates.

Host	Symptoms induced by indicated isolate[2]						
	SL-25	WA-1	WI-1	P202(ST)	E210	E224	PsS_2(ST)
Pisum sativum L.							
cv. 447	a /LR,VC	a /LR,VC	a /LR,VC	a /LR,VC	– / –	a /lr,vc	a /LR,VC
P.I. 140297	a /LR,VC	a /LR,VB	a /n,ST	a /N!	a /n,ST	a /LR,VB	a /n,ST(N)
P.I. 206823	a /lr,vb	a /LR,VB	a /LR,VB	a /LR,VB	a /LR,VB	a /LR,VB	a /LR,VB
Tetragonia tetragonoides (Pall.) Ktze	– / –	– / –	– / –	a / –	– / –	– / –	– / –
Vicia faba var. *minor* (Peterm.) Beck	a /M,LD	a /M,LD	a /M,LD	a /M,LD	a /M,LD	a /M,LD	a /M,LD
Phaseolus vulgaris L. cv.							
Black Turtle Soup	a / –	– / –	a / –	a / –	– / –	– / –	– / –

[1] Symptoms on inoculated leaves / symptoms from systemic infection.
Symptom symbols are: LR, lr = leafroll (severe, mild); VC, vc = vein clearing (severe, mild); VB, vb = vein banding (severe, mild); n = localized tissue necrosis; N! = rapid, whole-plant necrosis; St = plant stunting; M = leaf mottle; LD = leaf deformity; a = latent infection; – = no virus detectable by bioassay on *Chenopodium amaranticolor* Coste & Reyn.
[2] Comparison made at Corvallis, Oregon, USA.

Tabel 2. Symptomen geïnduceerd door erwterolmozaïekvirus in geselecteerde waardplantesoorten.

Mink et al., 1974) were satisfactory, SDS-gel serology with antiserum to PSbMV-SL25 was more convenient and reliable for direct comparisons among isolates.

Particle-length frequency distributions for isolates gathered at Corvallis were plotted from photomicrographs of infected-leaf-dip preparations fixed with glutaraldehyde (Hampton, et al., 1974), with tobacco mosaic virus particles as an internal calibration standard (Bos, 1975). Photomicrographs were produced with a Philips EM-300 electron microscope operating at 60 kV.

Results

Pisum reactions to virus isolates. Responses in 19 *P. sativum* differentials inoculated with the seven isolates presumed to be PSbMV ranged from rapid development of whole-plant necrosis to immunity (Table 1). The differentials were arrayed into five groups based on this range of responses. The proportion of plants developing specific symptoms is presented for four of the seven isolates to show the degree of uniformity within *Pisum* differential lines.

Group I. Inoculation of these lines with five of the seven isolates uniformly produced rapid whole-plant necrosis. Isolate WA-1 induced symptoms in lines of this group that were intermediate in severity between those of atypical isolate E224 and those of the other five isolates. Isolate E224 tended to induce mild leafroll and vein clearing in all lines of Groups I through IV.

Group II. Pisum lines of this group had been considered members of other groups in preliminary tests at Corvallis, but they were regrouped after results from all authors had been analyzed. In overall severity, responses of lines of this group appeared intermediate between whole-plant necrosis (Group I) and severe leafroll (Group III) types. However, the seven isolates induced very diverse symptoms on these lines, which thus appeared to distinguish among PSbMV isolates. Interestingly, isolate E224 induced latent infection in most of the P.I. 206823 plants inoculated.

Group III. Virus isolates induced generally severe leafroll and vein clearing in lines of this group, although isolate E210 tended to induce whole-plant necrosis or late-developing systemic necrosis, not followed by plant death, in some plants of P.I. 175877 and 204724.

Group IV. Lines of this group, in which inoculation with isolate C-4-24 had produced characteristically mild leafroll symptoms, responded with a mixture of mild and severe symptoms when inoculated with other isolates. Despite nonuniformity of reactions to PSbMV isolates similar to that within Group II lines, the overall reaction severity of these lines was intermediate between those of the Group III and Group V lines.

Group V. The majority of plants in lines P.I. 193586 and 269774 were immune to all virus isolates. Line P.I. 269818 contained only plants that were immune to all PSbMV isolates.

Supplementary host reactions. Isolates obtained from each author were tested on selected hosts at Corvallis (Table 2). Isolate SL-25, obtained from an imported seedstock, resembled isolate C-4-24 and was used in its place. Isolates P202-ST and PsS_2-ST were obtained from Inouye and Musil, respectively, in infected seed. The

remaining isolates, supplied by authors in desiccated infected tissue, were as presented in Table 1.

Pea cultivar 447 was included because it had been a standard test and propagation host in previous work of the first two authors (Hampton et al., 1974; Knesek et al., 1974; Mink et al., 1974) and yet was quite resistant in the present study to inoculation with isolate E210. In two tests, plant of 447 did not become infected when inoculated with buffered homogenate of desiccated E210- or E224-infected pea tissue. Bell bean plants, however, were readily infected with this same inoculum. Mild PSbMV symptoms were finally induced in plants of 447 inoculated with isolate E224 from infected Bell bean, whereas parallel passages from E210-infected Bell bean plants to 447 plants caused no infection.

Symptoms induced by the various isolates on plants of P.I. 140297 were similar in the multilaboratory tests (Table 1) and tests at Corvallis (Table 2). This line, and probably line P.I. 263033 (see Table 1), may indeed be useful in distinguishing PSbMV isolates or strains. Particularly, Japanese isolate P202 and P202-ST behaved similarly in the two trials, as did the Czechoslovakian isolate PsS_2 and PsS_2-ST. Plants of P.I. 206823, which appeared to have distinguished isolates by means of leaf symptom intensities in the first trial (Table 1), reacted almost indistinguishably to inoculation by all isolates at Corvallis.

Only isolate P202-ST was recoverable from inoculated leaves of *Tetragonia tetragonoides* (Pall.) Ktze; otherwise the selection of this host tested at Corvallis was not susceptible to PSbMV isolates. Another selection of this host was susceptible to isolate P202 in the fourth author's laboratory. Bell bean plants were uniformly susceptible to all isolates tested, producing similar symptoms in each case. Bean (*Phaseolus vulgaris* L.) cultivar Black Turtle Soup was not susceptible to four isolates. Inoculated leaves of this host, however, became infected with three of the isolates.

Serological results. Four PSbMV antisera prepared at three locations were tested by microprecipitin serology against purified antigens from most of the isolates presented in Table 1 (Table 3). Homologous and heterologous modal titers in most

Table 3. Microprecipitin titers of antisera to selected isolates of pea seed-borne mosaic virus.

Antisera	Antigens[a]						
	C4-26	WA-1	WI-1	P202	E224	LRM-1	BYMV[b]
PSbMV-551	512	512	256	512	512	512	32
PSbMV-SL25	512	512	512	512	512	512	0
PSbMV-WA-1	512	512[c]	512	512	NT	NT	16
PSbMV-P202	256	512	512	512	NT	NT	NT

[a] Antigens purified (Knesek et al., 1974) and standardized to 100 μg/ml; titers represent the modal value from at least three tests. NT = no test.

[b] Isolate Gil-6 of the bean yellow mosaic virus severe strain was included as a distantly related control antigen.

[c] Homologous titers underlined.

Tabel 3. Microprecipitatie-titers van antisera tegen isolaten van het erwterolmozaïekvirus.

Fig. 1. SDS-gel double-diffusion test showing conterminous precipitin bands between pea seed-borne mosaic virus (PSbMV) isolates and PSbMV-SL25 antiserum. 1 through 7 are crude extracts from plants infected with individual PSbMV isolates. 1 = E210, 2 = E224, 3 = WA-1, 4 = WI-1, 5 = P202, 6 = LRM-1, 7 = SL25, 8 = healthy plant extract. AS7 = antiserum to PSbMV-SL25, diluted 1:2.

Fig. 1. SDS-gel-dubbele-diffusietoets met doorlopende precipitatiebanden van verschillende isolaten van het erwterolmozaïekvirus (putjes 1 tot en met 7) en antiserum tegen het isolaat SL25; putje 8 bevatte sap van een gezonde plant.

cases were 512. Isolates were indistinguishable from each other by microprecipitin reactions against these four antisera. One of the four antisera, PSbMV-SL25, produced ideally visible bands when tested by SDS-gel serology against purified virus or infected-plant extracts. Crude extracts from desiccated and or freshly harvested plants infected with each of the seven isolates were therefore compared against this antiserum in SDS-treated gels (Fig. 1). Conterminous bands were produced by these isolates in all adjacent-well arrangements, indicating a very close serological relationship among the isolates from four countries. No bands were produced when antiserum was tested against extracts from healthy plants or when preimmune serum was tested against sap from infected plants.

Particle lengths. Particles of all seven isolates, when prepared from leaf sap and fixed with 2% glutaraldehyde, had length modes of 750 to 780 nm, as compared with the 300 nm length mode for tobacco mosaic virus, mixed into each sample as an internal standard.

Discussion

Despite reports of major differences in viral properties, we have shown that the reported isolates are variants of the same virus. Among the reported conflicts, the ability of two isolates to infect *Tetragonia tetragonoides* (= *T. expansa* Murr.) (Bos, 1970; Inouye, 1967), the particle-length variability (Hampton, 1969; Hampton et al., 1974; Stevenson and Hagedorn, 1969), and the failure of the pea aphid to transmit the virus (Inouye, 1967) were most notable.

In the present study, only one of seven PSbMV isolates (P202) was infectious to *T. tetragonoides* (Northrup King Lot No. 807, 1978), and that infection was limited to inoculated leaves in three separate tests. Similarly, 'Black Turtle Soup' bean was only locally susceptible to three of seven isolates tested. Latent, localized infection of bean cultivar Bataaf was previously reported for isolate E210 by Bos (1970).

Upon re-evaluation of PSbMV transmissibility by the pea aphid, Inouye (unpublished results) achieved transmission when aphids were given acquisition access periods of less than 15 min, and specifically when access was limited to 30-second periods.

Discrepancies in reported particle lengths for PSbMV were partially resolved by evidence that PSbMV particles were subject to specific-point breakage and required glutaraldehyde fixation for reliable photomicrographic measurements (Hampton et al., 1974). Also, some of the initially reported particle lengths for PSbMV isolates may have been subject to electron microscope calibration error, now minimized by use of appropriate internal standards such as tobacco mosaic virus (Bos, 1975).

The serological relatedness previously reported for Japanese and U.S. isolates (Mink et al., 1974) has now been extended to all comparable isolates reported in the world between 1966 and 1970.

Evaluation of seven virus isolates presumed to be PSbMV provided the opportunity to evaluate the suitability of 19 *P. sativum* lines as PSbMV strain differentials and simultaneously examine the magnitude of variation among several isolates. Such variation fell within the limits typical for other well-defined legume potyviruses, e.g. bean common (Drijfhout, 1978) and bean yellow mosaic (Jones and Diachun, 1977) viruses. The U.S. isolates varied about as much as those from the other three countries. Ultrastructurally discernible differences among U.S. isolates were reported previously (Hampton, 1973). Isolates E210 and E224 were least typical by the criterion of symptoms induced in plant hosts but were serologically indistinguishable from other isolates. Isolate E210 induced uniform, moderate symptoms in the lines of *P. sativum* Host Groups II, III, and IV and failed to infect pea cultivar 447, which was found to be susceptible to all other PSbMV isolates. This isolate also induced systemic infection in *Chenopodium quinoa* (Bos, 1970), a characteristic not found in other PSbMV isolates. Isolate E224 induced unusually mild symptoms in all susceptible *P. sativum* differentials, including those that had died rapidly when inoculated with all other isolates. Likewise, the 'pea latent strain' of PSbMV reported in Yugoslavia by Miličić & Plavšić (1978) presumably represents a significant departure from characteristics previously described for other PSbMV isolates. The extent to which plant breeders may be forced to broaden the genetic base of PSbMV immunity, due to virus strain diversity, is unknown at this time.

Although PSbMV and bean yellow mosaic virus (BYMV) are both infectious to peas and are similar in several respects, the two viruses are readily distinguishable. Specifically, they are serologically distinct and the host range of PSbMV is markedly different and much more narrow than that of BYMV. In addition, symptoms induced in pea cultivars consist typically of marked leaf rolling by PSbMV and conspicuous mosaic without leaf deformity by BYMV.

The segment of *P. sativum* germplasm represented by the 19 plant introduction accessions examined appears suitable for PSbMV strain comparisons. Reactions to the PSbMV isolates examined seemed best characterized by Host Groups I, III, and V. Simplified sets of differential lines may therefore be preferable for future studies of PSbMV strain relationships.

Genetically, the range of *P. sativum* symptoms is interesting, because resistance to PSbMV has been accepted to be conditioned by a single recessive gene, *sbm* (Hagedorn and Gritton, 1973). Whole-plant necrosis (Host Group I) would therefore presumably be caused by modifying genes of a unique germplasm that enhance host sensitivity to PSbMV, whereas tendencies toward latent infection or infection with very mild symptoms would presumably be caused by modifying genes that reduce host sensitivity. We consider the entire range of *P. sativum* reaction types to be an

interesting demonstration of susceptibility-gene modification.

Recent evidence (Hampton, 1980) indicates that mixtures of PSbMV-resistant and -susceptible germplasm are not uncommon within *P.sativum* lines of the USDA Plant Introduction collection. Line P.I. 193586, in the present study, consisted of a mixture of resistant (80%) and susceptible (20%) plants. Line P.I. 269774 contained a small proportion (2%) of susceptible plants, whereas P.I. 269818 contained only immune plants.

Samenvatting

Waardplantonderscheid en serologische overeenkomst tussen isolaten van het erwterolmozaïekvirus

Tussen 1966 en 1970 zijn in verschillende landen virussen gerapporteerd, die bij erwt met zaad overgaan, maar in verschillende opzichten leken te verschillen. Isolaten uit Japan en de USA bleken serologisch nauw aan elkaar verwant, zo niet identiek te zijn. Daarom werd de internationale naam 'pea seed-borne mosaic virus' voorgesteld. In Nederland was het virus beschreven onder de naam erwterolmozaïekvirus.

Zeven isolaten van het virus uit de USA, Japan, Tsjechoslowakije en Nederland zijn nader met elkaar vergeleken in reactie op geselecteerde differentiërende rassen van erwt *(Pisum sativum)* en op enkele andere plantesoorten, en in serologische eigenschappen zowel als in deeltjeslengte.

Serologisch waren de isolaten niet van elkaar te onderscheiden, wel echter van het verwante bonescherpmozaïekvirus. De voor alle vormen van het laatste virus onvatbare 'Perfection'-type erwterassen bleken al eerder alle vatbaar te zijn voor het erwterolmozaïekvirus. Ook verschillen de isolaten niet in deeltjeslengte (750 nm).

Bij toetsing in zes verschillende over de wereld verspreide laboratoria bleek de reactie van de differentiërende erwterassen te variëren van een snelle, de hele plant dodende necrose (groep I) tot onvatbaarheid (groep V). Ook tussen de virusisolaten bestonden kleine verschillen in reactie. Het Nederlandse isolaat E224 gedroeg zich opvallend mild. Ook in de directe vergelijkingsproeven op enkele toetsplantesoorten bleken kleine biologische verschillen te bestaan. De geconstateerde verschillen overschrijden echter niet die tussen stammen van eenzelfde virus. Wellicht gaat het bij het optreden van necrose en van zwakke symptomen om genen die het vatbaarheidsgen *sbm* modificeren.

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