Characterization and Molecular Detection of the P4 Pathotype of Pea Seedborne Mosaic Potyvirus

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ABSTRACT

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The New York isolate of pea seedborne mosaic potyvirus (PSbMV) pathotype P4 was differentiated from the more prevalent PSbMV-P1 pathotype on selected pea cultivars, serologically and by sequence-specific reverse transcription-polymerase chain reaction (RT-PCR). PSbMV-P4 was seed-transmissible at much lower rates than P1 (0 to 0.7% versus 0 to 32%) in selected pea cultivars and was not detectable by enzyme-linked immunosorbent assay (ELISA) in reproductive tissues (pollen or embryo axes) of pea, whereas P1 was readily detectable in these tissues. P4 also was aphid-transmitted at lower rates (4 to 16%) than those of P1 (11 to 40%) and had distinct virus acquisition-time optima. RT-PCR detected approximately 10 pg of P4 RNA per 1.0 µg of total nucleic acids from P4-infected tissues and provided positive pathotype identification in single or mixed infections. RT-PCR and pathotype-selective ELISA were used to determine seed-transmission rates of the two pathotypes in seedling progeny of pea (*Pisum sativum*) cultivars inoculated with P1, P4, or P1 + P4. There were slight variations in the frequency of P1 seed transmission in the presence of P4; P4 seed-transmission rates did not increase in the presence of P1.

Additional keywords: viral pathotype interactions.

Although three pathotypes of pea seedborne mosaic potyvirus (PSbMV) have been reported, only two (P1 and P2) were well characterized previously (16). The third PSbMV pathotype, P4, was isolated from seedlings of USDA *Pisum sativum* L. Plant Introduction (PI) 471128 and partially characterized (2,19). A selected isolate, referred to here as P4-NY, was defined as a PSbMV pathotype by Provvidenti and Alconero (19) who discovered *P. sativum* gene *sbm-4* and demonstrated that it conferred specific resistance to PSbMV-P4-NY. By definition, therefore, P4-NY is acknowledged as the type isolate of PSbMV-P4. On this premise, we compared the pathogenicity and seed transmission (i.e., economic significance) of P4-NY with pathotype P1, PSbMV-DPD1, in selected U.S. pea cultivars.

Polymerase chain reaction (PCR) application to PSbMV-P1 detection was reported previously (17) and was adapted here to sequence-specific detection of P4-NY. For this purpose, we developed P4-NY-sequence-specific primers, compared detection sensitivity by reverse transcription (RT) PCR and by pathotype-selective enzyme-linked immunosorbent assay (ELISA), and investigated interactions between the P4-NY and P1-DPD1 pathotypes during seed transmission in pea. We were unable to derive other PSbMV-P4 isolates from seedlings of PI 471128 or from nine other selected North India *P. sativum* landraces, thus, precluding comparisons of P4-NY with other P4 isolates.

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MATERIALS AND METHODS

Virus sources. The PSbMV-P1 isolate DPD1 (15) used in this study was originally obtained from J. Jorgensen, Danish Plant Directorate, Lyngby, Denmark. Isolate PSbMV-P4-NY, the P4-type isolate, was provided by R. Provvidenti, New York State Agricultural Experiment Station, Geneva (2,19). Both P1-DPD1 and P4-NY were sequenced by E. Johansen et al. (13,14,15; E. Johansen, K. Keller, W. Dougherty, and R. Hampton, *unpublished data*). Our genome diagram of PSbMV (Fig. 1) also includes most of the potyviral genome features described by Riechmann et al. (20).

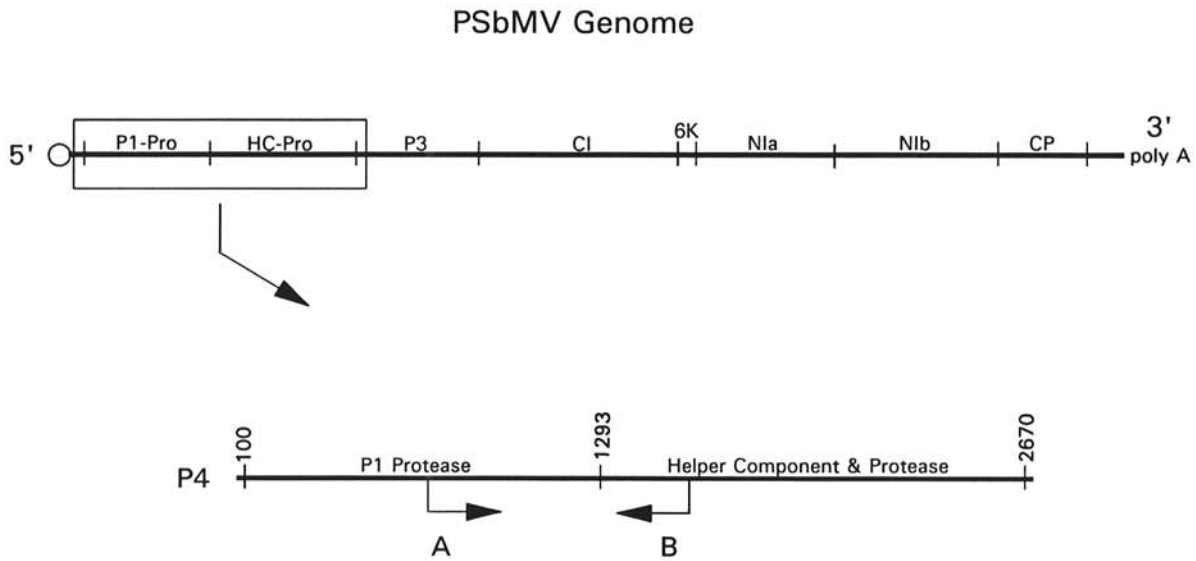
Purification. Several conventional methods were evaluated for deriving high yields of intact, purified virions of P4. The method published for white lupin mosaic potyvirus (12) proved satisfactory, typically yielding 4 to 6 mg of purified virus per 50 g of infected pea (cv. Early Freezer 680) tissues, harvested 3 weeks after plant inoculation. Purified P4 preparations were used to establish detection thresholds of P4-selective ELISA and as a source of viral RNA for testing and standardizing P4-NY sequence-specific assays.

Symptomatology in pea cultivars. P4-NY–induced symptoms were characterized in 46 selected cultivars susceptible to PSbMV-P1. Of these, 10 cultivars were chosen for detailed comparisons of symptoms induced by each pathotype. The symptomatology of PSbMV-P1 in susceptible pea (*P. sativum*) genotypes was described and illustrated previously (8). Symptom severity was graded as mild, moderate, and severe mosaic with stunting, and very severe stunting with leaf or plant necrosis. Symptoms induced by P4 and P1 also were compared in prospective local-lesion hosts (7), including five species of *Chenopodium* and three selections of *Tetragonia tetragonioides*.

Aphid transmission. Aphid-transmission efficiency of PSbMV-P4 was determined using third and fourth instar *Acyrthosiphon pisum* Harris originally collected by R. Hampton in Idaho and reared on faba bean (*Vicia faba*) under controlled conditions. Aphids were collected and fasted 2 h prior to each of three experiments. In the first experiment, aphids were allowed 3-, 30-, and 60-min acquisition-access periods (AAP) on young pea plants systemically infected with PSbMV-P1 (cv. 447) or P4 (cv. Early Freezer 680) and then transferred to healthy 12- to 14-day-old 'Early Freezer 680' seedlings (3 aphids per plant). Aphids remained on test plants for approximately 24 h until killed by fumigation. Test plants were grown in the greenhouse for 3 weeks, examined for symptoms, and individually assayed for the presence of virus by standard double-antibody sandwich (DAS)-ELISA. The same procedures were repeated in two subsequent experiments, except that AAP were shortened to 1, 3, 5, and 7 min. All trials included aphid-transmitted P1 as a standard for comparison.

Serology and seed transmission. *Trial 1.* Sets of approximately 60 seedlings of 10 selected cultivars were each inoculated with extracts from P1-, P4-, or P1 + P4-infected plants and grown to maturity. Infected seed-source plants in this trial and in trial 2 were grown under standard greenhouse conditions (day/night temperature 22/18°C, 14-h photoperiod, supplemented with fluorescent or sodium vapor illumination, 100 to 120 μmol m^{-2} s^{-1}) free of aphids. Each seedling was inoculated twice with the respective inoculum 12 and 26 days after emergence. For dually inoculated plants, P1 and P4 were inoculated simultaneously. Seeds from these sources were planted in a greenhouse, and the resultant seedlings were tested for PSbMV seed transmission (Table 1) by non-pathotype-selective DAS-ELISA (4). The DAS-ELISA procedure employed rabbit immunoglobulin G (IgG) raised against a combination of PSbMV pathotype P1 and P2 virions (9). The IgG reacted strongly with P1 and P2, as well as P4, and negligibly with extracts from healthy plants. The standardized DAS-ELISA system included reagent concentrations that maximized the distinction between extracts from P1- or P4-infected pea tissues and healthy plant extracts and detected viral antigen at concentrations of ≥250 pg/ml.

Seedlings from P1 + P4-inoculated mother plants containing ELISA-detectable PSbMV were tested again by a P4-selective indirect-ELISA procedure that was developed to detect PSbMV-P4 in the presence of P1. The procedure was as follows. Leaf-tissue samples were ground with mortar and pestle and diluted 50-fold (wt/vol) in 0.05 M carbonate ELISA coating buffer, pH 9.6. Crude sap (200 μl per well) was loaded into microtiter plates (Falcon 3915, Beckman & Dickenson Labware, Sumpter, NC) and allowed to incubate overnight at 4°C. Plates were subsequently blocked with 0.1% nonfat powdered milk in PBS (2.68 mM KCl, 1.47 mM KH_2PO_4, 0.14 M NaCl, 8.10 mM Na_2HPO_4, 3.07 mM NaN_3) for 2 h at 37°C. Polyclonal antiserum produced to pathotype P4 virions was diluted 120,000-fold in P1-infected plant sap, which in turn was diluted 50-fold in virus buffer (2% polyvinylpyrrolidone [MW 10,000], 0.24% chicken egg ovalbumin [Sigma Chemical Co., St. Louis], 0.1% Tween-20 in PBS, pH 7.4) and preincubated 3 h at 37°C before being added as primary antibody to antigen-coated wells of microtiter plates. The plates were incubated 2 h at 37°C and then blocked with the powdered milk mixture, as before. Alkaline-phosphatase–conjugated goat anti-



Primers	Position
A (5')TGCGTGAAGGTTAAACAAAACAGTAG(3')	703-728
B (5')ATTCAAGTCCTGTATTGCCAGATGT(3')	Complementary to 1530-1554

Fig. 1. Diagrammatic representation of the pea seedborne mosaic virus (PSbMV) genome (top horizontal bar), showing the coding regions associated with particular gene products: P1-Pro = P1-proteinase; HC-Pro = helper component and proteinase; P3 = P3 protein; CI = cytoplasmic inclusion protein; 6K = 6-kDa protein; VPg-Pro = genome-linked protein and proteinase; Pol = putative RNA-dependent RNA polymerase; CP = capsid protein. The region between nucleotides 100 and 2671 of PSbMV pathotype P4 (E. Johansen, K. Keller, W. Dougherty, and R. Hampton, *unpublished data*) is expanded to show the positions and orientations of primers A and B. Primer sequences and nucleotide positions are indicated.

rabbit IgG (Sigma) (1:2,000) in virus buffer was added and allowed to incubate for 2 h at 37°C, followed by the addition of substrate (1.0 mg of *p*-nitrophenyl phosphate per ml). Plates were washed after each step (0.1% Tween-20 in PBS). A_{405} values were recorded by BioTek Instruments microplate reader EL309 (BioTek, Winooski, VT), typically after 10 to 24 h at room temperature.

Trial 2. Approximately 80 seedlings of four selected pea cultivars were inoculated with pathotypes P1, P4, or P4 followed by P1. Seedlings inoculated with either P1 or P4 were inoculated only once 10 days after emergence. Those receiving both pathotypes were inoculated first with P4 10 days after seedling emergence and on separate leaves with P1 3 days later. The efficacy of P4-NY-sequence RT-PCR detection, described below, was tested with tissues from selected P4- and P4 + P1-inoculated cv. Early Freezer 680 seed-source mother plants. All mother plants were observed for typical PSbMV symptoms and grown to maturity, after which seeds were harvested and sown in a greenhouse. Two weeks after sowing these seeds, resultant seedlings were assayed for seed transmission of PSbMV by DAS-ELISA. Seedlings from mother plants inoculated with either P4 or P1 + P4 with ELISA-detectable PSbMV were reassayed by P4-sequence-specific RT-PCR. For this assay, an oligonucleotide primer pair corresponding to the P4 RNA sequence (Fig. 1) was synthesized, and a P4-specific RT-PCR system was developed, tested, and standardized.

P4-NY-specific RT-PCR. The methodology of Kohnen et al. (17) for PSbMV-P1 was followed, except that primer B, (5′) ATTCAAGTCCTGTATTFCCAGATGT(3′) (Fig. 1), was used to prime the reverse-transcription reaction and as the downstream primer for PCR, and primer A, (5′)TGCGTGAAGGTTAAACAA AACAGTAG(3′) (Fig. 1), was used at the upstream primer. Amplification products were electrophoretically analyzed on 1% agarose gels and visualized by staining with ethidium bromide. Product size was determined by comparison to commercially available ΦX174 dsDNA replicative form markers. The identity of a P4-specific PCR product was verified by digestion with *Nru*I (nucleotide position 1094) and *Sph*I (nucleotide position 929), which resulted in two products of the predicted size (data not shown). A blind test involving nucleic acid extracts from coded desiccated tissue samples prepared from healthy, P1-infected, or P4-infected plants was used to confirm P4-assay accuracy and precision.

Viral distribution in reproductive tissues. The presence or absence of PSbMV-P4 antigen in reproductive tissues of *P. sativum* cvs. Dual and Early Freezer 680 was compared with that of P1 by standardized DAS-ELISA, described above. The following tissues from P1- or P4-infected plants were tested: viable pollen, embryo axes from immature and mature seeds, and testae from immature and mature seeds. Twenty samples per cultivar of each tissue type were sampled from P1-infected plants. Samples (122) were taken from P4-infected Dual plants and 20 samples from P4-infected plants of cv. Early Freezer 680. Mature seeds were soaked in water overnight before separating seed parts for assays.

TABLE 1. Trial 1 of pea seedborne mosaic virus pathotypes 4 and 1 (PSbMV-P4 and -P1) seed-transmission frequencies

Cultivar	PSbMV-infected seedlings (%)[a]			P4-selective ELISA[b]
	P1	P4	P1 + P4	
Abador	0 (0/37)	0 (0/254)	5 (1/19)	0/1
Aldot	14 (13/95)	<1 (1/146)	15 (18/122)	0/18
Ceras	28 (33/119)	0 (0/160)	15 (21/142)	2/21
Challis	7 (8/113)	0 (0/113)	8 (11/135)	0/11
Charo	15 (23/150)	0 (0/127)	9 (14/156)	1/14
Dual	32 (37/114)	0 (0/210)	49 (41/83)	0/41
Popet	0 (0/38)	0 (0/31)	>1 (1/69)	0/1
Scout	23 (38/162)	0 (0/134)	13 (11/87)	0/11
Sounder	5 (6/119)	0 (0/169)	5 (4/79)	0/4
Tempter	33 (20/60)	0 (0/183)	33 (36/110)	0/36

[a] Numbers in parentheses indicate number of infected seedlings per number of assayed seedlings in 10 commercial pea cultivars.

[b] Progeny of P1 + P4-infected mother plants that tested positive for PSbMV were retested by P4-selective enzyme-linked immunosorbent assay (ELISA).

RESULTS

Comparative symptomatology of pathotypes P1 and P4. Symptoms induced in most U.S. pea cultivars by PSbMV-P4 were comparable to those of PSbMV-P1, from mild mosaic with little effect on plant growth to very severe mosaic with leaf distortion, severe stunting, and necrosis. Cultivars in which the two pathotypes induced equivalent mild symptoms included Alaska Sweet and Aldot; equivalent moderate symptoms, Charo, Dark Grey Sugar, Quincy, and Small Sieve Alaska; equivalent severe symptoms, Ceras and Dual; and equivalent very severe symptoms, Scout. Cultivars in which distinctions between pathotypes were observed included: i) P4 more severe on Abador, Avon, Early Freezer 680, Little Marvel, Popet, Regal, and Tempter; and ii) P1 more severe on Challis, Sounder, and 447.

Comparisons of P4 and P1 on 20 known or experimental PSbMV local-lesion hosts produced two limited distinctions. Both *Chenopodium album*, PI 433378, and *C. quinoa*, Corvallis strain, produced typical local lesions only to P1. *C. album* PI 433379 produced large red lesions when inoculated with P1 and much smaller, water-soaked lesions when inoculated with P4; however, this distinction likely depends on test-plant age and condition and optimal greenhouse temperature, lighting, and photoperiod. *C. amaranticolor*, Corvallis strain, produced equivalent local lesions to both pathotypes. The remaining 17 strains or accessions of these three species and of *C. berlandieri* (3), *C. pallidicaule* (2), and *Tetragonia. tetragonioides* (3) remained free of symptoms after inoculation.

Comparative aphid-transmission efficiencies of P1 and P4. In three successive trials, PSbMV-P4 was aphid (*A. pisum*)-transmissible at substantially lower rates than was P1. Third- or fourth-instar apterae transmitted P4 at rates of 4 to 16%. Parallel rates of P1 aphid transmission were 11 to 40%. A separate distinction of the pathotypes was demonstrated by different aphid AAP required for maximal transmission rates. P4 was not transmitted when aphids were provided feeding access of more than 7 min; maximum transmission (16%, twice) occurred after 3 to 5 min AAP. P1 was transmitted by *A. pisum* at maxima of 21, 33, and 40% after 1 to 7 min AAP and 12% after 30 min AAP.

Comparative seed-transmission rates of P1 and P4. Pathotype P4 was not ELISA detectable in the pollen or embryo axes

TABLE 2. Trial 2 of pea seedborne mosaic virus pathotypes 4 and 1 (PSbMV-P4 and -P1) seed-transmission for singular PSbMV pathotypes and mixed pathotypes in four commercial pea cultivars

Cultivar	PSbMV-infected seedlings (%)[a]			Isolate-specific RT-PCR[b]	
	P1	P4	P1 + P4	P1	P4
Ceras	13 (22/171)	0 (0/287)	0 (0/9)	0[c]	0
Charo	5 (5/94)	0 (0/246)	6 (7/124)	7/7	0/7
Dual	18 (18/101)	<1 (1/167)[d]	5 (3/60)	3/3	0/3
Early Freezer 680	31 (5/16)	0 (0/169)	0 (0/27)	0	0

[a] Numbers in parentheses indicate the number of infected seedlings per number of assayed seedlings, determined by double-antibody sandwich–enzyme-linked immunosorbent assay (DAS-ELISA). Seedling progeny of P1-, P4-, or P1 + P4-inoculated mother plants were assayed initially by DAS-ELISA for the presence of PSbMV, irrespective of pathotype.

[b] Progeny of P1 + P4-infected mother plants containing ELISA-detectable PSbMV were retested by P1-DPD1– or P4-NY–specific reverse transcription-polymerase chain reaction (RT-PCR).

[c] Most samples that were ELISA-negative were not assayed by PCR. Such samples, when assayed by RT-PCR as "negative controls," never contained PCR-detectable PSbMV-P1 or -P4.

[d] Seed transmission of pathotype P4 in pea cv. Dual was first detected by DAS-ELISA and then verified by P4-specific PCR.

of either immature or mature seeds of cvs. Dual or Early Freezer 680. PSbMV antigen was readily ELISA detectable in testae from both immature and mature seeds, but no virus was mechanically transmissible from any of several bioassayed testae. In parallel studies, P1 was readily ELISA detectable in all tested reproductive tissues. The presence of P1 RNA in all reproductive tissues, except testae, was verified by P1-specific RT-PCR.

In seed-transmission trial 1, P4 was measurably seed-transmissible only in a single seed (0.7%) of one cultivar, Aldot. The seed-transmission rate of P1 when inoculated alone ranged from 0% in Abador and Popet to >30% in Dual and Tempter. Moreover, P4 was rarely transmissible (i.e., 3/298) in seeds from dually inoculated plants and was detectable by pathotype-selective ELISA in seeds from only two cultivars, Ceras and Charo (Table 1). Both of these cultivars, accordingly, were selected for a second trial.

In seed-transmission trial 2, P1 was seed-transmitted at rates ranging from 5% (Charo) to 31% (Early Freezer 680), whereas the frequency of P4 seed transmission was <1% and only in cv. Dual (Table 2). In plants first inoculated with P4 and then with P1, total PSbMV seed transmission varied between 0% (Ceras and Early Freezer 680) and 6% (Charo), markedly less than in seeds from these cultivars when inoculated with P1 alone.

In mixture with P4, pathotype P1 was seed-transmitted only in seedlings from P1 + P4-inoculated Charo and Dual mother plants, whereas it was seed-transmissible in all four cultivars when inoculated alone. P4 was not detected in any of these same plants using the P4-NY-specific RT-PCR assay. This assay also verified ELISA-detectable seed transmission (0.6%) of P4 in progeny from P4-inoculated Dual mother plants.

In trial 1, total PSbMV seed transmission from mother plants inoculated with P1 versus P1 + P4 was greater in cvs. Ceras, Charo, and Scout, lesser in cv. Dual, and equal in the remaining cultivars (Table 1). In trial 2, in which P4 was introduced prior to P1, the presence of P4 uniformly suppressed P1 seed transmission (Table 2).

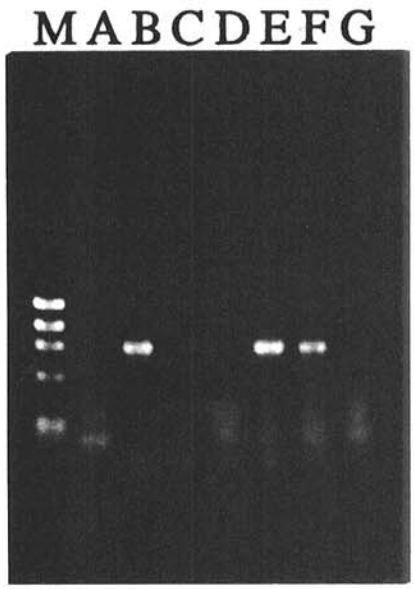


Fig. 2. Representative agarose-gel electrophoretic analyses of reverse transcription-polymerase chain reaction (RT-PCR)–generated products. Trials were replicated several times with identical results. Viral RNA preparations and infected pea leaf-tissue nucleic acid extracts were selectively amplified by RT-PCR using primers A and B to generate a predicted 850-bp product. Two microliters of a 100-µl PCR sample were analyzed in each lane. Lane A, 10 ng of pea seedborne mosaic virus pathotype 1 (PSbMV-P1) RNA; lane B, 10 ng of PSbMV-P4 RNA; lane C, water blank; lanes D and E, 1.0 µg of nucleic acid extracted from P1- (lane D) or P4-infected (lane E) pea leaves; lanes F and G, 1.0 µg of nucleic acid extracted from P1- and P4-infected leaf tissue combined in equal amounts (lane F) or noninfected pea leaves (lane G). DNA size markers (*Hae*III digest of ΦX174 replicative form DNA) of 1,353, 1,078, 872, 603, and 310 bp are shown in lane M.

In cases in which no seed transmission was detectable by DAS-ELISA (Table 2), detection of PSbMV RNA in all ELISA-negative samples was not attempted. However, such samples were often used as RT-PCR "negative controls." In such ELISA-negative samples, PSbMV was never detected by P1- or P4-specific RT-PCR.

In both seed-transmission trials, disease symptoms in P1 + P4-inoculated plants were generally more severe than in P4- or P1-inoculated plants. Seed numbers yielded per cultivar, consequently, were lower when plants of Abador, Dual, Scout, and Sounder were inoculated with both pathotypes (Table 1). However, seed numbers were not lower in P1 + P4-inoculated plants of other cultivars, particularly Challis, Charo, and Popet. Similarly, in trial 2, cvs. Ceras and Charo were very severely affected by P4- or P4 + P1-induced disease but yielded larger seed numbers than cvs. Dual and Early Freezer 680.

Evidence for P4-sequence-specific RT-PCR effectiveness was demonstrated in trial 2 (e.g., seed transmission of P4 in cv. Dual) by the generation of a predicted 850-bp product only in the presence of P4 RNA (Fig. 2B, E, and F), relative to the molecular marker, a 872-bp digestion product of ΦX174. No RT-PCR product of similar size was ever observed as nonspecific RT-PCR products. Blind tests of coded healthy, P1-infected, and P4-infected tissue samples established complete accuracy and reliability of the assay, i.e., each pathotype was identified with 100% accuracy.

DISCUSSION

Based on DAS-ELISA serology and capsid protein and 3′-untranslated region sequences (>90% sequence identity to PSbMV-P1, EMBL Accession No. X87938), P4 is, indeed, a pathotype of PSbMV but is distinguishable from PSbMV-P1 by crucial biological properties (i.e., extremely limited seed-transmissibility, absence from reproductive tissues in selected pea cultivars, and distinct aphid-transmission attributes). The present study and the reported analyses of the *sbm-4* gene conferring resistance to P4 in *P. sativum* (19) provide complementary evidence of the PSbMV identity of "P4-NY."

Our results indicate that P4-NY is seed-transmitted in *Pisum* at much lower rates than in either P1 or P2 (5,6 [lentil strain = P2], 10,11,16). This suggests that, in comparison with the other PSbMV pathotypes, P4 is less well adapted to U.S. *P. sativum* cultivars and that this could limit i) its dissemination in commercial seeds, ii) its pathological significance in commercial pea cultivars, and, possibly, iii) its long-term survival in agro-ecosystems. Our purpose was to characterize P4 behavior in commercial pea cultivars, alone and under the influence of P1. Thus, perpetuation of P4 in North India landraces, although certainly relevant, was beyond the scope of this study.

Seed-transmission rates of P4 did not limit its detection in *Pisum* germ plasm by Alconero and Hoch (1), who classified 42 of 189 seedborne PSbMV isolates from exotic pea genotypes as P4. Isolate P4-NY was regarded (R. Provvidenti, *personal communication*) as a representative of the 42 P4 isolates detected (1).

Isolates S4 and S6, reported as the P4 pathotype by Ligat and Randles (18), were seed-transmitted at rates of 90% but failed to induce disease symptoms (pea cv. Dundale) after successive transmissions through seeds. Asymptomatic mother plants, when assayed by either dot immunobinding or cDNA dot hybridization, contained no detectable PSbMV. Seeds from such plants, however, contained detectable PSbMV, and inoculum prepared from seeds was infectious. These characteristics of S4 and S6 isolates differ profoundly from those of P4-NY. We are currently making sequence comparisons between P4-NY and isolate S4.

PSbMV-P1 seed-transmission rates obtained in our studies approximated those observed in other studies (16). In two trials of P4-P1 interactions, the influence of P4 on P1 seed-transmissibility varied from neutral to suppressive or enhancing, depend-

ing on the pea cultivar, whereas P1 had no measurable influence on P4 seed-transmissibility. Thus, as measured in commercial pea cultivars, the more prevalent pathotype P1 does not appear to assist survival or perpetuation of the less common pathotype P4.

The sequence and timing of plant inoculations with P1 and P4 could readily influence resulting virus-virus and virus-host interactions. Plants in trial 1 were inoculated simultaneously with both pathotypes, whereas in response to trial 1 data plants in trial 2 were first inoculated with P4, anticipating that P4 seed-transmissibility might be enhanced if introduced first. However, the seed-transmission rate of P4 relative to P1 was similar in both trials and was not enhanced by dual inoculation with P1 in either trial.

Variations in viral seed-transmission rates would be expected to result from interactions involving host genotype, age of plant at infection, viral isolate characteristics, plant vigor/nutrition, ambient temperatures (3), and, possibly, virus-pathotype interactions. Thus, our data should be understood to represent only selected segments within a gamut of such variables.

Detection of PSbMV isolates P1-DPD1 and P4-NY by RT-PCR was based on sequence-specific priming and subsequent amplification of reverse-transcribed viral RNA. The primer pair for P1, nucleotide positions 6179 through 6204 and 7370 through 7393, respectively (17), facilitated amplification of a P1-specific 1,200-bp product. By comparison, a predicted 850-bp product unique to P4 in the current study was consistently generated by P4-sequence–determined primers A and B (Fig. 1) in tissue samples containing ≥10 pg of P4 RNA per 1 μg of tissue-extracted nucleic acid (Fig. 2). P4-specificity was indicated by the formation of this RT-PCR product only in the presence of P4 RNA (Fig. 2). The methods presented appear to have potential in other applications requiring sensitivity exceeding ELISA by at least 25-fold and a high degree of precision in virus or pathotype (P1-DPD1 and P4-NY) detection and identification.

In this study, we have been able to distinguish between the DPD1 isolate of P1 and a single isolate of P4. The specific nucleotide sequences conferring PSbMV pathogenicity are unknown. Therefore, pathotype-specific detection per se must await the identification of sequences or their translation products as determinants of the PSbMV pathotype.

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