Diversity Among Isolates Within the PAV Serotype of Barley Yellow Dwarf Virus

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ABSTRACT

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Several barley yellow dwarf virus (BYDV) isolates collected from viruliferous aphids in upstate New York were identified as PAV serotypes, based on their reaction with a polyclonal antiserum to NY-PAV. Four of six isolates examined were distinguished from the NY-PAV type isolate of BYDV by their failure to react with a PAV-specific monoclonal antibody in enzyme-linked immunosorbent assay (ELISA) and by restriction fragment length polymorphisms of polymerase chain reaction-amplified viral sequences. The capsid protein amino acid sequence of one of these four isolates, designated PAV-129, was less similar to that of NY-PAV (86.5% similar) than NY-PAV is to two other isolates, serotyped as PAV, from Indiana (98% similar) or Australia (97% similar). In biological comparisons of PAV-129 and NY-PAV, the efficiency of transmission by two aphid species, *Rhopalosiphum padi* and *Sitobion avenae*, was not significantly different; however, PAV-129 caused more severe symptoms when inoculated to a variety of oat genotypes. In addition, the growth and grain yield of 'Ogle', a spring oat considered resistant to the BYDV PAV serotype was significantly reduced when infected with PAV-129.

Barley yellow dwarf is an economically damaging disease of cereals worldwide (16). The disease is caused by a group of luteoviruses known collectively as barley yellow dwarf virus (BYDV). Five type isolates of BYDV, collected in New York and identified on the basis of their specific aphid vectors (22,28,30), have been used as antigens to produce polyclonal and monoclonal antibodies. These antibodies are frequently used to assign BYDV isolates from wide geographical areas to one of five BYDV serotypes (PAV, MAV, RPV, RMV, and SGV).

Serological, cytological, and, more recently, nucleic acid sequence data have been used to separate the five serotypes into two distinct subgroups (18); subgroup I includes the PAV, MAV, and SGV serotypes and subgroup II includes the RPV and RMV serotypes. Comparison of capsid protein gene sequences of subgroup II isolates suggest that considerable diversity may exist among isolates within the same serotype from different geographical areas. The capsid protein amino acid sequence of isolates within the RMV serotype from New York and Illinois are only 80% identical, whereas those of isolates from Illinois and nearby Minnesota are 97% identical (7). In addition, serological differences, detectable using polyclonal antisera, have been reported among isolates within the RMV serotype from New York and Montana (37).

Conversely, isolates within the subgroup I serotypes appear to have a higher degree of capsid protein sequence similarity regardless of geographical origin. Capsid protein sequences of isolates in the SGV serotype from Texas and New York share 96% similarity (15), and isolates within the PAV serotype from New York, Indiana, and Australia share at least 96% similarity (26,34). Serological variants within the SGV and MAV serotypes have been identified using monoclonal antibodies (15,17), but no serological differences have been found among isolates within the PAV serotype (2,5,19). The data from the aforementioned sequence comparison and serological studies suggest a high degree of similarity among isolates belonging to the PAV serotype from diverse geographical areas. However, considerable variability can exist in aphid transmission phenotype (8) and in the disease severity and symptom expression (2,21) induced by isolates within the PAV serotype. Sequence and serological variability of isolates from a single location have not been examined. The objective of this study was to examine the degree of diversity among members of the PAV serotype from a single location using biological, serological, and molecular methods of classification.

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MATERIALS AND METHODS

Virus isolates, aphid transfer, and virus purification. The New York PAV isolate of BYDV (NY-PAV) and its propagation in oat (*Avena byzantina* K. Koch cv. Coast Black) have been described (28). Field isolates were obtained from migrating alate *Rhopalosiphum padi* (Linnaeus) Kaltenbach aphids that were alighting on winter wheat grown at the Musgrave Research Farm, Aurora, NY. Aphids were collected during October and November of 1992, returned to the laboratory, and individually placed on oat seedlings. Virus was subsequently recovered from the oats that became infected. Six isolates identified as 83, 88, 129, 228, 251, and 257 were selected for further study on the basis of their ability to induce more severe symptoms on 'Coast Black' oat than those induced by NY-PAV. Isolate 129 induced the most severe symptoms. A PAV isolate from Illinois, IL-PAV (13), provided by A. Hewings, USDA-ARS, was also used in several experiments. All isolates were maintained in oat by repeated transmission with *R. padi*. Virions were purified from plants as described by Hammond et al. (12).

Enzyme-linked immunosorbent assay (ELISA). Double antibody sandwich (DAS)-ELISA was performed essentially as described by Gray et al. (10). Polyclonal antisera to NY-PAV, NY-

MAV, and NY-RPV were described by Rochow and Carmichael (29). Polyclonal antisera to NY-SGV and NY-RMV were described by Webby and Lister (36). Serotype identification of all field isolates was based on positive reactions using polyclonal antisera to PAV and negative reactions with polyclonal antisera to RPV, RMV, and SGV in DAS-ELISA. Antisera to NY-MAV and NY-PAV cross-react with NY-PAV and NY-MAV antigens, respectively (29); therefore, monoclonal antibodies (described below) were used to distinguish between PAV and MAV serotypes.

Triple antibody sandwich (TAS)-ELISA was performed essentially as described by D'Arcy et al. (4), using the antiserum to NY-PAV as the primary antibody. Monoclonal antibodies used as secondary antibodies included PAV-1C2 (6), which recognizes a linear epitope on the NY-PAV capsid protein previously mapped to a domain within amino acids 45 to 64 (26); PAV-IL-1, which is specific to an epitope of the undisrupted capsid of IL-PAV (4); and MAV-3, which was produced against the NY-MAV isolate and cross-reacts with NY-PAV (14). Monoclonal antibodies specific to the NY-MAV isolate, MAV-1 and MAV-4 (14), were also used in TAS-ELISA to rule out the possibility that an isolate belonged to the MAV serotype. The tertiary antibody used in all TAS-ELISA was goat-anti-mouse conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis).

Reverse transcriptase-polymerase chain reaction (RT-PCR) of field isolates and cloning and sequencing the capsid protein gene of isolate 129. Nucleic acid extraction from leaf tissue and RT-PCR using primers specific for luteovirus capsid protein sequences were performed using oligonucleotides Lu-4 and Lu-1 as described in Robertson et al. (27). DNA products of approximately 530 bases pairs were digested with restriction enzymes *Sau*3AI, *Hae*III, and *Taq*I (New England Biolabs, Beverly, MA) and restriction fragments were separated on 5% polyacrylamide gels and stained with ethidium bromide.

To amplify the entire capsid protein coding sequence of isolate 129, the same procedure described above was followed except that oligonucleotide P3 (5′-GGTGAAATGAATTCAGTAGG-3′), identical to bases 2,853 through 2,871 of Vic-PAV (20), was used in place of oligonucleotide Lu-1. The resulting PCR product was ligated into the "TA" cloning vector (Invitrogen, San Diego, CA), and the nucleotide sequences of the plasmid inserts were determined using an automated sequencer (Applied Biosystems, Inc., Foster City, CA). Sequence was determined from two different plasmids derived from independent PCR reactions. Sequence comparisons were performed with "PILEUP" from the Genetics Computer Group (GCG) software program (Madison, WI).

Virus transmission studies. The transmission efficiency of NY-PAV and PAV-129 by *R. padi* or *Sitobion avenae* Fabricius was measured in four independent experiments. Seedlings of 'Coast Black' oat were inoculated with NY-PAV or PAV-129 and allowed to grow in the greenhouse for 3 to 4 weeks for use as virus source tissue. Fourth instar aphids were allowed a 48-h acquisition access period on detached leaves. Single aphids were then transferred to individual, healthy oat seedlings, 17 to 20 per treatment, for a 72- to 96-h inoculation access period. Aphids were killed by fumigation with *O,O*-dimethyl-*O*-(2,2-dichlorovinyl) phosphate (DDVP) in a closed chamber and test plants were placed in an insect-free greenhouse, observed for symptoms, and, in some cases, tested for infection by ELISA. Transmission efficiency was calculated as the proportion of aphids transmitting virus from each source leaf.

Host response to virus infection. Disease induced by each of the virus isolates was examined on several oat genotypes including two spring oat cultivars, Astro and Clintland 64, and a winter oat cultivar, Coast Black, all of which are susceptible to BYDV (11,32). In addition, disease was evaluated on the spring oat cultivar Ogle and a spring oat breeding accession IL86-5262, both of which are reported to be resistant to BYDV (11). Plants at the first leaf stage were inoculated using three to five aphids per plant as described above, and subsequently grown in either a glasshouse or a growth chamber at 20°C during the day and 15°C at night with a 16-h light, 8-h dark cycle. DAS-ELISA was used to determine the infection status of individual plants.

RESULTS

Serological characterization of field isolates. NY-PAV, IL-PAV, and the six field isolates obtained from viruliferous *R. padi* aphids reacted with PAV and MAV antisera, but not with antisera to RMV, RPV, and SGV, with the exception of isolate 88, which also reacted with antisera to RPV (Table 1). Isolate 88 was later found to be the product of a mixed infection of a PAV serotype and a RPV serotype. None of the isolates reacted with MAV-1 and MAV-4 in ELISA (data not shown), indicating all were of the PAV serotype. The field isolates were subsequently labeled PAV-83, PAV-88, PAV-129, PAV-228, PAV-251, and PAV-257.

Plants infected with the field isolates or NY-PAV were compared by TAS-ELISA using three different monoclonal antibodies known to react with PAV serotypes MAV-3, IL-1, and 1C2. All isolates were detected by monoclonal antibodies MAV-3 and IL-1, but only two of the six field isolates, PAV-251 and PAV-257,

TABLE 1. Reactivity of various barley yellow dwarf virus (BYDV)-specific polyclonal and monoclonal antibodies to PAV serotype field isolates in enzyme-linked immunosorbent assays (ELISA)[a]

Virus isolate	Polyclonal antisera[b]					Monoclonal antisera[c]		
	PAV	MAV	SGV	RPV	RMV	IL-1	MAV-3	1C2
Healthy	0.03[d]	0.05	0.05	0.00	0.01	0.02	0.01	0.04
Control[e]	–	0.30	0.29	0.46	0.42	0.02	0.06	0.01
NY-PAV[f]	0.68	0.19	0.08	0.00	0.02	0.42	0.19	0.46
PAV-83	0.27	0.12	0.09	0.00	0.03	0.10	0.16	0.02
PAV-88	0.26	0.13	0.05	0.26	0.03	0.22	0.16	0.02
PAV-129	0.38	0.14	0.03	0.00	0.03	0.38	0.16	0.02
PAV-228	0.66	0.21	0.11	0.00	0.03	0.20	0.26	0.03
PAV-251	0.32	0.13	0.07	0.00	0.03	0.24	0.09	0.48
PAV-257	0.34	0.11	0.08	0.00	0.09	0.22	0.10	0.78

[a] Polyclonal antibodies were used in double antibody sandwich (DAS)-ELISA (homologous coating and conjugating antibodies) and monoclonal antibodies were used in triple antibody sandwich (TAS)-ELISA. The PAV polyclonal antibody was used as the coating antibody in all TAS-ELISA.

[b] The five type isolates of BYDV from New York were used as antigens to produce the polyclonal antisera which are described in Rochow and Carmichael (29) or Webby and Lister (36).

[c] The monoclonal antibodies IL-1 and 1C2 are PAV-specific; MAV-3 reacts with both MAV and PAV serotypes.

[d] Absorbance values (A_{405}) were the means of duplicate wells using one sample. Samples were tissue extracts diluted 1:5 in phosphate-buffered saline (PBS).

[e] Positive control samples used with polyclonal antisera were from plants infected with the homologous isolate. Sap from MAV-infected oat was used as a control for the monoclonal antibodies.

[f] The PAV serotype isolate from New York.

reacted with monoclonal antibody 1C2 (Table 1). Thus, isolates PAV-83, PAV-88, PAV-129, and PAV-228 were serological variants within the PAV serotype.

To examine the relative affinity of several antibodies for representative virus isolates, virions were purified from plants infected with NY-PAV and two field isolates; one that reacted with monoclonal 1C2, PAV-251, and one that did not, PAV-129. Polyclonal antisera to NY-PAV had similar affinities for these three isolates; however, the monoclonal antibody IL-1 had a lower affinity for PAV-129 relative to PAV-251 or NY-PAV (Table 2). Monoclonal antibody 1C2 failed to react with PAV-129, but had a similar affinity for both NY-PAV and PAV-251.

Restriction fragment length polymorphism (RFLP) analysis of isolates. A portion of the capsid protein gene was amplified by RT-PCR using nucleic acid samples extracted from plants infected with NY-PAV or one of the six field isolates. RT-PCR products of the expected size (approximately 530 nucleotides) were obtained from all samples and were digested with three different restriction enzymes. Restriction maps based on these data are shown in Figure 1. All isolates had identical RFLP patterns when digested with *Taq*I, while digestion with *Sau*3AI separated the field isolates into two groups. Group A contained isolates PAV-251, PAV-257, and NY-PAV. Group B yielded an additional restriction fragment and included isolates PAV-83, PAV-88, PAV-129, and PAV-228. Digestion with *Hae*III identified group A, but further subdivided group B; PAV-129 generated a RFLP pattern that differed from PAV-83, PAV-88, and PAV-228. IL-PAV generated a *Hae*III RFLP pattern that was identical to NY-PAV (data not shown). The RFLP patterns expected from sequence data would be identical to NY-PAV for P-PAV (PAV isolate from Indiana); whereas Vic-PAV (PAV isolate from Australia) would generate RFLP patterns unlike any of those shown in Figure 1.

Preliminary field surveys were conducted to investigate the relative occurrence of virus isolates within the PAV serotype that generated the various *Hae*III digestion patterns shown in Figure 1. Fourteen symptomatic winter wheat plants were collected at Aurora, NY, in October 1993. The RFLP patterns from nine samples were identical to that of NY-PAV, two samples were identical to that of PAV-228, three samples had NY-PAV patterns plus additional bands characteristic of PAV-228, and no samples matched the pattern of 129 (data not shown). Furthermore, 32 isolates were obtained from viruliferous alate aphids collected in October 1994. All were identified as PAV serotypes using polyclonal antisera and all were evaluated for their ability to react with monoclonal antibody 1C2. Twenty-three did not react in TAS-ELISA with 1C2. RFLP data were obtained for 7 of the 23; six generated RFLP patterns similar to PAV-228 and one similar to PAV-129.

TABLE 2. Reaction of purified virions with polyclonal and monoclonal antisera

	DAS-ELISA[b]		TAS-ELISA[c]		
Virus isolate[a]	PAV	MAV	IL-1	MAV-3	1C2
NY-PAV[d]	0.358[e]	0.060	0.194	0.326	0.450
PAV-251	0.477	0.071	0.280	0.292	0.530
PAV-129	0.533	0.211	0.051	0.378	0.011

[a] Fifty nanograms of purified virions were diluted in healthy plant sap. A_{405} of healthy plant sap for each antibody was: pcPAV = 0.005, pcMAV = 0.008, mcIL-1 = 0.002, mcMAV-3 = 0.000, and mclC2 = 0.012.

[b] Double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) used polyclonal antibodies raised against the NY-PAV or NY-MAV isolates.

[c] Triple antibody sandwich-enzyme-linked immunosorbent assay (TAS-ELISA) used virions trapped with PAV polyclonal antisera. Monoclonal antibodies IL-1 and IC2 were raised against PAV serotypes and were PAV-specific. Monoclonal MAV3 was raised against NY-MAV, but cross-reacts with PAV serotypes.

[d] The PAV serotype isolate from New York.

[e] Absorbance values (A_{405}) were averages of samples from duplicate wells.

Isolate PAV-129 capsid protein sequence. The capsid protein gene of PAV-129 coded for a protein of 200 amino acids (Fig. 2), which was identical to the number of amino acid residues in the capsid proteins of all other PAV isolates that have been sequenced. The capsid protein of isolate PAV-129 shared no greater than 87% amino acid identity with PAV isolates from New York (NY-PAV) (26), Indiana (P-PAV) (33), and Australia (Vic-PAV) (20) (Table 3).

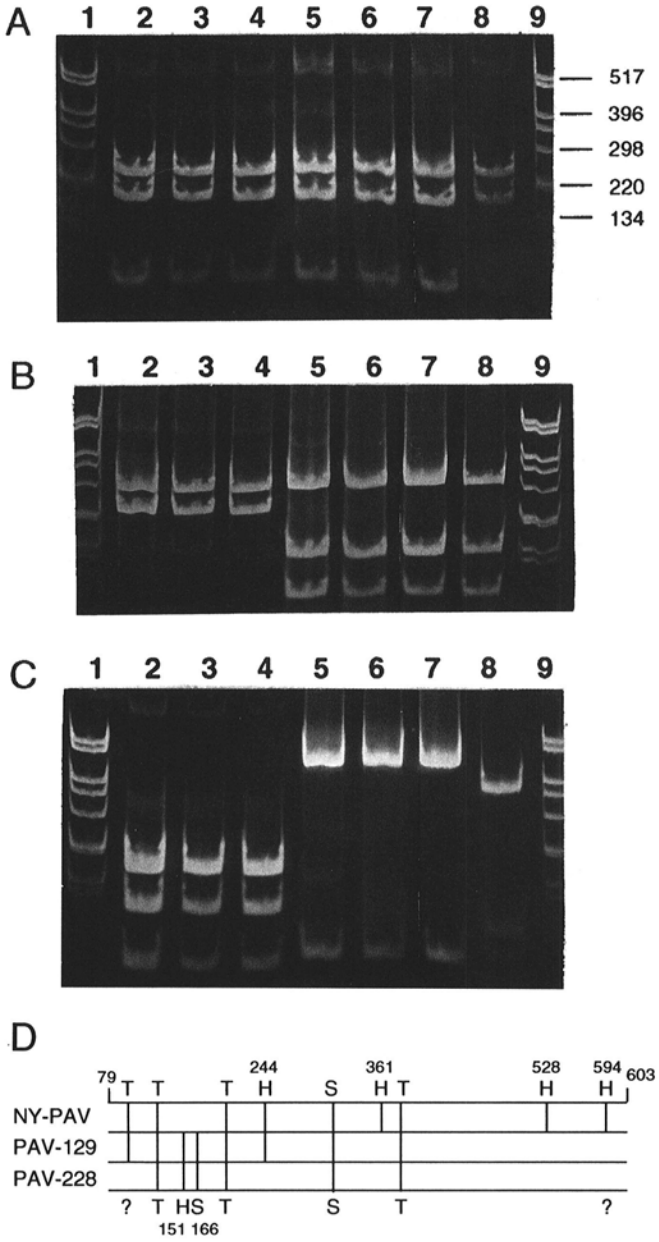

Fig. 1. A, Restriction fragment length polymorphism (RFLP) of polymerase chain reaction (PCR) products from the PAV isolate from New York (NY-PAV) and six PAV-like field isolates digested with *Taq*I. **B,** RFLP of PCR products from NY-PAV and six PAV-like field isolates digested with *Sau*3AI. **C,** RFLP of PCR products from NY-PAV and six PAV-like field isolates digested with *Hae*III. Samples were analyzed on 5% polyacrylamide gels. Lanes 2 to 8: NY-PAV, PAV-251, PAV-257, PAV-83, PAV-88, PAV-228, and PAV-129, respectively. Lanes 1 and 9: DNA size markers (BRL; Bethesda Research Laboratories, Gaithersburg, MD). **D,** Restriction maps of the PCR products amplified from the capsid protein of NY-PAV, PAV-129, and PAV-228. The maps for the NY-PAV and PAV-129 were determined from their nucleotide sequence, and the map for PAV-228 was deduced from RFLP analysis. The restriction sites indicated are *Hae*III (H), *Taq*I (T), and *Sau*3AI (S); question marks indicate sites that yield fragments were too small to detect in this RFLP analysis. The nucleotide positions of the termini of the PCR product and of sites that differed in the three groups are given, with numbers corresponding to the capsid protein sequence of NY-PAV (23).

Of the 12 nonconserved changes between PAV-129 and NY-PAV, six were clustered between amino acid residues 46 and 60 and four were between residues 109 and 120 (Fig. 2). Residues 46 to 60 overlapped with the proposed surface epitope of monoclonal antibody 1C2, which has been mapped to a segment containing amino acid residues 45 to 64 (26). Amino acid changes within this region, therefore, provided an explanation for the failure of monoclonal antibody 1C2 to bind to isolate 129 in ELISA. The coding sequence for three of the six nonconserved amino acid changes between NY-PAV and PAV-129 within the 1C2 epi-

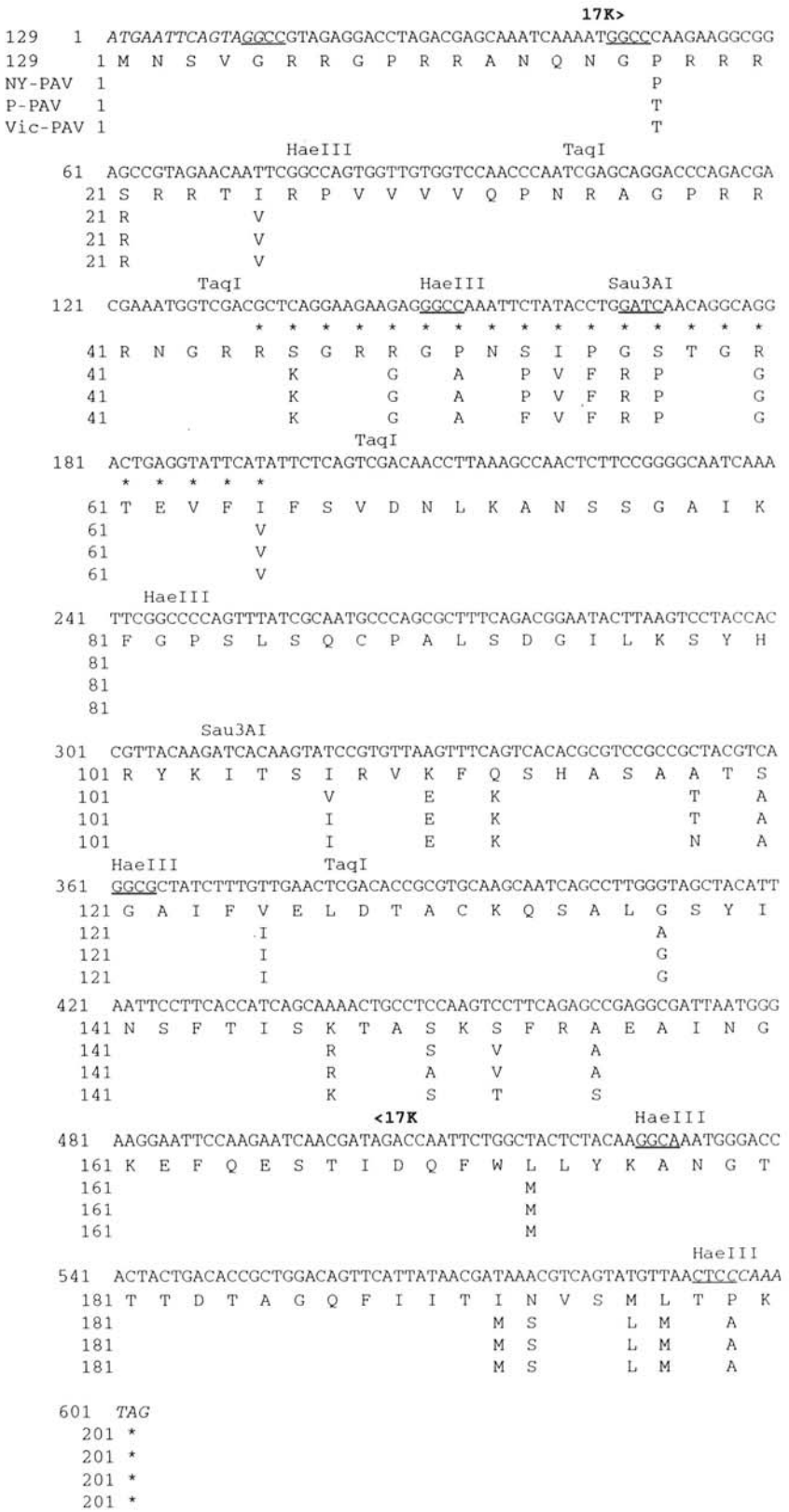

Fig. 2. Nucleotide sequence of the capsid protein gene of PAV-129. The predicted amino acid sequence for the capsid protein of PAV-129 is shown and differences from this sequence within the capsid protein of PAV serotype isolates from New York (NY-PAV), Indiana (P-PAV), and Australia (Vic-PAV) are designated below the PAV-129 sequence. The nucleotide and amino acid sequence in italics was derived from the primers used to clone the gene. Restriction sites for *Sau*3AI (GATC) and *Hae*III (GGCC) that differed between the PAV-129 and NY-PAV sequence are underlined. The epitope of monoclonal antibody 1C2, previously mapped to the polypeptide, is indicated by "*" symbols. References for previously published capsid protein sequence are Vic-PAV (20), P-PAV (33), and NY-PAV (26).

tope corresponded to nucleotide changes in PAV-129 that created a *Hae*III (nucleotide position 151) and a *Sau*3AI (nucleotide position 167) restriction site. It is interesting to note that isolates that failed to react with 1C2 shared these two restriction sites (Fig. 1D), suggesting that they may have similar amino acid changes at these nucleotide positions. This would explain the consistent correlation between the RFLP pattern of these four isolates with *Sau*3AI and their failure to bind to monoclonal 1C2 in TAS-ELISA.

PAV-129 was distinguished from PAV-83, PAV-88, and PAV-228 in RFLP analysis by the presence of a *Hae*III site at nucleotide position 244. Comparison of PAV-129 and NY-PAV capsid protein sequences showed that PAV-129 and NY-PAV shared this site, and it was, therefore, not unique to PAV-129.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). BYDV particles contain two protein species, a capsid protein and a capsid protein-readthrough product, the carboxyl-terminus of which is truncated (35). The capsid protein from PAV-251 comigrated with that of NY-PAV in SDS-PAGE, while the capsid protein from isolate 129 migrated faster than expected (Fig. 3). The predicted M_r values of the NY-PAV and NY-RPV capsid proteins, shown for comparison, are 21,993 (26) and 22,190 (34), respectively. The predicted M_r value of the PAV-129 capsid protein was 21,911. Therefore, one would expect it to migrate similar to the NY-PAV capsid protein. The difference in the expected and observed mobility of the PAV-129 capsid protein may be because of differences in amino acid composition, although the overall charge of the different PAV coat proteins was similar. The Coomassie-stained protein that migrated slower than the truncated readthrough protein was not detectable in Western blots of purified virion samples using virion-specific antibodies (data not shown; 35) and was, therefore, believed to be a host protein. The host protein was not observed in the PAV-129 preparation used to generate Figure 2, but was visible in other preparations.

TABLE 3. Comparison of the nucleotide and deduced amino acid sequences of the coat proteins from isolates within the PAV serotype and the NY-MAV isolate of barley yellow dwarf virus (BYDV)

Serotype isolates[a]	NY-PAV	P-PAV	Vic-PAV	PAV-129
	Deduced amino acid identity (%)			
NY-MAV	73.7	73.2	73.7	71.2
NY-PAV	–	98.0	97.0	86.5
P-PAV	–	–	97.0	86.6
Vic-PAV	–	–	–	87.1
	Nucleotide identity (%)			
NY-MAV	78.6	78.7	78.2	77.7
NY-PAV	–	94.0	94.0	89.6
P-PAV	–	–	95.0	90.2
Vic-PAV	–	–	–	89.6

[a] The PAV serotype isolates were from New York (NY), Indiana (P), and Australia (Vic). References for coat protein sequences are: NY-MAV (25), NY-PAV (26), P-PAV (33), and Vic-PAV (20).

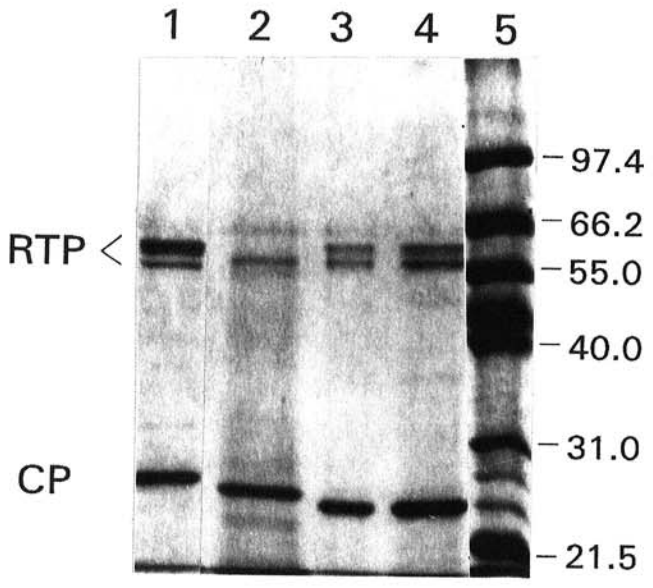

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of capsid proteins from the PAV isolate from New York (NY-PAV) and two PAV-like field isolates of barley yellow dwarf virus (BYDV). Virions were disrupted with SDS and the capsid proteins were separated through 15% polyacrylamide. Lane 1, NY-RPV; lane 2, isolate 129; lane 3, isolate 251; lane 4, NY-PAV; and lane 5, molecular weight standards. Position of the coat protein (CP) and coat protein-readthrough proteins (RTP) are indicated.

Aphid transmission phenotype of isolates. Results from four independent transmission tests indicated that the transmission efficiencies of NY-PAV and PAV-129 by single adults of either *R. padi* or *S. avenae* were not significantly different. Mean transmission efficiency (± standard deviation) by *R. padi* was 53% ± 24% and 50% ± 23% for NY-PAV and PAV-129, respectively. *S. avenae* transmitted NY-PAV or PAV-129 to 17% ± 10% and 13% ± 5% of the test plants, respectively.

Symptom severity on oat genotypes. Symptoms induced in 'Coast Black' oat by all field isolates were more severe than those induced by NY-PAV, and the symptom severity remained stable in multiple passages of isolates over 2 to 3 years. In three independent glasshouse experiments, the fresh weight of plants infected with PAV-251 or PAV-129 was significantly lower than that of plants infected with NY-PAV. In one experiment, at 7 weeks postinoculation the mean fresh weight ± standard deviation (n = 5) of plants infected with NY-PAV was 7.8 ± 0.6 g, whereas that of plants infected with PAV-251 or PAV-129 was 3.2 ± 0.3 g and 3.0 ± 0.2 g, respectively. The fresh weight of mock-inoculated plants was 12.8 ± 3.9 g (n = 4).

The consistent difference in the symptom phenotype induced by PAV-251 or PAV-129 on 'Coast Black' oat in the greenhouse was also observed in growth chamber-grown plants. Symptoms induced by PAV-251 appeared earlier and spread faster to upper leaves than those induced by NY-PAV. Qualitatively, however, the symptoms were similar to those induced by NY-PAV, and were characterized by progressive yellowing of leaves from the leaf tip to the base and spread of yellowing from first leaf to older leaves. In contrast, plants infected with PAV-129 developed yellowing symptoms later than plants infected with NY-PAV or PAV-251, but the PAV-129-infected plants were more stunted. Emergence of the third leaf in PAV-129-infected plants was consistently delayed and development of this leaf was abnormal; notches in the leaf margins and/or corkscrew-like twisting of the leaf blade were common (Fig. 4).

To test whether the symptom severity and phenotype caused by PAV-129 was specific to 'Coast Black' oat, we inoculated this isolate to four additional oat genotypes. Grain and straw weight of 'Astro' and 'Clintland 64' plants (susceptible to BYDV) and 'Ogle' plants (resistant to BYDV) infected with PAV-129 were significantly reduced compared with that of NY-PAV (Fig. 5). These hosts developed severe symptoms that were characteristic of PAV-129 infection in 'Coast Black' oat. Grain and straw weight of IL86-5262 plants resistant to BYDV, were not significantly reduced by infection with PAV-129 or NY-PAV (Fig. 5), and plants did not develop symptoms (Fig. 4).

DISCUSSION

Investigations of PAV isolates from wide geographical areas (4) and of PAV isolates that differ in virulence (2), though limited, failed to detect serological variation within this serotype. Our

results demonstrated that there was serological variability among isolates within the PAV serotype. The serological variants were not readily distinguished using polyclonal antisera, but could be identified using monoclonal antibodies. Similar findings have been reported for isolates belonging to the MAV and SGV serotypes (15,17). Based on these results, we would caution against use of monoclonal antibodies for field survey work or to initially group BYDV isolates into serotypes. Our limited field surveys in 1993 and 1994 indicated that 30 to 70% of the PAV-positive plants identified by the polyclonal antibody would have been missed if we had used the monoclonal antibody 1C2 as the diagnostic antibody.

The RFLP analyses of amplified capsid protein sequences provided an alternative method to distinguish variants within the PAV serotype and to indicate the diversity among isolates from a single geographical location and different geographical locations, e.g., Vic-PAV and NY-PAV. Based on our preliminary field surveys, the RFLP variants that differed from the NY-PAV were commonly found infecting winter wheat. Furthermore, a field isolate of unknown serotype obtained from winter wheat in Nebraska (27) displayed a *Sau*3AI RFLP pattern identical to PAV variants in this study (Fig. 1B, lanes 5 to 8) suggesting that this variant may be widely distributed in BYDV populations.

The correlation between the *Sau*3AI restriction pattern of PCR products and the failure of field isolates to bind to monoclonal 1C2 raises the possibility that this restriction site serves as an indicator for loss of the 1C2 epitope. A majority of amino acid changes between PAV-129 and NY-PAV occurred between amino acid positions 46 and 60 of the capsid protein. The epitope for the 1C2 monoclonal antibody is located within amino acids 45 to 64 (26), and the sequence of nucleotides encoding these amino acids contains both a *Hae*III and a *Sau*3AI restriction site. It is interesting to note that in a previous comparison of luteovirus capsid proteins, this same region (amino acids 52 to 61) was less conserved than other regions (34). Therefore, this portion of capsid protein may constitute a variable region on the surface of the virion, although the significance of such variation awaits further information on the structure of the luteovirus particle.

The capsid protein is the major component of the virus particle and is presumably required for aphid transmission (35). Our results suggested that the amino acid differences between the capsid proteins of PAV-129 and NY-PAV did not affect the efficiency or vector specificity of aphid transmission. It is possible, however, that the aphid transmission phenotype of the two isolates may differ with aphid species other than the two used in this study.

Differences in symptom severity or virulence of the isolates on different host plants may also be related to the variation in capsid protein sequences. Infection of several oat genotypes by the field isolates used in this study resulted in increased symptom severity and decreased yield, relative to the NY-PAV. Two of these isolates, PAV-251 and PAV-257, were indistinguishable from NY-PAV; therefore, we cannot correlate the RFLP patterns shown in Figure 1 with symptom phenotype. It is possible that subtle changes in the capsid protein, undetected in this study, may be related to changes in symptom severity. Minor changes in amino acid sequences of the capsid proteins of several viruses have been associated to alterations in symptom phenotype (1,3,31). PAV-129 was of particular interest because it exhibited a unique symptom phenotype, which was similar to that of a subculture of MAV reported in Indiana, called "notched" (23,24). PAV-129 also differed from NY-PAV in its ability to overcome the resistance/tolerance

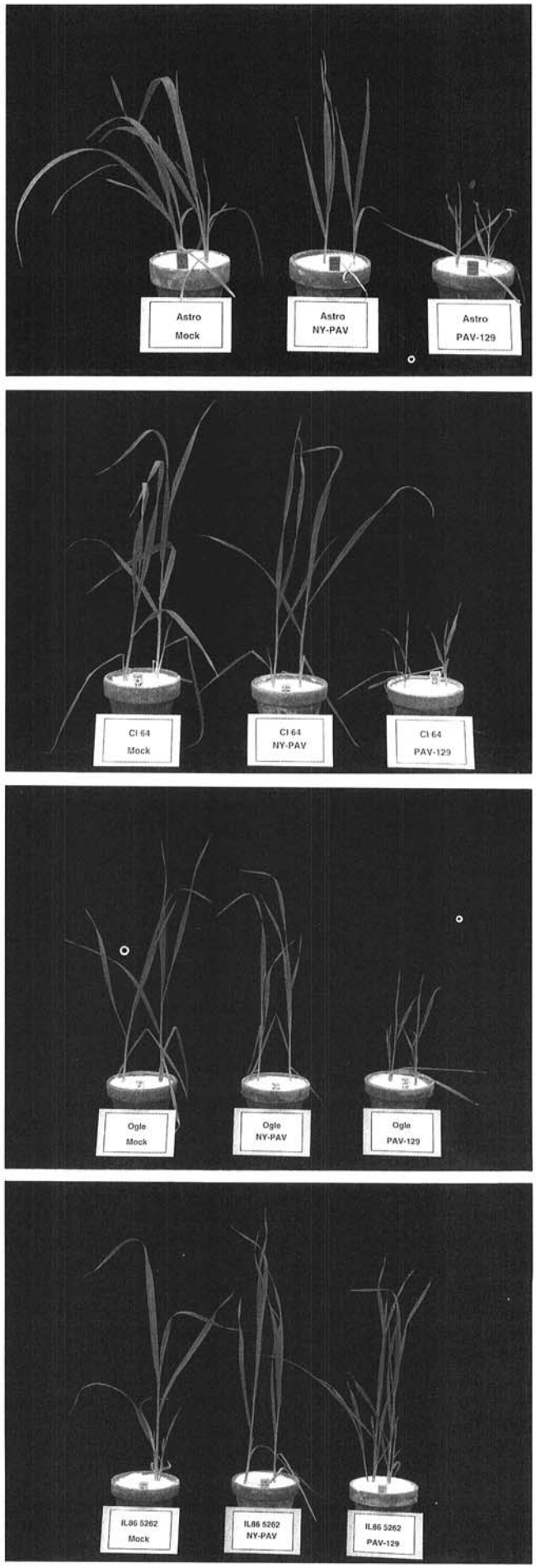

Fig. 4. Comparison of plants of four oat genotypes infected with PAV-129 or the PAV isolate from New York (NY-PAV) or mock inoculated. Plants were inoculated at the one- to two-leaf stage by allowing 10 to 15 viruliferous aphids a 72-h inoculation access period. Photographs were taken 4 weeks postinoculation.

mechanisms in 'Ogle' and cause severe disease and yield loss. Additional work is required to determine whether changes in capsid protein sequences are responsible for these altered virulence and symptom phenotypes, or if symptom determinants are located in other regions of the viral genome. However, these results indicated that the ability of PAV serotypes to cause disease on host plants could not be predicted by NY-PAV or any single isolate, and biological variation among isolates should be considered when screening plants for resistance or tolerance.

The degree of capsid protein sequence variation between PAV-129 and NY-PAV was surprising, since a high degree of amino acid sequence similarity (at least 97% amino acid identity) was previously found in comparisons of the capsid proteins of PAV isolates collected from a wide geographical region, e.g., New York, Indiana, and Australia (26,34). Sequence divergence of capsid protein genes of BYDV isolates within a serotype is not unprecedented, although the divergence has been among isolates from different geographical regions rather than within a region. Domier et al. (7) reported that the nucleotide sequence of capsid protein genes of RMV serotypes from Illinois and Minnesota were 99% similar, whereas they were only 80% similar to an isolate from New York. The nucleotide sequence of the capsid protein gene from a fourth RMV serotype from Montana further supports geographical diversity by having only 76% similarity to the Illinois and Minnesota isolates and 78% similarity to the New York isolate (9). The Illinois and Minnesota isolates were obtained from infected maize, the Montana isolate from barley, and the New York isolate from oat. It is interesting to note that we identified the PAV isolates that differed from NY-PAV only from infected maize plants, aphids moving from maize into winter wheat, or fall-infected winter wheat plants. Only isolates similar to NY-PAV were found in infected spring oat plants or aphids alighting on cereals in the spring. NY-PAV was isolated from an infected oat plant, while the very similar P-PAV and Vic-PAV were isolated from infected wheat plants. Currently, we do not know whether heterogenous populations of BYDV isolates belonging to the same serotype are supported by all hosts, but our observations, along with the data on RMV serotype diversity, suggested that the host plant, or the aphid vector population, may play a role in the diversity, selection, and maintenance of variants within a common BYDV serotype.

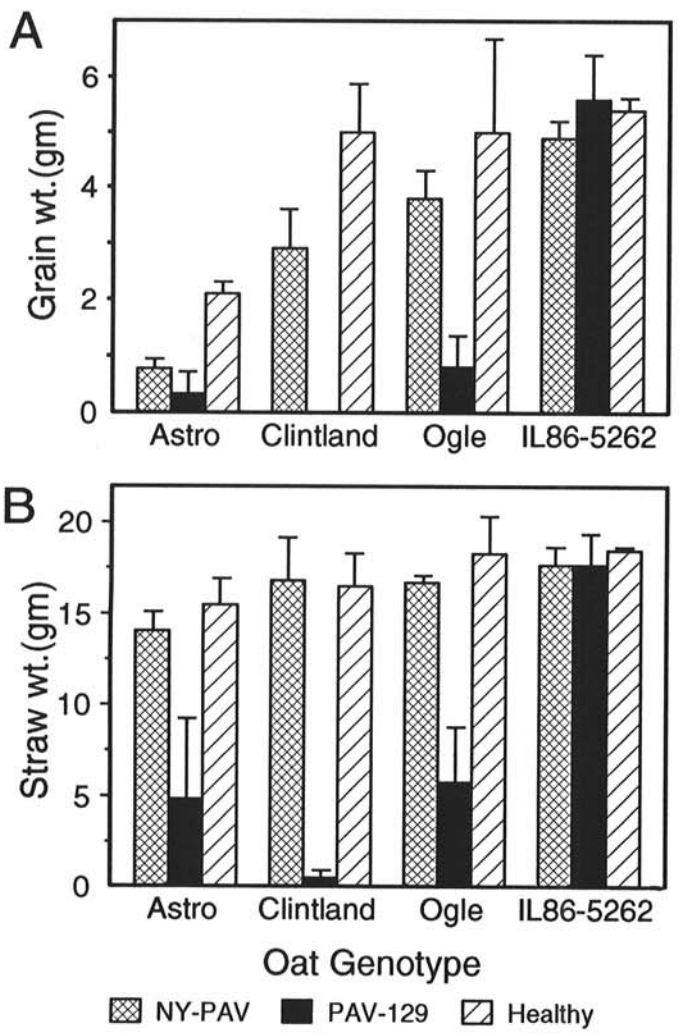

Fig. 5. A, Grain weight of four oat genotypes infected with PAV-129 or the PAV isolate from New York (NY-PAV). **B,** Straw weight of four oat genotypes infected with PAV-129 or NY-PAV. Plants were inoculated at the one- to two-leaf stage and grown in a growth chamber at 20°C during the day and 15°C at night with a 16-h light, 8-h dark cycle. Error bars represent the standard error for the mean of three plants.

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