SGV Serotype Isolates of Barley Yellow Dwarf Virus Differing in Vectors and Molecular Relationships

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The nucleotide sequence data reported in this paper will appear in the DDJB, EMBL, and GenBank nucleotide sequence databases under the accession number U06865 for BYDV (NY-SGV) and the accession number U06866 for BYDV (TX-SGV).
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ABSTRACT

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Two SGV serotype isolates of barley yellow dwarf virus (BYDV), NY-SGV from New York State and TX-SGV from Texas, exhibited differences in aphid transmissibility that could significantly influence their relative occurrence and epidemiology; i.e., TX-SGV was readily transmitted by a range of vector aphids, whereas NY-SGV showed a much greater vector specificity. In serological assays, TX-SGV differed from NY-SGV but resembled an SGV serotype isolate from Idaho that shares similar vector relationships with TX-SGV. Dot blot hybridization assays using cloned cDNA probes distinguished the SGV isolates from the P-PAV, MAV-PS1, NY-RPV, and NY-RMV isolates of BYDV and from each other. Nucleotide sequences were determined for the 22-kDa coat protein gene, the associated 17-kDa internal open reading frame, and a 50-kDa protein gene of the NY-SGV and TX-SGV isolates. The deduced amino acid sequences of these proteins shared approximately 96% similarity between isolates but had only about 71% similarity with comparable regions from the MAV-PS1 and P-PAV serotype isolates and approximately 57% similarity with those of the NY-RPV isolate. These comparisons did not identify obvious differences in primary structure that might be related to differences in vector relationships of NY-SGV and TX-SGV. The results demonstrate that the SGV serotype is distinct from other BYDV serotypes and that it includes sequence-distinguishable variants that differ in epidemiologically significant properties, such as transmissibility by various vectors.

The barley yellow dwarf luteoviruses (BYDVs) were originally differentiated as five biotypes (MAV, PAV, SGV, RPV, and RMV) by their aphid transmissibility (18). Isolates representative of each biotype, designated on the basis of vector relationships and their origin in New York state (19), were named: NY-RPV, transmitted readily by *Rhopalosiphum padi* (L.); NY-RMV, transmitted readily by *R. maidis* (Fitch); NY-MAV, transmitted readily by *Sitobion avenae* (Fabricius); NY-PAV, transmitted readily by *R. padi* and *Sitobion avenae*; and NY-SGV, transmitted readily by *Schizaphis graminum* (Rondani) (18). Subsequent investigations of serological relationships showed a parallel separation into serotypes (28). However, although both serological properties and vector specificities involve capsid protein (CP) properties (6), recent studies have shown that vector specificity need not correspond to serotype groupings among isolates (1,2,8,14).

Vector specificities, serotyping, and other characteristics have been used to group MAV, PAV, and SGV serotypes into BYDV subgroup 1 and RPV and RMV serotypes into BYDV subgroup 2 (5,15,18,28). This grouping is supported by the recently published nucleotide sequences of isolates MAV-PS1 (25), Vic-PAV (16), P-PAV (25), and NY-RPV (26). Sequences for the MAV and PAV serotype isolates are similar and contain five large open reading frames (ORFs), now conventionally numbered 1 to 5, plus a small ORF, numbered 6, whereas the sequence for the RPV serotype contains six large ORFs, now conventionally numbered 0 to 5 (15). Moreover, comparisons of the CP and other genes in the 3′ region of the genomes of MAV-PS1, P-PAV, and NY-RPV indicate that MAV and PAV isolates resemble each other more than they resemble NY-RPV (26,27). This region includes genes important in encoding proteins that may determine aphid-transmission specificity.

BYDVs are obligately transmitted by aphids, and their vector relationships can be relatively specific. Virus-aphid specificity seems to result from recognition between virions of a specific isolate and virus receptors in the salivary gland of a particular aphid species (6). ORFs for two proteins putatively involved in virion structure have been identified for BYDVs: ORF 3, encoding the 22-kDa CP, and ORF 5, encoding a 50-kDa protein. This latter ORF is contiguous with the CP ORF and is probably translated by a translational readthrough of the CP termination codon, producing a readthrough protein. A third ORF (ORF 4), corresponding to a 17-kDa protein, is embedded within the coding region for the CP but in a different reading frame. If the virus-receptor hypothesis applies, the determinants for specificity of aphid transmission must be part of the structures formed by one or more of these proteins and should be reflected in specific genomic characteristics. Recently, as reported here, we found that an isolate of the SGV serotype from Texas (TX-SGV) is readily transmissible not only by *Schizaphis graminum* but also by *Sitobion avenae*, *R. padi*, and *R. maidis*, whereas the NY-SGV type isolate is readily transmitted only by *Schizaphis graminum*. We report here on a study of other features differentiating them from each other and other BYDV isolates, including diagnostic serological properties, dot blot hybridization tests, and differences in nucleic acid and deduced amino acid sequences.

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MATERIALS AND METHODS

Virus isolates and enzyme-linked immunosorbent assay (ELISA). TX-SGV, from a wheat plant collected in Texas (supplied by D. Marshall, Texas A&M University, College Station), and NY-SGV (supplied by W. F. Rochow from the Cornell University collection) were maintained in barley (*Hordeum vulgare* L.) cv. Moore or oat (*Avena sativa* L.) cv. Clintland 64 in constant environment chambers at 20°C. They were transferred regularly with *Schizaphis graminum*, and virions were purified from Moore barley roots as described by Webby and Lister (29). ID-SGV (8), a SGV isolate from Idaho supplied by S. Halbert, was not maintained regularly, but virions were purified once from a culture maintained in conditions similar to those for TX-SGV and NY-SGV. ID-SGV is transmissible by several vector aphids (8) and is similar in this respect to other SGV isolates previously reported in Idaho collections (20). Similar procedures were used to propagate MAV-PS1 (14), P-PAV (9), NY-RPV, and NY-RMV, which were transferred by *Sitobion avenae*, *R. padi*, *R. padi*, and *R. maidis*, respectively. Double-antibody sandwich-ELISA (DAS-ELISA) (13) with serotype-specific antisera (29) was used to check that cultures remained uncontaminated and in tests to compare the BYDV isolates. Triple-antibody sandwich-ELISA (TAS-ELISA) with monoclonal antibodies (MAbs) (29) also was used to assess serological relatedness among the isolates.

Aphid-transmission experiments. In experiments on vector specificity, nonviruliferous *Schizaphis graminum*, *Sitobion avenae*, *R. padi,* or *R. maidis* were allowed 5- to 10-day acquisition access periods on Moore barley plants infected with TX-SGV or NY-SGV and then were transferred in groups of three to five aphids to Clintland 64 oat plants or, in the case of *R. maidis*, to Moore barley test plants, for inoculation access periods of 2 to 3 days. Aphid survival was recorded, and aphids then were killed with malathion. The plants were grown for 2 weeks in the greenhouse before being tested for virus infection by DAS-ELISA. Only test plants on which aphids survived for at least 2 days were included in the results. Another set of two similar experiments tested the transmissibility of a culture of TX-SGV that had been transmitted sequentially through Clintland 64 oat or Moore barley plants by batches of 10 *R. padi*, *Sitobion avenae*, *R. maidis,* and *Schizaphis graminum*, respectively. In further experiments, nymphs and adults of *Schizaphis graminum* from aphid cultures that had been raised on Moore barley plants infected with TX-SGV or NY-SGV were transferred singly to Moore barley or Clintland 64 oat test plants, as above, to assess their ability to transmit the virus.

RNA extraction, cDNA library construction, and polymerase chain reaction (PCR). Purified virions were pelleted by ultracentrifugation, and the RNA was obtained by phenol/chloroform extraction and ethanol precipitation after the virions were disassociated in 200 mM Tris-HCl (pH 9.0), 2% (wt/vol) sodium dodecyl sulfate (SDS), and 5 mM EDTA at 60°C for 10 min. cDNA libraries were constructed from the genomic RNAs of NY-SGV and TX-SGV, respectively, by the method of Gubler and Hoffman (7) and cloned into the *Hinc*II site of pGEM-3Z (Promega, Madison, WI).

cDNA clones also were prepared by PCR with synthetic oligomers containing BYDV sequences. One microgram of genomic RNA was reverse transcribed (Superscript, Life Technologies [LT], Richmond, CA) and treated with RNase H (LT). The DNA product was ethanol precipitated and dissolved in 20 µl of distilled water. PCR was performed under the following conditions: 1 µl of cDNA template; 200 µM dNTPs; 100 pM each of the primers; 2 mM $MgCl_2$; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.01% (wt/vol) gelatin; and 2.5 units of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, CT) in 100 µl. The reaction mixture was covered with 50 µl of mineral oil and, after an initial 5-min incubation at 95°C, was subjected to 25 or 30 cycles of 95°C for 1 min, 50°C for 2 min, and 72°C for 2 min.

Sequencing and screening libraries. Purified recombinant plasmid DNA was sequenced by the dideoxy chain termination method (23) with a modified T7 DNA polymerase (Sequenase, U.S. Biochemical, Cleveland). Nucleotide sequence analyses were performed with Microgenie software, version 4.0 (Beckman Instruments, Inc., Fullerton, CA), and Genetics Computer Group

TABLE 1. Double-antibody sandwich (DAS) and triple-antibody sandwich (TAS) enzyme-linked immunosorbent assay (ELISA) with purified virions of the NY-SGV, TX-SGV, and ID-SGV isolates of barley yellow dwarf virus (BYDV)

Antibody	ELISA value with SGV serotype isolate[a]		
	NY-SGV	TX-SGV	ID-SGV
DAS-ELISA with polyclonal antiserum			
MAV-PS1	0.08	0.26	0.16
P-PAV	0.02	0.09	nt[b]
NY-SGV	1.44	1.59	1.37
NY-RPV	0.03	0.02	nt
NY-RMV	0.01	0.01	nt
TAS-ELISA with MAb[c]			
MAV1	0.08	0.08	nt
MAV3	0.18	2.10	1.75
AF8	0.56	0.71	0.49
MAFF2	0.04	0.05	0.06
MAC91	0.03	0.03	0.04

[a] Means of duplicate wells, 100 ng per well. Virions of other BYDV serotype isolates (MAV-PS1, P-PAV, NY-RPV, and NY-RMV for DAS-ELISA; MAV-PS1 and P-PAV for TAS-ELISA) and buffer without virions also were included and used as positive and negative controls, respectively, for checking the reactivity of each antibody used.

[b] Not tested.

[c] Monoclonal antibodies (MAbs) used were MAV1, MAV3 (10), AF8 (12; C.-H. Lei and R. M. Lister, *unpublished data*), MAFF2, and MAC91 (24).

TABLE 2. Percent transmission of the TX-SGV and NY-SGV isolates of barley yellow dwarf virus by vectors *Schizaphis graminum, Rhopalosiphum padi, Sitobion avenae,* and *R. maidis*[a]

Isolate	Vector			
	S. graminum	*R. padi*	*S. avenae*	*R. maidis*
TX-SGV[b]	100	81	67	58
TX-SGV[c]	91	72	68	88
NY-SGV[d]	56	0	0	0
NY-SGV[e]	82	14	5	<1

[a] Acquisition access period 5 to 10 days; test feed 2 to 3 days; 5 to 10 aphids per plant. Vector clones supplied by W. F. Rochow from the Cornell collection.

[b] Determined in present work in two experiments, involving totals of 36 to 49 plants for each vector, with the original TX-SGV isolate.

[c] Determined in present work in two experiments, involving totals of 38 to 47 plants for each vector, with a culture of TX-SGV that had been transmitted sequentially through Clintland 64 oat or Moore barley plants by batches of *R. padi*, *S. avenae*, *R. maidis,* and *S. graminum*, respectively.

[d] Determined in present work in one experiment involving 50 plants for each vector.

[e] Determined by Johnson and Rochow (11).

TABLE 3. Transmission of the TX-SGV and NY-SGV isolates of barley yellow dwarf virus by single *Schizaphis graminum* nymphs and adults raised on infected Moore barley

Exp.	Test plant	Percent transmission (number of tests)			
		Nymph		Adult	
		TX-SGV	NY-SGV	TX-SGV	NY-SGV
1	Clintland 64 oat	82(28)	nt[a]	72(36)	nt
2	Clintland 64 oat	92(50)	18(49)	82(49)	4(49)
3	Moore barley	70(47)	14(34)	56(46)	3(34)
	Totals	80(125)	17(83)	70(131)	4(83)

[a] Not tested.

(Madison, WI) sequence analysis software, version 7.2 (4). Clones that contained CP sequences homologous to those found in other BYDV isolates (16,25,27) were assumed to contain part of the putative SGV CP gene, and these sequences were the basis for primers to amplify the SGV CP coding region by PCR. Some clones recovered after PCR were used as hybridization probes, ^{32}P-labeled with a multipriming labeling kit (Ambion, Inc., Austin, TX), to screen the libraries for additional CP cDNA clones. Clones containing the RTP gene sequences were identified by probing the cDNA libraries for clones overlapping the CP gene.

Dot blot hybridization. In one set of experiments, dot blot hybridization tests (22) were applied to purified virions at a range of concentrations (1.0, 0.1, 0.01, and 0.001 µg/ml), estimated from E_{260nm} (0.1%) = 8 (3). Virion preparations in 0.1 M sodium phosphate, pH 7.0, were immobilized on a nylon membrane (Nytran, Schleicher and Schuell, Inc., Keene, OH) pretreated with 20× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0). The same virion preparations also were examined by DAS-ELISA at the same range of concentrations to confirm that virion concentrations were consistent among dilutions and to compare isolates serologically. Blots were baked under vacuum for 3 to 4 h at 80°C and then incubated for 30 min to 1 h at 68°C in 10% formamide, 1.5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]), 1% SDS, and 5× Denhardt's solution. Hybridization was in the same solution plus 50 µg of calf thymus DNA per ml with the [α-^{32}P]dCTP-labeled probe (1.5×10^7 cpm) at 68°C for 16 h. Blots were washed sequentially with 2× SSC plus 0.1% SDS, 0.5× SSC plus 0.1% SDS, 0.1× SSC plus 0.1% SDS, and 0.1× SSC plus 1% SDS, each for 15 min at room temperature.

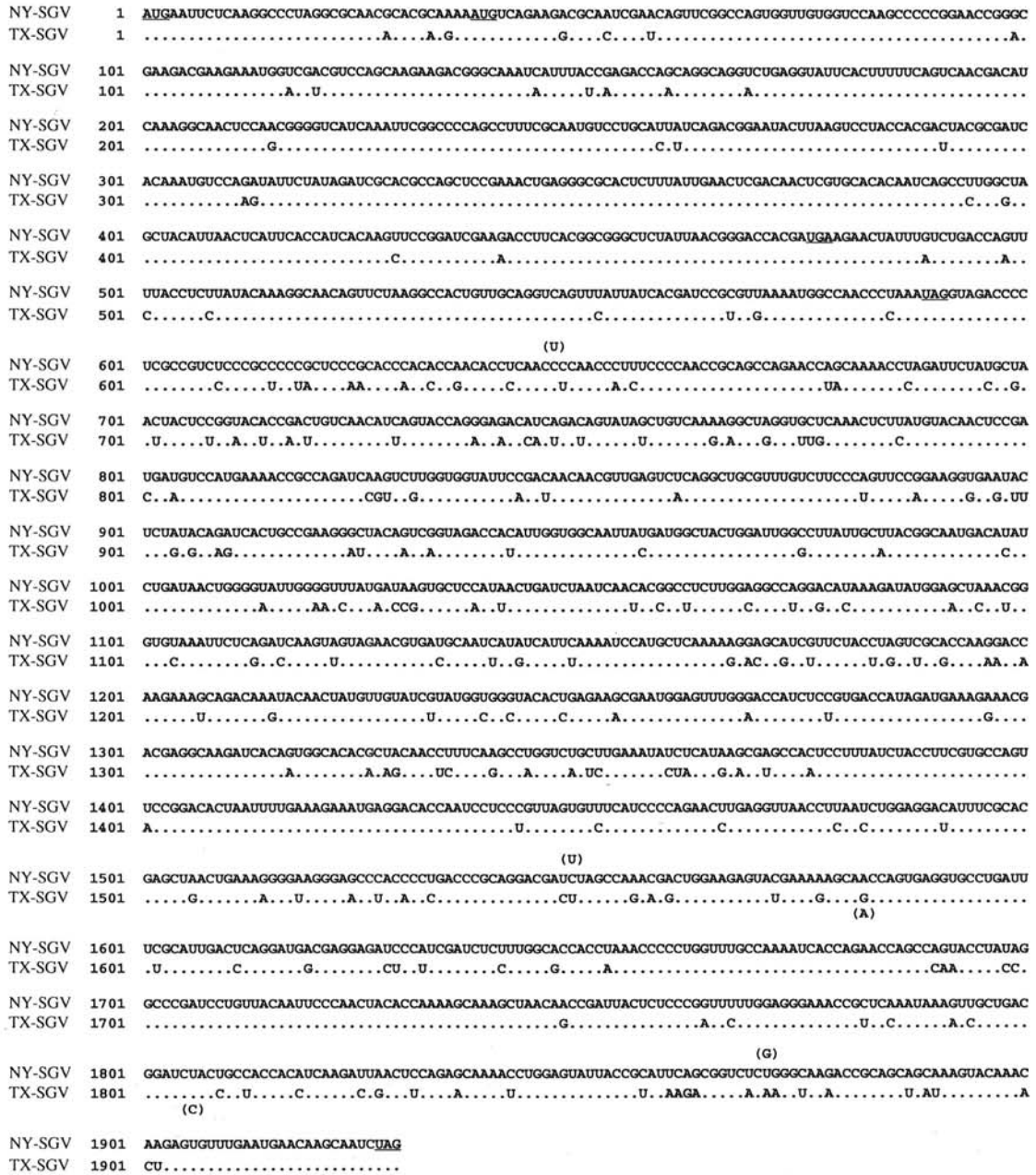

Fig. 1. Comparative nucleotide sequences coding for the coat proteins and 50-kDa proteins of barley yellow dwarf virus isolates NY-SGV and TX-SGV. Nucleotides that are identical between NY-SGV and TX-SGV are indicated by dots; only the nucleotides that differ in TX-SGV are listed. Variant nucleotides identified from sequencing multiple clones are noted in parentheses above or below the consensus sequence for NY-SGV and TX-SGV, respectively. The first underlined AUG indicates the coat protein initiation codon; the second underlined AUG indicates the 17-kDa internal reading frame initiation codon; the first stop codon, UGA, and the second and third stop codon, UAGs, indicate the stop codons for the coat protein, the 17-, and the 50-kDa proteins, respectively.

RESULTS

ELISA comparisons. DAS-ELISA tests with polyclonal antisera (Table 1) indicated that all three SGV isolates were mutually related but were unrelated to the subgroup 2 isolates NY-RPV and NY-RMV. Both TX-SGV and ID-SGV showed a small degree of cross-reactivity with the NY-MAV antiserum, similar to that previously reported for PAV serotype isolates (30; Table 1), but such cross-reactivity was much less evident for NY-SGV. Table 1 also summarizes typical results obtained in TAS-ELISA tests with SGVs and other virus isolates and various MAbs. TX-SGV and ID-SGV both reacted much more strongly than NY-SGV with Mab MAV3, but all the isolates reacted similarly with other MAbs tested (Table 1).

Aphid-transmission experiments. Comparisons of the transmissibility of TX-SGV by aphids showed that whereas only *Schizaphis graminum* transmitted NY-SGV, *Sitobion avenae*, *Schizaphis graminum*, *R. padi*, and *R. maidis* all transmitted TX-SGV quite efficiently (Table 2). Similar results were obtained in transmission experiments carried out with the TX-SGV culture after passaging it sequentially through *R. padi*, *Sitobion avenae*, *R. maidis*, and *Schizaphis graminum*, respectively, by transmission to Clintland 64 oats or Moore barley plants (Table 2). In further experiments, both single nymphs and single adults of *Schizaphis graminum* raised on TX-SGV infected Moore barley plants transmitted the virus efficiently, whereas those raised on NY-SGV–infected plants transmitted this virus poorly (Table 3). For each isolate, transmission by adults was less efficient than transmission by nymphs, but the differences in transmission efficiency were much greater for NY-SGV than for TX-SGV (Table 3). These effects were similar with both Clintland 64 oats and Moore barley test plants.

Clones and characteristics of the CP, 17-, and 50-kDa ORFs of SGV genomes. ORFs corresponding to ORFs 3, 4, and 5 of other BYDVs (15), i.e., the CP, 17-, and 50-kDa ORFs, respectively, were identified and sequenced as follows. Nucleotide sequencing of cDNA clones from the TX-SGV library identified a clone (TSGV171) containing the sequence (5′-CGTATTTTATTTAC-3′). This sequence (RNA form) is conserved in the 5′ untranslated region flanking the MAV and PAV CP genes (25,26) and, thus, was chosen as one PCR primer. The second PCR primer (5′-GGATCCGTCTACCTATTTGG-3′), which was similar to that designed by Robertson et al. (17), represented a 14-nucleotide sequence located (RNA form) at the 5′ end of the MAV and PAV CP

TABLE 4. Percent nucleotide identity among the coat protein and 50-kDa open reading frames (ORFs) of NY-SGV, TX-SGV, and other barley yellow dwarf virus isolates[a]

	TX-SGV[b]	MAV-PS1[c]	P-PAV[c]	NY-RPV[d]
NY-SGV[b]	94.6	68.4	69.2	52.6
	(84.8)	(51.8)	(50.8)	(43.9)
TX-SGV	...	69.5	70.7	52.4
	...	(52.0)	(52.4)	(45.8)
MAV-PS1	...	...	76.7	52.8
	...	...	(60.2)	(45.0)
P-PAV	...	...	...	55.0
	...	...	...	(45.3)

[a] Values for the 50-kDa ORFs are in parentheses.
[b] This work.
[c] Ueng et al. (25).
[d] Vincent et al. (26,27).

TABLE 5. Percent amino acid similarity among the deduced coat proteins and 50-kDa proteins (in parentheses) of NY-SGV, TX-SGV, and other barley yellow dwarf virus isolates

	TX-SGV[a]	MAV-PS1[b]	P-PAV[b]	NY-RPV[c]
NY-SGV[a]	95.9	70.4	71.4	56.9
	(93.7)	(61.7)	(66.3)	(51.0)
TX-SGV	...	71.9	72.4	58.5
	...	(62.0)	(61.3)	(52.2)
MAV-PS1	...	...	80.8	59.1
	...	...	(74.0)	(52.4)
P-PAV	...	...	...	60.9
	...	...	...	(52.8)

[a] This work.
[b] Ueng et al. (25).
[c] Vincent et al. (26,27).

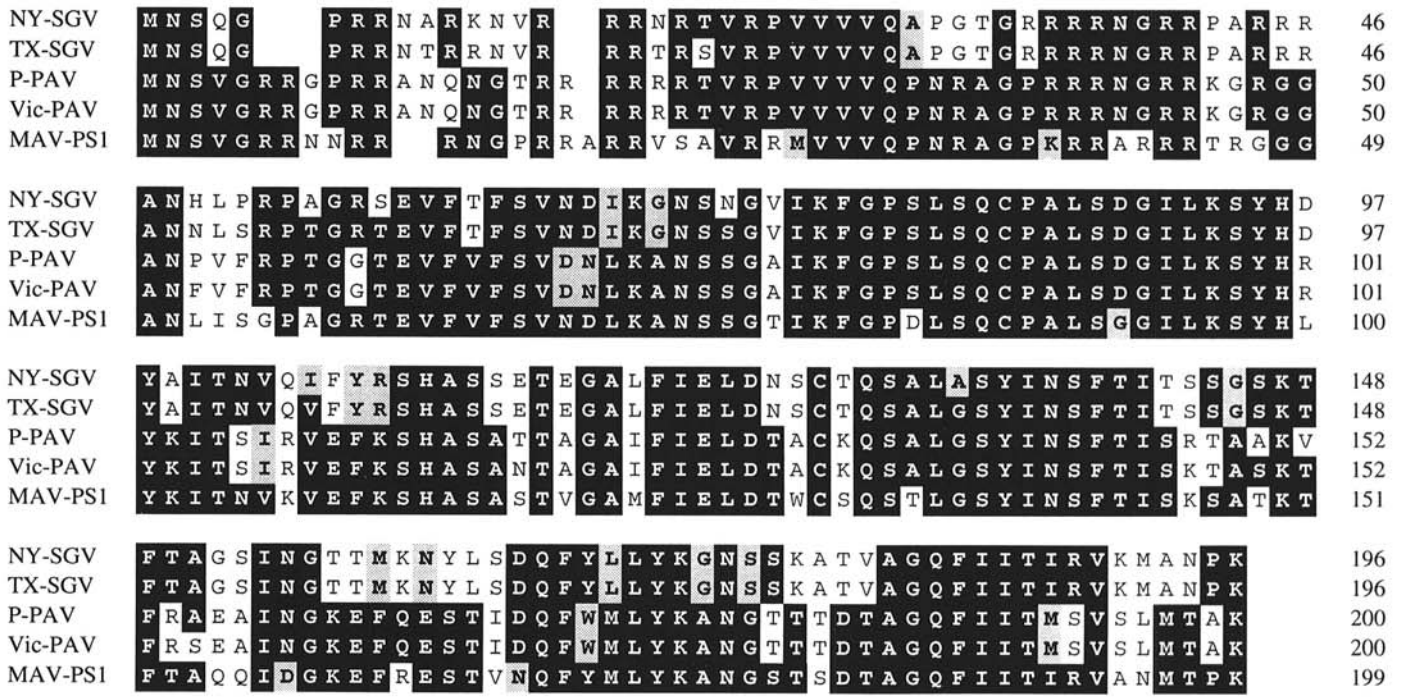

Fig. 2. Comparison of the deduced amino acid sequences of the putative coat proteins of barley yellow dwarf virus isolates NY-SGV and TX-SGV with those of MAV-PS1, P-PAV (27), and Vic-PAV (16). Identical amino acids are represented against a black background; chemically related amino acids are represented against a shaded background.

coding regions with an introduced *Bam*HI restriction site (underlined). Three clones, including one designated as TSGV-PCR-CP7, were obtained from cDNA fragments produced by PCR. Screening the cDNA library with TSGV-PCR-CP7 as the hybridization probe identified other cDNA clones. The CP nucleotide sequence was determined solely from the cDNA clones, such that the reported sequence represents at least two separate clones. Similar strategies were used for NY-SGV; thus, clone NY-SGV7 was identified by its sequence homology with the 5′ end of the TX-SGV CP gene. Within NY-SGV7, the sequence 5′-GTCCAAGCCCCCGGAACCG-3′ was identified beginning 79 nucleotides downstream from the NY-SGV CP ATG. This 19-mer was used in PCR with the downstream primer described above.

Two clones were obtained by PCR, and five overlapping cDNA clones were obtained from the NY-SGV library. The putative CP genes of both TX-SGV and NY-SGV contain 591 nucleotides (Fig. 1) and encode a protein with 196 amino acid residues and a predicted M_r of 21,700. Another ORF, corresponding to ORF 4 (15), is embedded in the CP gene in a different frame, starting at nucleotide 38 and consisting of 441 nucleotides, potentially encoding a protein with 146 amino acid residues and predicted M_r of 16,400 for TX-SGV or 16,600 for NY-SGV. Some clones extended downstream from the CP region to overlap a 50-kDa ORF. This enabled sequencing of this region by methods similar to those described above. The putative protein encoded by the 50-kDa ORF for both NY- and TX-SGV contains 1338 nucleotides and 445 amino acid residues, with a predicted M_r of 49,700 for NY-SGV and 49,900 for TX-SGV (Fig. 1).

Sequence comparison of the CP, 17-, and 50-kDa ORFs. When the TX-SGV and NY-SGV sequences were aligned and gapped to maximize homology, the percent similarity of NY-SGV to other BYDV isolates was TX-SGV > MAV and PAV isolates > NY-RPV (Table 4). The deduced CP amino acid sequence of NY-SGV had higher similarity to that of TX-SGV than to those of other serotype isolates that were compared (Table 5). Similarly, the putative 17-kDa ORF proteins had higher similarity (95.9%) to each other, than to those of the MAV and PAV isolates (approximately 70%) or NY-RPV (50%). Although the percentages varied, TX-SGV showed similar relationships in such comparisons (Tables 4 and 5). Comparisons of the 50-kDa ORFs showed similar relationships among the isolates compared. When deduced amino acid sequences for the CPs (Fig. 2) and 50-kDa proteins (Fig. 3) of both SGVs were compared with other subgroup 1 BYDV isolates for which both the CP and 50-kDa protein sequences are known, i.e., MAV-PS1, P-PAV (25), and Vic-PAV (16), no differences in primary structure could be correlated with differences

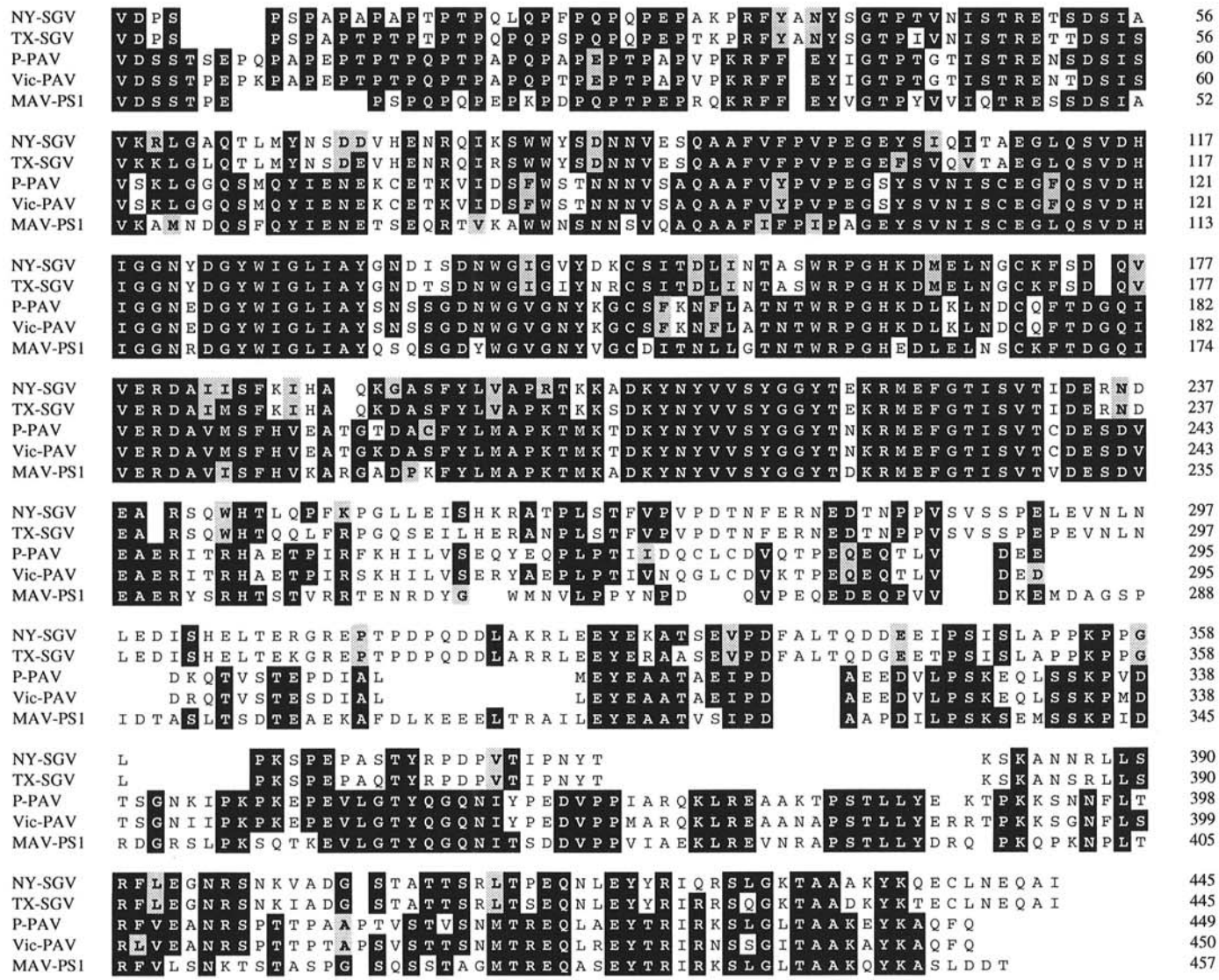

Fig. 3. Comparison of the deduced amino acid sequences of the 50-kDa proteins of barley yellow dwarf virus isolates NY-SGV and TX-SGV with those of MAV-PS1, P-PAV (25), and Vic-PAV (16). Identical amino acids are represented against a black background; chemically related amino acids are represented against a shaded background.

in vector relationships.

Dot blot hybridization. Clone TSGV-PCR-CP7, which contains the whole CP gene plus 50 base pairs of the 5′ noncoding region, hybridized strongly with TX-SGV, ID-SGV, and NY-SGV, weakly with P-PAV, and did not hybridize with MAV-PS1, NY-RPV, and NY-RMV (Fig. 4A). However, clone TSGV-PCR-CP7 did differentiate NY-SGV from TX-SGV and ID-SGV because hybridization with NY-SGV over the range of dilutions used was weaker than with the other two SGV isolates. Similar results were obtained with clone NSGV-PCR-CP51, which contains the entire CP gene of NY-SGV, except for the 78 base pairs 3′ of the ATG, as the probe. This clone hybridized very weakly, if at all, with P-PAV, but in contrast to TSGV-PCR-CP7, it hybridized more strongly with NY-SGV than with the other two SGV isolates (Fig. 4B). Variations in these results over the range of dilutions used were internally consistent with the spectrophotometric estimates of RNA concentration and with ELISA results (Table 1) that confirmed that virion concentrations were similar among the preparations used for hybridization.

DISCUSSION

Our serological test results clearly differentiated the SGV isolates from other subgroup 1 isolates and also differentiated NY-SGV from TX-SGV and ID-SGV. Aphid-transmission experiments also indicated clear differences between NY-SGV and TX-SGV. Thus, in experiments with groups of about 10 aphids that were allowed a 1- to 2-week acquisition access period and a 3- to 6-day transmission feed, Johnson and Rochow (11) showed that *Schizaphis graminum* transmitted NY-SGV to 82% of test plants, *R. padi* to 14%, *Sitobion avenae* to 5%, and *R. maidis* to fewer than 1% (Table 2). In our similar experiments with groups of three to five aphids and somewhat shorter acquisition and inoculation access times, we confirmed that of these vectors only *Schizaphis graminum* transmitted NY-SGV efficiently, but we found that all the species tested readily transmitted TX-SGV. This was true even after TX-SGV had been passaged sequentially through all of the vectors, a treatment designed to separate mixtures of viruses having different vector specificities. Another interesting difference was that both single nymphs and adults of *Schizaphis graminum* transmitted TX-SGV quite efficiently, whereas in our experiments and in those of Johnson and Rochow (11) and Zhou and Rochow (31), nymphs were much more efficient as vectors of NY-SGV than were adults.

Nucleotide and deduced amino acid sequence comparisons have not been available for SGV serotype isolates. The information reported here supports the inclusion of the SGV serotype in subgroup 1 on the basis of the nucleotide and deduced amino acid sequence similarity of the putative CP genes of NY-SGV and TX-SGV with those of MAV-PS1, NY-MAV, Vic-PAV, P-PAV, and NY-RPV (Tables 4 and 5; Fig. 2). At the nucleotide level, NY-SGV and TX-SGV have 94.6% identity in the CP regions (Table 4). Such homology is comparable to homologies between paired isolates of other serotypes (25). For example, in the same region, P-PAV and Vic-PAV have 95.0% identity, and MAV-PS1 and NY-MAV have 99.3% identity. The two SGV isolates share essentially the same CP coding region similarities with MAV-PS1 and P-PAV (both 70%) and have a low similarity with NY-RPV (52%) (Table 4). Nucleotide sequence comparisons between the CP coding regions appear to be a good index of relationships among BYDV isolates. That it is reasonable to classify the SGV serotype in BYDV subgroup 1 is confirmed by comparisons of the deduced amino acid sequences of RTPs (Tables 4 and 5; Fig. 3).

Although NY-SGV differs significantly in vector relationships from TX-SGV, the putative CPs of both isolates share about 96% homology (Tables 3 and 4), respectively, with most of the variability located in the N-terminal domains (Fig. 2). Attempting to correlate differences in the deduced amino acid sequences with

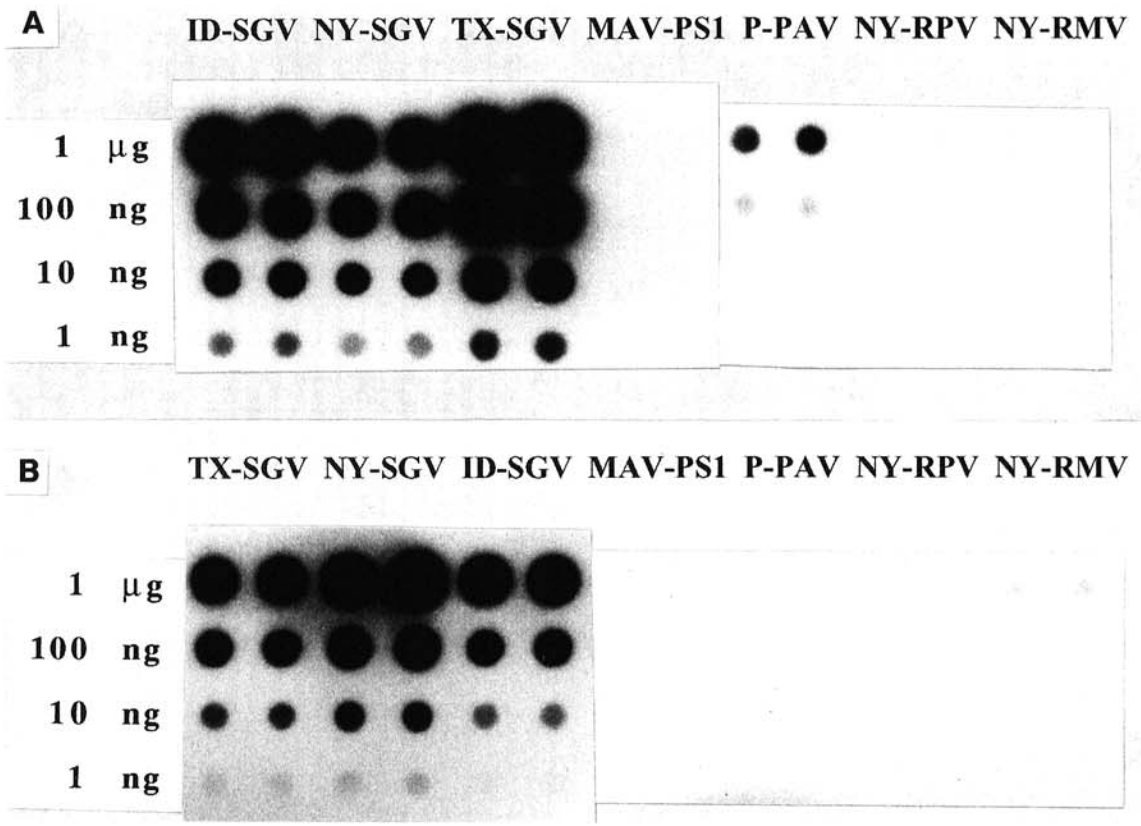

Fig. 4. Detection of various concentrations of purified preparations (1 ng/ml to 1 µg/ml, indicated to the left) of seven isolates of barley yellow dwarf virus by dot blot hybridization, probed with cDNA clones from **A,** TX-SGV (clone TSGV-PCR-CP7) and **B,** NY-SGV (clone NSGV-PCR-CP51).

differences in vector relationships must remain speculative for the present, until structural models for BYDV become available. However, for icosahedral RNA viruses, the N-terminal regions of the CPs are generally located in the interior of the virus particle (21). If this also is true for the BYDV CP, then differences in primary structure between the two SGV isolates in these regions are unlikely to be responsible for their different vector relationships. However, mismatches are much more abundant in the 50-kDa ORFs, which is consistent with the notion that this protein could account for vector specificity, as discussed by Miller et al. (16) and Vincent et al. (27).

In the set of experiments done here, clones covering the CP region from either NY-SGV or TX-SGV clearly hybridized with their respective homologous and heterologous SGV isolates but only weakly, if at all, with other BYDV isolates (Fig. 4), confirming the results of our serological tests, which indicated that it is reasonable to regard SGVs as distinct. In fact, the results of both the serological and nucleic hybridization tests indicate a diagnostic basis for differentiating NY-SGV and TX-SGV, not only from the other subgroup 1 isolates but also from each other, in ecological and epidemiological studies. Interestingly, in this regard, the nucleic acid hybridization experiments indicated that TX-SGV resembles ID-SGV more than NY-SGV (Fig. 4). Like other SGV serotype isolates from Idaho, ID-SGV is transmitted not only by *Schizaphis graminum* but also by other vectors (8,20). BYDV serotypes in general comprise variants differing in properties that can be significant in their pathology and epidemiology; it is important to try to correlate such properties with differential diagnostic and molecular characteristics.

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