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Characterization of a new curtovirus, pepper yellow dwarf virus, from chile pepper and distribution in weed hosts in New Mexico

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Abstract

Over 4,950 asymptomatic weed samples from more than 20 weed species that are host plants for curtoviruses were collected from ten chile pepper fields in southern New Mexico (NM) during 2003, 2004 and 2005 to identify whether they were infected with curtoviruses and to determine which curtoviruses were distributed in the weed population. Polymerase chain reaction using primers designed to detect a portion of the coat protein (*cp*) gene were used to detect curtoviruses, and infected plants were further tested for specific curtoviruses using primers designed to detect a portion of the replication-associated protein (*rep*) gene. Amplification of the *cp* gene was successful from 3.7, 1.17, and 1.9% of the weed samples in 2003, 2004, and 2005, respectively. Seventy-three amplicons from those samples were sequenced and compared to well-characterized curtoviruses. Analysis of the *rep* nucleotide sequences showed that ~32.9% of the weed isolates tested were closely related to beet mild curly top virus (BMCTV). Approximately 12.4% were closely related to beet severe curly top virus (BSCTV). The rest of the weed isolates (54.7%), which shared a very high level of nucleotide sequence identity to each other, represent a new curtovirus species. Using eight primers designed for PCR, complete genomes of three curtoviruses isolated from chile pepper samples representing the three groups of curtoviruses in southern New Mexico were sequenced. Comparisons of whole sequences of the genomes revealed that the DG2SW171601 isolate (2,929 nucleotides) was nearly identical to BMCTV-W4 (~98% nucleotide sequence identity). The LRME27601 isolate (2,927 nucleotides) was most closely related to BSCTV (~92% nucleotide sequence identity). The LNJ17601 isolate (2,959 nucleotides) shared only from 49.9 to 88.8% nucleotide sequence identity with other well-characterized curtoviruses. Based on the accepted cut-off of 89%, we propose that the LNJ17601 isolate is a member of a new curtovirus species. Chile peppers infected with this virus in the field express chlorotic stunting symptoms, so we propose the name pepper yellow dwarf virus (PeYDV). This new curtovirus species may be the result of mutations in the genome and recombination between BMCTV-W4 and BSCTV.

Introduction

Curly top disease is caused by members of several different curtovirus species. These viruses infect a broad range of host plants that includes over 300 species of plants in 44 plant families including chile pepper (*Capsicum annuum* L.), sugarbeet (*Beta vulgaris* L.), tomato (*Lycopersicon esculentum* Mill.), and weed hosts such as mustards (London rocket and flaxweed), pigweed, spurred anoda, *Kochia* sp., and *Chenopodium* sp. [4].

Curtoviruses are known to have appeared in North America before the twentieth century, and they now occur throughout the western United States, including California, Iowa, Idaho, Colorado, Illinois, Maryland, Michigan, Minnesota, Nebraska, North Carolina, Ohio, South Dakota, Texas, Virginia, Washington, Wisconsin, and also in southwestern Canada and Mexico [4, 6, 29]. Infrequent occurrences of curtovirus epidemics have been reported in the southern New Mexico chile-pepper-growing areas since 1927 [8]. Currently, curtoviruses cause one of the most important diseases of chile in southwestern New Mexico [15]. Because of curly top, the chile crop in New Mexico experiences substantial losses. For example, in 1999, curtoviruses infected from 20 to 50% of the plants in chile fields, depending on location [14]. In 2000, curtoviruses infected less than 5% of plants, with the result that chile production increased 68% compared to 1999 [22]. In 2001, a year with high curly top incidence, the chile yield per acre was 12% lower than that in 2000 or 2002, years with minimal curly top incidence. In 2003, the chile crop on many farms had decreased by one-third due to curly top disease outbreaks [9]. The symptoms of curly top disease in chile pepper include stunting, chlorosis and thickened, stiff plants. It causes premature fruit ripening and deformity, reducing both quality and yield of chile peppers [9].

Curtoviruses are transmitted from plant to plant by the beet leafhopper, *Circulifer tenellus*. The mode of transmission is circulative, persistent, and nonpropagative [4, 18]. In California, in the late fall, adult beet leafhoppers migrate to perennial weeds in the foothills of the coastal range and then, in the late winter or early spring, lay eggs on annual weeds. The nymphs are presumed to acquire virus from these hosts. In mid- to late spring, when these weeds dry, viruliferous mature leafhoppers move to cultivated areas, where they feed on crop plants or weeds concentrated along roadsides and irrigation ditches, transmitting the virus from winter weeds to agricultural crops. The leafhoppers reproduce for several generations before migrating back to overwintering areas [7]. The movement of beet leafhoppers in southern New Mexico has not been studied fully. However, a limited survey has shown that the leafhoppers build up to high numbers in the summer chile pepper crops, and they are thought to survive on weeds during the winter, as occurs in California [9].

Curtoviruses are a complex of monopartite geminiviruses belonging to the genus *Curtovirus* within the family *Geminiviridae*. The viruses have circular single-stranded DNA genomes, approximately 3.0 kb in length and encapsidated within twinned, icosahedral particles. Curtovirus genomes commonly contain seven open reading frames (ORFs). The sense strand encodes ORFs V1 to V3, while ORFs C1 to C4 are encoded by the complementary or anti-sense strand [18]. The capsid or coat protein (CP) is encoded by the V1 ORF and is necessary for both virion formation and for systemic infection of the host. Moreover, the CP is an important component of the specificity between the virus and its leafhopper vector [5]. The CP is highly conserved among curtoviruses. ORFs V2 and V3 are unique to the curtoviruses. ORF V2 plays a role in regulating genomic single-strand and double-strand DNA production and accumulation, and ORF V3 facilitates cell-to-cell movement of the virus [16, 18]. ORF C1 encodes the replication-associated protein (Rep), which begins rolling-circle replication by introducing a strand-specific nick within the origin of DNA replication [18, 26]. A replication enhancer protein encoded by ORF C3 optimizes virus genome replication. ORF C2 encodes a protein that plays a role in movement of the virus, resulting in reduced symptom severity [17, 18]. Recent research has shown that C2 plays an important role in severe curly top virus pathogenesis [2], while ORF C4 affects cell division, symptom expression, and host range [17, 18].

There are three species of curtoviruses whose members have been reported to cause disease in sugar beet (*Beta vulgaris* L.), and are distinct in terms of their host range, symptom severity, nucleotide sequence, and rep complementation [13, 25]: Beet curly top virus (BCTV), Beet severe curly top virus (BSCTV), and Beet mild curly top virus (BMCTV) [13,

25]. A comparison of nucleotide sequences among laboratory isolates of the curtoviruses has shown that BCTV isolates have greater than 95% similarity [23, 28]. In contrast, the nucleotide sequences of BSCTV isolates show 80% similarity to those of BMCTV genomes. The nucleotide sequences of BSCTV and BMCTV genomes vary between 79 and 83% similarity with that of BCTV [25, 27]. Another curtovirus has been identified in horseradish. Studies have revealed that this virus differs from other curtoviruses because it lacks the replication enhancer gene. Therefore, this virus is known as a separate virus called horseradish curly top virus (HrCTV) [19, 20, 27]. Recently, additional curtoviruses have been reported: spinach curly top virus (SCTV), infecting spinach in Texas [1], and beet curly top Iran virus (BCTIV), infecting sugar beet, tomato, spinach, and turnip in Iran [30].

A limited survey of sugarbeets and peppers in eastern New Mexico found BSCTV infecting only sugar beets. Conversely, only BMCTV was isolated from peppers [24]. A small survey of curtoviruses in weeds in New Mexico showed that both BMCTV and BSCTV were present, but BMCTV was predominant [9]. However, comparing amino acid sequences of the *Rep* genes of 201 infected chile pepper samples collected in many fields in New Mexico by Creamer et al. [10] showed that 18.5% of the New Mexico field isolates tested shared 98–100% amino acid identity in the *rep* gene with beet mild curly top virus. Forty-eight percent of the NM field isolates shared 96–97% amino acid identity in the *rep* gene with beet severe curly top virus, and 32% shared 93–97% amino acid identity in the *rep* gene with both BMCTV and BSCTV. But sequences from cloned amplicons from the third group of viruses revealed that the high nucleotide sequence identity with both BSCTV and BMCTV had not occurred due to a mixture of virus isolates within the samples. The alternative *rep* sequence was suggested to be the result of a new virus in the genus *Curtovirus* [10].

This work was conducted to determine whether the virus previously referred to as the New Mexico unknown strain is a new virus by sequencing the whole genome and comparing it with those of other curtoviruses. In addition, we determined which curtoviruses infect weeds in southern New Mexico that were collected from different fields during 2003, 2004 and 2005.

Materials and methods

Collection of weed samples

Weeds were collected from the margins of 10 chile pepper fields located in Luna and Doña Ana counties in southern New Mexico during 2003, 2004, and 2005. Approximately ten samples were collected from each field every 2 weeks. Samples were composed of randomly collected leaves from more than 20 weed species including *Amaranthus* (pigweed), *Anoda cristata* (spurred anoda), bindweed, *Chenopodium* sp., *Datura* sp., *Descurainia sophia* (flixweed), globe mallow, *Kochia scoparia*, *Malva parviflora*, morning glory, *Physalis wrightii* (wrights ground cherry), Portulaca, puncture vine, purslane, *Sasola iberica* (Russian thistle), *Sisymbrium irio* (London rocket), and *Solanum elaeagnifolium* (silver leaf night shade).

DNA extraction and polymerase chain reaction

After the plants were identified to species, leaf tissue (2 g) was ground in liquid nitrogen with a mortar and pestle. Powdered material was either immediately extracted or stored at -20°C . Total nucleic acid was extracted using a modified Dellaporta extraction method [11], followed by a phenol/chloroform extraction. The total DNA was stored in Tris-EDTA, pH 7.0, at -20°C . All weed samples initially were tested for curtoviruses by polymerase chain reaction (PCR) using a set of primers (BCTV CP4f and CP6r) to amplify a 576-bp fragment of the CP region [10]. All weed samples that showed a positive PCR result for CP gene

screening were also tested for curtovirus identity using two sets of primers. One set of primers amplified a 488-bp portion of the rep region (BCTV rep1f, 5'-ACCACCTTTAATGACACGT-3', corresponding to nucleotides 2,043–2,061 of BSCTV, and BCTV rep2r, 5'-GCATTAAATGCAGGTAATGCA-3', complementary to nucleotides 2,511–2,531 of BSCTV). Another set of primers amplified a 723-bp fragment of the rep region (BCTV 2repf1, 5'-ATAGTCCAGGACTTAAGGGCTTC-3', corresponding to nucleotides 1,857–1,880 of BSCTV, and BCTV 2repr1, 5'-CAACTTCCAGGGAGCAAAATC-3', complementary to nucleotides 2,552–2,573 of BSCTV). These primers were also used for DNA sequencing.

PCR reactions were carried out in a 50- μ l reaction mixture containing 1 $\times$ PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin), 200 μ M each dNTP, 1.5 mM MgCl₂, 500 μ M each primer, 4 U *Taq* DNA polymerase, and 1 μ l of purified DNA. PCR amplification consisted of 33 cycles of 94°C for 30 s, 51°C for 60 s, and 72°C for 90 s, followed by a final extension of 72°C for 10 min. Amplification products were separated by electrophoresis on a 1% agarose gel, and the bands were visualized with ethidium bromide.

Amplification of whole genomes of curtoviruses

Total DNA extracted from three chile pepper isolates collected in southern New Mexico in 2001 were used as templates to amplify whole genomes of curtoviruses. LRME27601, DGSW171601, and LJN17601 were reported by Creamer et al. [10] with tentative identities as BSCTV, BMCTV, and NM unknown, respectively. The whole genomes of these three isolates were amplified by PCR using two sets of primers. One set of primers (rep1f and cp6r) amplified a 2,042-bp fragment and another set (cp4f and rep2r) amplified a 2,000-bp fragment. For DNA sequencing, eight primers were used, including rep1f, rep2r, cp4f, cp6r, seq1f (5'-CCGCAATCTGAACATGAGGACTA-3', corresponding to nucleotides 1,204–1,227), seq2f (5'-CTATTGGGGGCTCTCGTAAACTT-3', corresponding to nucleotides 2,848–2,871), seq1r (5'-AACCACAACCTCCGCAAATC-3', complementary to nucleotides 1,541–1,561), and seq2r (5'-ATATTTGTTCGCCACAACCTTTTGA-3', complementary to nucleotides 121–145).

Sequencing and phylogenetic analysis

Polymerase chain reaction products were precipitated to remove excess primers and nucleotides using isopropanol (100 μ l of 90% isopropanol/0.75 M ammonium acetate). Sequencing was carried out on the PCR products using the Big Dye version 3.1 terminator system and an ABI 3100 sequencer. Sequences were aligned by DNASTAR-Megalign software using the Slow-Accurate CLUSTALW method with those of the following curtoviruses obtained from GenBank: BCTV (M24597), BSCTV (U02311), BMCTV-W4 (AY134867), BMCTV (U56975), spinach curly top virus (SCTV, AY548948), and horseradish curly top virus (HrCTV, U49907). The parameters were Gap penalty: 15.00, Gap length penalty: 6.66, Delay Divergent Seq (%): 30, and DNA transition weight: 0.50. The phylogenetic trees were generated by analysis of 1,000 bootstrap replications using DNASTAR-Megalign software. The pairwise analysis sequence comparison (PASC) program, which is available online at <http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=main> was used for deciding whether an isolate is a member of a new species by pairwise global sequence alignment of all complete genomes in the family *Geminiviridae* [3]. A recombination detection program (RDP, version 3 beta 26) was used to detect and analyze recombination signals in a set of aligned DNA sequences [21].

Results

Detection of curly top virus in weed samples by PCR

The 576-bp fragments of the CP region were amplified from 114 weed samples from a total of 4,965 weed samples using the CP primer set (cp4f and cp6r). Of these, 68 of 1881 weed samples (3.7%), 17 of 1,574 weed samples (1.1%) and 28 of 1,510 weed samples (1.9%) collected during 2003, 2004 and 2005, respectively, showed positive results for amplifying the CP region. These virus-infected weed samples were distributed in all collected weed species except bindweed and morning glory (Table 1). However, the largest numbers of infected weed samples were *Sisymbrium irio* and *Kochia scoparia*.

Ninety-two PCR amplicons of the CP region from these 114 weed samples were sequenced and aligned with the homologous sequences from well-characterized curtoviruses in GenBank. The 92 isolates shared at least 98% nucleotide identity with BMCTV-W4 or BSCTV. These 92 weed samples that were identified as positive for CP sequences were used to amplify the fragments of the rep region. The rep1f/rep2r set of primers was successful in amplifying a 488-bp fragment of the *rep* region for 18 samples. The 2repf1/2repr1 set of primers was successful in amplifying a 723-bp fragment of the *rep* region for 62 samples. Both these primer sets failed to amplify the rep region for 12 samples. Seventy-three of these fragments (488-bp and 723-bp fragments) were sequenced. After alignment, the isolates were separated into three major groups based on nucleotide sequence identity (Fig. 1). The first group of 24 isolates (see isolates DR3S121503, DSSE172203, LMCSNE17505, and DR2NE261003 as examples) was most closely related to BMCTV. The isolates shared 96.4–99.2% nucleotide sequence identity with BMCTV-W4, and only 80.5–90.7% with BSCTV (Table 2; Fig. 1). Within the group, these isolates shared 95.1–100% nucleotide sequence identity with each other. Two of these isolates (DSSE172203 and DSW272203) collected from different plants (spurred anoda and *Sasola iberica*) shared the same nucleotide sequence. The second group of 9 isolates (see isolates DR3S23103, DGS161204, LKN38803, and DR2N151203 as examples) shared 89.7–95.3% and 89.3–94.8% nucleotide sequence identity with BSCTV and BMCTV-W4 (Table 2; Fig. 1) and shared 90.9–100% nucleotide sequence identity with each other. Two of these isolates (DR2W161003 and DGS161204) shared the same nucleotide sequence although they were collected from different fields in different years. Based on the phylogenetic tree, this group was most closely related to BSCTV. The third group consisted of 40 isolates (see isolates DSW372203, LMCCNW1121704, LWS2101803, and DRE1111503 as examples) that shared a high level of nucleotide sequence identity with one another (from 98.3 to 100%). Eighteen of these isolates (see DR2W111103 and LMCCNW1121704 as examples) shared the same nucleotide sequence although they were collected from different plants and different fields in different years. These isolates shared 89.9–91.5% and 88.6–90.1% nucleotide sequence identity with BSCTV and BMCTV, respectively (Table 2; Fig. 1). This group included 22 of 61 weed samples isolated in 2003 (see DR2W111103 as an example), 13 of 16 samples isolated in 2004 (see LDN212404 as an example), and one of seven samples isolated in 2005 (see DR1S12505 as an example). Based on this result, we concluded that this group represents a new curtovirus strain that exists stably in the weed population.

Identifying curtovirus strains in New Mexico

To amplify the full nucleotide sequences of curtoviruses in New Mexico, including DG2SW171601, LRME27601, and LJN17601, 8 primers were designed for use in the PCR. The complete nucleotide sequences of DG2SW171601, LRME27601, and LJN17601 are 2,929, 2,927, and 2,959 bp long, respectively. The nucleotide sequences of LJN17601 and LRME27601 isolates are available in the GenBank under accession nos. EU921828 and

FJ545686, respectively. A comparison of nucleotide sequence identities based on multiple alignments of the sequences of the three New Mexico isolates with previously characterized curtoviruses available in GenBank (Table 3) indicated that DG2SW171601 shared a high level of nucleotide sequence identity with BMCTV-W4 (97.9%) and BMTCV (97.2%). This isolate shared only 84.5% nucleotide sequence identity with BSCTV and 63.9–80.0% with other curtoviruses. LRME27601 isolate shared a high level of nucleotide sequence identity with BSCTV (92.1%). This isolate shared only 87.5% nucleotide sequence identity with BMCTV-W4 and 64.8–87.3% with other curtoviruses. The LJN17601 isolate shared a low level of nucleotide sequence identity with all previously characterized curtoviruses (from 63.3 to 88.8%).

Comparison of nucleotide sequence identities resulted in the phylogenetic tree shown in Fig. 2. In this figure, the DG2SW171601 isolate is located in the group containing BMCTV-W4 and BMCTV. The LRME27601 isolate is in the same group with BSCTV. The LJN17601 isolate is located between these two groups.

The complete nucleotide sequences of DG2SW171601, LRME27601, and LJN17601 isolates were also analyzed using the pairwise analysis sequence comparison (PASC) program. The result showed that the DG2SW171601 and LRME27601 sequences have the highest level of nucleotide sequence identity to BMCTV-W4 (97.85%) and BSCTV (92.23%), respectively. The highest level of nucleotide sequence identity of the LJN17601 isolate is only 88.18% to BMCTV-W4.

To determine the relative levels of conservation of amino acid sequences among New Mexico curtovirus isolates and other characterized curtoviruses, the seven ORFs of LJN17601, DG2SW171601, and LRME27601 isolates were compared. A comparison of amino acid sequence identities based on multiple alignments of the sequences of three New Mexico isolates with previously characterized curtoviruses (Table 4) indicated that the sense strand encoding the three overlapping ORF V1, V2 and V3 of the LJN17601 isolate showed high levels of amino acid sequence similarity to other isolates (from 93.3 to 99.2%). The complementary sense strand encoding the four overlapping ORF C1 to C4 of the LJN17601 isolate shared low levels of amino acid sequence similarity to other isolates (from 50.6 to 91.1%).

To identify whether recombination had occurred between New Mexico chile pepper curtovirus field isolates, Recombination Detection Program (RDP, version 3 beta 26) was used. Analysis of the nucleic acid sequences of LJN17601, DG2SW171601 and LRME27601 showed that there are two potential recombinant regions between them (Fig. 3). The first potential recombinant region begins at nucleotide 904 and ends at 1,025. The *P*-Val value of this potential recombination is 4.509×10^{-2} . The second potential recombinant region begins at nucleotide 2,286 and ends at 2,479 with *P*-Val = 2.354×10^{-2} . In both these recombinations, LJN17601 is the possible progeny.

Discussion

In 2003, Creamer et al. [9] reported that curtoviruses were found in only nine weed samples of a total of 686 samples collected in 2001 (1.3%) and one weed sample of total 1,768 samples collected in 2002 in southern New Mexico (0.06%). Infected weed samples belonged to four species: *Amaranthus* sp. (pigweed), *Sisymbrium* irio (London rocket), *Chenopodium* sp. (lambsquarters), *Kochia scoparia* L. (Schrader). In this report, we found that curtoviruses existed in 3.7, 1.1, and 1.9% of 1,881, 1,574, and 1,510 weeds, respectively, collected during 2003, 2004 and 2005. The high percentage of virus infection (3.7%) found in weeds in 2003 was no surprise because it corresponded with curtovirus

outbreaks in chile pepper [9]. Many infected weed species were identified, including Kochia, portulaca, pigweed, puncture vine, *Chenopodium*, spurred anoda, bindweed, *Datura*, ground cherry, flixweed, and globe mallow. This result revealed that curtoviruses were maintained in the weed population. All of the weeds found infected have previously been reported as hosts of curtoviruses [4]. Identifying curtoviruses infecting weeds collected in southern New Mexico in 2003, 2004 and 2005 also revealed that members of at least three different curtovirus species were present in the weed population—BMTCV, BSCTV and the new curtovirus. This conclusion corresponds to previous research reported in 2005 [10] about curtoviruses in chile pepper crops.

According to previous researchers [12, 13, 25], a value of ~90% nucleotide sequence identity has been established as the demarcation threshold between two curtovirus species. Complete genome comparisons among curtoviruses using the DNASTar-Megalign and PASC programs revealed that the DG2SW171601 isolate was virtually identical to BMCTV-W4 (~98% nucleotide sequence identity); the LRME27601 isolate was most similar to BSCTV (~92% nucleotide sequence identity). However, the LNJ17601 isolate was not closely related to any previously characterized curtoviruses. The highest level of nucleotide sequence identity of this isolate to BMCTV-W4 is only 88.8%. These results again corresponded to our previous research [10] and the result of curtovirus identification in weed samples reported in this paper. Because the accepted cut-off is 89% [13], we propose that the LNJ17601 curtovirus isolated from chile pepper and described in this report represents a new species. Based on the chlorotic stunting symptoms observed in all curtovirus-infected chile peppers in the field, we propose the name Pepper yellow dwarf virus (PeYDV). This new curtovirus species may be the result of recombination between BMCTV-W4 and BSCTV, because there are two potential recombinant regions located in ORF V1 and C1 encoding the CP (from nucleotide 904 to 1025) and replication protein (from nucleotide 2,286 to 2,479), respectively. When the amino acid sequences of seven ORFs of LNJ17601 were aligned with other curtoviruses, we found alterations in many amino acids, especially in the region of ORFs C1 to C4. The alternations may be the result of mutations that contributed to producing the new curtovirus species, Pepper yellow dwarf virus.

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Fig. 1.

Phylogenetic comparison of nucleotide sequences of the replication-associated protein gene of curtovirus isolates from selected New Mexico weeds with those of beet severe curly top virus (BSCTV), beet mild curly top virus W4 (BMCTV-W4), beet mild curly top virus (BMCTV), beet curly top virus (BCTV) and horseradish curly top virus (HrCTV)

Fig. 2.

Phylogenetic comparison of complete nucleotide sequences of curtovirus isolates from New Mexico chile pepper with beet severe curly top virus (BSCTV), beet mild curly top virus-Worland 4 (BMCTV-W4), beet mild curly top virus (BMCTV), beet curly top virus (BCTV), spinach curly top virus (SCTV), and horseradish curly top virus (HrCTV)

Fig. 3.

The plots display potential recombinant regions between nucleotide sequences of DG2SW171601, LRME27601, and LJN17601 isolates. The potential recombinant regions located in ORF V1 and C1 encode the coat protein (from nucleotide 904 to 1025) and replication protein (from nucleotide 2,286 to 2,479), respectively

Table 1

Curtovirus-infected weeds collected 2003–2005 in New Mexico

Weed plant	2003	2004	2005
<i>Sisymbrium irio</i>	30/360	8/231	4/245
<i>Kochia scoparia</i>	12/147	3/154	5/164
<i>Chenopodium</i> sp.	6/135	0/148	2/139
<i>Amaranthus</i>	0/233	0/198	0/155
<i>Anoda cristata</i>	9/76	1/114	1/20
<i>Datura</i> sp.	2/21	1/36	1/21
<i>Salsola iberica</i>	5/283	1/194	7/264
<i>Physalis wrightii</i>	1/91	0/108	1/58
<i>Solanum elaeagnifolium</i>	1/170	0/137	1/105
<i>Malva parviflora</i>	1/15	0/13	0/7
<i>Descurainia sophia</i>	1/85	2/17	0/100
Globe mallow	N/A	1/45	N/A
Total	68/1616	17/1395	22/1278

Table 2

Comparison of percent nucleic acid sequence identity among *rep* regions of New Mexico chile pepper curtovirus isolates with previously characterized curtoviruses available in GenBank

	Isolate	BMCTV-W4	BSCTV	BCTV	SCTV
Group 1	DR3S121503	99.2	87.6	73.9	59.5
(Level of nucleic acid identity in the group from 95.1 to 100%)	LMCCW191005	98.9	87.3	74.0	59.3
	LMCSNE17505	98.5	87.1	73.6	59.5
	DR2E151204	98.2	86.7	74.0	59.7
	DSW272203	98.1	87.8	74.1	59.1
	DSSE172203	97.7	88.6	71.9	61.4
	DR2NE261003	96.4	89.2	69.5	58.4
Group 2	DR3S23103	89.3	95.3	67.4	58.4
(Level of nucleic acid identity in the group from 90.9 to 100%)	DR2W161003	92.7	93.4	71.7	60.2
	DGS161204	92.7	93.4	71.7	60.2
	LKN38803	92.7	92.9	71.9	60.1
	DR2NE262403	92.6	91.8	69.5	57.6
	DR2N151203	94.8	89.7	74.1	59.4
Group 3	DSW372203	91.5	89.4	74.7	59.4
(Level of nucleic acid identity in the group from 98.3 to 100%)	DR2W11103	90.7	89.5	74.4	59.4
	LMCCNW1121704	90.7	89.5	74.4	59.4
	DR1S12505	90.8	89.6	74.6	59.8
	LDN212404	90.6	89.4	74.2	59.2
	LWS2101803	90.3	89.1	74.5	59.5
	DRE1111503	89.9	88.6	74.0	59.4

Isolate designations include the county, field, location in the field, and date (i.e., DR2NE261003 = Doña Ana County, Rincon Field 2, northeast quadrant, sample 2, collected 6/10/03)

BMCTV-W4 beet mild curly top virus-Worland 4 (AY134867), *BSCTV* beet severe curly top virus (U02311), *BCTV* beet curly top virus (M24597), *SCTV* spinach curly top virus (AY548948)

Table 3

Comparison of percent complete nucleic acid sequence identity among New Mexico chile pepper curtovirus isolates with previously characterized curtoviruses available in GenBank

	BMCTV-W4	BMTCV	BSCTV	BCTV	SCTV	HrCTV
DG2SW171601	97.9	97.2	84.5	80.0	78.6	63.9
LRME27601	87.5	87.3	92.1	79.7	82.1	64.8
LJN17601	88.8	88.3	82.2	77.0	79.9	63.3

BMCTV-W4 beet mild curly top virus, Worland 4 (AY134867), *BMTCV* beet mild curly top virus (U56975), *BSCTV* beet severe curly top virus (U02311), *BCTV* beet curly top virus (M24597), *SCTV* spinach curly top virus (AY548948), and *HrCTV* horseradish curly top virus (U49907)

Table 4

Comparison of percent amino acid identity among open reading frames of New Mexico curtovirus isolates from chile pepper with two characterized curtoviruses available in GenBank BSCTV and BMCTV-W4

Isolate	<u>LJN17601</u>						
	V1	V2	V3	C1	C2	C3	C4
LRME27601	94.1	92.9	95.5	87.9	75.9	79.6	80.2
BSCTV	93.3	95.2	95.5	80.3	70.2	72.8	50.6
DG2SW171601	93.7	99.2	98.9	90.8	74.8	83.1	81.2
BMCTV-W4	94.1	96.8	96.6	91.1	73.5	82.4	80.0