Genotypic Variability and the Occurrence of Less than Genome-Length Viral DNA Forms in a Field Population of Beet Curly Top Geminivirus

Drake C. Stenger

Department of Biological Sciences and Plant Molecular Biology Center, Northern Illinois University, DeKalb 60115.
This research was supported by funds provided by the Northern Illinois University Plant Molecular Biology Center.
I thank C. M. Rush and G. B. Heidel for kindly providing field-collected beet curly top geminivirus samples and B. A. McDonald, I.-R. Choi, and J. E. Duffus for helpful discussions.
Accepted for publication 2 August 1995.

ABSTRACT

Stenger, D. C. 1995. Genotypic variability and the occurrence of less than genome-length viral DNA forms in a field population of beet curly top geminivirus. Phytopathology 85:1316-1322.

During October 1994, a field population of beet curly top geminivirus (BCTV) from the Texas panhandle was sampled and examined for genotypic variability and the accumulation of viral DNA forms. Twelve BCTV-infected sugar beet samples collected from six fields were analyzed by Southern hybridization and found to contain typical genome-length, single- and double-stranded forms of the viral DNA. Most samples also contained less than genome-length viral DNA forms, which varied in size, abundance, and complexity among samples and were likely defective-interfering DNA molecules produced under natural conditions. The extent of genotypic variability among the field isolates was determined by restriction endonuclease mapping of 35 full-length BCTV clones directly obtained from 11 individual plant samples. A total of six genotypic variants were identified from the Texas panhandle population. All the variants were closely related to the previously characterized CFH strain of BCTV and possessed only minor variability at the level of endonuclease restriction sites. Sequence comparisons of the origin of DNA replication (*ori*) and 5′-proximal flanking region revealed that the six Texas panhandle variants possessed few (one to three per variant) nucleotide substitutions relative to the CFH strain, and none of the substitutions occurred within elements of the *ori* implicated as strain-specific determinants of replication. While minor genotypic heterogeneity was observed at all levels of population examined (single plant, field, or locality), these results indicate that CFH genotype represents the dominate BCTV strain infecting sugar beet grown in the Texas panhandle.

The old literature concerning beet curly top geminivirus (BCTV) contains reports of isolates differing in pathogenic properties widely dispersed throughout the western United States and occasionally extending eastward in range (1). Characterization of these isolates was confined to phenotypic analyses of pathogenic properties since the etiology of curly top disease was then undefined, although it was presumed to be caused by a virus.

More recently, molecular analyses have defined BCTV as the type member of subgroup II geminiviruses and revealed considerable genotypic and phenotypic variation in a few of the surviving historic isolates that were recovered from diseased sugar beet (*Beta vulgaris* L.) and maintained in the laboratory by serial leafhopper transmissions. Stanley et al. (37) first cloned and sequenced the California isolate of BCTV. Three additional beet-infecting isolates of BCTV (Logan, CFH, and Worland) have been cloned and their genotypic and pathogenic properties characterized (24,41,42). The California isolate (37) was recovered by J. H. Freitag, probably during the 1930s, and was subsequently maintained by A. H. Purcell upon the retirement of Freitag (A. H. Purcell, *personal communication*). The Logan isolate was collected near Logan, Utah, prior to 1975 (5) and is independent of the Logan 66-10 isolate collected in northern Utah in 1966 (45). The Worland isolate (41) was recovered by J. E. Duffus near Worland, Wyoming, in 1986. The origins of the CFH isolate are more obscure. A full-length DNA clone of the CFH genome was fortuitously obtained (41) during cloning of the genome of the narrow-host-range horseradish curly top virus (HrCTV). Since the wide-host-range phenotype of the cloned CFH genome was inconsistent with the phenotypic properties of HrCTV and an infectious HrCTV clone (41), it was assumed by the authors that the cloned CFH genome was derived from a wide-host-range BCTV isolate of the Duffus collection (recovered from the field prior to 1986) that was inadvertently introduced as a contaminant of the HrCTV culture during propagation in the common host, shepherd's-purse (*Capsella bursa-pastoris* (L.) Medik.).

Although the cloned genomes of the California and Logan isolates of BCTV are indistinguishable by pathogenic properties (41), the two genomes may be distinguished as variants of a single strain on the basis of minor differences in restriction endonuclease maps (41) and nucleotide sequence (19,37). In contrast, the cloned genomes of the CFH and Worland isolates of BCTV are distinct from one another in both genotype and pathogenic properties and also may be distinguished from the California and Logan isolates (41). An analysis of the nucleotide sequence of the CFH isolate (39) indicates that the DNA origin of replication (*ori*) and complementary sense open reading frames (ORFs) C1, C2, C3, and C4 have diverged from the California and Logan genomes. In contrast, the virion sense ORFs (V1, V2, and V3) remain highly conserved among the three sequenced isolates. The retention of conserved restriction endonuclease cleavage sites in the cloned Worland genome (41) mapping to the region of DNA encoding the virion sense ORFs and concomitant lack of conserved restriction endonuclease cleavage sites in the region of DNA encoding the complementary sense ORFs suggest that the Worland genome also has divergent complementary sense ORFs while retaining conserved virion sense ORFs. The nucleotide sequence of the *ori* region obtained for the Worland genome (40) indicates that the Worland isolate is more closely related to the CFH isolate than to the California or Logan isolates.

Corresponding author: D. Stenger; E-mail address: DSTENGER@NIU.EDU

Evidence has been presented indicating that distinct strains of BCTV possess both *cis*- and *trans*-replication elements, which are strain specific and not separately interchangeable. The *cis*-specificity element has been mapped to sequences of the intergenic region containing the *ori*. This conclusion is based upon the strain-specific mobilization and amplification of a Logan-derived defective-interfering DNA (DI-DNA) integrated into the genome of transgenic *Nicotiana benthamiana* Domin as a tandem repeat (40). Further evidence of a strain-specific *cis* element was obtained through the analysis of self-replication competency and DI-DNA mobilization by a series of recombinant BCTV genomes constructed from the Logan and CFH genomes (2). In these experiments, replication of a recombinant genome or DI-DNA molecule was dependent upon the compatible interaction of the *ori* with a viral encoded C1 rep protein containing the cognate *trans*-specificity element residing within the 89 N-terminal amino acid residues of the BCTV C1 rep protein. The geminivirus rep protein-*ori* interaction has been demonstrated to be a multistep process in which the rep protein first binds to a directly repeated sequence element of the *ori* (6,8–10) and subsequently mediates a rolling circle replication mechanism (43) initiated at a strand-specific nick in the conserved stem-loop element of the *ori* (16, 22,36).

Despite the accumulation of genotypic and phenotypic information obtained from the four cloned BCTV genomes derived from laboratory-maintained isolates, the occurrence and dispersion of BCTV genotypes and the complexity of BCTV populations in the field today have not been examined. Furthermore, although BCTV genomes consistently produce less than genome-length DI-DNAs de novo upon inoculation of plants with cloned DNA under laboratory conditions (11,40,44), it remains to be established whether DI-DNAs are associated with BCTV under natural conditions in the field or are simply artifacts of the laboratory. Both of these questions are relevant to a better understanding of BCTV diversity and biology in the field. In this report, a simple and efficient method is presented for analyzing BCTV diversity and the accumulation of viral DNA forms in a field population of the virus.

MATERIALS AND METHODS

Sample acquisition and processing. Two plants expressing leaf curling, vein swelling, and enation symptoms typical of BCTV infection were sampled from each of six sugar beet fields in the Texas panhandle during October 1994. Two of the fields were located near Bushland, one near Hereford, and the remaining three near Wildorado (Table 1). Leaf samples (5 g) from individual sugar beet plants were extracted for total DNA after being ground to a fine powder in liquid N_2. Ground leaf tissue was resuspended and ground further in 4.5 ml of STE (50 mM Tris-HCl, 100 mM NaCl, 10 mM EDTA, and 1% β-mercaptoethanol, pH 8.0), 0.5 ml of 10% sodium dodecyl sulfate, 2.5 ml of equilibrated phenol, and 2.5 ml of chloroform-isoamyl alcohol (24:1). After centrifugation (10 min at 5,000 × *g*), the aqueous phase was recovered, extracted with 5 ml of chloroform-isoamyl alcohol, and centrifuged (10 min at 5,000 × *g*). Total nucleic acids were then concentrated from the aqueous phase by ethanol precipitation. The pellet was resuspended in 0.5 ml of TE (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and RNA was removed by RNaseA digestion (25 µl of 1 $mg \cdot ml^{-1}$) for 30 min at 37°C. Samples were then extracted with an equal volume of phenol-chloroform, and total DNA was concentrated from the aqueous phase by ethanol precipitation. Pellets containing total DNA were washed with ethanol, air dried, resuspended in 0.5 ml of TE, and stored frozen at –20°C. DNA concentration was estimated spectrophotometrically for each sample on the basis of absorbance at 260 nm.

Characterization of BCTV DNA forms in field-collected samples. Total DNA samples (5 µg) were analyzed by Southern hybridization, with or without prior digestion with *Eco*RI. Single- and double-stranded native viral DNA forms were detected with a complementary sense riboprobe transcribed with SP6 RNA polymerase and *Eco*RI-digested pCFH-CSP812 as a template. *Eco*RI-digested, double-stranded viral DNA forms were detected with a strand-specific riboprobe of virion sense transcribed with T7 RNA polymerase and *Hin*dIII-digested pACFH-R as a template. pCFH-CSP812 was constructed by insertion of the 812-bp *Csp*45I fragment of CFH (nucleotides 302 to 1,114) from pCFH (41) into pGEM7Zf+ (Promega, Madison, WI); pACFH-R was constructed by insertion of the genome-length CFH *Eco*RI insert of pCFH (41) into pALTER-1 (Promega).

DNA cloning and analysis. Total DNA samples (50 µg) extracted from individual field-collected plants were digested with *Eco*RI and size fractionated by agarose gel electrophoresis. Size-fractionated DNA (2.5 to 3.5 kbp) was recovered from agarose gels with Gene Clean II (BIO 101, Vista, CA), ligated to *Eco*RI-digested pUC8, and used to transform *Escherichia coli* strain DH5α (GIBCO-BRL, Gaithersburg, MD). Ampicillin-

TABLE 1. Sugar beet samples infected with beet curly top geminivirus and summary of cloning experiments

Sample	Locality	Field	Representative clone	Number of clones	Variant[a]
94-1	Bushland	1	pT94-1-25	5	IV
94-4	Bushland	1	pT94-4-59	6	III
			(pT94-4-197)[b]	(1)	(V)
94-6	Bushland	2	pT94-6-9	5	I
94-7	Bushland	2	pT94-7-214	2	I
94-12	Wildorado	3	pT94-12-87	3	I
94-14	Wildorado	3	pT94-14-157	1	VI
94-16	Wildorado	4	pT94-16-17	4	I
94-19	Wildorado	4	pT94-19-109	4	I
			(pT94-19-154)	(1)	(II)
94-22	Wildorado	5	ND[c]	ND	ND
94-23	Wildorado	5	pT94-23-7	1	II
94-28	Hereford	6	pT94-28-124	1	III
94-30	Hereford	6	pT94-30-188	1	III

[a] Based upon restriction endonuclease site variability as presented in Figure 3.
[b] Parentheses denote data for distinct variants in cases in which more than one variant was cloned from an individual plant sample.
[c] Not determined.

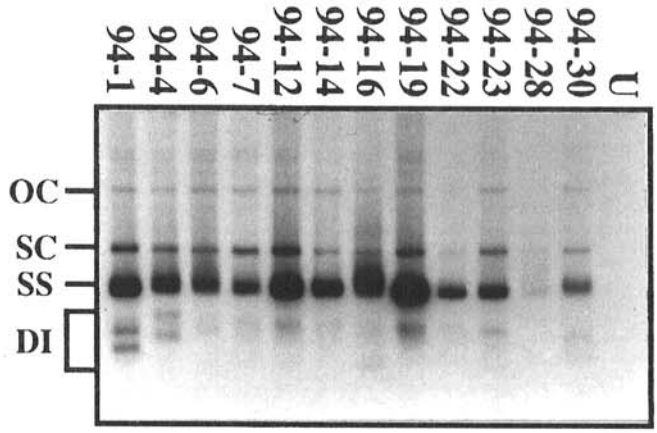

Fig. 1. DNA forms present in field-collected beet curly top geminivirus (BCTV) isolates. Presented is an autoradiograph of a Southern blot of total DNA (5 µg) extracted from 12 BCTV-infected sugar beet samples (94-1 to 94-30) collected from the Texas panhandle population during October 1994. The mobility of genome-length single-stranded (SS) and supercoiled (SC) and open circle (OC) double-stranded DNA forms are indicated at left. The range of mobility of heterogeneous, less than genome-length defective-interfering (DI) DNAs is indicated at left. Hybridization was conducted with a riboprobe of complementary sense transcribed from pCFH-CSP812. Lane U contains DNA extracted from an uninfected sugar beet sample.

resistant transformants lacking β-galactosidase activity (200 to 400 colonies per transformation) were screened for BCTV inserts by colony hybridization (28) with a full-length Logan riboprobe of complementary sense transcribed from pCT10 (44). Clones identified as containing BCTV DNA inserts by colony hybridization were verified by Southern hybridization of *Eco*RI-digested plasmid DNA with a riboprobe transcribed from pCT10. The viral DNA inserts of each plasmid were characterized by restriction endonuclease mapping and partial DNA sequencing with Sequenase 2.0 (USB/Amersham, Cleveland, OH).

RESULTS

BCTV DNA forms in field-collected samples. All 12 BCTV-infected sugar beet samples obtained from the Texas panhandle population (Table 1) contained variable amounts of typical genome-length, single- and double-stranded DNA forms of the viral genome (Fig. 1). Most of the samples also contained less than genome-length DNA forms, which varied in size, abundance, and complexity from sample to sample (Fig. 1). The detection of less than genome-length viral DNA forms in the undigested sample 94-1 with a strand-specific riboprobe of virion sense (Fig. 2) demonstrates that at least some of the less than genome-length viral DNA forms are double-stranded replicative intermediates.

Characterization of cloned BCTV genomes. Genome-length, double-stranded DNA in all 12 BCTV field samples was linearized by digestion with *Eco*RI, suggesting that each isolate contained a single, although not necessarily identical, *Eco*RI cleavage site (Fig. 2). A total of 35 recombinant clones containing 2.9-kbp *Eco*RI inserts of BCTV DNA were obtained from 11 of the field samples (Table 1 and Fig. 3). Endonuclease restriction mapping was used to classify the cloned BCTV genomes, and six variants were identified on the basis of the presence or absence of polymorphic sites (Table 1 and Fig. 4). A comparison of the endonuclease restriction maps generated for each cloned genome derived from the Texas panhandle population indicated that all the variants recovered were most closely related to the previously characterized CFH isolate of BCTV. Of 10 restriction endonuclease sites present on the CFH genome, 34 cloned genomes representing five variants retained all 10 sites. A single clone representing a sixth variant was obtained that retained nine of the 10 endonuclease restriction cleavage sites conserved in the CFH genome. In contrast, only three of the restriction endonuclease cleavage sites mapped on the cloned Texas variants (the *Sna*BI, *Csp*45I, and *Bst*XI sites within the coat ORF V1) were conserved relative to the California, Logan, or Worland genomes. Although the *Ssp*I site conserved within the stem-loop element of all previously characterized geminiviruses was not mapped, sequence obtained for the *ori* (Fig. 5) indicated that this site (AATATT) was retained within all the Texas variants examined. The six Texas variants could be distinguished from one another on the basis of the presence or absence of polymorphic *Xho*I, *Sal*I, *Pvu*II, or *Kpn*I sites, which are not present within the California, Logan, CFH, or Worland genomes, or the absence of a *Csp*45I site in variant VI (Fig. 4).

A comparison of the nucleotide sequence of the *ori* (CFH nucleotides 33 to 100) and 5′-proximal flanking region (CFH nucleotides 1 to 32) revealed that each of the six Texas variants retained a high degree of conservation with the CFH genotype (Fig. 5). Nucleotide changes were limited to a total of five positions, with no more than three substitutions occurring within a single variant. Sequence elements of the *ori* implicated as essential *cis* elements of DNA replication (the directly repeated motif of the putative C1 rep protein binding site, and the inverted repeats and invariant motif of the stem-loop) of five Texas variants were identical to the CFH genome. Although the single nucleotide substitution in pT94-14-157 (variant VI) within the 3′-proximal inverted repeat of the stem-loop would result in the potential stem structure being 10 rather than 11 bases in length, this region of the *ori* maps outside the replication specificity *cis* element (2), and chimeric BCTV genomes containing the same base-pairing mismatch in the stem as variant VI retain infectivity in *N. benthamiana* (42,43). Similarly, the single nucleotide substitution of pT94-12-87 (variant I) present between the 5′-proximal inverted repeat and invariant motif of the loop occurs at a position not implicated in viral replication competency or specificity. Nucleotide substitutions in the 5′-proximal flanking region adjacent to the direct repeats of the *ori* (Fig. 5) occur in a region of the viral genome that has not been assigned any specific function apart from likely being transcribed in complementary sense as part of the C1 ORF mRNA leader. Since the *ori* and 5′-proximal flanking region have undergone considerable divergence among phenotypically distinct BCTV strains (39,40), the high degree of sequence conservation among the Texas variants and

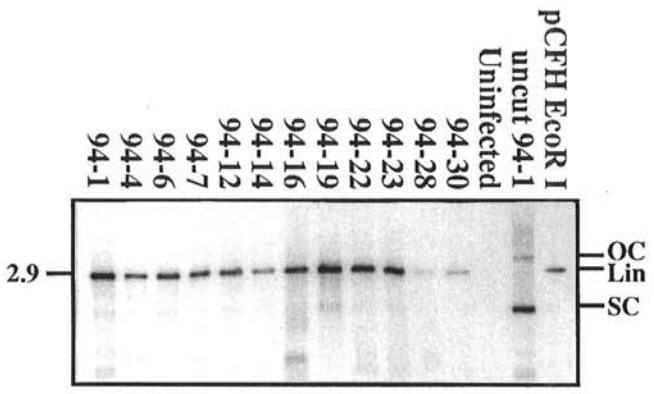

Fig. 2. Linearization of double-stranded beet curly top geminivirus (BCTV) DNA by *Eco*RI digestion. Presented is an autoradiograph of a Southern blot of total DNA (5 µg) extracted from 12 BCTV-infected sugar beet samples (94-1 to 94-30) recovered from the Texas panhandle population and incubated with *Eco*RI prior to electrophoresis. The mobilities of genome-length supercoiled (SC), open circle (OC), and linear (Lin) double-stranded DNA forms of the viral genome are indicated at right. The size (2.9 kbp) of linearized viral double-stranded DNA resulting from *Eco*RI digestion is indicated at left. The three lanes on the extreme right contain *Eco*RI-digested DNA isolated from an uninfected sugar beet grown in the greenhouse (5 µg), undigested total DNA isolated from Texas sample 94-1 (5 µg), or *Eco*RI-digested pCFH (2 ng) containing a genome-length *Eco*RI insert of the CFH genome (40). Hybridization was conducted with a genome-length riboprobe of virion sense transcribed from pACFH-R capable of detecting only double-stranded BCTV DNA forms. Unlabeled faint heterogeneous bands likely represent cleaved or native double-stranded defective-interfering DNA forms.

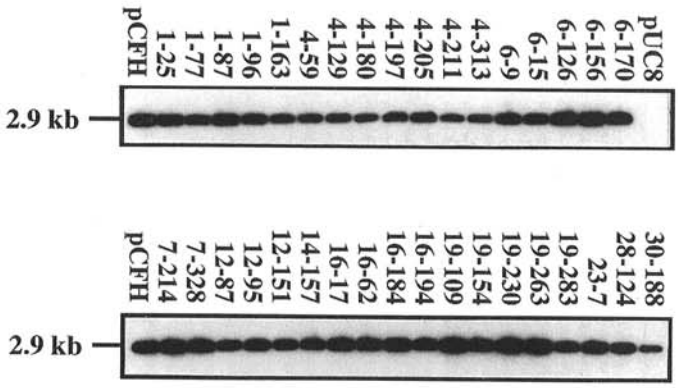

Fig. 3. Verification of 35 beet curly top geminivirus genomes cloned from the Texas panhandle population. Presented is an autoradiograph of a Southern blot of each cloned Texas genome (10 ng) released from the pUC8 plasmid vector by digestion with *Eco*RI prior to electrophoresis. Hybridization was conducted with a genome-length Logan riboprobe transcribed from pCT10. The size (2.9 kb) of linearized double-stranded DNA resulting from *Eco*RI digestion is indicated at left.

CFH supports the conclusion that the Texas panhandle population is dominated by minor variants of the CFH genotype.

Examination of the dispersion pattern of genetic variability indicates that the Texas panhandle BCTV population contained heterogeneity at all levels of organization examined: single plant, field, and locality (Table 1). Two plant samples yielded more than one cloned variant, demonstrating that more than one BCTV variant may exist within a single plant in the field. Although it cannot be determined unequivocally whether the occurrence of more than one variant within a single plant was the result of the inoculation of two previously existing variants or arose via mutation of a single variant after infection, the occurrence of variant II (Table 1 and Fig. 4) in a mixed infection with the most common variant (I) in Wildorado field 4 and the independent recovery of variant II from a separate field (Wildorado field 5) suggest that both genomes were likely preexisting and transmitted (individually or as a mixture) to result in a mixed infection. Up to three variants were recovered from a single field (Bushland field 1), and variability among separate fields was observed for both localities (Bushland and Wildorado) for which more than one field was sampled. Collectively, these results define a minimum degree of genetic variability within the Texas panhandle BCTV population and demonstrate that a single strain of BCTV exists in the field as a constellation of variants that may be identified as individuals.

DISCUSSION

Although other geminiviruses have less than genome-length DI-DNA forms associated with uncloned cultures that have been serially passaged in the laboratory (4,26,27,38), only in the case of BCTV have DI-DNA molecules been demonstrated to be produced consistently from cloned inocula on first passage (11, 40,44). The high degree of variation in size and complexity of BCTV DI-DNAs is also atypical relative to other geminiviruses. The presence of less than genome-length viral DNA forms varying in size, abundance, and complexity in field-collected material closely resembles results obtained in laboratory experi-

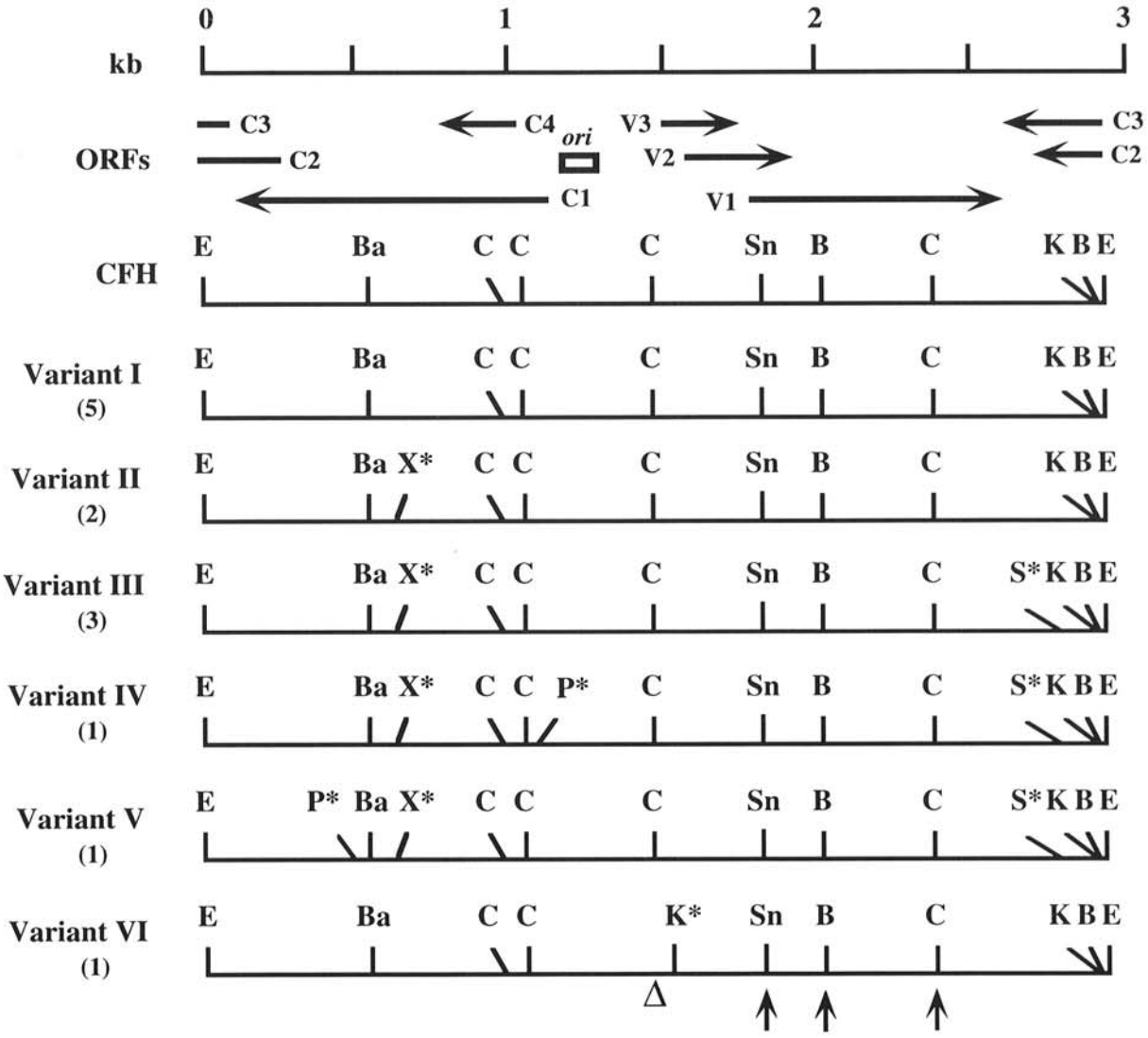

Fig. 4. Beet curly top geminivirus (BCTV) variants cloned from the Texas panhandle population. Presented is an alignment of restriction endonuclease maps of the cloned *Eco*RI inserts of the CFH genome and six distinct variants (I–VI) recovered from BCTV-infected sugar beets collected from the field. Horizontal arrows denote the location and polarity of open reading frames (ORFs) deduced from the CFH genome sequence. The location of the DNA origin of replication is denoted as a box, and scale is represented in kilobase pairs. Restriction endonuclease cleavage sites conserved among CFH and six Texas variants are indicated by one-letter abbreviations; asterisks denote restriction endonuclease cleavage sites present in one or more of the Texas variants that are not present in CFH. Triangle denotes location of a *Csp*45I site that is conserved in all variants depicted except variant VI. Vertical arrows denote location of three restriction endonuclease cleavage sites within the coat protein V1 ORF conserved among all the Texas variants and the previously characterized CFH, California, Logan, and Worland genotypes. The number of individual plant samples from which each variant was recovered is in parentheses. E = *Eco*RI; Ba = *Bam*HI; C = *Csp*45I; Sn = *Sna*BI; K = *Kpn*I; B = *Bst*XI; X = *Xho*I; P = *Pvu*II; and S = *Sal*I.

ments with cloned BCTV DNA inocula and strongly suggests that BCTV readily produces DI-DNAs in the field under natural conditions. In addition to geminiviruses, a number of distinct plant viruses belonging to diverse taxa have associated DI molecules, at least under experimental conditions in the laboratory (17,21,25,34,35). However, there is but one other report demonstrating that DI-like molecules may be associated with a plant virus in the field. Coutts and Buck (3) examined cassava naturally infected with the bipartite African cassava mosaic geminivirus (ACMV; formerly cassava latent virus) containing a half-unit length form of DNA B similar in size to a well-characterized DI-DNA form of ACMV DNA B present in a laboratory isolate of the virus maintained by serial passage in *N. benthamiana* (38). However, in the case of ACMV, DI-DNAs do not appear upon inoculation of *N. benthamiana* with cloned DNA, and ACMV DI-DNAs were not generated during a serial passage experiment in which a viral culture derived from cloned inocula was passaged five times (38). Despite the differences in DI-DNA production observed in BCTV and ACMV, DI-DNAs of both viruses may be produced by a common mechanism, which may differ only in frequency of occurrence. This hypothesis is based upon an examination of the deletion boundaries present in DI-DNA molecules of both viruses, which are demarcated by 2- to 7-base, directly repeated sequences (11,38,44). Therefore, establishing that molecules resembling DI-DNAs accumulate in plants naturally infected with BCTV in the field and the consistent de novo production of BCTV DI-DNAs in the laboratory may facilitate experimentation concerning the mechanism of geminivirus DI-DNA production.

Geminiviruses encompass a diverse collection of plant-infecting agents intensively studied during the past 15 years. Presently, more than 36 distinct geminiviruses and strains have been completely sequenced and classified on the basis of phylogenetic analyses of sequence data (7,31). Diversity within and among geminiviruses has been examined in a number of cases. Genomic variation among subgroup I geminiviruses infecting monocots in Africa has been assessed by biological and serological properties (29) or by physical properties of the genome (20). Isolates of squash leaf curl virus that differ in host range have been described (23), and field isolates of the virus have been examined with probes that are able to discriminate among cucurbit-infecting geminiviruses (33). A recent examination of the nucleotide sequences of geminiviruses that cause cassava mosaic disease in Africa and India indicate that at least three distinct geminiviruses

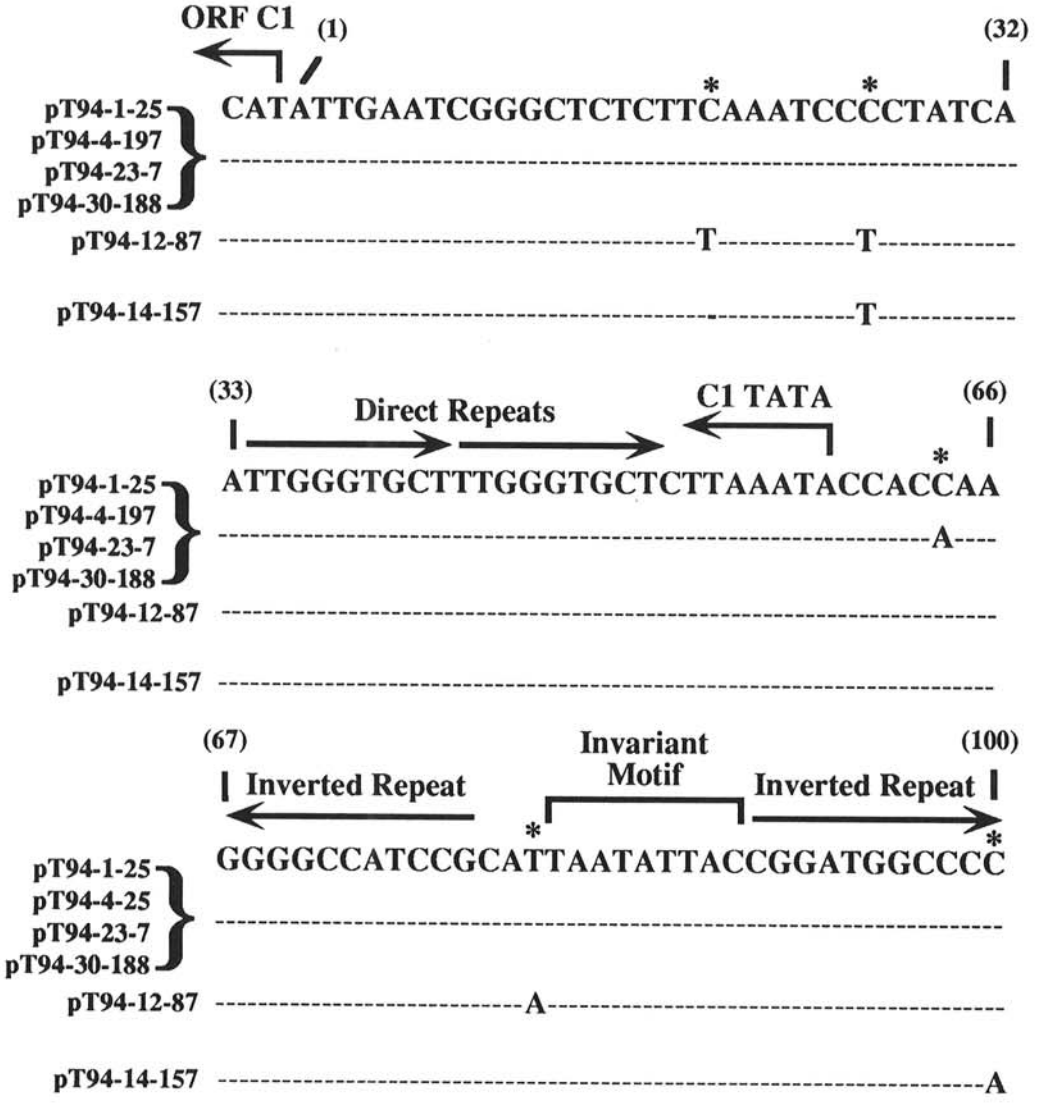

Fig. 5. Nucleotide sequence variation within the *ori* and 5′-proximal flanking region. The positions of nucleotide substitutions (asterisks) occurring in six variants of beet curly top geminivirus recovered from the Texas panhandle population are depicted and compared with the CFH virion sense sequence of the *ori* (CFH nucleotides 33 to 100) and 5′-proximal flanking region (CFH nucleotides 1 to 32). The locations of *ori* elements (direct repeats, C1 TATA box, stem-loop inverted repeats, and invariant motif) are indicated by arrows. The location of the complementary sense C1 open reading frame (ORF) initiation anticodon is indicated.

each have the potential to cause the disease (18). Bean golden mosaic virus (BGMV) isolates recovered from the Caribbean Basin or South America exhibit genotypic variability correlated with location (7,12–15). The inability of certain BGMV isolates to form viable pseudorecombinants (12) suggests that like BCTV, BGMV may have developed strain-specific replication elements. The phylogenetic characterization of genotypically distinct, whitefly-transmitted geminiviruses that infect tomato (31) is yet another example of geminivirus diversity, while the recent introduction of tomato yellow leaf curl virus into the Western Hemisphere (30,32) provides documentation indicating that geminivirus populations are not static. Thus, when genotypic variability of BCTV is compared with variability in geminiviruses at large, it is perhaps not surprising to find that isolates of BCTV possess distinct genotypic and phenotypic properties.

All of the Texas panhandle BCTV isolates examined in this study may be considered minor variants of the CFH genotype, establishing that the CFH strain of BCTV is currently a significant pathogen of sugar beet that must be considered relevant with respect to the development of strategies for disease resistance. It is particularly interesting to note that no genotypes resembling the BCTV isolates California, Logan, or Worland were encountered in the Texas panhandle population. While the current survey was far from exhaustive and does not preclude the occurrence of other genotypes at low levels, the CFH genotype certainly appears to dominate the population in the region surveyed.

The genetic complexity of BCTV populations present in other regions of the country needs to be examined. Such a study would address the following questions: Are other BCTV genotypes (i. e., California, Logan, and Worland) still present in the virus population? If so, what is the current distribution of BCTV genotypes throughout the endemic range of the virus? Are there other distinct BCTV genotypes that have not yet been discovered? These questions may be addressed through a comprehensive and methodical survey of natural BCTV populations with the cloning strategy employed in this investigation. As a result, a sufficiently large enough sample size may be obtained to facilitate quantitative population genetic studies on a geminivirus without the logistical problems associated with maintaining laboratory cultures of each isolate and with minimal probability of introducing uncontrolled biases.

LITERATURE CITED

1. Bennett, C. W. 1971. The Curly Top Disease of Sugarbeet and Other Plants. Monogr. 7. American Phytopathological Society, St. Paul, MN.
2. Choi, I.-R., and Stenger, D. C. 1995. Strain-specific determinants of beet curly top geminivirus DNA replication. Virology 206:904-912.
3. Coutts, R. H. A., and Buck, K. W. 1987. Cassava latent virus specific DNAs in mosaic diseased cassava of Nigerian origin. Neth. J. Plant Pathol. 93:241-244.
4. Czosnek, H., Ber, R., Navot, N., Antignus, Y., Cohen, S., and Zamir, D. 1989. Tomato yellow leaf curl virus DNA forms in the viral capsid, in infected plants, and in the insect vector. J. Phytopathol. 125:47-54.
5. Duffus, J. E., and Skoyen, I. O. 1977. Relationship of age of plants and resistance to a severe isolate of beet curly top virus. Phytopathology 67:151-154.
6. Eagle, P. A., Orozco, B. M., and Hanley-Bowdoin, L. 1994. A DNA sequence required for geminivirus replication also mediates transcriptional regulation. Plant Cell 6:1157-1170.
7. Faria, J. C., Gilbertson, R. L., Hanson, S. F., Morales, F. J., Ahlquist, P., Loniello, A. O., and Maxwell, D. P. 1994. Bean golden mosaic geminivirus type II isolates from the Dominican Republic and Guatemala: Nucleotide sequences, infectious pseudorecombinants, and phylogenetic relationships. Phytopathology 84:321-329.
8. Fontes, E. P. B., Eagle, P. A., Sipe, P. S., Luckow, V. A., and Hanley-Bowdoin, L. 1994. Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. J. Biol. Chem. 269:8459-8465.
9. Fontes, E. P. B., Gladfelter, H. J., Schaffer, R. L., Petty, I. T. D., and Hanley-Bowdoin, L. 1994. Geminivirus replication origins have a modular organization. Plant Cell 6:405-416.
10. Fontes, E. P. B., Luckow, V. A., and Hanley-Bowdoin, L. 1992. A geminivirus replication protein is a sequence-specific DNA binding protein. Plant Cell 4:597-608.
11. Frischmuth, T., and Stanley, J. 1992. Characterization of beet curly top virus subgenomic DNA localizes sequences required for replication. Virology 189:808-811.
12. Gilbertson, R. L., Faria, J. C., Ahlquist, P., and Maxwell, D. P. 1993. Genetic diversity in geminiviruses causing bean golden mosaic disease: The nucleotide sequence of the infectious cloned DNA components of a Brazilian isolate of bean golden mosaic geminivirus. Phytopathology 83:709-715.
13. Gilbertson, R. L., Faria, J. C., Hanson, S. F., Morales, F. J., Ahlquist, P., Maxwell, D. P., and Russell, D. R. 1991. Cloning of the complete DNA genomes of four bean-infecting geminiviruses and determining their infectivity by electric discharge particle acceleration. Phytopathology 81:980-985.
14. Gilbertson, R. L., Hidayat, S. H., Martinez, R. T., Leong, S. A., Faria, J. C., Morales, F., and Maxwell, D. P. 1991. Differentiation of bean-infecting geminiviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Dis. 75:336-342.
15. Gilbertson, R. L., Rojas, M. R., Russell, D. R., and Maxwell, D. P. 1991. Use of asymmetric polymerase chain reaction and DNA sequencing to determine genetic variability of bean golden mosaic geminivirus in the Dominican Republic. J. Gen. Virol. 72:2843-2848.
16. Heyraud, F., Matzeit, V., Kammann, M., Schaeffer, S., Schell, J., and Gronenborn, B. 1993. Identification of the initiation sequence for viral-strand DNA synthesis of wheat dwarf virus. EMBO J. 12:4445-4452.
17. Hillman, B. I., Carrington, J. C., and Morris, T. J. 1987. A defective interfering RNA that contains a mosaic of a plant virus genome. Cell 51:427-433.
18. Hong, Y. G., Robinson, D. J., and Harrison, B. D. 1993. Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. J. Gen. Virol. 74:2437-2443.
19. Hormuzdi, S. G., and Bisaro, D. M. 1993. Genetic analysis of beet curly top virus: Evidence for three virion sense genes involved in movement and regulation of single- and double-stranded DNA levels. Virology 193:900-909.
20. Hughes, F. I., Rybicki, E. P., and von Wechmar, M. B. 1992. Genome typing of southern African subgroup 1 geminiviruses. J. Gen. Virol. 73:1031-1040.
21. Ismail, I. D., and Milner, J. J. 1988. Isolation of defective interfering particles of sonchus yellow net virus from chronically infected plants. J. Gen. Virol. 69:999-1006.
22. Laufs, J., Traut, W., Heyraud, F., Mazeit, V., Rogers, S. G., Schell, J., and Gronenborn, B. 1995. In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Proc. Natl. Acad. Sci. USA 92:3879-3883.
23. Lazarowitz, S. G. 1991. Molecular characterization of two bipartite geminiviruses causing squash leaf curl disease: Role of viral replication and movement functions in determining host range. Virology 180:70-80.
24. Lee, S., Stenger, D. C., Bisaro, D. M., and Davis, K. R. 1994. Identification of loci in *Arabidopsis* that confer resistance to geminivirus infection. Plant J. 6:525-535.
25. Li, X. H., Heaton, L. A., Morris, T. J., and Simon, A. E. 1989. Turnip crinkle virus defective interfering RNAs intensify viral symptoms and are generated de novo. Proc. Natl. Acad. Sci. USA 86:9173-9177.
26. Macdonald, H., Coutts, R. H. A., and Buck, K. W. 1988. Characterization of a subgenomic DNA isolated from *Triticum aestivum* plants infected with wheat dwarf virus. J. Gen. Virol. 69:1339-1344.
27. MacDowell, S. W., Coutts, R. H. A., and Buck, K. W. 1986. Molecular characterisation of subgenomic single-stranded and double-stranded DNA forms isolated from plants infected with tomato golden mosaic virus. Nucleic Acids Res. 14:7967-7984.
28. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
29. Mesfin, T., Bosque-Pérez, N. A., Buddenhagen, I. W., Thottappilly, G., and Olojede, S. O. 1992. Studies of maize streak virus isolates from grass and cereal hosts in Nigeria. Plant Dis. 76:789-795.
30. Nakhla, M. K., Maxwell, D. P., Martinez, R. T., Carvalho, M. G., and Gilbertson, R. L. 1994. Widespread occurrence of the eastern Mediterranean strain of tomato yellow leaf curl geminivirus in tomatoes in the Dominican Republic. Plant Dis. 78:926.
31. Padidam, M., Beachy, R. N., and Fauquet, C. 1995. Classification and identification of geminiviruses using sequence comparisons. J. Gen. Virol. 76:249-263.
32. Polston, J. E., Bois, D., Serra, C.-A., and Concepción, S. 1994. First report of a tomato yellow leaf curl-like geminivirus in the Western Hemisphere. Plant Dis. 78:831.

33. Polston, J. E., Dodds, J. A., and Perring, T. M. 1989. Nucleic acid probes for detection and strain discrimination of cucurbit geminiviruses. Phytopathology 79:1123-1127.
34. Rochon, D. M., and Johnston, J. C. 1991. Infectious transcripts from cloned cucumber necrosis virus cDNA: Evidence for a bifunctional subgenomic mRNA. Virology 181:656-665.
35. Romero, J., Huang, Q., Pogany, J., and Bujarski, J. J. 1993. Characterization of defective interfering RNA components that increase symptom severity of broad bean mottle virus infections. Virology 194:576-584.
36. Stanley, J. 1995. Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs within the conserved nonanucleotide motif during the initiation of rolling circle DNA replication. Virology 206:707-712.
37. Stanley, J., Markham, P. G., Callis, R. J., and Pinner, M. S. 1986. The nucleotide sequence of an infectious clone of the geminivirus beet curly top virus. EMBO J. 5:1761-1767.
38. Stanley, J., and Townsend, R. 1985. Characterisation of DNA forms associated with cassava latent virus infection. Nucleic Acids Res. 13:2189-2206.
39. Stenger, D. C. 1994. Complete nucleotide sequence of the hypervirulent CFH strain of beet curly top virus. Mol. Plant-Microbe Interact. 7:154-157.
40. Stenger, D. C. 1994. Strain-specific mobilization and amplification of a transgenic defective-interfering DNA of the geminivirus beet curly top virus. Virology 203:397-402.
41. Stenger, D. C., Carbonaro, D., and Duffus, J. E. 1990. Genomic characterization of phenotypic variants of beet curly top virus. J. Gen. Virol. 71:2211-2215.
42. Stenger, D. C., Davis, K. R., and Bisaro, D. M. 1994. Recombinant beet curly top virus genomes exhibit both parental and novel pathogenic phenotypes. Virology 200:677-685.
43. Stenger, D. C., Revington, G. N., Stevenson, M. C., and Bisaro, D. M. 1991. Replicational release of geminivirus genomes from tandemly repeated copies: Evidence for rolling-circle replication of a plant viral DNA. Proc. Natl. Acad. Sci. USA 88:8029-8033.
44. Stenger, D. C., Stevenson, M. C., Hormuzdi, S. G., and Bisaro, D. M. 1992. A number of subgenomic DNAs are produced following agroinoculation of plants with beet curly top virus. J. Gen. Virol. 73:237-242.
45. Thornley, W. R., and Mumford, D. L. 1979. Intracellular location of beet curly top virus antigen as revealed by fluorescent antibody staining. Phytopathology 69:738-740.