Phytopathology® • XXXX • XX:X-X • https://doi.org/10.1094/PHYTO-09-25-0297-R

Virology

Beet Curly Top Virus Genetic Diversity, Impact on Cannabinoids, Potential Seed Transmission, and Vector Biology in Hemp

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Accepted for publication 16 January 2026.

Abstract

Beet curly top virus (BCTV) has emerged as a major threat to hemp production in the western United States. Despite this concern, little is known about BCTV biology in hemp. This study investigated the incidence and genetic variability of BCTV strains, as well as the impact of BCTV infection on cannabinoid profiles, potential for seed transmission, and vector survival on hemp. Field surveys across five states revealed four distinct BCTV strains, with BCTV-CO and BCTV-Wor being the most prevalent. In addition, BCTV-PeCT was detected for the first time in hemp samples from Oregon, Colorado, and New Mexico, whereas BCTV-PeYD was detected exclusively in New Mexico. Genetic analysis showed high nucleotide diversity and widespread recombination among hemp-associated BCTV strains, consistent with active genetic exchange in BCTV. Furthermore, BCTV was detected in surface-disinfected seeds (77% in the Elite genotype, 18% in the 791 genotype) and in a small number of seedlings in grow-out experiments (0.98% in Elite, 0.87% in 791). Additional seed dissection experiments in Elite revealed BCTV presence in 41% of embryos, 72% of endosperm, and 5% of seed coats, demonstrating potential for seed transmission in hemp rather than surface contamination. BCTV infection reduced cannabinoid levels in one hemp genotype but not in another, indicating genotype-specific effects of BCTV on cannabinoid production. Finally, beet leafhoppers, the exclusive vector of BCTV, were unable to survive on hemp beyond 7 days, indicating that the insect cannot complete its life cycle on hemp. These findings provide a foundational understanding of BCTV evolutionary dynamics and host interactions in hemp, with implications for disease management.

Keywords: BCTV, beet leafhopper, cannabinoids, *Cannabis sativa*, geminivirus, recombination, viral strains

The legalization of hemp (*Cannabis sativa* L., <0.3% tetrahydrocannabinol [THC]) at the federal level has introduced a new and profitable crop into the agricultural landscape (Agricultural Act of 2014; Agriculture Improvement Act of 2018). The latest National Hemp Report indicates that the total planted area for industrial hemp in the United States reached 45,294 acres in 2024, a 64% increase from 2023, and total harvested acres rose by 55% to 32,694 acres (USDA-NASS 2024). Hemp is attractive to growers in many western U.S. states because of its lower water requirements, and the dry climate in the region naturally restricts most foliar fungal pathogen issues seen in more humid regions. Despite the widespread adoption of hemp, there is little guidance on disease identification and management for growers.

Recent reviews on cannabis pathology identified some major concerns, although viruses infecting hemp are understudied (Miotti et al. 2023; Punja 2021; Punja et al. 2024a). Chiginsky et al. (2021) published the first comprehensive virome report for hemp, observing seven viruses and one viroid infecting hemp in Colorado. More recently, surveys of viruses infecting hemp in Colorado during 2021 and 2022 identified beet curly top virus (BCTV) and several novel viruses (Hackenberg et al. 2025). BCTV is a circular single-stranded DNA virus (approximately 3.0-kb genome) that belongs to the genus *Curtovirus* in the family *Geminiviridae* (Gilbertson et al. 2021; Harrison 1985; Varsani et al. 2014), and it is currently considered the most damaging viral pathogen of hemp in the western United States (Bennett et al. 2024; Chiginsky et al. 2021; Giladi et al. 2020; Hu et al. 2021; Jarugula et al. 2023; Melgarejo et al. 2022; Miotti et al. 2023; Rivedal et al. 2022). The symptoms of BCTV in hemp include stunting, leaf curling, yellowing, vein swelling, and necrosis (Gilbertson et al. 2021; Hackenberg et al. 2025; Hu et al. 2021; Schoener and Wang 2023). In addition to hemp, curly top

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Funding: Support was provided by the U.S. Department of Agriculture-National Institute of Food and Agriculture Crop Protection and Pest Management (2022-70006-37996) and the Foundation for Food and Agriculture Research (Rapid Outcomes from Agricultural Research Program).

e-Xtra: Supplementary material is available online.

The author(s) declare no conflict of interest.

disease (CTD) has been of major importance to agriculture since the late 1800s, impacting the yield and quality of multiple major crops, including sugar beet, common bean, spinach, pepper, squash, and tomato (Chen and Gilbertson 2016; Creamer 2020; Gilbertson et al. 2021).

Since its initial detection in hemp in Colorado (Chiginsky et al. 2021; Giladi et al. 2020), BCTV has been reported across several western states, including Arizona (Hu et al. 2021), Nevada (McGue et al. 2021), California (Melgarejo et al. 2022), Oregon (Frost and Ocamb 2023), Washington (Jarugula et al. 2023), and New Mexico (Creamer et al. 2024). Fulfillment of Koch's postulates with an infectious clone of the BCTV-CO strain further confirmed its pathogenicity in hemp (Melgarejo et al. 2022). There are 11 recognized strains of BCTV (Chen and Gilbertson 2016; Strausbaugh et al. 2017), the occurrence of which varies across time and region, with common co-infections (Gilbertson et al. 2021; Lam et al. 2009; Strausbaugh et al. 2017). BCTV strains identified in hemp include BCTV-CO, BCTV-Wor, and BCTV-PeYD (Chiginsky et al. 2021; Creamer et al. 2024; Giladi et al. 2020; Hu et al. 2021; Jarugula et al. 2023; Melgarejo et al. 2022).

Multiple strains have been identified, originally classified as mild- and severe-type variants based on the pathogenicity and symptom expression in sugar beet (Gilbertson et al. 2021; Strausbaugh et al. 2017). Current strain classification uses 77 and 94% genome-wide pairwise identity as species and strain thresholds, respectively (Strausbaugh et al. 2017; Varsani et al. 2014). The BCTV genome is prone to genetic recombination, and with co-infections, the emergence of novel virus variants can be accelerated (Gilbertson et al. 2021; Strausbaugh et al. 2017; Varsani et al. 2014). Its monopartite genome encodes up to seven genes across two DNA strands: three virion-sense open reading frames (ORFs) for the capsid protein (V1), a regulatory protein (V2), and a movement protein (V3) and four complementary-sense ORFs for the replication-associated protein (C1), a silencing suppressor (C2), a replication enhancer (C3), and a symptom determinant (C4) (Frischmuth and Stanley 1992; Frischmuth et al. 1993; Guerrero et al. 2020).

The virus is transmitted exclusively by the beet leafhopper (BLH), *Neoaliturus tenellus* (Baker) (also referred to as *Circulifer tenellus*), in a circulative, non-propagative manner, and historically, it was reported that the virus cannot be transmitted mechanically or via seed (Bennett 1962, 1971; Chen and Gilbertson 2016; Gilbertson et al. 2021; Han et al. 2024). However, one study demonstrated that BCTV has a 38 to 78% seed transmission rate in a local petunia (Anabestani et al. 2017). Given the emergence of BCTV in hemp, understanding the evolutionary dynamics and seedborne potential of this pathogen in this new host is essential, as it could have implications for CTD management.

BCTV has emerged as a major pathogen threatening hemp in the western United States. Nevertheless, more knowledge is needed regarding the viral genetic diversity in hemp, effects on plant phytochemistry, and potential for seed transmission. This study aimed to address some of these aspects of BCTV biology in hemp by further examining the prevalence and genetic variability of BCTV strains in the western United States, impact on cannabinoid production, potential for seed transmission, and survivorship of BLHs on hemp.

Materials and Methods

BCTV source and inoculation of hemp plants

BLHs, viruliferous for BCTV, were collected from sugar beet (*Beta vulgaris*) fields in Boise, Idaho, and transported to the laboratory at Colorado State University under an approved USDA permit (P526P-19-04080). This colony was maintained on sugar beet (*B. vulgaris* cultivar BPA9000). Several leaf tissues of CTD symptomatic plants and a pooled sample of 10 BLHs (comprising five adults and five nymphs) were collected from the colony and tested bimonthly using PCR to confirm that the colony remained 100% viruliferous. The colony consisted of a mix of BCTV-CA/Logan and BCTV-Svr strains and was used for all experiments in this study. DNA was extracted using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, U.S.A.), following the manufacturer's instructions. DNA was then diluted to 25 ng/µl and used for PCR with the following primers to target BCTV coat protein (expected amplicon size: 496 bp), BCTV2-F: GTGGAT CAATTTCCAGACAATTATC and BCTV2-R: CCCATAAGAGC CATATCAAACTTC (Strausbaugh et al. 2008). The PCR comprised 10 µl of DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, U.S.A.), 1 µl of the forward and reverse primers, 7 µl of molecular biology-grade water, and 2 µl of diluted sample DNA. The PCR thermocycling conditions were as follows: 94°C for 2 min, [94°C for 45 s, 58°C for 45 s, and 72°C for 45 s] × 35 cycles, 72°C for 5 min, and a 4°C hold. PCR amplicons were stained with GelRed (Biotium, Fremont, CA, U.S.A.) and visualized by gel electrophoresis using an Azure 200 Gel Imager (Azure Biosystems, Dublin, CA, U.S.A.).

To infect hemp plants with BCTV, plants at the three- to five-leaf stage were inoculated with a cohort of five; mixed stages of nymph and adult BLHs viruliferous for BCTV were attached to the most developed mature leaves using foam clip cages (36.5 mm in diameter) (BioQuip, Compton, CA). BLHs were allowed to feed for 48 h before being removed from the plants. Three weeks after the inoculation access period, approximately 50 mg of hemp leaf tissues was collected from the new growth of each plant and tested for BCTV infection using PCR. DNA extraction and PCR were performed as described above.

High-throughput sequencing of field hemp samples from the western United States

A total of 64 DNA samples from hemp plants with CTD symptoms and infected with BCTV were acquired from across the western United States from 2019 to 2023. Samples were obtained from hemp fields in Washington (*n* = 11), Oregon (*n* = 36), California (*n* = 5), Colorado (*n* = 8), and New Mexico (*n* = 4). DNA quality was evaluated using a NanoDrop One Spectrophotometer and a Qubit 4 Fluorometer (Thermo Fisher Scientific). The DNA samples were then sent to Azenta Life Sciences (Chelmsford, MA, U.S.A.) for high-throughput sequencing. Libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs, Ipswich, MA, U.S.A.) and sequenced on the Illumina MiSeq Series platform with paired-end (2 × 150 bp) reads. The MiSeq sequencing was used for initial assessment of the BCTV strain diversity and genome coverage. To obtain complete or near full-length BCTV genome sequences for robust phylogenetic, nucleotide identity and diversity, and recombination analyses, deeper sequencing was performed on the same libraries from 25 selected samples from Washington (*n* = 5), Oregon (*n* = 5), California (*n* = 5), Colorado (*n* = 6), and New Mexico (*n* = 4) using the NovaSeq X Plus platform with paired-end (2 × 150 bp) sequencing. MiSeq and NovaSeq sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject accession number PRJNA1312043.

BCTV strain and hemp virome analyses

A bioinformatic workflow for BCTV strain identification and hemp virome characterization is illustrated in Supplementary Figure S1. Raw paired-end reads from MiSeq sequencing were quality-trimmed using CLC Genomics Workbench v.25.0.1 (Qiagen Sciences, Germantown, MD, U.S.A.) to remove adapter sequences and low-quality bases (Supplementary Table S1). Host-derived reads were filtered by mapping trimmed reads to the *Cannabis sativa* genome cs10 (NCBI RefSeq GCF_900626175.2). Unmapped reads were then aligned to the complete BCTV nucleotide database retrieved from NCBI. Reads mapping to BCTV were used for de novo assembly of BCTV contigs. The assembled contigs were validated through BLASTn and BLASTx analyses against the NCBI nt and nr databases. The BLAST top hit of the longest BCTV contig from each sample was used to determine the BCTV strain (Supplementary Table S2), with strain identity retrieved either directly from NCBI or inferred from phylogenetic analyses in previous studies (Chiginsky et al. 2021; Strausbaugh et al. 2017). For samples with uncharacterized BCTV strains or insufficient read coverage to yield BCTV contigs, strain identification was determined based on results from NovaSeq sequencing, as described below. MiSeq samples with uncharacterized BCTV strains that were not selected for deeper NovaSeq sequencing, thereby precluding strain identification, were labeled as unknown strains (or strains not determined).

NovaSeq sequencing data were processed similarly using CLC Genomics Workbench (Supplementary Table S3). Reads mapping to the BCTV nucleotide database were assembled de novo using both CLC Genomics Workbench and SPAdes v.4.1.0 (Prjibelski et al. 2020) with default parameters. Consensus sequences were generated by merging contigs from both assemblers using CLC Genomics Workbench and manually curated to ensure accuracy and confirm that the sequences covered full-length or near full-length (>90% coverage) BCTV genomes. These consensus sequences were further validated via BLASTn and BLASTx analyses against the NCBI nt and nr databases (Supplementary Table S4). Assembled BCTV genome sequences were submitted to the NCBI GenBank database, and their associated accession numbers are provided in Supplementary Table S4. Additionally, virome analysis of the 25 hemp samples was performed by assembling all non-host reads de novo using CLC Genomics Workbench with default parameters. The resulting contigs were subjected to BLASTn and BLASTx analyses against the NCBI nt and nr databases to identify viral sequences.

Pairwise nucleotide identity and phylogenetic analyses of BCTV genome sequences

A total of 28 BCTV genome sequences, assembled from NovaSeq sequencing data of the 25 hemp samples, were included in phylogenetic and pairwise nucleotide identity analyses. Among these samples, three were infected by two different BCTV strains. Full-length sequences representing each of the 11 BCTV strains were selected based on previous studies (Melgarejo et al. 2022; Strausbaugh et al. 2017), including 10 BCTV-CA/Logan, 8 BCTV-CO, 2 BCTV-Kim1, 10 BCTV-LH71, 5 BCTV-Mild, 10 BCTV-Svr, 8 BCTV-Wor, 6 BCTV-SpCT, 1 BCTV-PeYD, 2 BCTV-PeCT, and 1 BCTV-SvrPep. Additionally, seven complete BCTV genomes identified from *C. sativa* were retrieved from NCBI GenBank, comprising four Worland, one Colorado, and two uncharacterized strains (Supplementary Table S5). A horseradish curly top virus sequence (NCBI GenBank accession U49907) was included as an outgroup.

Pairwise nucleotide identities of full-length BCTV sequences (total 99 sequences) were analyzed using SDT v.1.3 with the MAFFT alignment option (Muhire et al. 2014), with pairwise identity calculated across aligned nucleotide positions. The classification was performed following the recommended criteria by Varsani et al. (2014), with 77 and 94% genome-wide pairwise identity as species and strain demarcation thresholds, respectively (Varsani et al. 2014). A phylogenetic tree was constructed using the maximum likelihood method in IQ-Tree v.2.4.0 with the GTR + F + I + G4 model for nucleotide substitution (Minh et al. 2020). The multiple sequence alignment was performed using MAFFT v.7.505 (Katoh and Standley 2013). The best-fit model was determined by ModelFinder within IQ-Tree. Branch support was assessed via 1,000 bootstrap replicates. The resulting phylogenetic tree was visualized in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Genetic diversity of hemp BCTV genome sequences

Nucleotide diversity (π), which measures the average number of nucleotide differences per site between sequences, was calculated for hemp-derived BCTV sequences from this study using DnaSP v.6.12.03 (Rozas et al. 2017). The sequences were aligned using MAFFT, as described previously. To assess genetic variation across the viral genome of each strain, a sliding window analysis was performed with a window size of 100 nucleotides and a step size of 25 nucleotides.

Phylogenetic network and recombination analyses

Initial assessment for non-tree-like evolution of BCTV sequences, which signal recombination, was performed using the Neighbor-Net method in SplitsTree v.6.4.16 (Huson and Bryant 2006). Recombination events were then detected using RDP4 v.4.101 with seven algorithms: RDP, GENECONV, Chimaera, MaxChi, BootScan, Siscan, and 3Seq (Martin et al. 2015). Statistical significance was assessed using Bonferroni-corrected *P* values with $\alpha = 0.05$. Recombination events detected by at least four different methods were considered reliable. The multiple sequence alignment from the maximum likelihood phylogeny was used for both analyses.

Evaluation of BCTV effects on cannabinoid production in two hemp genotypes

To evaluate the effects of BCTV on cannabinoids, two different fiber/grain dual-purpose hemp genotypes, 4394 and 4681, were analyzed. Both were shown to be susceptible to BCTV in preliminary experiments. These genotypes were derived from a bi-parental F2 population obtained from the hemp breeding company New West Genetics (Fort Collins, CO, U.S.A.). Hemp seeds were germinated in plug trays filled with a PRO-MIX HP BIOFUNGICIDE + MYCORRHIZAE growing medium (Premier Tech Horticulture, Quakertown, PA, U.S.A.) and placed inside a growth chamber set to a 16-h light/8-h dark photoperiod at 28°C during the day and 24°C at night. After root systems were established, seedlings were transplanted into 1-gallon pots and moved into large PVC pipe cages (51 × 56 × 152 cm) enclosed with organza netting. The cages were housed in a greenhouse maintained under a 16-h light/8-h dark photoperiod at 23°C during the day and 18°C at night. Plants were fertilized bimonthly with Jack's Professional 15N-16P-17K fertilizer (JR Peters, Allentown, PA, U.S.A.) at a rate of 1.3 ml/liter. Plants were infected by cohorts of viruliferous BLHs with a mix of BCTV-CA/Logan and Svr strains as described above. For each line, there were six female plants: three infected with BCTV and three noninfected control plants. Hemp plants were grown to full flowering maturity in the greenhouse. At maturity, flowers were harvested from a 10-cm section of the cola, placed in 50-ml conical tubes, and stored at −80°C until cannabinoid analysis.

Cannabinoid analysis

Hemp samples, including three BCTV-infected and three noninfected flower samples from the 4394 and 4681 hemp lines, were lyophilized at −80°C under <0.02 mBar for 24 h using a FreeZone Plus 4.5 Liter Freezer Dryer (Labconco, Kansas City, MO, U.S.A.). After lyophilization, samples were homogenized for 5 min using a bead beater (Next Advance, Troy, NY, U.S.A.). Approximately 40 mg of homogenized tissue per sample was placed into 2-ml Eppendorf tubes. One milliliter of cold 80% methanol was added to each sample, followed by a 30-min vigorous vortex at 4°C. Samples were then sonicated in an ice bath for 15 min and vortexed again for 30 min at 4°C. Following DNA extraction, samples were centrifuged at 15,000 × *g* at 4°C for 10 min. The supernatants were collected and diluted 10- and 100-fold with cold 50% methanol. A 100-µl aliquot of the diluted sample was mixed with 20 µl of an internal standard and stored at −20°C until analysis. Additionally, an aliquot (10 µl) from each sample was pooled to generate a quality control sample. The authentic standards of cannabinoids and labeled standard THC-d3 were purchased from Cerilliant (Round Rock, TX, U.S.A.). The internal standard, 100 ng/ml of THC-d3, was prepared in 50% methanol.

Liquid chromatography-tandem mass spectrometry analysis was performed on a Waters ACQUITY UPLC coupled to a Waters Xevo

TQ-S triple quadrupole mass spectrometer. Chromatographic separations were carried out on an ACQUITY Premier HSS T3 column (2.1 × 100 mm, 1.8 μm; Waters, Milford, MA, U.S.A.). Mobile phases included water with 0.1% formic acid (A) and acetonitrile (B). Samples were held at 6°C in the autosampler, and the column was operated at 45°C. The injection volume was 2 μl. The capillary voltage of the mass spectrometer detector was set to 0.7 kV in positive mode, with an inter-channel delay of 3 ms. The source temperature was set to 150°C and the desolvation temperature to 450°C. The desolvation gas flow was 1,000 liters/h, cone gas flow (nitrogen) was 150 liters/h, and collision gas flow (argon) was 0.15 ml/min. The nebulizer pressure (nitrogen) was set to 7 Bar. The autodwell feature was set for the collection of 12 points-across-peak. The cone voltage and collision energy of each multiple reaction monitoring were optimized. The cannabinoids in samples were analyzed using "de-optimized" cone and collision energy voltage. Samples were injected in a random order. Raw data files were imported into the Skyline open-source software package (MacLean et al. 2010). Visual inspections were performed for each target analyte for retention time and peak area integration. Peak areas were extracted for target compounds detected in biological samples and normalized to the peak area of the appropriate internal standard or surrogate in each sample. Absolute quantitation (μg/g) was calculated using the linear regression equation generated for each compound from the calibration curve. The data were log-transformed and confirmed for normality using the Shapiro–Wilk test ($P > 0.05$). A general linear model analysis was conducted to evaluate cannabinoid concentrations (16 compounds) in response to BCTV infection, followed by Fisher's least significant difference test ($P < 0.05$) for pairwise comparisons between two treatment groups (infected versus noninfected). The correlation between viral copy numbers and cannabinoid levels was assessed using Spearman's correlation analysis. All statistical analyses were performed using Minitab software (version 22.1.0).

Potential BCTV seed transmission in hemp

Seed collection from BCTV-infected plants. Hemp seeds from two different fiber/grain dual-purpose genotypes were used: Elite, a commercial open-pollinated line, and 791, a line from a bi-parental cross F2 population developed by New West Genetics. These genotypes are susceptible to BCTV (Hackenberg et al. 2025). Seeds were germinated, transplanted, and grown following the methods described previously. Each genotype was placed in separate, large PVC pipe cages (50 cm × 50 cm × 1.6 m) enclosed with organza netting. After 3 weeks, hemp plants were inoculated with BCTV-viruliferous BLHs with a mix of BCTV-CA/Logan and Svr strains as described earlier. BCTV infection of each plant was confirmed at 4 weeks postinoculation by testing the new growth of both symptomatic and asymptomatic plants using PCR as described above, except that the template concentration was increased to 100 ng/μl for better detection of low virus titer in these samples. Plants that were not infected with BCTV were discarded. Only BCTV-infected plants (both male and female) were grown to maturity (12 weeks), and seeds were collected from the infected female flowers for the two genotypes. This experiment was repeated three times independently across different months in 2024 and 2025.

Detection of BCTV in seeds. The testing of seed infection included three independent replicates corresponding to the three seed collection experiments as described above. A total of 90 and 50 seeds collected from BCTV-infected Elite and 791 were surface disinfected in 10% bleach (final active ingredient, sodium hypochlorite, concentration: 0.75%) for 5 min, respectively. Seeds were then rinsed with distilled water for 1 min to remove excess bleach, transferred individually into 1.5-ml microcentrifuge tubes, and ground in liquid nitrogen using disposable pestles. Seeds collected from BCTV-free Elite and 791 plants were included as negative controls. DNA was extracted using the Wizard Genomic DNA Purification Kit, and BCTV infection was determined by PCR using BCTV coat protein primers as detailed above. A separate PCR was conducted to generate amplicons for sequencing, using the same DNA extracts from BCTV-positive hemp samples from both genotypes. The resulting PCR amplicons (496 bp) were purified with the DNA Clean and Concentrator-5 Kit (Zymo Research, Irvine, CA, U.S.A.) or GeneJET PCR Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions and subsequently characterized with Sanger sequencing (Azenta Life Sciences).

Detection of BCTV in dissected seed tissues. To confirm BCTV presence in progeny tissues and rule out contamination from maternal tissues, a seed dissection experiment was conducted using the Elite genotype. Seeds were first surface disinfected according to the protocol described above and dissected with a sterile scalpel blade and forceps. Each seed was carefully separated into its three main components: seed coat, endosperm, and embryo (Supplementary Fig. S4A). To mitigate any potential surface contamination incurred during the dissection process, each individual tissue component was immediately subjected to a second surface disinfection step. The dissected tissues were individually processed for DNA extraction and PCR detection of BCTV as described previously. The seed dissection experiment was performed independently three times across different days, yielding a total of 20, 11, and 51 tissue samples for seed coat, endosperm, and embryo tissues, respectively. Seed tissues dissected from healthy Elite plants were included as negative controls.

Detection of BCTV in seedlings. Similarly, seedling infection testing included three independent replicates corresponding to the three seed collection experiments. The seeds were surface disinfected as described above. A total of 204 and 115 seeds from BCTV-infected Elite and 791 hemp genotypes were germinated in 72-well seed plug trays (54 × 28 × 4.5 cm) with a PRO-MIX BX growing medium (Premier Tech Horticulture). All plants were grown in an insect-free growth chamber at 25°C under a 16-h light/8-h dark photoperiod. Sugar beet plants (cultivar BPA9000) were also reared in the same growth chamber as a negative control to monitor for any unintended infestation by BLHs. Four weeks postgermination, the new growth (fourth node) from each plant was collected using metal or disposable plastic forceps. Metal forceps were thoroughly cleaned between each sample collection with 10% bleach. Plastic forceps were not reused. Samples were stored at −20°C after collection. DNA was extracted, and the presence of BCTV was determined by two separate PCR assays prior to Sanger sequencing as described previously. Leaf tissues of sugar beet plants grown in the same growth chamber were also collected and tested to confirm the absence of BCTV.

BLH survival assay on hemp and sugar beet plants

To assess the survivorship of BLHs on hemp, a survival assay was performed using 1-day-old adult BLHs on hemp (cultivar Elite) and the preferred host sugar beet (cultivar BPA9000). Both BCTV-viruliferous individuals harboring a mix of BCTV-CA/Logan and Svr strains and non-viruliferous BLH individuals were tested. A cohort of 10 viruliferous and non-viruliferous BLHs were confined to 5-week-old hemp or sugar beet plants within transparent cylindrical enclosures, and survival was recorded daily for 7 days. The experiment was independently repeated three times, including a total of 120 insects (10 insects × 4 treatment combinations × 3 biological replicates), at 25°C under a 16-h light/8-h dark photoperiod. Kaplan–Meier survival curves were generated for each treatment group and compared using the Mantel–Cox log-rank test, with the P value adjusted for multiple comparisons using the Benjamini–Hochberg method. The statistical analysis was performed in R v.4.4.3.

Results

BCTV strain diversity in hemp fields across the western U.S. states

The analysis of BCTV diversity in hemp plants across five western U.S. states revealed multiple strains and notable geographic differences in virus incidence (Fig. 1; Supplementary Table S2). Of 64 BCTV-infected samples, BCTV-CO was the most common strain (60%), followed by BCTV-Wor (21%), with both strains being frequently detected across all surveyed states. BCTV-PeCT was detected for the first time in Colorado and Oregon, but at a relatively low incidence (11 and 3%, respectively). In New Mexico, BCTV-PeYD was the most common strain (60%), followed by BCTV-PeCT and BCTV-Wor. Notably, a subset of samples from Washington (1 out of 11) and Oregon (6 out of 36) yielded BCTV sequences that could not be definitively assigned to known strains. This ambiguity does not necessarily indicate novel BCTV variants and may reflect the inability to retrieve identification from existing NCBI databases and published literature or limitations in the diagnostic resolution of this study.

Pairwise nucleotide identity and phylogenetic analyses of hemp-associated BCTV strains

The analysis of 25 hemp samples using NovaSeq technology yielded 28 complete or near-complete BCTV genome sequences, with three samples (OR12, CO2, and NM2), each infected with two BCTV strains (Supplementary Table S4). A pairwise nucleotide identity matrix, displaying the identity between each pair of BCTV sequences, revealed clear strain-specific clustering (Fig. 2). Isolates from the same strain consistently show high nucleotide identities, with an average identity of >96% for isolates of different strains (Supplementary Table S6). Conversely, inter-strain comparisons displayed relatively lower pairwise identity values, ranging from 74 to 93% in average identities. Moreover, the Colorado strain group showed the lowest internal identity level, with an average percentage identity of 96%, compared with others, which ranged from 98 to 100% (Supplementary Table S6).

To further resolve evolutionary relationships among BCTV isolates, a maximum likelihood phylogenetic tree was constructed (Fig. 3). In the tree, isolates of each strain were placed into separate clades. Sequences from hemp samples in the present study clustered within previously characterized strains, including BCTV-CO, BCTV-Wor, BCTV-PeYD, and BCTV-PeCT. Notably, hemp-derived BCTV-CO and BCTV-Wor sequences, along with those from other studies, predominantly clustered together, generally separating from sequences originally isolated from sugar beet and pepper plants. Among the strains identified in this analysis, BCTV-CO was the most common (56%), followed by BCTV-Wor (22%), BCTV-PeYD (11%), and BCTV-PeCT (11%). All BCTV sequences from Washington and California hemp samples were placed in the BCTV-CO strain, except for one from Washington that was identified as BCTV-Wor. Of the four hemp samples from New Mexico, three were BCTV-PeYD, whereas one was infected with the BCTV-PeCT and BCTV-Wor strains. Oregon and Colorado hemp samples showed a mix of BCTV-CO, BCTV-Wor, and BCTV-PeCT.

In addition to BCTV strain identification, metagenomic analysis of NovaSeq sequencing data was performed to investigate the broader DNA virome in hemp. Besides BCTV, this analysis did not find any other plant DNA viruses.

Recombination and genetic diversity of hemp-associated BCTV strains

To detect recombination, we created a phylogenetic network with the Neighbor-Net method using all hemp-derived BCTV sequences and 11 previously characterized BCTV strains (Fig. 4A). The resulting network delineated the major BCTV strain clusters corresponding to previously described BCTV strains. The hemp-derived sequences were grouped within the CO, Wor, PeCT, and PeYD clusters, consistent with prior maximum likelihood tree-based strain classifications (Fig. 3). Consistent with others, widespread reticulated structures were observed among the sequences (Melgarejo et al. 2024; Strausbaugh et al. 2017). This non-tree-like pattern is indicative of potential recombination events shaping the evolution of naturally occurring BCTV strains.

Recombination analysis identified 16 distinct putative recombination events among all BCTV strains except BCTV-Mld (Table 1), with most likely breakpoints occurring in the C1, C2, and C3 genes (events 1 to 8, 11 to 14, and 16) and the intergenic region (events 2, 4, 9 to 11, and 15). The widespread inter-strain recombination is consistent with the network analysis in this study, which revealed extensive reticulations among BCTV strains (Fig. 4A). All the identified putative recombinant strains identified were previously reported (Melgarejo et al. 2024; Strausbaugh et al. 2017; Varsani et al. 2014). Notably, BCTV-PeCT showed the highest number (5) of independent recombination events, a trend consistent with the previous report identifying four recombination events in this strain (Varsani et al. 2014). Additionally, a great number of hemp-derived isolates were identified as recombinants (9 out of 16 events), particularly those belonging to BCTV-CO and BCTV-Wor strains.

To further explore the genetic variation among BCTV strains, the nucleotide diversity (π) was calculated across the BCTV genome sequences of each strain identified in hemp, including 18 BCTV-CO, 11 BCTV-Wor, 3 BCTV-PeCT, and 3 BCTV-PeYD sequences. Notable variation in nucleotide diversity was observed, with several pronounced peaks exceeding the average genome-wide π value of these strains (0.031; horizontal dotted line) (Fig. 4B). These diversity hotspots were most prominent at approximately 200 nt in the intergenic region and between genomic positions of approximately 1,300 to 1,600 nt, approximately 1,600 to 1,800 nt,

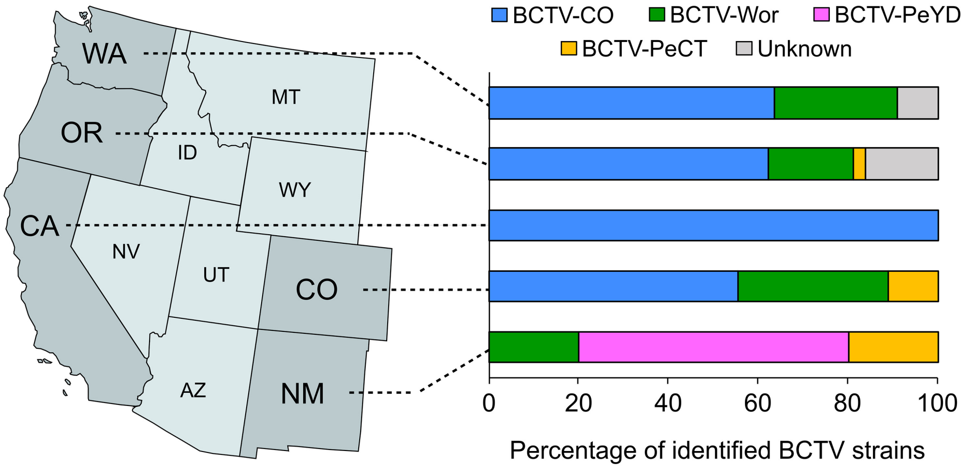

Fig. 1. Percentages of beet curly top virus (BCTV) strains identified in hemp fields across five western U.S. states: Washington (WA), Oregon (OR), California (CA), Colorado (CO), and New Mexico (NM). BCTV contigs were assembled in CLC Genomics Workbench using MiSeq sequencing data and searched (BLASTn) against the NCBI nt databases. Hemp samples were considered infected by BCTV strains based on the top hit of the longest contig sequence.

and approximately 2,000 to 2,400 nt overlapping with regions encoding the C2/C3, C2, and C1 ORFs, respectively. These regions coincide with previously identified recombination breakpoints (Table 1), confirming that these areas are important sites of genetic exchange. The nucleotide diversity varied across tested strains, with the BCTV-CO strain showing the highest genome-wide π value (0.044), followed by BCTV-PeCT (0.040), BCTV-Wor (0.027), and BCTV-PeYD (0.013). The BCTV-CO strain showed a pronounced spike in diversity around positions of approximately 1,300 to 1,600 nt and approximately 2,200 to 2,400 nt, corresponding to C2/C3 and C1 ORFs. Similarly, the BCTV-Wor strain also showed its highest spike within the C2/C3 ORFs (approximately 1,300 to 1,600 nt). The BCTV-PeYD strain showed low nucleotide diversity throughout the genome, with its highest spike occurring at approximately 2,200 nt within the C1 ORF. The highest spike across the strains was observed in the BCTV-PeCT strain around approximately 1,600 to 1,800 nt, corresponding to the C2/C3 ORFs. All strains displayed minimal diversity across the V1, V2, V3, and C4 coding regions.

BCTV impact on hemp cannabinoids

BCTV infection had no impact on cannabinoid concentrations in flower tissues in line 4394 (Table 2). However, in line 4681, virus infection led to a reduction in cannabinoids, with a significant decrease in eight compounds: CBDV ($P = 0.009$), THCV ($P = 0.005$), CBD ($P = 0.003$), CBCV ($P = 0.009$), CBN ($P = 0.0002$), delta9THC ($P = 0.013$), CBC ($P = 0.023$), and CBT ($P = 0.032$). The greatest reductions were observed for CBD (11.2-fold), followed by CBN (8-fold) and CBDV (7.7-fold). Neither genotype exhibited visible CTD symptoms associated with BCTV infection.

Seedborne detection and potential seed transmission of BCTV in hemp

To determine whether BCTV is seedborne in hemp, seeds collected from BCTV-infected plants of both the Elite and 791 genotypes were tested by PCR (Supplementary Fig. S2A). In the Elite genotype, 77% (69 out of 90) of seeds tested positive for BCTV, whereas 18% (9 out of 50) of seeds from the 791 genotype were BCTV-positive. Seeds from noninoculated Elite and 791 plants were included as negative controls, and none tested positive for BCTV.

To more precisely characterize the localization of BCTV within hemp seeds and differentiate between infection of maternal and embryonic tissues, we performed additional seed dissection experiments on the Elite genotype and tested for the presence of BCTV in the embryo, endosperm, and seed coat using PCR (Supplementary Fig. S4). Our results confirmed the positive detection of BCTV in

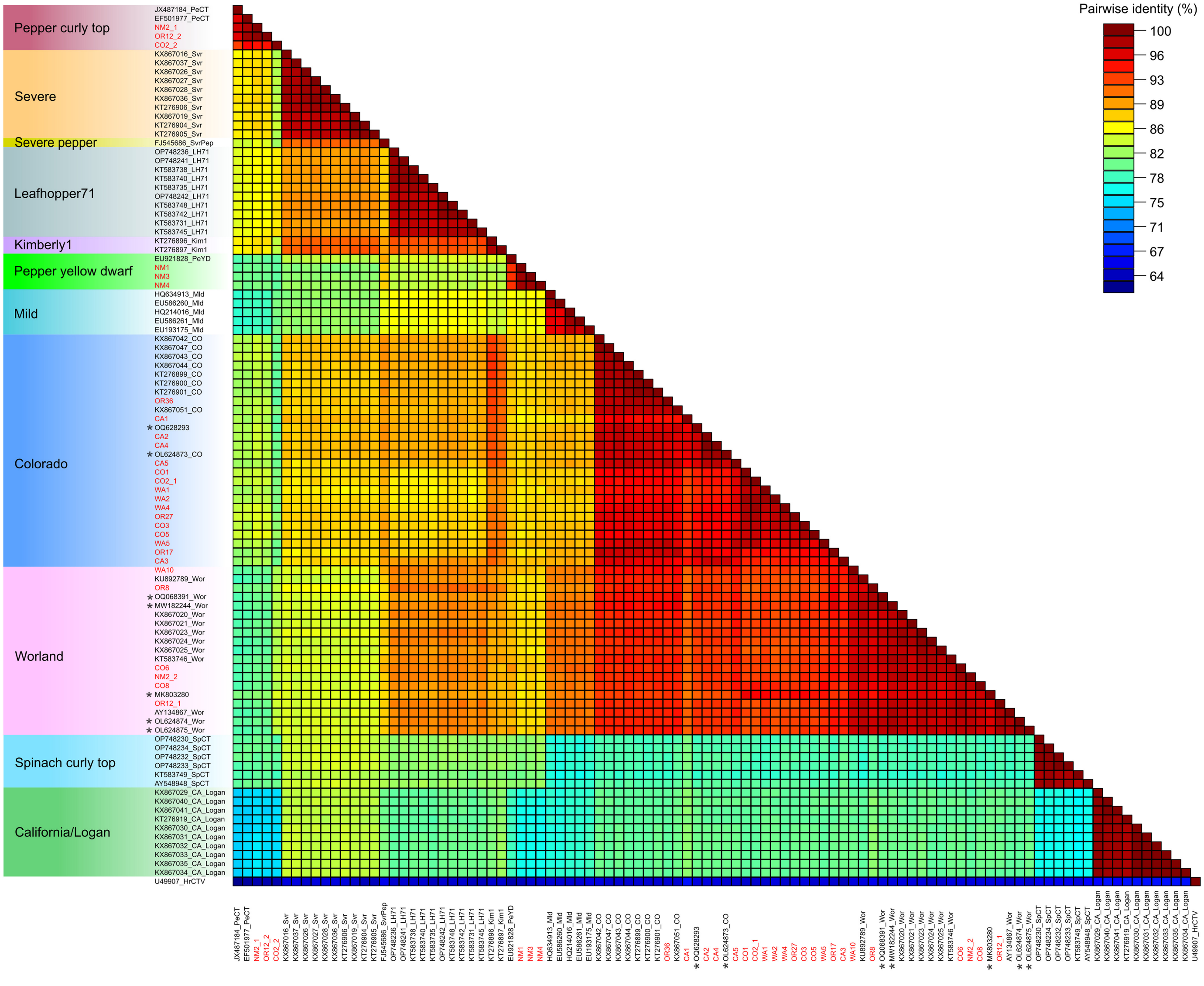

Fig. 2. Pairwise nucleotide identity matrix of full-length beet curly top virus (BCTV) genomes. The matrix includes newly assembled BCTV sequences from this study and representative sequences of the 11 known BCTV strains. Sequences were aligned using the MAFFT program and analyzed using SDT v.1.3. An asterisk indicates that the host species of the virus is *Cannabis sativa*. Samples from this study are marked in red.

41.2% (21 out of 51) of embryos, 72.7% (8 out of 11) of endosperm tissues, and 5% (1 out of 20) of seed coats. None of the seed tissues dissected from control Elite plants tested positive for BCTV.

To further assess the potential seed transmission of BCTV in hemp, the presence of the virus was tested by PCR in grow-out tests from BCTV-infected plants (Supplementary Fig. S2B). BCTV was detected in the seedling leaves of both Elite and 791 genotypes, which showed severe stunting (Supplementary Fig. S3). Both genotypes had similar infection levels, with 0.98% in Elite (2 out of 204) and 0.87% in 791 (1 out of 115). All BCTV-positive seed

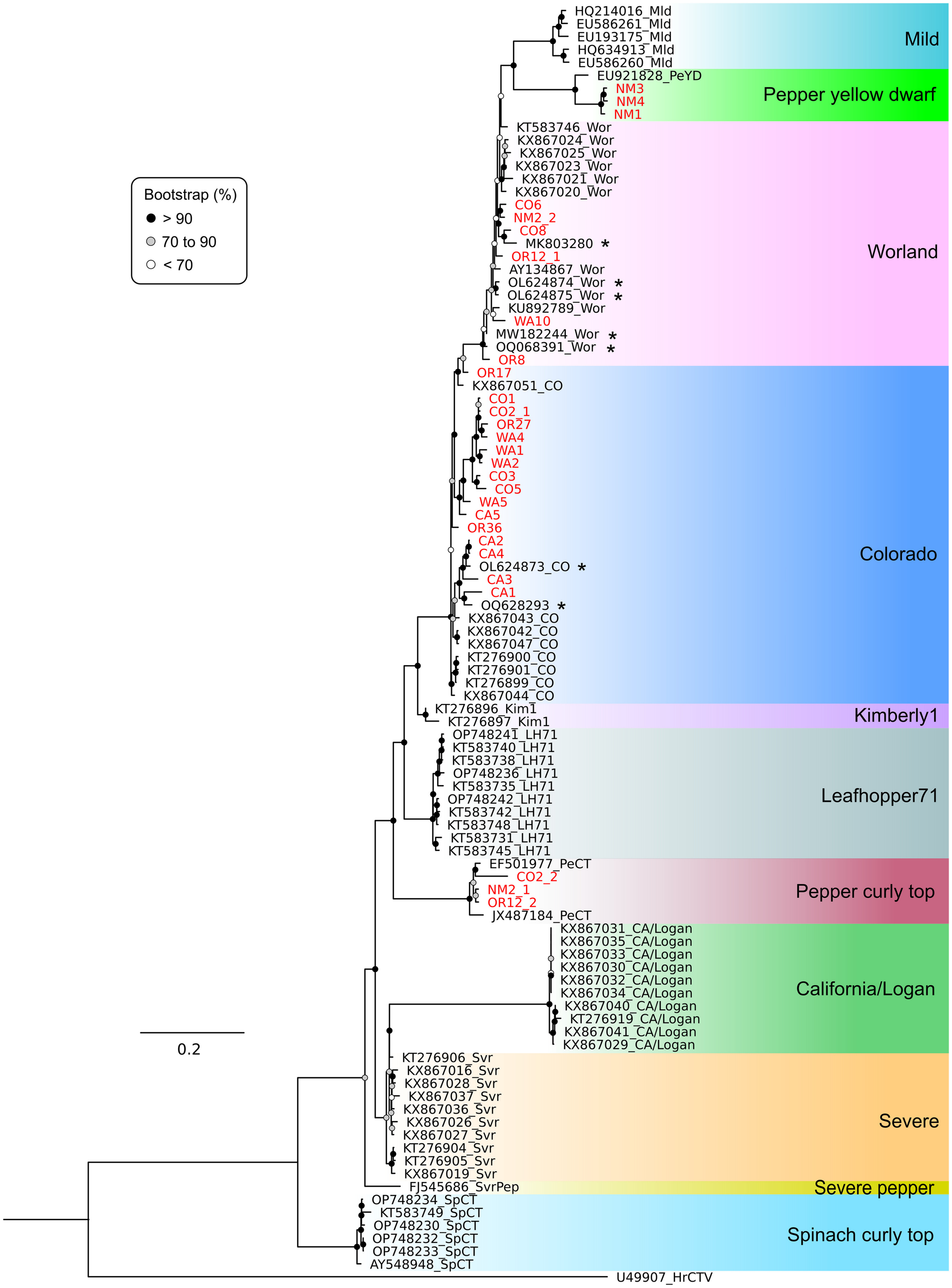

Fig. 3. Phylogenetic analysis of full-length sequences of beet curly top virus (BCTV) assembled from NovaSeq sequencing data in this study and other BCTV sequences retrieved from GenBank, representing the 11 BCTV strains. Multiple sequence alignment was performed using MAFFT. The maximum likelihood phylogenetic tree was constructed using IQ-Tree v.2.4.0 with a GTR + F + I + G4 model for nucleotide substitution. An asterisk indicates that the host species of the virus is *Cannabis sativa*. Samples from this study are marked in red.

and seedling samples were verified using separate PCR assays using the same hemp DNA extracts from BCTV-positive plants from both genotypes, and the resulting amplicons were confirmed to be BCTV sequences by Sanger sequencing. The grow-out assays were conducted in an insect-free growth chamber under controlled greenhouse conditions, and sugar beet plants maintained in the same chamber served as negative controls and tested negative for BCTV infection, ruling out the possibility of vector-mediated transmission.

Survivorship of BLHs on hemp plants

On sugar beet, both viruliferous and non-viruliferous BLHs showed high survival rates, with over 83% of individuals alive by day 7 and no significant difference observed between the two groups ($P = 0.71$) (Fig. 5). In contrast, BLH survival on hemp was markedly reduced ($P < 0.0001$), with both viruliferous and non-viruliferous leafhoppers showing continuous declines in survival from day 2 onward. By day 7, survival rates on hemp dropped to 0% for both viruliferous and non-viruliferous BLH groups.

Discussion

To date, three BCTV strains are known to infect hemp: BCTV-CO, BCTV-Wor, and BCTV-PeYD (Chiginsky et al. 2021; Creamer et al. 2024; Jarugula et al. 2023; Melgarejo et al. 2022; Rivedal et al. 2022). In addition to these, we detected BCTV-PeCT for the first time in hemp samples from Oregon, Colorado, and New Mexico. These findings are consistent with earlier studies, in which BCTV-CO and BCTV-Wor were found at high incidence in hemp samples in Colorado (Chiginsky et al. 2021), Oregon (Rivedal et al. 2022), California (Melgarejo et al. 2022), and New Mexico, where BCTV-Wor and BCTV-PeYD were found infecting hemp (Creamer et al. 2024). These results expand the known strain composition of BCTV infecting hemp and underscore the dynamic and evolving landscape of BCTV populations across western U.S. hemp fields.

Phylogenetic analysis clearly grouped the hemp-derived BCTV genome sequences within clades of known strains. This classification was supported by both the pairwise nucleotide identity matrix, generated using recommended identity threshold for strain demarcation (Varsani et al. 2014), and the phylogenetic network analysis. Among the strains, BCTV-CO showed slightly lower intra-strain identity (96%) compared with others (98 to 100%), suggesting greater genetic variability within this dominant lineage. Notably, hemp-derived sequences of BCTV-CO, BCTV-Wor, BCTV-PeYD, and BCTV-PeCT tended to cluster together within their respective strain groups, generally separating from isolates collected from traditional crops, such as sugar beet and pepper. This observation echoes earlier, more localized findings in Colorado, where all hemp-derived BCTV sequences formed a distinct group that included BCTV-Wor and BCTV-CO (Chiginsky et al. 2021). The consistent phylogenetic separation of hemp-associated strains suggests an ongoing process of divergent evolution driven by host-specific selection pressures.

BCTV has a highly recombinant nature, capable of generating novel variants with altered pathogenicity, host range, and vector interactions (Chen and Gilbertson 2016; Creamer 2020; Gilbertson et al. 2021; Strausbaugh et al. 2017; Varsani et al. 2014). The analyses revealed extensive evidence of recombination in BCTV infecting hemp, with the phylogenetic network showing widespread reticulations among the different strains, indicative of frequent historical recombination events among BCTV strains (Strausbaugh et al. 2017; Varsani et al. 2014). Most of the likely recombination breakpoints were located within the coding regions of C1, C2, and C3 and the intergenic region, areas previously reported as recombination hotspots in the BCTV genome and other geminiviruses (Lefeuvre et al. 2009; Strausbaugh et al. 2017; Varsani et al. 2014). All the putative recombinant strains identified had been previously reported in other host plants (Melgarejo et al. 2024; Strausbaugh et al. 2017; Varsani et al. 2014). For instance, Strausbaugh et al. (2017) identified BCTV-Kim1 as a recombinant, with Wor as the potential major parent strain and Svr as the minor parent strain and breakpoints within the C2/C3 and C1 genes. The same recombination event (event 7) was detected in this study. The identification of a substantial number of hemp-derived isolates as recombinants, particularly those belonging to the BCTV-CO and BCTV-Wor strains, underscores the dynamic nature of BCTV evolution in hemp and highlights recombination as a key driver of BCTV genetic diversity. Nucleotide diversity analyses further supported these findings. The regions with the highest levels of genetic variation overlapped with recombination hotspots, particularly in the C1, C2, C3, and intergenic regions. These regions are functionally important for viral replication and pathogenicity (Frischmuth and Stanley 1992; Frischmuth et al. 1993; Guerrero et al. 2020) and are highly conserved among BCTV strains across different species (Chen and Gilbertson 2016). These results are consistent with BCTV populations in other host plants and reflect the highly recombinant nature and established evolutionary patterns of BCTV. Together, the co-occurrence of high nucleotide diversity and recombination events in these regions suggests ongoing adaptive evolution in BCTV populations infecting hemp.

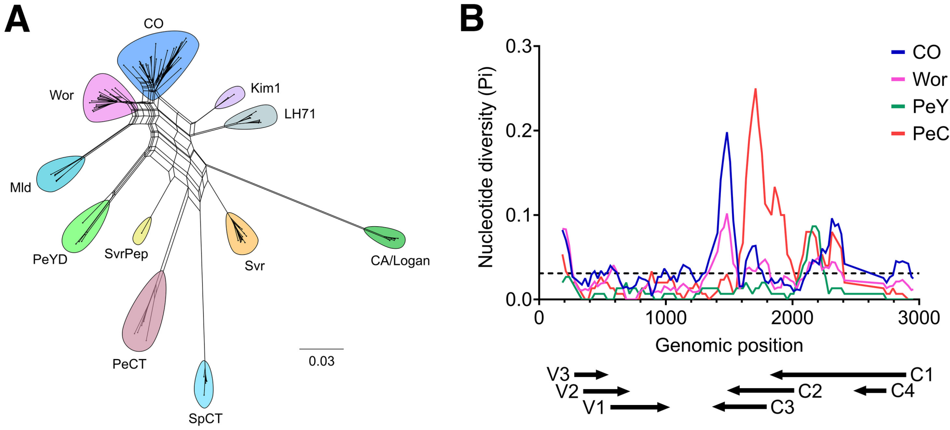

Fig. 4. Phylogenetic network and genetic diversity of full-length beet curly top virus (BCTV) genomes. **A,** Phylogenetic network of BCTV genome sequences. Branch lengths reflect the number of nucleotide substitutions across the genome. Strain abbreviations: Mld, mild; Wor, Worland; LH71, leafhopper71; CO, Colorado; Kim1, Kimberly1; CA/Logan, California/Logan; Svr, severe; SvrPep, severe pepper; PeCT, pepper curly top; SpCT, spinach curly top; PeYD, pepper yellow dwarf. **B,** Nucleotide diversity (π), measured as the average number of pairwise differences per site, was calculated using a 100-nt sliding window with a 25-nt step across the hemp-derived BCTV genomes. Color-coded solid lines indicate nucleotide diversity for individual strains identified in this study. The horizontal dotted line represents the average diversity across all four strains. Annotated BCTV coding regions are shown below the graph.

Plants, as sessile organisms, rely on secondary metabolites to mediate responses to diverse biotic and abiotic stressors, including pathogens. Among these metabolites, cannabinoids are hypothesized to play a role in hemp defense against stresses, such as UV radiation, herbivory, and pathogens (Gorelick and Bernstein 2017; MacWilliams et al. 2023; Tanney et al. 2021). In this study, BCTV infection had no impact on cannabinoid levels in genotype 4394. However, in genotype 4681, infection led to a reduction in all major cannabinoids, suggesting a genotype-dependent response. There were no observable differences in symptoms exhibited by the BCTV-infected genotypes 4394 and 4681 (data not shown). Similar findings were reported in another study, in which total cannabinoid content was significantly reduced in the TropB cultivar but not in the ABSLH cultivar (Foust 2023). The impact on cannabinoids also aligns with earlier reports documenting changes in cannabinoid levels in response to herbivore feeding (Kostanda and Khatib 2022; Park et al. 2022) and infection by another plant pathogen, hop latent viroid (Punja et al. 2024b). As the genotypes used in this study were fiber/grain types, future research should evaluate the impact of BCTV on cannabinoid levels in hemp genotypes cultivated for CBD production.

Seed transmission of viruses is important in disease epidemiology, as it enables virus persistence across seasons, long-distance dispersal, and primary inoculum establishment for secondary spread (Escalante et al. 2024). Whereas the terms seedborne and seed-transmitted are often used interchangeably, they are not synonymous. Seedborne refers to the presence of a virus in seed tissues, such as the seed coat, endosperm, or embryo, whereas seed-transmitted specifically describes pathogens that successfully infect and establish in the emerging seedling. The distinction is important, as not all seedborne viruses lead to transmission (Agarwal and Sinclair 1997). It has been generally accepted that curtoviruses, such as BCTV, are not vertically transmitted through seed to the next generation of plants. Phloem-limited viruses, like curtoviruses, stay inside the plant's phloem tissue. When seeds form, they develop from special tissues that lack symplastic connection to the phloem. This means the virus is unable to easily move from the infected plant into the developing seed. However, BCTV and beet curly top Iran virus were reported to be transmitted via seeds in a local petunia variety obtained from a local florist, with BCTV having a 38 to 78% seed transmission rate, compared with beet curly top Iran virus, with an 8 to 18% transmission rate (Anabestani et al. 2017).

TABLE 1. Recombination events detected in beet curly top virus (BCTV) isolates

Event	Sequences detected with recombination event	Recombinant(s)[a]	Hemp-derived recombinant isolate(s)	Recombination breakpoints (begin–end)[b]	Major parent(s)	Minor parent(s)	Methods[c]	*P* value[d]
1	10	BCTV-LH71	–	2,892–1,952	BCTV-PeCT	BCTV-Wor	RGBMCS**3**	6.91E-79
2	5	BCTV-PeCT	NM2_1, OR12_2	179–1,505	BCTV-Svr	Unknown	R**G**BMCS3	1.25E-77
3	23	BCTV-CO	CA2, CA3, CA4, CA5, CO1, CO2_1, CO3, CO5, OR27, OR36, WA2, WA4, WA5, OQ628293, OL624873	1,487*–2,382	BCTV-Wor BCTV-CO BCTV-CO	BCTV-Kim1 BCTV-Svr BCTV-PeCT BCTV-CO	RGBMCS**3**	7.16E-68
4	6	BCTV-SpCT	–	272–1,492	BCTV-Svr BCTV-SvrPep	Unknown	**G**MCS3	3.37E-61
5	8	BCTV-CO	OR17, CA5, OR36, WA4	2,048–2,926	BCTV-Kim1	BCTV-Wor	GBMS**3**	1.26E-54
6	1	BCTV-PeCT	CO2_2	2,464–2,909	BCTV-Wor BCTV-CO BCTV-Mld	BCTV-Svr BCTV-SpCT	GBMCS**3**	3.63E-47
7	6	BCTV-Kim1 BCTV-PeCT	– NM2_1, OR12_2	1,521*–2,822	BCTV-Wor	BCTV-Svr BCTV-PeCT BCTV-SvrPep	GBMC**S**3	8.50E-43
8	16	BCTV-Svr BCTV-Kim1 BCTV-PeCT	– – NM2_1, OR12_2	1,502*–2,453	BCTV-SpCT	BCTV-PeYD	GBMCS**3**	7.40E-26
9	1	BCTV-SvrPep	–	251–1,321	BCTV-Svr	Unknown	GMCS**3**	5.01E-19
10	17	BCTV-Wor	CO6, CO8, NM2_2, OR12_1, OR8, WA10, OQ068391, OL624874, OL624875, MW182244	280–1,347	BCTV-Mld	BCTV-Svr BCTV-CA/Logan	GMC**S**3	4.15E-27
11	10	BCTV-CA/Logan	–	195–1,504*	BCTV-Mld	BCTV-Svr	GBMC**S**3	1.04E-34
12	1	BCTV-Wor	WA10	1,960–2,211	BCTV-Wor	BCTV-PeYD	GMC**3**	2.30E-10
13	1	BCTV-SvrPep	–	2,592–2,690	BCTV-Svr BCTV-SpCT BCTV-LH71	BCTV-PeYD BCTV-Wor BCTV-CO BCTV-Mld	**G**MCS3	4.13E-09
14	10	BCTV-CA/Logan	–	2,539–2,865	Unknown	BCTV-SpCT BCTV-LH71	BMC**S**3	3.25E-11
15	1	BCTV-PeYD	–	79–168*	BCTV-PeYD	Unknown	**G**MC3	1.42E-05
16	1	BCTV-PeCT	CO2_2	1,547*–2,155	Unknown	BCTV-Wor	GM**S**3	1.75E-16

[a] Strain abbreviations: Mld, mild; Wor, Worland; LH71, leafhopper71; CO, Colorado; Kim1, Kimberly1; CA/Logan, California/Logan; Svr, severe; SvrPep, severe pepper; PeCT, pepper curly top; SpCT, spinach curly top; PeYD, pepper yellow dwarf.

[b] Recombination breakpoints are defined relative to the first nucleotide after the intergenic region cleavage site at the origin of replication. Asterisk (*) indicates the uncertainty of the precise breakpoint position.

[c] Recombination events and their putative parental viruses are identified using the RDP (R), GENCONV (G), BOOTSCAN (B), MAXCHI (M), CHIMERA (C), SISCAN (S), and 3SEQ (3) methods in RDP4.

[d] The presented *P* values correspond to the lowest *P* values determined by the programs highlighted in bold and underlined.

In addition to curtoviruses, seed transmission has also been reported in phloem-limited begomoviruses and mastreviruses in the family *Geminiviridae* (Sandra and Mandal 2024). We found a high rate of BCTV presence in surface-sterilized seeds of one genotype, Elite (76%), compared with the 791 genotype (18%), highlighting the genotype-dependent response. Additionally, we further confirmed the presence of BCTV in progeny tissues of seeds, including embryo and endosperm, indicating that the virus is not restricted to maternally derived tissues, such as the seed coat. The infection rate of endosperm tissues was also found to be comparable to the whole-seed infection rate. These new data suggest virus entry into internal seed tissues during development rather than surface contamination. In contrast, the grow-out assays detected low rates of BCTV presence in seedlings for both genotypes (<1%), with positive plants exhibiting severe stunting. Although geminiviruses were long thought to be excluded from seeds due to a lack of vascular connectivity, recent studies suggest that the embryonic suspensor may serve as a critical "window of opportunity" for the virus to move from maternal tissues into the embryo proper during early development (Renukadevi et al. 2022). Future research involving larger seed numbers and extensive field-level monitoring is required to address the broader epidemiological implications and the biological relevance of potential seed transmission in commercial hemp production.

Plant viruses are known to affect the performance and behavior of their insect vectors. The persistently transmitted geminiviruses have demonstrated mixed positive, negative, or neutral effects on whitefly and leafhopper vectors (Eigenbrode et al. 2018). Previously, we reported that viruliferous BLHs produced more nymphs on sugar beets compared with non-viruliferous BLHs, whereas other life history traits were unaffected. This elevated nymphal production increased adult survival, potentially facilitating virus transmission (Han et al. 2024). In the current study, there were no differences in adult survival between viruliferous and non-viruliferous BLHs on sugar beet plants, and adults continued to survive past 7 days. In contrast, BLH survival on hemp was significantly reduced, with 0% survival by day 7 in both viruliferous and non-viruliferous groups. Although BLHs feed on a wide range of crops, they cannot complete their life cycle on all hosts (Chen and Gilbertson 2016). For example, Hudson et al. (2010) reported that leafhoppers lay few eggs on chili pepper and tomato, and those eggs rarely hatch; additionally, adult leafhoppers confined to chili pepper plants for 24 h exhibited over 90% mortality. Similarly, Munyaneza and Upton (2005) observed high mortality rates when leafhoppers were caged on bean (95%) and tomato (65%) for less than a week. More recently, Creamer et al. (2024) demonstrated that BLHs did not prefer to settle on hemp, instead choosing the cage walls over hemp plants in choice assays. Although BLHs cannot reproduce on hemp, brief phloem-feeding periods (2 to 20 min) can still result in low levels of virus transmission (Bennett 1971). Although host suitability cannot be inferred solely from a single no-choice greenhouse assay, the goal of this study was to determine whether BLHs could survive on hemp long enough to support virus transmission. Future research should consider multi-genotype host use by BLHs at the landscape level to address vector movement, host preference, and population-level dynamics under field conditions.

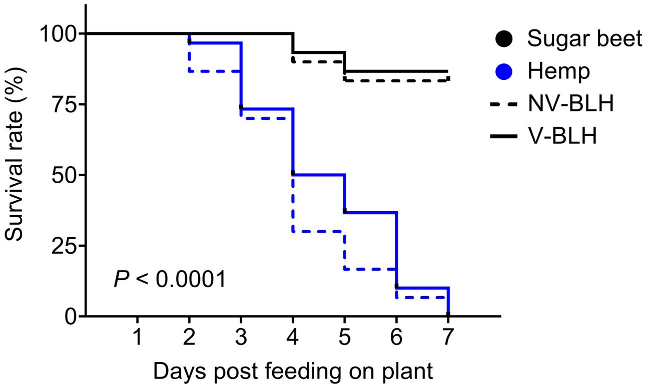

Fig. 5. Survivorship of beet leafhoppers (BLHs) on sugar beet and hemp plants. Survival of beet curly top virus (BCTV)-viruliferous (V) and non-viruliferous (NV) BLHs. BLHs were monitored over 7 days on sugar beet and hemp plants. Each line represents a total of 30 insects from three biological replicates. Statistical differences between survival curves were assessed using the Mantel–Cox log-rank test, followed by pairwise comparisons with Benjamini–Hochberg false discovery rate correction (adjusted $P < 0.05$).

In the current study, all hemp inoculations were performed using viruliferous BLHs maintained on sugar beet plants infected with a mixed population of the CA/Logan and Svr strains. Notably, these strains were not detected in field surveys of hemp. Previous research has shown that the amount of virus acquired by BLH depends on the viral titer in the acquisition host, regardless of strain. For example, BLHs that acquired BCTV-Svr or Wor from sugar beet or shepherd's purse plants exhibiting mild symptoms (indicative of low viral titers)

TABLE 2. Impact of beet curly top virus (BCTV) infection on cannabinoid levels in two hemp lines

		Average concentration ± standard error (µg/g)		
Line	Cannabinoid[a]	Noninfected	Infected	*P* value[b]
4394	CBC	27.16 ± 15.03	25.28 ± 8.6	0.860
	CBCV	0.75 ± 0.44	0.66 ± 0.4	0.808
	CBD	187.55 ± 22.14	829.28 ± 285.81	0.054
	CBDA	48,235.85 ± 60,93.39	70,914 ± 30,078.89	0.765
	CBDV	1.77 ± 0.21	7.98 ± 6.18	0.226
	CBDVA	818.44 ± 318.39	1,088.7 ± 502.45	0.860
	CBG	20.64 ± 5.57	27.1 ± 10.35	0.778
	CBGA	3,055.51 ± 549.24	6,289.16 ± 5,006.94	0.797
	CBLA/CBCA	2,123.37 ± 268.74	2,772.52 ± 1,108.75	0.888
	CBN	0.1 ± 0.02	0.25 ± 0.03	0.182
	CBNA	2.35 ± 0.27	2.91 ± 0.66	0.792
	CBT	0.56 ± 0.41	0.25 ± 0.09	0.628
	delta9THC	12.87 ± 2.27	47.68 ± 18.88	0.080
	THCA	741.74 ± 97.41	950.51 ± 376.21	0.905
	THCV	0.27 ± 0.05	1.02 ± 0.69	0.189
	THCVA	24.31 ± 9.04	26.18 ± 13.61	0.988
4681	**CBC**	50.4 ± 15.31	9.09 ± 5.22	**0.023**
	CBCV	3.09 ± 0.58	0.67 ± 0.52	**0.009**
	CBD	1,096.33 ± 391.24	97.74 ± 41.48	**0.003**
	CBDA	25,402.1 ± 2,745.92	17,412.16 ± 4,034.22	0.611
	CBDV	33.47 ± 10.24	4.34 ± 3.51	**0.001**
	CBDVA	1,874.96 ± 1,277.67	730.37 ± 546.57	0.234
	CBG	14.54 ± 2.26	4.03 ± 1.19	0.100
	CBGA	320.64 ± 112.99	94.29 ± 48.31	0.075
	CBLA/CBCA	959.67 ± 56.66	1,113.93 ± 585.84	0.883
	CBN	0.46 ± 0.12	0.06 ± 0.05	**0.0002**
	CBNA	1.87 ± 0.09	0.74 ± 0.45	0.110
	CBT	0.42 ± 0.08	0.19 ± 0.16	**0.032**
	delta9THC	72.8 ± 20.31	9.6 ± 3.83	**0.013**
	THCA	326.39 ± 20.05	382.62 ± 206.48	0.877
	THCV	3.29 ± 0.51	0.52 ± 0.38	**0.005**
	THCVA	80.03 ± 56.69	29.56 ± 21.15	0.235

[a] Cannabinoids shown in bold indicate a significant difference ($P < 0.05$) in levels between BCTV-infected and noninfected plants. Abbreviations: CBC, cannabichromene; CBCV, cannabichromevarin; CBD, cannabidiol; CBDV, cannabidivarin; CBG, cannabigerol; CBN, cannabinol; CBT, cannabitriol; delta9THC, delta-9-tetrahydrocannabinol; THCV, tetrahydrocannabivarin. The acidic precursors to these compounds, which are often the dominant forms in raw plant material, are cannabidiolic acid (CBDA), cannabidivarinic acid (CBDVA), cannabigerolic acid (CBGA), cannabicyclolic acid/cannabichromenic acid (CBLA/CBCA), cannabinolic acid (CBNA), tetrahydrocannabinolic acid (THCA), and tetrahydrocannabivarinic acid (THCVA).

[b] Statistical analysis was performed using a general linear model followed by pairwise comparisons between two treatments with Fisher's least significant difference test on log-transformed data. Data normality was confirmed using a Shapiro–Wilk test.

harbored lower virus titers than BLHs that acquired the virus from sugar beet or shepherd's purse plants exhibiting severe symptoms and high viral titers (Chen and Gilbertson 2009). Future studies should investigate how variation in viral titer among hemp plants influences BCTV acquisition and transmission by BLHs, including the potential for virus acquisition from potential seed-transmitted BCTV.

In conclusion, this study improves our understanding of the genetic variability of BCTV strains infecting hemp in the western United States and confirms that recombination is a key driver of BCTV evolution. The findings reveal genotype-dependent reductions in cannabinoid production caused by BCTV infection, underscoring the potential for a direct economic impact of this viral pathogen. We also confirmed the seedborne nature of BCTV in hemp, albeit at a low rate in seedlings. Furthermore, this study reinforces that hemp does not support BLH survival, though short feeding periods likely remain sufficient for virus transmission. Collectively, these insights enhance our understanding of BCTV evolutionary dynamics and host interactions in hemp and provide useful information for future research and management strategies to safeguard hemp production.

Acknowledgments

We thank Cesar Augusto Diniz Xavier from North Carolina State University for his invaluable discussions and insights on genetic analysis and the hemp growers of Colorado for allowing us to sample their fields, and New West Genetics (Fort Collins, CO, U.S.A.) for providing the hemp seeds used in this study.

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