Viral small RNA sequencing of Pepperplant (Capsicum annuum) reveals presence of Tomato leaf curl New Delhi virus infection in China

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Date: 2025-01-08T00:00:00+00:00

Abstract

Pepper (Capsicum annuum), a crucial economic crop worldwide (Tang et al. 2020), has been observed in multiple fields depicting susceptibility symptoms across Anhui Province, China, a primary region for pepper cultivation. Data obtained in November 2023, revealed that infected pepper plants exhibited yellowing leaves with wrinkling, curling, and mottling, while fruits displayed more severe symptoms, including browning, blackening, and necrosis. The infection significantly impaired the appearance and internal quality of pepper fruits, adversely affecting local production. Surveys indicated that approximately 25% to 35% of pepper plants showed these symptoms. We hypothesize that a viral disease is causing these symptoms (Wu et al. 2024).

To determine the pathogenic virus, we mixed the collected samples and sent them to Lianchuan Co., Ltd. (Hangzhou, China) for small RNA sequencing (Wu et al. 2015). The library preparation was carried out using the TruSeq™ Small RNA Sample Prep Kits (Illumina, San Diego, USA). Subsequently, the constructed library was sequenced on the Illumina Hiseq2000/2500 platform. From the 18059475 raw readings obtained, we removed 3' adapters and low-quality sequences, resulting in 14020232 clean readings, accounting for 77.63% of the initial raw readings. After removing the host genome, align the sequence with the virus database and analyze the distribution of hot-spots. Additional sequence assembly was conducted using virus detection software to generate longer contigs. Later, the concatenated sequences were annotated with viral sequences from the NT and NR databases. The raw data had been deposited in GenBank (PRJNA1194353). Specific sequence analysis showed that the average number of times each base in the sequence of tomato leaf curl New Delhi virus (ToLCNDV, HM159454.1) was sequenced and covered was 59.92%, and the sequence homology was 100%. ToLCNDV is classified within the genus Begomovirus of

the family Geminiviridae and are known to be transmitted by the whitefly Bemisia tabaci (Lopez et al. 2015). To further validate the presence of ToLCNDV in Pepper. The expected size 771-base pair (bp) RT-PCR product of ToLCNDV coat protein (CP) gene was obtained from all 24 tested samples using the pair of specific primer (ToLCNDV 5 -ATGGCGAAGCGACCAGCAGATATC-3 /5 -TTAATTTGTGGCCGAATCATAAAAG-3). Each set of sequences showed 100% identity within their respective groups. The sequence had been deposited in GenBank with accession numbers PQ638334 for the CP gene of ToLCNDV. When compared with nucleotide sequences in the NCBI database, the CP gene of ToLCNDV in pepper exhibited 99.87% nucleotide homology with the Tomato leaf curl New Delhi virus isolate Ningbo6 segment DNA-A (OP585370).

Field investigations were conducted across various regions of Anhui Province in China, where 46 pepper samples displaying symptoms such as yellowing, mottling, and wrinkling of leaves were collected. These samples were then subjected to RT-PCR and dot enzyme-linked immunosorbent assay (Dot-ELISA) testing, utilizing ToLCNDV-specific primers and viral antibodies for detection. 35 of these symptomatic samples, tested positive for ToLCNDV, the infection rate of up to 76%.

The above results show that the pepper leaves in Anhui Province exhibit symptoms of wrinkling and mottling are most likely caused by ToLCNDV infection. To verify this assumption, healthy Nicotiana benthamiana plants were inoculated with above mentioned crude extract of the samples containing the virus. Wrinkled and mottled leaves, short plant stature and some other similar symptoms were observed in 5 out of 10 inoculated Pepper plants 22 days post inoculation which was later confirmed with RT-PCR and sequencing results.

These findings indicate that the symptoms observed in pepper leaves, characterized by wrinkling, mottling, and stunted plant growth, are most probably the result of infection by ToLCNDV. To our knowledge, this is the first report of presence ToLCNDV in pepper plants in China.

Full Text

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Funding: Funding was provided by The Excellent Youth Research Program of Anhui Universities (2023AH030047), National Natural Science Foundation of China (32302297), and Scholars of Anhui Province (2308085Y25).

Pepper (Capsicum annuum), a crucial economic crop worldwide (Tang et al. 2020), has been observed in multiple fields showing susceptibility symptoms across Anhui Province, China, a primary region for pepper cultivation. Data obtained in November 2023 revealed that infected pepper plants exhibited yellowing leaves with wrinkling, curling, and mottling, while fruits displayed more severe symptoms, including browning, blackening, and necrosis. The infection significantly impaired the appearance and internal quality of pepper fruits, adversely affecting local production. Surveys indicated that approximately 25% to 35% of pepper plants showed these symptoms. We hypothesize that a viral disease is causing these symptoms (Wu et al. 2024).

To determine the pathogenic virus, we mixed the collected samples and sent them to Lianchuan Co., Ltd. (Hangzhou, China) for small RNA sequencing (Wu et al. 2015). The library preparation was carried out using the TruSeq™ Small RNA Sample Prep Kits (Illumina, San Diego, USA). Subsequently, the constructed library was sequenced on the Illumina Hiseq 2000/2500 platform. From the 18,059,475 raw reads obtained, we removed 3' adapters and low-quality sequences, resulting in 14,020,232 clean reads, accounting for 77.63% of the initial raw reads.

After removing the host genome, we aligned the sequences with the virus database and analyzed the distribution of hot-spots. Additional sequence assembly was conducted using virus detection software to generate longer contigs. Later, the concatenated sequences were annotated with viral sequences from the NT and NR databases.

The raw data have been deposited in GenBank (PRJNA1194353). Specific sequence analysis showed that the average coverage of each base in the ToLCNDV sequence (HM159454.1) was 59.92%, with 100% sequence homology. ToLCNDV is classified within the genus Begomovirus of the family Geminiviridae and is known to be transmitted by the whitefly *Bemisia tabaci* (Lopez et al. 2015). To further validate the presence of ToLCNDV in pepper, the expected 771-base pair (bp) RT-PCR product of the ToLCNDV coat protein (CP) gene was obtained from all 24 tested samples using a pair of specific primers (ToLCNDV 5 -ATGGCGAA GCGACCAGCAGATATC-3 /5 -TTAATTTGTGGCCGAATCATAAAAG-3). Each set of sequences showed 100% identity within their respective groups. The sequences have been deposited in GenBank with accession number PQ638334 for the CP gene of ToLCNDV. When compared with nucleotide sequences in the NCBI database, the CP gene of ToLCNDV in pepper exhibited 99.87% nucleotide homology with the Tomato leaf curl New Delhi virus isolate Ningbo6 segment DNA-A (OP585370).

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Thirty-five of these symptomatic samples tested positive for ToLCNDV, with an infection rate of up to 76%.

The above results indicate that the wrinkling and mottling symptoms observed in pepper leaves in Anhui Province are most likely caused by ToLCNDV infection. To verify this assumption, healthy *Nicotiana benthamiana* plants were inoculated with the above-mentioned crude extract of the samples containing the virus. Wrinkled and mottled leaves, short plant stature, and other similar symptoms were observed in 5 out of 10 inoculated *N. benthamiana* plants 22 days post-inoculation, which were later confirmed with RT-PCR and sequencing results.

These findings indicate that the symptoms observed in pepper plants, characterized by wrinkling, mottling, and stunted growth, are most probably the result of infection by ToLCNDV. To our knowledge, this is the first report of the presence of ToLCNDV in pepper plants in China.

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Declarations

The author(s) declare no conflict of interest.

Keywords: First report, Tomato leaf curl New Delhi virus, Pepper (Capsicum annuum), China

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Note: Figure translations are in progress. See original paper for figures.