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Short communication

Turnip curly top virus, a highly divergent geminivirus infecting turnip in Iran

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ARTICLE INFO

Article history: Received 21 February 2010 Received in revised form 6 May 2010 Accepted 31 May 2010 Available online 8 June 2010

Keywords: Geminivirus Curtovirus Turnip

ABSTRACT

From 2006 onwards turnip crops in Fars province, Iran, have been noted with unusual leaf curling and vein swelling symptoms which are characteristic of the leafhopper-transmitted viruses of the genus Curtovirus (family Geminiviridae). Rolling circle amplification was used to clone viruses from five turnip isolates exhibiting leaf curl symptoms. Analysis of the sequences showed them to have >93% sequence identity and to be distinct from all other geminiviruses previously characterised. Analysis of the sequence of this virus, for which we propose the name Turnip curly top virus (TCTV), showed it to have a genome arrangement in the complementary-sense similar to that of curtoviruses (consisting of four overlapping genes) but only two open reading frames in the virion-sense (the curtoviruses encode three). The complementarysense genes are homologous to those of curtoviruses but show little sequence identity to their curtovirus homologs, with the exception of the product of the C4 open reading frame (ORF) which shows ~70.6% amino acid sequence identity to the C4 of the North American curtoviruses, Pepper curly top virus and Beet mild curly top virus. For curtoviruses the C4 protein is a symptom determinant, which likely explains the similarity of TCTV symptoms to those of curtoviruses. In the virion-sense the predicted product of the V2 ORF of TCTV shows no significant similarity with any proteins in the databases whereas the product of the V1 ORF (encoding the coat protein [CP] of geminiviruses) shows low levels of sequence identity to the CPs of curtoviruses. These findings show TCTV to be a highly divergent geminivirus with similarities to viruses of the genus curtovirus. The significance of these findings, particularly the taxonomic implications are discussed.

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Viruses of the family *Geminiviridae* are distinct in having genomes of circular, single-stranded (ss) DNA that are packaged within twinned quasi-isometric ("geminate") virions (Stanley et al., 2005). Geminiviruses are divided into four genera based on genome organisation and biological properties, most important of which are the type of insect vector (either whitefly, leafhopper or treehopper) and host range (either mono- or dicotyledonous hosts; Stanley et al., 2005). Whitefly (*Bemisia tabaci*)-transmitted geminiviruses, with either bipartite or monopartite genomes, are included in the genus *Begomovirus*. Those having monopartite genomes that are transmitted by leafhopper vectors, primarily to monocotyledonous plants, are included in the genus *Mastrevirus*. Viruses that have

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monopartite genomes distinct from those of the mastreviruses and that are transmitted by leafhopper vectors to dicotyledonous plants are placed in the genus *Curtovirus*, with *Beet curly top virus* (BCTV) as the type species. The most recently established of the present four genera of the *Geminiviridae*, *Topocuvirus* holds just a single species: *Tomato pseudo-curly top virus* (TPCTV). TPCTV is a monopartite virus with a complementary-sense genome arrangement typical of, and homologous to, viruses of the *Begomo-* and *Curtovirus* genera, with four overlapping genes (Briddon et al., 1996). The main factor leading to the establishment of this fourth genus was the fact that TPCTV is the only known geminivirus transmitted by a treehopper (*Micrutalis malleifera*).

Over the past year two highly unusual geminiviruses have been identified in the Middle East and Africa. The first is Beet curly top Iran virus (BCTIV) isolated from sugar beet, tomato, spinach, and turnip originating from Iran (Yazdi et al., 2008). This virus has a virion-sense genome arrangement typical of curtoviruses (three overlapping genes) and the predicted amino acid sequences of each

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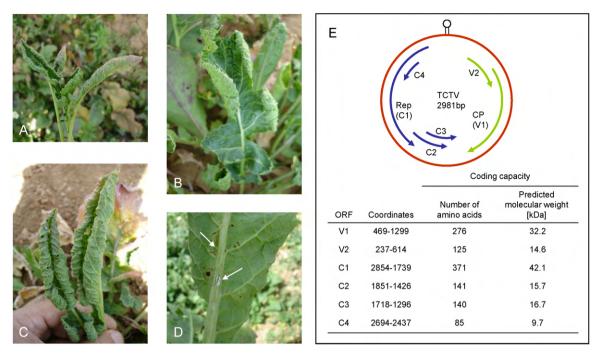


Fig. 1. Symptoms caused by TCTV on Turnip (*Brassica rapa*) leaves are inward rolling of the leaves resulting in characteristic cup shaped morphology (A and B). Additional symptoms include thick and brittle leaves, swelling of veins on the lower leaf surfaces (C) and fluid exudation from the petioles or midribs on the lower leaf surfaces (D). The genome of TCTV contains six major open reading frames (ORFs), two in the virion-sense ORFs and four in complementary-sense (E).

show the highest levels of identity to the corresponding gene products of curtoviruses. In contrast, the complementary-sense gene arrangement (2 genes) is more similar to mastreviruses and the gene products show greater levels of identity to the corresponding mastrevirus gene products. In addition, earlier studies have shown the virus to be transmitted by the leafhopper *Circulifer hematocepts* (Hosseini Abhari et al., 2005), whereas the other curtoviruses, including that from Iran, are transmitted by a related species, *Circulifer tenellus* (Soto and Gilbertson, 2003; Soto et al., 2005; Chen et al., 2010). Although provisionally placed in the genus *Curtovirus*, awaiting the demonstration of infectivity of the clones to satisfy Koch's Postulates, these features (a combination of those seen in curto- and mastreviruses) are sufficiently distinct for the virus to be the founding member of a new genus in the family *Geminiviridae*.

The other unusual geminivirus, Eragrostis curvula streak virus (ECSV), was isolated from a monocotyledonous weed originating from South Africa (Varsani et al., 2009). ECSV has two virionsense genes most closely related to those of mastreviruses and, although the complementary-sense gene arrangement is similar to that of mastreviruses, the replication associated protein (Rep) and C2 gene products are more closely related to those of topocu-, begomo-, and curtoviruses. The presence in the ECSV genome of a C2 open reading frame (ORF) that is potentially homologous to the transcription activator protein (TrAP) genes of topocu-, begomo-, and curtoviruses is interesting in that mastrevirus genomes

do not encode a TrAP. These features will similarly require the establishment of a new genus once Koch's postulates have been satisfied.

Turnip (Brassica rapa) leaves with inward rolling of the leaf margins resulting in characteristic cup shaped leaves (Fig. 1A and B) were first observed in 2006 in Zafar-abad (lat.: 29.401°, long.: 52.583°; 20 km south of Shiraz, Fars Province, Iran). In 2009, a high incidence of similarly symptomatic plants were noted in Homayejan (lat.: 29.693°, long.: 52.175°; 50 km west of Shiraz). Disease progression resembled that of geminivirus infections with diseased plants initially displaying symptoms confined to the youngest leaves with all subsequently emerging leaves also exhibiting symptoms. Other symptoms included, thick and brittle leaves, swelling of veins on the lower leaf surfaces (Fig. 1C) with fluid being exuded from the petioles or midribs on the lower leaf surfaces (Fig. 1D). These symptoms are very similar to those induced by the curtovirus Beet severe curly top virus (BSCTV) in sugarbeet (Briddon et al., 1998). BSCTV has previously been shown to occur in this region of Iran suggesting that the turnips were possibly infected with a curtovirus related to this species.

Rolling circle amplification (RCA) was used to amplify circular DNA molecules, as described by Shepherd et al. (2008), from total nucleic acids extracted from 10 symptomatic turnip leaf samples from the Fars province of Iran (one collected in 2006 from Zafar-abad and nine in 2009 from Homayejan). Of the ten samples

Table 1Pairwise Hamming-distance (also known as p-distance) comparisons of TCTV genome sequences with those of all available curtovirus and curtovirus-like species. The values in parentheses indicate numbers of isolates used in the comparisons.

	TCTV (5)	BCTV (2)	BMCTV (5)	BSCTV (3)	HrCTV (1)	SpCTV (1)	PCTV (1)	PYDV(1)	BCTIV (3)
TCTV (5)	93.5-100	56.6-57.6	55.4-56.5	57.3-58.0	48.7-49.2	56.7-57.2	56.0-56.3	56.4-57.1	43.9-45.1
BCTV (2) BMCTV (5)		97.2	75.1–79.1 90.7–97.2	79.5-82.3 81.1-87.4	63.4-63.9 62.4-63.4	75.2–75.3 77.0–78.6	72.8–73.2 78.2–79.5	76.2–76.5 85.0–88.2	54.1-54.6 54.0-54.5
BSCTV (3)				83.5-98.3	63.0-63.8	82.1-83.7	84.1-86.6	82.1-88.1	54.8-55.4
HrCTV (1)					100	63.8	60.9	63.0	54.4-54.5
SpCTV (1)						100	80.0	80.1	54.9
PCTV (1)							100	79.3	53.6-53.9
PYDV (1)								100	53.9-54.4
BCTIV (3)									96.0–98.2

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