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Fulfilling Koch's postulates confirms the monopartite nature of tomato leaf deformation virus: A begomovirus native to the New World

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ABSTRACT

The monopartite nature of the begomovirus tomato leaf deformation virus (ToLDeV) reported in Peru is demonstrated here. The DNA molecule cloned from an infected plant was shown to be fully infectious in tomatoes inducing leaf curling and stunted growth similar to that observed in field-infected plants. The viral DNA was reisolated from systemically infected tissues of inoculated plants, thus fulfilling Koch's postulates. ToLDeV was demonstrated, therefore, as the causal agent of the disease syndrome widespread in tomato crops in Peru. This virus was shown to be present throughout the major tomato-growing regions of this country, both in tomatoes and wild plants. Analyses of the sequences of 51 ToLDeV isolates revealed a significant genetic diversity with three major genetic types co-circulating in the population. A geographical segregation was observed which should be taken into account for virus control. Constraints to genetic divergence found for the C4 gene of ToLDeV isolates suggest a relevant function for this protein. The results obtained confirm ToLDeV as a monopartite begomovirus native to the New World, which is a significant finding for this region.

1. Introduction

Begomoviruses (genus *Begomovirus*) are small plant viruses with twinned, quasi-isometric virions encapsidating genomes of circular single-stranded DNA (ssDNA) characteristic of members of the family *Geminiviridae* (Brown et al., 2012). Begomoviruses are an emerging threat worldwide (Varma et al., 2011) and are transmitted in nature by the whitefly *Bemisia tabaci* Genn. (*Hemiptera*: *Aleyrodidae*). The genus *Begomovirus* consists of viruses with either monopartite or bipartite genomes. Globally, begomoviruses are grouped into two major phylogenetic clades named Old World (OW) and New World (NW) (Briddon et al., 2010). Most begomoviruses have bipartite genomes with components designated DNA-A and DNA-B, each of which is 2.5–2.8 kilobases. Both components are required for infectivity (Stanley, 1983). Demonstration of the infectivity of a single component was done for the first time for the begomovirus tomato yellow leaf curl virus (TYLCV), thus showing that begomoviruses with a single genomic component exist (Navot et al., 1991). Remarkably, no monopartite begomovirus native to the NW has been identified to date, although recently the OW monopartite begomovirus TYLCV was inadvertently introduced and has spread in the NW (Duffy and Holmes, 2007; Polston et al., 1999). Another exception is monopartite begomoviruses that infect sweet potato [*Ipomoea batatas* (L.) Lam], and probably are spreading worldwide through the exchange of infected propagating material (tubers). These are known as sweepoviruses and cluster in a monophyletic clade separated from OW and NW begomoviruses (Albuquerque et al., 2012).

Coding sequences are present in both the virion (V) and complementary (C) sense strands of genome components of monopartite and bipartite begomoviruses, separated by an intergenic non coding region (IR). In bipartite begomoviruses, DNA-A component has five or six genes. These are AV1 (which encodes the coat protein, CP), AV2 (precoat protein), AC1 (replication associated protein, Rep), AC2 (transcriptional activator protein, TrAP), AC3 (replication enhancer protein, Ren), and AC4. Interestingly, DNA-As of NW begomoviruses are characterized for the absence of AV2. The DNA-B component has two genes that encode proteins directly involved in movement, BV1 (which encodes the nuclear shuttle protein, NSP), and BC1 (movement protein, MP), on the V-sense and C-sense strands respectively (Brown et al., 2012). The genomes

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of monopartite begomoviruses have a similar organization and genes homologous to DNA-A of bipartite begomoviruses (Brown et al., 2012). These genes include V1 (CP protein), V2 (precoat), C1 (Rep), C2 (TrAP), C3 (Ren), and C4. Different gene requirements exist for monopartite and bipartite begomoviruses. Thus, monopartite begomoviruses absolutely require a functional CP for systemic infection and C4 as a pathogenicity factor, whereas both proteins can be dispensable in bipartite begomoviruses; also, V2 is essential for virus-host interaction and pathogenicity, whereas it is absent in NW bipartite begomoviruses (Luna et al., 2012; Pooma et al., 1996; Pooma and Petty, 1996; Rojas et al., 2001; Wartig et al., 1997). The IR contains the promoters for transcription of the V- and C-sense genes (Hanley-Bowdoin et al., 1999), the stem-loop structure with the nonanucleotide sequence conserved in geminiviruses, TAATATTAC (Jeske, 2007; Lazarowitz et al., 1992), and virus-specific repeated sequences (iterons) where the Rep binds to initiate replication (Argüello-Astorga et al., 1994).

The spread of begomoviruses causing severe damage to important food crops in tropical and subtropical regions in the last two decades is associated with the spread of their insect vector *B. tabaci* and the global movement of plant materials (Navas-Castillo et al., 2011; Seal et al., 2006; Varma et al., 2011). Emergence of these viruses is especially important in Latin America in regions where reproduction of *B. tabaci* is favored by high temperatures (Morales, 2010; Morales and Jones, 2004; Navas-Castillo et al., 2011; Rojas and Gilbertson, 2008). Control of begomoviruses is mainly based on intensive insecticide treatment programs to control vector transmission, with limited success to reduce virus spread (Nauen and Denholm, 2005). The use of host genetic resistance if available, therefore, is the best control option, such as for the *Ty-1* gene widely used commercially to control damage caused in tomato (*Solanum lycopersicum* L.) by TYLCV (Michelson et al., 1994). Durable control based on genetic resistance, however, requires knowledge about the genetic diversity of the virus population (García-Arenal and McDonald, 2003).

A new begomovirus named tomato leaf deformation virus (ToLDeV) has been recently described infecting tomato crops in Peru (Márquez-Martín et al., 2011). Tomato plants infected with ToLDeV exhibited a disease syndrome consisting of upward curling of leaflet margins, leaflet deformation and growth stunting with dramatic yield losses when infections occur in early growth stages (Márquez-Martín et al., 2011). The complete nucleotide sequence was obtained for a circular ssDNA molecule associated with symptomatic plants (GenBank accession number GQ334472), showing a genome organization typical of DNA-A of NW begomoviruses (with no AV-2 gene present) and phylogenetic relationships with this group of viruses (Márquez-Martín et al., 2011). No DNA-B could be isolated from symptomatic plants suggesting that ToLDeV might be a monopartite begomovirus (Márquez-Martín et al., 2011). Demonstration of the infectivity of the cloned DNA, however, was lacking as was fulfilling of Koch's postulates for this molecule as the causal agent of the disease observed in tomato. The presence of a monopartite begomovirus would be a significant finding for the New World. Also, no information was available about the genetic diversity of the ToLDeV population in Peru, which is essential to implement durable control strategies.

In the present study, we demonstrated the monopartite nature of ToLDeV and that it is the causal agent of the disease spreading in tomato crops in Peru. Isolates of this virus were found throughout this country. The genetic diversity of ToLDeV was characterized suggesting the presence of a locally evolved population with three major genotypes. Also, infection of ToLDeV was shown in wild hosts and a geographical segregation of the population is suggested. This demonstrates ToLDeV as a monopartite begomovirus native to the New World.

Table 1
Presence of tomato leaf deformation virus (ToLDeV) in samples exhibiting begomovirus-like symptoms collected in Peru during a survey conducted between 2003 and 2010.

Host plants	Species	No. ToLDeV infected samples/total no. of plants analyzed (% infected)[a]
Cultivated hosts[b]		
Tomato resistant cv.	*Solanum lycopersicum* L.	30/71 (42.2)
Tomato susceptible cv.	*Solanum lycopersicum* L.	18/44 (40.9)
Sweet pepper	*Capsicum annuum* L.	0/10 (0)
Habanero pepper	*Capsicum chinense* Jack	0/3 (0)
Red Peruvian chile	*Capsicum bacatum* cv. *Pendulum* (Willd.) Eshbaugh	0/4 (0)
Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	0/3 (0)
Common bean	*Phaseolus vulgaris* L.	0/5 (0)
Pallar bean	*Phaseolus lunatus* L.	0/5 (0)
Wild hosts		
Weeds	21 species	2/60 (3.3)[c]
Wild relatives of tomato	4 species	1/45 (2.2)[d]
Total		51/250 (20.4)

[a] Samples were analyzed by dot-blot hybridization using a digoxigenin-labeled DNA probe specific to the tomato leaf deformation virus.
[b] Tomato resistant cvs. are commercial tomato cultivars with tolerance to tomato yellow leaf curl disease (TYLCD), the most widespread: 'Dominator' (Seminis, Saint Louis, USA) and 'Tyson' (Hazera Genetics, Shikmim, Israel). Tomato susceptible cvs. are several commercial tomato cultivars not tolerant to TYLCD.
[c] Samples positive to ToLDeV were from a plant of the species *Anoda cristata* (L.) Schltdl. (family *Malvaceae*) and from a plant of *Tanacetum parthenium* L. (family *Compositae*).
[d] The sample from a plant of a wild relative of tomato infected with ToLDeV corresponds to the species *Solanum pennellii* Correll.

2. Materials and methods

2.1. Virus isolates

A total of 250 samples were collected from cultivated and wild host plants exhibiting begomovirus-like symptoms throughout vegetable growing regions of Peru (departments of Lambayeque, La Libertad, Lima, Ica and Arequipa) during 2003 and 2008–2010. Surveys comprised plants from cultivated hosts such as tomato, pepper (*Capsicum annuum* L., *Capsicum chinense* Jack, *Capsicum bacatum* cv. *Pendulum* (Willd.) Eshbaugh), bean (*Phaseolus vulgaris* L., *Phaseolus lunatus* L.), and cucurbit (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), wild relatives of tomato indigenous to Peru, and weeds (Table 1). A sample from each plant consisted of apical young leaves that were stored dried at 4 °C until used. After confirming ToLDeV presence by dot-blot hybridization (see below), 48 isolates from tomato representing all growing areas of Peru along with the three additional isolates detected in wild hosts were included in the analysis (Table 1).

2.2. Sample analysis

Field samples and agroinoculated plants were analyzed for the presence of ToLDeV by squash blot or by dot-blot hybridization using 1 μl of total DNA (see below), on positively charged nylon membranes (Roche Diagnostics, Mannheim, Germany). For hybridization, a digoxigenin (DIG)-labeled DNA probe, specific to the ToLDeV IR region was used. The probe was prepared by PCR according to the DIG-labeling detection kit (Roche Diagnostics) and as described by Navas-Castillo et al. (1999), using the primer