

Distribution and molecular variability of begomoviruses affecting economically important crops in Cuba: epiphytiological elements

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Introduction

The results presented here describe studies on the distribution, molecular variability and epiphytiology of begomoviruses in Cuba, mainly focused on those affecting tomato. The work presents prospection results that have allowed the examination of the distribution of these viruses among the main areas of tomato culture in the country, identifying those with the highest affectation and examining the main epidemiological factors influencing on the large rates of incidence and the high severity of the infestations, as well as monitoring and demonstrating the adaptability of TYLCV-IL(CU) to the agricultural ecosystems of Cuban tomato. Further knowledge on the molecular evolution of begomoviruses has been provided, evidencing their potential colonization of other ecological habitats such as pepper, bean and pumpkin cultures, which may function as natural, active reservoirs for TYLCV-IL(CU). Importantly, two new species of bipartite begomoviruses infecting tomato, pepper and tobacco crops are described, under the proposed names of Tobacco Yellow Crinkle Virus (TbYCV, infecting tobacco and pepper) and Tomato Yellow Leaf Distortion Virus (ToYLDV, infecting tomato crops).

The use of total DNA sequencing in symptomatically infected tobacco plants also detected the presence in this crop of another begomovirus which, although representing a variant of the previously known *Euphorbia* mosaic virus instead of a new species of the genus, is however detected in Cuba for the first time.

The identification and partial sequencing of regions of taxonomic relevance demonstrated the presence and diversity of begomoviral populations in weeds, a very important topic given the potential interaction of these populations with economically important crops and their possible contribution to genetic diversity through recombination events that may give rise to new, more aggressive or better adapted viral species.

The results obtained here arise from an integral approach to the study of the alterations to agricultural systems that includes both, biological and molecular changes shaping the emergence of a viral disease and its evolution towards increased severity in the interface between natural ecosystems and productive ecosystems, which are completely managed by man.

Results

Distribution, molecular variability and epiphytiological elements of TYLCV-IL(CU) in Cuba

Prospective studies were performed in the most important areas for tomato agriculture in Cuba during

the 1997-1998, 1999-2000, 2001-2002 and 2004-2005 harvests, in order to monitor the distribution of TYLCV-IL (CU) and Tomato Mosaic Havana Virus (ToMHV). The results evidence a progressive adaptation of TYLCV-IL(CU) to the conditions of the agricultural ecosystem of tomato plantations, to the point of partial or total displacement of ToMHV as judged by the data for both single or mixed infections (Figure 1). They therefore illustrate the predominance and adaptation of TYLCV-IL(CU) to the productive conditions prevalent in our country, which have been favorable for its instauration and dissemination in both, established production varieties and newly introduced cultivars.

The wide dissemination of TYLCV-IL(CU) and the resulting displacement of ToMHV is an important aspect that has to be taken into account when monitoring the whitefly-geminivirus complex, given its vital influence in the nature of control measures and the selection of resistant or tolerant varieties for further introduction. Similar situations have been described in Spain, where TYLCV-Sar was gradually displaced by the Israeli isolate of TYLCV from 1992 onwards, and in Florida where Tomato Mottle Virus has also been displaced [1, 2].

High infestation rates (46-93.6%) were found among the evaluated varieties and hybrids. Although no symptoms of the disease were detected in the ARO8484 hybrid, it supported high percentages of viral replication. This finding is highly relevant given

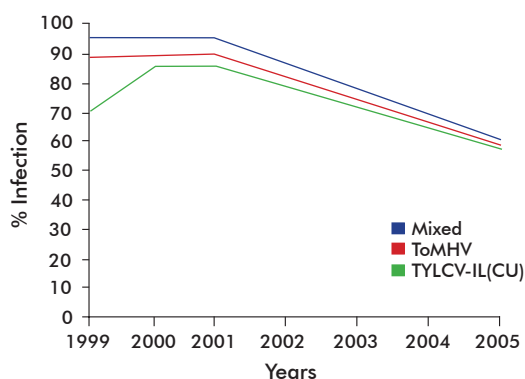


Figure 1. Diagram showing the behavior of single and mixed infections of TYLCV-IL(CU) and ToMHV during prospectations performed in different years.

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the significant importation of TYLCV-resistance hybrids by Cuba, therefore requiring the implementation of strict management practices to avoid the conversion of these plantations into natural reservoirs and sources of propagation for this viral disease.

Phylogenetic analyses of the 5' terminal region of the RC and *rep* proteins using the DNA genomic sequencing data of TYLCV-IL(CU) isolates collected in different geographic zones between 1995 and 2002 showed an identity percentage of 97-99% when compared to the Israeli variant previously identified by Martínez et al. [3]. This evidences a low rate of molecular variability, with a divergence average of 0-4% that falls within the accepted limits for genetically stable populations, even when studying the non-coding regions of RNA viruses [4]. The results, therefore, lead to the conclusion that the TYLCV-IL(CU) samples represent a single population with low genetic diversity in the process of expansion into new ecological niches; and therefore the aggressiveness and wide distribution of this virus in our country are not caused by genetic variations or molecular alterations of this strain.

A probability of 2.5-3% for the occurrence of recombination events with ToMHV was determined for 18 of the studied isolates. Additionally, the sites of possible inter-species recombination events were located with the aid of bioinformatics software, also finding 3 different secondary structures with a high thermodynamic stability for the first 200 nucleotides of the 5' end of RC during the course of this analysis.

The sequencing data and phylogenetic analyses for the isolates from symptomatic pepper, bean and pumpkin plants at the prospected areas allowed for the first time the detection in Cuba of the presence of natural infections of TYLCV-IL(CU), with isolates sharing a sequence identity of 95-99%.

In addition to the prospecting work, during the 2004-2005 harvest, producers from 28 fields in 11 localities of the Granma, Holguín, Las Tunas, Camagüey and Ciego de Ávila provinces were also surveyed so as to determine the main factors associated to the epiphytotics caused by the whitefly-geminivirus complex in this culture. The analyses of symptomatic plants, adult counts, and the collection of plants were performed following the English flag method, using the modality of surveys for the producers and monitoring in the fields.

The results of the statistical analysis showed that the factors with the strongest association with a high incidence were the combination of previous hosts, the continuity of the culture and an increase in the density of *Bemisia tabaci* (whitefly). In contrast, factors associated with a low incidence were the presence of good management practices, the phenological stage of the culture at the time of observation, the date of seeding, the geographical position (incidence was lower in Western locations) of the field and its altitude above sea level (the higher the altitude, the lower the incidence). The lower incidence in western locations was associated to better management practices or other prevalent local conditions not taken into account in the study.

Epidemiological and ecological factors in tomato systems were identified as external forces influen-

cing the incidence and severity of TYLCV-IL(CU) infestations, as well as the increase in *B. tabaci* biotype B populations. These factors are simultaneously doubling as intermediates for the passage of new begomoviral species from natural niches to managed productive ecological niches, resulting in the formation of favorable scenarios for the evolution and emergence of diseases, as has been referred by some authors [5].

Identification and characterization of new species of begomovirus in Cuba

Natural recombination events are the main factor behind the emergence of new begomoviral species. During the prospection work performed in the 2007-2008 harvest, a set of symptoms unlike those typical of TYLCV-IL(CU) infections began to be detected in tomato fields. Simultaneously, symptoms similar to those reported for whitefly-transmitted viruses began to be detected in pepper, tobacco and weeds located in the neighboring fields.

After performing viral DNA extractions followed by amplification with generic primers for the A genomic fragment and the central region of the gene for the capsid protein, the presence of an isolate of the *Jatropha* Mosaic Virus in *Jatropha gossipifolia* was detected, and plants of *Asteraceae* family (*Pseudelephantopus spicatus*) were reported as a new host for the *Macropitium* Yellow Mosaic Virus (MaYMV, [6]).

Total DNA samples from economically relevant plants were subjected to rolling circle amplification (RCA) with the aim of obtaining copies of the complete viral genome. Cloning and sequencing of these amplified genomes allowed the identification of two new viruses, which were proposed as new species of bipartite begomoviruses and received the names of Tomato yellow leaf distortion virus (ToYLDV, infecting tomato), and Tobacco yellow crinkle virus (TbYCV, infecting tobacco and pepper). Additionally, the results allowed the detection of the *Euphorbia* mosaic virus infecting tobacco, which does not constitute a new taxonomic finding but is yet the first report of this virus in Cuba.

Results confirm the potential of RCA for studies of begomoviral genomics in Cuba. RCA has previously been proposed as a fast and efficient alternative for the analysis of the diversity of circular genome DNA viruses, and has widely been used in studies of the begomoviral genus [7].

The diversity of bipartite begomoviruses interacting in natural agricultural ecosystems uncovered by this work is relevant not only for Cuba, but for countries of the whole Caribbean basin, where a large number of begomoviruses has been detected in economically important crops as well as in wild plants. At least 4 begomoviral species are shared between Jamaica and Cuba, which have very similar phytosanitary environments and ecosystems [8].

The studies performed for this work, in turn, are coherent with the idea that for agricultural management the constant monitoring of all factors contributing to the development and spread of pests, using rational identification and diagnostic systems and implementing proper measurements for the ecological management of agricultural systems such as the production

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of healthy seedlings, taking into account the biodiversity of the specific geographic area and including the introduction of resistant varieties, constitutes a lower cost, environmentally friendly alternative that must be employed for better control and minimization of pest-induced losses [9-11].

Relevance of the study

Knowledge on which productive areas are most affected by begomoviruses and, in particular, by TYLCV-IL(CU), as well as the determination of the main factors influencing the infestations, are important elements for local decision taking during pest management and has evidenced the importance of a regionalization strategy for tolerant and/or resistant varieties of tomato in the most affected areas.

The prevalence and genetic stability of TYLCV-IL(CU) evidenced by this work have become indispensable tools for the formulation of strategies to obtain resistant tomato varieties. Likewise, the detection of new begomoviral species in weeds and crops such as tomato, pepper and even tobacco, with common hosts and a high probability for the appearance of recombinations, confirms the intense evolutive activity of this genus and underlines the sustained need for continuing crop improvement programs that try to find new sources of resistance with higher stability and robustness.

Conclusions

TYLCV-IL(CU), with a high molecular stability and infecting naturally crops such as bean, pepper and pumpkin, has undergone a fast and devastating adaptation to the environmental conditions of Cuba that has resulted in the displacement of ToMHV. Factors both environmental and epidemiological in the current productive ecosystems have influenced the severity and incidence of TYLCV-IL(CU) on the field, favoring changes in the evolutive structure of begomoviral populations and evidencing the need for new genetic materials with longer lasting resistance. The study of the complete genome of begomoviral isolates has allowed the detection of new species in economically relevant crops such as tomato, pepper and tobacco, as well as in wild plants from natural ecosystems.

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