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Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: Evidence for the presence of a new virus species of recombinant nature

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Abstract

We examined the native plant host *Solanum nigrum* as reservoir of genetic diversity of begomoviruses that cause the tomato yellow leaf curl disease (TYLCD) emerging in southern Spain. Presence of isolates of all the species and strains found associated with TYLCD in this area was demonstrated. Mixed infections were common, which is a prerequisite for recombination to occur. In fact, presence of a novel recombinant begomovirus was demonstrated. Analysis of an infectious clone showed that it resulted from a genetic exchange between isolates of the ES strain of *Tomato yellow leaf curl Sardinia virus* and of the type strain of *Tomato yellow leaf curl virus*. The novel biological properties suggested that it is a step forward in the ecological adaptation to the invaded area. This recombinant represents an isolate of a new begomovirus species for which the name *Tomato yellow leaf curl Axarquia virus* is proposed. Spread into commercial tomatoes is shown.

Keywords: Begomovirus; *Bemisia tabaci*; Genetic diversity; *Lycopersicon esculentum*; Recombination; *Solanum nigrum*; Tomato yellow leaf curl disease; Tomato yellow leaf curl virus; Wild reservoir; Whitefly transmission

Introduction

Emergence of viral diseases can cause considerable damage (Chua et al., 2000; Hahn et al., 2000; Rybicki and Pietersen, 1999; Schrag and Wiener, 1995). Despite this, the attributes responsible for the establishment and spread of specific invaders are often difficult to pinpoint or are unknown. Ecological studies have provided a body of information concerning factors that are important for invasive parasites (Kolar and Lodge, 2001; Schrag and Wiener, 1995). However, genetic approaches have received far less attention, even though genetic variation may determine the success of invaders. For successful emergence, introduced parasites need to evolve rapidly to circumvent loss of genetic variation normally associated with founder effects, and adapt to the novel environmental conditions. Key forces driving evolution are mutation, recombination, genetic drift, natural selection, and migration (Charlesworth and Charlesworth, 2003). Gene flow provided by sex and/or recombination is exploited by parasites to increase their evolutionary potential and to enhance local adaptation (Bürger, 1999), viruses are not an exception (García-Arenal et al., 2001; Michalakis and Roze, 2004; Moya et al., 2004; Roossinck, 1997).

Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) constitute a group of plant viruses that exploit gene flow provided by recombination (Chatchawankanphanich and Maxwell, 2002; Monci et al., 2002; Padidam et al., 1999; Pita et al., 2001; Preiss and Jeske, 2003; Sanz et al., 2000; Zhou et al., 1997). They are transmitted in nature by the whitefly (*Hemiptera:Aleyrodidae*) *Bemisia tabaci* and have small twinned (geminate) icosahedral virions that encapsidate circular single-stranded (ss) DNA genomes (Stanley et al., 2005). Most begomoviruses have bipartite genomes comprising two DNA components (DNAs A and B). The DNA A encodes a replication-associated protein (Rep), the coat protein (CP), and proteins such as replication enhancer protein (REn), and transcription activator protein (TrAP), that participate in the

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control of replication and gene expression. The DNA B encodes proteins that are required for virus movement in plants. Open reading frames (ORFs) are organized bi-directionally in both genome components, separated by an intergenic region (IR) that contains key elements for the replication and transcription of the viral genome, including the origin of replication (reviewed in Hanley-Bowdoin et al., 2000). Several begomoviruses have been reported that consist of a single genomic component that resembles DNA A, among them most of the viruses associated with tomato yellow leaf curl disease (TYLCD) (Moriones and Navas-Castillo, 2000).

During the last two decades, begomoviruses have emerged worldwide as a result of the spread of their insect vector *B. tabaci* (Rybicki and Pietersen, 1999), causing diseases to a wide variety of plant species, some of them of great agricultural importance (Chatchawankanphanich and Maxwell, 2002; Czosnek and Laterrot, 1997; Ribeiro et al., 2003; Stanley et al., 2005; Stonor et al., 2003). Recent introduction of begomoviruses into new areas provides an ideal model to analyze aspects of genetic adaptation and evolution of an invading virus. The spread of begomoviruses in Spain is well documented (Sánchez-Campos et al., 1999, 2002). The first reports of infections were of the early 1990s, associated with the presence of *Tomato yellow leaf curl Sardinia virus* (TYLCSV). Subsequent introductions of *Tomato yellow leaf curl virus* (TYLCV) strains were reported, which provided the substrate for interactions, and spread to new host species (Monci et al., 2002; Morilla et al., 2003, 2005; Navas-Castillo et al., 1999).

Native species, acting as reservoirs can play an important role in the emergence of plant virus epidemics (Hull, 2002). For begomoviruses, studies are available, for example, in the *Eupatorium makinoi*–begomovirus system, that have described the effects of virus infections in natural plant communities (reviewed by Funayama-Noguchi, 2001). However, studies to understand the genetic structure and dynamics of begomovirus populations in wild reservoirs and possible effects on epidemics of cultivated species are scarce and less detailed (Frischmuth et al., 1997; Jovel et al., 2004; Ooi et al., 1997; Roye et al., 1997, 1999; Sanz et al., 2000). In this report, we provide information about a wild reservoir of TYLCD-associated viruses, that might help to get an insight into the bases of begomovirus epidemics and evolution. We examine the begomovirus population present in *Solanum nigrum* L., a wild host species (Bedford et al., 1998; Salati et al., 2002; Sánchez-Campos et al., 2000) widely distributed in the Mediterranean region. Data are presented that support the contribution of *S. nigrum* as reservoir of genetic diversity for TYLCD-associated viruses. Also, we provide evidence of the presence of a novel begomovirus of recombinant nature for which putative parents, spatial (geographical area, host), and temporal origin are suggested. This recombinant is a step forward in the ecological adaptation of begomoviruses emerging in the invaded area. Novel genetic and pathogenic characteristics made the International Committee on Taxonomy of viruses to consider it as a representative of a new begomovirus species, for which the name *Tomato yellow leaf curl Axarquia virus* was proposed.

Results

S. nigrum is a reservoir of genetic diversity for TYLCD epidemics

A total of 53 samples were collected between 2000 and 2003 in Málaga (southern Spain) from *S. nigrum* plants exhibiting symptoms of TYLCD-associated virus infection. All reacted positively in a hybridization analysis for detection of TYLCD-associated viruses. To identify the genetic diversity of the begomovirus population present in this host species, the nucleotide sequence of the IR was determined for a number of virus-related DNA fragments amplified from these samples. The 35 sequences thus obtained, have been deposited in the EMBL/GenBank/DDBJ Nucleotide Sequence Database under accession numbers DQ121456 to DQ121490. Phylogenetic reconstruction showed that sequences corresponding to all the TYLCD-associated begomoviruses species and strains reported to date in Spain were present in *S. nigrum*: the type and Mld strains of TYLCV, the ES strain of TYLCSV, and the type strain of *Tomato yellow leaf curl Málaga virus* (TYLCMalV) (Stanley et al., 2005) (Fig. 1). Similar phylogenetic relationships were deduced using either maximum-likelihood, parsimony, or neighbor-joining methods (data not shown). Therefore, these results suggested the relevance of this wild host as reservoir of viral genetic diversity for TYLCD epidemics. Interestingly, the presence of mixed infections in single *S. nigrum* plants was apparent, as observed for example, in sample Sn8:00, in which TYLCV-like and TYLCSV-like sequences were detected (ES: Sn8-1:00, ES:Sn8-2:00, and ES:Sn8-3:00, in Fig. 1). The pattern observed in the phylogenetic tree shows radial divergence of clades from an ancestral state. Also, phylogenetic reconstruction demonstrated that 34% of the sequences obtained from *S. nigrum* samples consistently grouped in a clade that could not be associated with any previously reported begomovirus. Furthermore, abnormally severe infection symptoms were observed in the field samples from which those sequences were derived. Therefore, an isolate representative of this group was further studied.

Isolate ES:Alg:00 represents a previously undescribed begomovirus

The complete genome sequence of an isolate representative of the clade discussed above (isolate named ES:Alg:00) was deduced from one full-length recombinant clone derived from sample Sn8:00. The sequence consisted of 2,772 nucleotides and has been deposited in the EMBL/GenBank/DDBJ Nucleotide Sequence Database under accession number AY227892. The genome organization is typical of monopartite begomoviruses, containing in the IR the stem–loop structure conserved in the geminiviruses that includes the Rep nick site (TAATATT↓AC) where rolling circle replication is initiated (Laufs et al., 1995). Detailed analysis of the genome of ES:Alg:00 revealed that it shares less than 89% nucleotide sequence identity relative to any previously characterized begomovirus; moreover, comparison of individual ORFs suggested that Rep and C4