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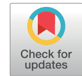
Lanai: A small, fast growing tomato variety is an excellent model system for studying geminiviruses



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ABSTRACT

Geminiviruses are devastating single-stranded DNA viruses that infect a wide variety of crops in tropical and subtropical areas of the world. Tomato, which is a host for more than 100 geminiviruses, is one of the most affected crops. Developing plant models to study geminivirus-host interaction is important for the design of virus management strategies. In this study, "Florida Lanai" tomato was broadly characterized using three begomoviruses (*Tomato yellow leaf curl virus,* TYLCV; *Tomato mottle virus,* ToMoV; *Tomato golden mosaic virus,* TGMV) and a curtovirus (*Beet curly top virus,* BCTV). Infection rates of 100% were achieved by agroinoculation of TYLCV, ToMoV or BCTV. Mechanical inoculation of ToMoV or TGMV using a microsprayer as well as whitefly transmission of TYLCV or ToMoV also resulted in 100% infection frequencies. Symptoms appeared as early as four days post inoculation when agroinoculation or bombardment was used. Symptoms were distinct for each virus and a range of features, including plant height, flower number, fruit number, fruit weight and ploidy, was characterized. Due to its small size, rapid growth, ease of characterization and maintenance, and distinct responses to different geminiviruses, "Florida Lanai" is an excellent choice for comparing geminivirus infection in a common host.

1. Introduction

Geminiviruses belong to a large, diverse family of plant infecting viruses (*Geminiviridae*) that are transmitted by insects and cause economically significant diseases worldwide (Zhang et al., 2001; Rojas et al., 2005; Hanley-Bowdoin et al., 2013). Geminiviruses are among the most economically important pathogens in a variety of crops including vegetables, fruits, root crops, cereals, spices and legumes (Morales and Anderson, 2001; Mansoor et al., 2003; Seal et al., 2006). The genomes of geminiviruses consist of either one (monopartite) or two (bipartite) circular, single-stranded DNA molecules, with the components of bipartite viruses known as DNA-A and DNA-B (Zhang et al., 2001; Brown et al., 2012; Hanley-Bowdoin et al., 2013). Geminiviruses are classified in nine genera according to their genome, host and insect vector (Zerbini et al., 2017).

Management of plant viruses is of vital importance to reduce the damage (Sastry and Zitter, 2014), especially in areas where food security is at risk due to high viral diversity and the emergence of more virulent strains (Damsteegt, 1999; Mansoor et al., 2003; Sastry and Zitter, 2014). In 2009, Rodrigues et al. (Rodrigues et al., 2009) concluded that disease management strategies need extensive knowledge of the virus infection, transmission, spread and their effects on host plants to select the best control measures. Studying viruses can be simplified if a tractable host system is available. The suitability of a host for studying the infection process is determined by its ability to become infected and to allow the virus to replicate and induce typical symptoms (Scholthof et al., 1996).

Geminiviruses, have been studied using model plant systems such as *Arabidopsis thaliana* (Muangsan et al., 2004; Ascencio-Ibáñez et al., 2008; Hanley-Bowdoin et al., 2013; Raja et al., 2014), *Nicotiana benthamiana* (Goodin et al., 2008), *Solanum nigrum* (Urbino et al., 2008), and *Datura stramonium* (Chen et al., 2013). These model plants have many advantages including small size, short life cycles, high seed germination rates and ease of genetic analysis (Meissner et al., 1997; Meinke et al., 1998; Matsukura et al., 2008). For example, *Arabidopsis* has one of the smallest genomes, making it useful for genetic

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Table 1
Infectious viral clones used to inoculate 'Florida Lanai' plants by agroinoculation or biolistics.

Virus[a]	Plasmid used for biolistics	Plasmid used for agroinoculation	References and comments
BCTV	BCTV in pMON521	BCTV in pMON521	Beet curly top virus (BCTV; strain Logan), a pMON525-based plasmid containing a BCTV DNA containing a partial tandem copy (provided by D. M. Bisaro of Ohio State University, Stenger et al., 1992).
TYLCV	pTYLCV2	pNSB1736	Partial tandem copy of Tomato yellow leaf curl virus (TYLCV; Dominican Republic isolate) cloned into pMON721 (Settlage et al., 2005; Reyes et al., 2013), from Acc. number AF024715.
ToMoV DNA A	pNSB1906	pNSB1906	Partial tandem copy of Tomato mottle virus (ToMoV) DNA-A cloned into pMON721 (Abouzid et al., 1992, Reyes et al., 2013)
ToMoV DNA B	pNSB1877	pNSB1877	Partial tandem copy of Tomato mottle virus (ToMoV) DNA-B cloned into pMON721 (Abouzid et al., 1992, Reyes et al., 2013)
TGMV DNA A	pMON1565	pMON337	Partial tandem copy of Tomato golden mosaic virus (TGMV) DNA-A (Fontes et al., 1994; Orozco and Hanley-Bowdoin, 1996; Elmer et al., 1988).
TGMV DNA B	pTG1.4B	pMON393	Partial tandem copy of Tomato golden mosaic virus (TGMV) DNA-B cloned in pTG1.4B (Fontes et al., 1994; Orozco and Hanley-Bowdoin, 1996).
CaLCuV DNA A	pCpCLCV A.003	pNSB1090	Cabbage leaf curl virus (CaLCuV) with a partial tandem copy (Turnage et al., 2002; Egelkrout et al., 2002).
CaLCuV DNA B	pCpCLCV B.003	pNSB1091	Cabbage leaf curl virus (CaLCuV) with a partial tandem copy (Turnage et al., 2002; Egelkrout et al., 2002).

[a] All clones have been designed to contain two viral origins of replication which allow the vector to release a functional viral monomer circularized by Rep and identical to wild-type viral DNA.

manipulation (Bevan and Walsh, 2006). Model plants are also usually inexpensive to study and readily accessible. However, information obtained using model plants may not always accurately reflect viral interactions or processes that occur in a non-model crop or reservoir plants in nature and disease can be the result of specific interactions between a virus and a host (Dawson and Hilf, 1992; Pallas and Garcia, 2011).

Of the 322 begomoviruses recognized by the International Committee on Taxonomy of Viruses, more than a third infect tomato and probably many others can infect solanaceous plants, underscoring the importance of having a suitable tomato variety for virus testing. Tomato (*Solanum lycopersicum* L., *Solanaceae*) is an herbaceous plant with hundreds of varieties that differ in size and generation time. Tomato has long been the preferred system for studying plant-pathogen interactions involving plants from the *Solanaceae* family (Arie et al., 2007; Meissner et al. 1997; Emmanuel and Levy, 2002). Tomato is susceptible to a wide range of viral diseases, many of which are associated with significant agronomic losses (Hanssen et al., 2010; Inoue-Nagata et al., 2016). As an example, tomato yellow leaf curl disease is caused by begomoviruses and has spread worldwide to become one of the most important viral diseases of tomato (Lefeuvre et al., 2010).

There is considerable physiological and genetic variation among tomato varieties that affects their suitability for laboratory studies. Among tomato varieties, Micro-Tom (TGRC accession # LA3911, UC Davis, Department of Plant Sciences, USA), a dwarf tomato cultivar derived from crossing cv. Florida Basket and Ohio 4013-3 (Scott and Harbaugh, 1989), is widely used in laboratory studies due to its small size (15–20 cm in height), rapid life cycle (70–90 days), and because it can be readily and efficiently transformed (Emmanuel and Levy, 2002; Meissner et al., 1997, Martí et al., 2006; Carvalho et al., 2011; Okabe et al., 2011; Sun et al., 2006). Studies require less time to complete because of its rapid life cycle that can accommodate up to four generations per year. Even though Micro-Tom has been widely adopted, its potential for molecular studies is limited because of its mutant genetic background, which results in brassinosteroid deficiency and deep green rugose leaves induced by the presence of the *dwarf (d)* and *miniature (mnt)* recessive genes (Bishop et al., 1996; Pnueli et al., 1998; Martí et al., 2006). The brassinosteroid pathway has been implicated in viral disease and symptom development, and alterations in the pathway may interfere with virus-plant interaction studies in Micro-Tom (Campos et al., 2010). Moreover, the gibberellin response is altered in Micro-Tom (Martí et al., 2006) and further interferes with data interpretation. In addition, Micro-Tom has a mutation in the *self-pruning (sp)* gene, which controls the regularity of the vegetative-reproductive switch along the compound shoot of tomato. This mutation is responsible for its determinate phenotype (Pnueli et al., 1998). Thus, it is important to look for new model systems that are either alternative or complementary to those currently used.

S. lycopersicum 'Florida Lanai' is also a small tomato variety that was developed for home gardens (Augustine et al., 1981). It has regular leaves and determinate growth, reaching a height of 60–90 cm. Flowers are open pollinated and produce a medium sized fruit (under 450 g) maturing approximately 60 days after transplanting or 90 days from seeding. Seed germination rate ranges from 82% to 96%. Even though 'Florida Lanai' plants are small and have a short generation time, they do not carry the recessive genes that place the use of Micro-Tom in doubt. 'Florida Lanai' has been used previously to characterize a new begomovirus species (Tomato yellow margin leaf curl virus) using biolistics to inoculate infectious clones (Nava et al., 2013). It has also been used to study geminivirus-insect interactions (McKenzie, 2002), although there has been no systematic characterization of its suitability as a model system for geminiviruses. In this study, we used three inoculation methods to examine 'Florida Lanai' as a model system for studying diverse geminiviruses that naturally infect tomato.

2. Materials and methods

2.1. Plant growth conditions and inoculation protocols

Florida Lanai seeds were kindly supplied by J. Scott (University of Florida, USA). 'Florida Lanai' plants were grown in sterile soil from seeds in a walk-in growth chamber at 25 °C, 80% humidity and a 16:8 light/dark (LD) cycle. After one week, the seedlings were transplanted into pots and propagated for two more weeks before inoculation. Virus inoculation was done by either *Agrobacterium*-mediated inoculation, low-pressure particle acceleration DNA delivery using a microdrop sprayer (Venganza, Inc.) or whitefly transmission from infected to healthy plant. The infectious clones corresponding to *Beet curly top virus* (BCTV), *Tomato yellow leaf curl virus* (TYLCV), *Tomato mottle virus* (ToMoV DNA-A and DNA-B), *Tomato golden mosaic virus* (TGMV DNA-A and DNA-B), *Cabbage leaf curl virus* (CaLCuV DNA-A and DNA-B), are described in Table 1. *E. coli* cultures for TYLCV, ToMoV, TGMV and CaLCuV DNA A and DNA B were prepared in LB broth containing 0.1 μg/ml carbenicillin, subsequently grown overnight at 37 °C with vigorous shaking. Similarly their corresponding *Agrobacterium* clones were prepared in LB broth containing 0.075 μg/ml Spectinomycin

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