Note

**Arabidopsis thaliana as a model host for Brevipalpus mite-transmitted viruses**

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Abstract: Brevipalpus-transmitted viruses (BTV) are a taxonomically diverse group of plant viruses which severely affect a number of major crops. Members of the group can be subclassified into cytoplasmic (BTV-C) or nuclear type (BTV-N) according to the accumulation sites of virions in the infected plant cells. Both types of BTV produce only local infections near the point of inoculation, by viruliferous mites. Features of BTV-plant interactions such as the failure of systemic spread in their natural hosts are poorly understood. In this study we evaluated *Arabidopsis thaliana*, a model plant commonly used for the study of plant-virus interactions, as an alternative host for BTV. Infection of Arabidopsis with the BTV-C Coffee ringspot virus and *Clerodendrum* chlorotic spot virus, and the BTV-N *Solanum* violaefolium ringspot virus, were mediated by viruliferous Brevipalpus mites collected in the wild. Upon infection, local lesions appeared in 7 to 10 days on leaves of, at least, 80% of the assayed plants. Presence of viral particles and characteristic cytopathic effects were detected by transmission electron microscopy (TEM) and the viral identities confirmed by specific reverse-transcriptase polymerase chain reaction (RT-PCR) and further amplicon sequencing. The high infection rate and reproducibility of symptoms of the three different viruses assayed validate *A. thaliana* as a feasible alternative experimental host for BTV.  

Keywords: *Clerodendrum* chlorotic spot virus, *Cleivirus*, *Solanum violaefolium* ringspot virus, *Coffee ringspot virus*, *Dichorhavirus*

Introduction

Plant viruses vectored by species of false spider mites of the genus *Brevipalpus* [Tenuippalpidae] are known as *Brevipalpus*-transmitted viruses [BTV] [Kitajima et al., 2014]. As a group, BTV affect, at least, 40 plant species ranging from major crops such as citrus and coffee, to economically important cultivated plants such as orchids and passion fruit, and other less significant ornamentals [Bastianel et al., 2010; Kitajima et al., 2010; Ramalho et al., 2014]. The BTV-caused citrus leprosis is the main viral disease affecting citrus production in Brazil and is acknowledged to be re-emergent in the Americas [Roy et al., 2015].  

BTV are classified as cytoplasmic (BTV-C) or nuclear (BTV-N) according to their replication and accumulation in plant cells. Taxonomically, BTV-C belong to at least one genus, *Cleivirus* [bipartite (+) single-stranded (ss) RNA] [Locali-Fabris et al., 2012], and possibly to *Higrevirus* [tripartite (+) ss RNA] [Melzer et al., 2012]. BTV-N are classified into the recently created genus *Dichorhavirus* [bipartite, [-] ss-RNA] [Afonso et al., 2016; Dietzgen et al., 2014]. Overall, BTV are intriguingly atypical since differently from other plant-virus systems in nature, viral long distance movement is not accomplished in any of their known hosts.

Although BTV were first identified in the early 20th century, molecular information concerning plant-BTV interaction remains barely known. Natural hosts of BTV show large, complex (e.g. citrus, coffee, orchids) [Cai et al., 2015; Xu et al., 2013; Wu et al., 2014; Denoeud et al., 2014] or unknown genomes (e.g. passion fruit) and may require customized installation for their growth and reproduction of BTV-caused diseases. Complexity of the research on the molecular processes involved in plant-BTV pathosystems may be partly bypassed by using appropriate experimental host systems. *Arabidopsis thaliana* appears as the primary alternative host model for plant-pathogen interaction studies, benefiting from a high-quality curated genome and several resources for reverse genetics approaches [Nishimura and Dangl, 2010].

Recently, we reported Arabidopsis as experimental host for *Citrus leprosis virus C* (CILV-C), a BTV-C [Arena et al., 2013; Ramos-González et al., 2016]. In this work, Arabidopsis plants were assessed for their capacity to host a wider range of BTV including another BTV-C, *Solanum violaefolium* ringspot virus (SvRSV), and two BTV-N, *Coffee ringspot virus* (CoRSV) and *Clerodendrum* chlorotic spot virus (CICSV) [Kitajima et al., 2010].

Materials and Methods

*Brevipalpus* spp. mites were collected from *Solanum violaefolium*, *Coffee arabica* and *Clerodendrum speciosum* infected with SvRSV, CoRSV and CICSV, respectively. Plants from wild type *A. thaliana* ecotype Columbia [Col-0] were obtained from the Arabidopsis Biological Resource Center [ABRC] and grown at 22 ± 2 °C with a 12-h light cycle in an environmental-controlled growth chamber Adaptis AR A1000 (Winnipeg, Canada).
Results and Discussion

During the last few decades, *A. thaliana* has become the major experimental model for plant biology, including plant-pathogen interactions [Nishimura and Dangl, 2010]. Besides the unique characteristics of the plant such as short generation time and small size (allowing for rapid growth and analysis of a large number of individuals in a minimum of space), its genome is compact and completely curated, and a wide mutant collection is available. Much of the current knowledge about the mechanisms underlying plant disease resistance and susceptibility has been discovered studying Arabidopsis patholgy and then translated to natural host systems [Nishimura and Dangl, 2010].

We have previously showed that CiLV-C is able to infect Arabidopsis, inducing localized chlorotic symptoms upon infestation with *Brevipalpus* viruliferous mites [Arena et al., 2013; Ramos-González et al., 2016]. In Arabidopsis, symptoms of CiLV-C infection occur in approximately 1/3 of the time they appear in sweet orange (*Citrus sinensis*), the virus’ natural host. Shorter incubation time represents a significant gain on experiments evaluating plant-virus interaction. In this study, we tested the susceptibility of Arabidopsis to SvRSV, a BTV-C and putative member of *Cilevirus*, CoRSV and CICSV, two BTV-N and possible members of the genus *Dichorhavirus*.

After 7 to 10 days of infestation with *Brevipalpus* spp. viruliferous mites, localized lesions appeared on the inoculated leaves of more than 80% of the plants in the sets corresponding to each evaluated virus [16 symptomatic /20 infested plants for CoRSV; 4/5 for CiCSV and 4/5 for SvRSV]. Arabidopsis plants infected with CoRSV exhibited brown patches not observable in the leaves infected either with CICSV or SvRSV. However, chlorotic spots on green leaves and green isolated islands on yellow senescent leaves were observed as a common response to the three inoculated viruses [Figure 1A, B, C]. This pattern of symptoms resembles that previously described for Arabidopsis infected with GILV-C [Arena et al., 2013] and also those occurring during infections of SvRSV, CoRSV and CICSV in their natural hosts [Figure 1D, E and F]. However, in natural hosts a necrotic area usually developed in the center of the chlorotic spots. Necrosis was not visible in any of the Arabidopsis plants infected with BTV assayed in this work. Probably, accelerated senescence that takes place on the Arabidopsis infected leaves hampers observation of such symptoms.

Table 1 − List of primers used to detect *Brevipalpus*-transmitted viruses (BTV).

<table>
<thead>
<tr>
<th>BTV</th>
<th>Primer sequence (5’–3’)</th>
<th>Target</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoRSV</td>
<td>GGACCAGTGAACAGAGAGGTG</td>
<td>ORF RdRp</td>
<td>389</td>
<td>Kitajima et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>CTCTGCGATGCTCTCAGTTG</td>
<td>RNA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SvRSV</td>
<td>TGGCGAATTGGGATGATCGG</td>
<td>ORF RdRp</td>
<td>596</td>
<td>Ferreira et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>CCGGTCTGCTTAATACTCC</td>
<td>RNA1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CICSV</td>
<td>ATATCACCATTAAACAAGGC</td>
<td>ORF RdRp</td>
<td>638</td>
<td>Unpublished</td>
</tr>
<tr>
<td></td>
<td>TCTGTGTGACATCTCTGCG</td>
<td>RNA2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CoRSV: *Coffee ringspot virus; SvRSV: Solanum violaefolium ringspot virus; CICSV: Clerodendrum chlorotic spot virus.*

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symptom. Furthermore, as expected, no disease symptoms were observed in the cauline systemic leaves of Arabidopsis, attesting to the typical non-systemic pattern of BTV infection under natural conditions.

Transmission electron microscopy analyses of plants inoculated with SvRSV allowed detecting enveloped bacilliform particles of ca. 50 × 100 nm in large pockets of the endoplasmic reticulum of cells of chlorotic lesions. Dense viroplasms were also seen in the cytoplasm of these cells (Figure 2A, B). In contrast, in leaf lesions from plants infected with CoRSV and ClCSV, non-enveloped bacilliform, rod-like virions of ca. 40 × 110 nm were observed in both the nucleoplasm and cytoplasm of palisade parenchyma cells. Most particles were arranged perpendicularly to the membranes of the endoplasmic reticulum and the nuclear envelope. Electron lucent viroplasms were detected in the nucleus of CoRSV and ClCSV infected cells, although they were more easily observed in the former case (Figure 2C, D, E, F). In general, virions and cytopathic effects seen in Arabidopsis were identical to those previously described in the natural hosts of SvRSV, CoRSV and ClCSV (Kitajima et al., 2014).

To confirm the identity of viruses present in symptomatic Arabidopsis, RT-PCR tests using specific primers were conducted. As expected, amplicons of approx. 600, 400 and 600 bp were obtained in samples from SvRSV, CoRSV and ClCSV, respectively (Figure 3). Consensus sequences of SvRSV and CoRSV amplicons showed identity values as high as 99 % with sequences obtained from the cognate viruses in the naturally infected hosts available under GenBank accessions number DQ514336 (Solanum violaefolium ringspot virus replicase-associated protein) and GQ979998 (Coffee ringspot virus isolate Cordeiropolis RNA-dependent RNA polymerase gene). For ClCSV, the
amplicons showed 100% with the viral sequence in the Clerodendrum plant used as the viral source. However, these sequences exhibited only 69% of identity with the sequence previously described for Citrus leprosis virus C (Kitajima et al., 2010) and 401564/2012-6). The authors wish to thank Luana Rogério, Alex Junior Soares and Thaís Sinico for their technical assistance.

In conclusion, the appearance of localized symptoms in the infected plants, the visualization of typical BTV particles and viroplasms in infected cells, and the confirmation of the identity of the viruses at nucleotide sequence level validated Arabidopsis as an alternative host for both BTV-C and -N. High susceptibility of this plant to mite-mediated transmission of BTV and its reduced time for symptom appearance will likely boost research on understanding the interactions involving this peculiar group of plant viruses and their hosts.

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