UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Agronomia Eliseu Maciel
Programa de Pós-Graduação em Fitossanidade

Tese

Aspectos da associação entre *Leonurus yellow spot alphasatellite* e begomovírus bissegmentados e identificação de *Colletotrichum karstii* em pitaia

Monique Bezerra Nascimento

Pelotas, 2019

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Tese apresentada ao Programa de Pós-Graduação em Fitossanidade da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Fitossanidade (área de conhecimento: Fitopatologia)

Orientador (a): Profª. Dra Danielle Ribeiro de Barros
Coorientador: Profº. Dr. Francisco Murilo Zerbini Junior

Pelotas, 2019

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

N244a Nascimento, Monique Bezerra

Aspectos da associação entre *leonurus yellow spot alphasatellite* e begomovírus bissegmentados e identificação de *colletotrichum karstii* em pitaia / Monique Bezerra Nascimento ; Danielle Ribeiro de Barros, orientadora ; Francisco Murilo Zerbini Junior, coorientador. — Pelotas, 2019.

78 f. : il.

Tese (Doutorado) — Programa de Pós-Graduação em Fitossanidade, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2019.

1. Begomovírus. 2. Alfassatélite. 3. Antracnose. 4. *Hylocereus*. I. Barros, Danielle Ribeiro de, orient. II. Zerbini Junior, Francisco Murilo, coorient. III. Título.

CDD : 634.0493

Elaborada por Dafne Silva de Freitas CRB: 10/2175

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Tese aprovada, como requisito parcial, para obtenção do grau de Doutora em Fitossanidade, Programa de Pós-Graduação em Fitossanidade, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas.

Data da Defesa: 27/08/2019

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Dedico a minha orientadora e coorientador, que me ensinaram sobre ciência e aos meus pais, Maria do Amparo e José Wilson, que me ensinaram sobre a vida. Ao meu noivo José Janderson, pelo carinho, paciência, apoio incondicional e pelo incentivo nas minhas decisões, em todos os momentos desta caminhada. E ao meu irmão Bryan Wilson pela amizade.

Agradecimentos

Agradeço primeiramente a Deus por ter me dado força, saúde e determinação para vencer esta etapa.

À Universidade Federal de Pelotas e ao Programa de Pós-Graduação em Fitossanidade pela oportunidade de realização do curso de doutorado.

À professora Drª. Danielle Ribeiro de Barros, pela orientação e ensinamentos.

Ao professor Dr. Francisco Murilo Zerbini Junior pela coorientação, pela oportunidade, dedicação e ensinamento, sendo exemplo de bom profissional.

À Universidade Federal de Viçosa (UFV) e ao Laboratório de Virologia Vegetal Molecular (LVVM), por oferecer o espaço e a infraestrutura adequada para a execução deste trabalho.

Aos familiares que me apoiaram e incentivaram em todos os momentos da minha vida, em especial minha amada mãe Amparo, meu pai Wilson e ao meu irmão Bryan Wilson. Aos meus padrinhos, Marlene Bezerra e Alexandre Fonseca, pelo apoio. E ao meu noivo Janderson, pelo companheirismo.

Aos amigos do laboratório de Virologia Vegetal da UFPel, Carolina Garcia, Ismail Teodoro, Johan e especialmente Silvia Paz por todo o apoio e amizade.

Aos amigos do laboratório de Virologia Vegetal Molecular da UFV, em especial aos amigos Ayane Fernanda, Tales Mendes, Angélica Nogueira e Tarsiane Barbosa, que tornaram os momentos de trabalho mais descontraídos e animados, além do companheirismo, amizade e carinho.

Aos colegas do laboratório de Virologia Vegetal Molecular da UFV, João Paulo, Roberta, Bernardo, César, Osvaldo, Baltazar, Marcos e Anelise, Guilherme e a querida técnica Patrícia.

Às amigas que sempre me apoiaram em todos os momentos, Aline Hipólito, Vanessa Gonçalves, Dayane Lopes, Iana Quadros e Izabel Figueiredo.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de estudos.

A todos aqueles, que de alguma forma, contribuíram direta e indiretamente para a realização deste trabalho.

Muito Obrigada!

“O saber a gente aprende com os mestres e os livros. A sabedoria, se aprende com a vida e os humildes”

(CORA CORALINA)

Resumo

NASCIMENTO, Monique Bezerra. **Aspectos da associação entre *Leonurus yellow spot alphasatellite* e begomovírus bissegmentados e identificação de *Colletotrichum karstii* em pitaia.** 2019. 78f. Tese (Doutorado) – Programa de Pós-Graduação em Fitossanidade. Universidade Federal de Pelotas. Pelotas, RS, Brasil.

As doenças de plantas são responsáveis por grandes perdas econômicas na agricultura. Dentre os vírus, o grupo que ressalta-se pela grande importância são os begomovírus que podem ser encontrados em associação com alfassatélites que dependem do begomovírus auxiliar para causar infecção sistêmica. O efeito da associação entre alfassatélites e begomovírus ainda são desconhecidos. No caso das doenças causadas por fungos destacam-se as espécies do gênero *Colletotrichum* por causarem doenças em uma ampla variedade de culturas. Deste modo, os objetivos desta tese foram: i) verificar a capacidade de interação de um alfassatélite, o *Leonurus yellow spot alphasatellite* (LeYSA), com três begomovírus (*Euphorbia yellow mosaic virus*, EuYMV; *Tomato yellow spot virus*, ToYSV; e *Tomato severe rugose virus*, ToSRV), investigando os efeitos da presença do alfassatélite na infectividade, sintomas, acúmulo de DNA viral em três hospedeiros (*Leonurus sibiricus*, *Nicotiana benthamiana* e tomateiro)), além de verificar se os alfassatélites interferem na transmissão de ToYSV e ToSRV pelo inseto vetor (*Bemisia tabacci*); ii) identificar o agente causal de antracnose em folhas de pitaia. Para verificar a capacidade de interação do alfassatélite em três begomovírus foram inoculados via biobalística nas plantas hospedeiras, a porcentagem de infectividade foi realizada através da detecção da presença e ausência dos vírus e do alfassatélite por PCR convencional e a quantificação do título viral e do alfassatélite foi realizado por qPCR. A interação de ToYSV e ToSRV com o LeYSA levou a uma maior severidade dos sintomas na presença do alfassatélite. A associação de LeYSA com ToYSV foi menos eficiente em tomateiro comparado a *L. sibiricus* e *N. benthamiana*, com base na menor porcentagem de plantas nas quais a presença do alfassatélite foi detectada. A associação de LeYSA com EuYMV foi menos eficiente em tomateiro do que em *N. benthamiana*, enquanto a interação com o ToSRV foi semelhantes em ambos os hospedeiros (EuYMV e ToSRV não infectaram *L. sibiricus*). Os resultados deste estudo fornecem novas informações que aumentam a compreensão das interações entre vírus, DNAs satélites e hospedeiros que contribuem para a adaptação e predominância de begomovírus no campo. Em agosto de 2017, observou-se plantas de pitaia no município de Pelotas (RS) com sintomas típicos de antracnose, as folhas foram submetidos a caracterização morfológica, molecular e ao teste de patogenicidade para identificação do agente causal em estudo. Confirmou-se que a antracnose em pitaia é causada por um fungo do gênero *Colletotrichum* a partir das características morfológicas observadas na massa conidial, conídios hialinos, asseptatos, retos, cilíndricos e obtusos no ápice. A análise das sequências de nucleotídeos das regiões ITS, ACT, TUB2, CAL, GAPDH, CHS-1 e HIS3 do fungo isolado de pitaia indicou 99-100% de identidade com sequências correspondente ao *Colleotrichum karstii*. Este é o primeiro relato de *C. karstti* infectando pitaia no Brasil.

Palavras-chave: Begomovírus. Alfassatélite. Antracnose. *Hylocereus.*

Abstract

NASCIMENTO, Monique Bezerra. **Aspects of the association between *Leonurus yellow spot alphasatellite* and bipartite begomoviruses, and identification of *Colletotrichum karstii* in pitaya.** 2019. 78f. Thesis (Doctorate) – Graduate Program in Plant Protection. Universidade Federal de Pelotas. Pelotas, RS, Brasil.

Plant diseases are responsible for large economic losses in agriculture. Among viruses, one group that stands out for its great importance are the begomoviruses. They can be found in association with alphassatellites, which depend on the helper begomovirus to cause systemic infection. The impact of the association between alphasatellites and begomoviruses remains largely unknown. In the case of fungal diseases, species of the genus *Colletotrichum* stand out because they cause disease in a wide variety of cultures. The main objectives of the thesis were: i) to verify the interaction of *Leonurus yellow spot alphasatellite* (LeYSA) with three begomoviruses (*Euphorbia yellow mosaic virus*, EuYMV; *Tomato yellow spot virus*, ToYSV; and *Tomato severe rugose virus*, ToSRV), investigating the effects of the presence of the alphasatellite in infectivity, symptoms, accumulation of viral DNA in three hosts (*Leonurus sibiricus*, *Nicotiana benthamiana* and tomato), in addition, it is possible to verify if alphasatellites interfere with the transmission of ToYSV and ToSRV by the vector insect (*Bemisia tabacci*). ii) to identify the causal agent of anthracnose on pitaya leaves. To verify the interaction ability of the alpha-satellite in three begomoviruses were inoculated via biobalistic in the host plants, the percentage of infectivity was performed by detection of the presence and absence of viruses and alphasatellite by conventional PCR and quantification of the viral titer and alphasatellite was performed by qPCR. The interaction of ToYSV and ToSRV with LeYSA was demonstrated, and showed a greater severity of symptoms in the presence of the alphasatellite. The association of LeYSA with ToYSV was less efficient in tomato plants compared to *L. sibiricus* and *N. benthamiana*, as measured by a lower percentage of plants in which the presence of the alphasatellite was detected. The association of LeYSA with EuYMV was less efficient in tomato than in *N. benthamiana*, while the interaction with ToSRV was similar in both hosts (EuYMV and ToSRV did not infect L. sibiricus).The results of this study provide new information that increases the understanding of the interactions between viruses, satellite DNAs, vector and host that contribute to the adaptation and predominance of begomoviruses in the field. In August of 2017, pitaya plants in the municipality of Pelotas (RS) were observed with typical symptoms of anthracnose. The leaves were submitted to morphological, molecular characterization and pathogenicity test to identify the causal agent under study. It has been confirmed that the anthracnose in pitaya was being caused by a fungus of the genus *Colletotrichum* from the morphological characteristics observed in the conidial mass, hyaline conidia, asseptates, straight, cylindrical and obtuse apex. Analysis of the nucleotide sequences of the ITS, ACT, TUB2, CAL, GAPDH, CHS-1 and HIS3 regions of the isolate pitaia fungus showed 99-100% identity with the sequences corresponding to *Colleotrichum karstii*. This is the first report of *C. karstti* infecting pitaya in Brazil.

Keywords: Begomoviruses. Alphasatellite. Anthracnose. *Hylocereus.*

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Sumário

1 Introdução geral

As doenças de plantas são responsáveis por grandes perdas econômicas na indústria agrícola mundial, representando mais de 40% das perdas totais de produção na maioria dos países em desenvolvimento (SHARMA, 2013). No Brasil, a agricultura representa um dos pontos fortes do setor econômico e as doenças de plantas são um dos maiores desafios enfrentados (MARTIN NETO et al., 2016).

Dentre as doenças de origem biótica que incidem nas colheitas e causam grandes perdas na produção, podemos destacar dois grandes grupos de agentes envolvidos, os fungos e os vírus. Os danos causados por estes patógenos variam de intensidade (brandos a severos) dependendo da adaptabilidade ao hospedeiro, bem como do ambiente propício para o desenvolvimento do patógeno. O monitoramento dos plantios e a detecção precoce dos agentes fitopatogênicos são medidas essenciais para reduzir a disseminação de doenças e facilitar as práticas de manejo de maneira mais eficaz (MARTINELLI et al., 2015).

Dentre as diferentes famílias e gêneros de vírus de plantas, os membros do gênero *Begomovirus* incluem um grande número de patógenos de relevância econômica em culturas de grande importância como o algodão, o feijão, a mandioca e o tomate (ROJAS et al., 2018).

Os begomovírus pertencem à família *Geminiviridae*, que é constituída por vírus com genoma composto por um ou dois componentes de DNA de fita simples circular encapsidados em partículas icosaédricas geminadas. A família é formada pelos gêneros *Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Eragrovirus, Grablovirus, Mastrevirus, Topocuvirus* e *Turncurtovirus*, definidos com base no tipo de inseto vetor, gama de hospedeiros, organização genômica e relacionamento filogenético (BROWN et al., 2012; VARSANI et al., 2014; VARSANI et al., 2017; ZERBINI et al., 2017).

Os begomovírus podem ser divididos em dois grupos, os do "Velho Mundo" (VM; Europa, África e Ásia) e os do "Novo Mundo" (NM; as Américas) (RYBICKI, 1994; PADIDAM et al., 1999; PAXIMADIS et al., 1999). A maioria dos begomovírus do NM possuem dois componentes genômicos conhecidos como DNA-A e DNA-B, e são transmitidos por moscas-brancas (*Bemisia tabaci*) a

plantas dicotiledôneas (BROWN et al., 2015). O DNA-A contém genes envolvidos na replicação e encapsidação da progênie viral. O DNA-B contem genes necessários para movimentos intra e intercelular na planta (LAZAROWTIZ et al., 1992). Ambos os componentes genômicos são necessários para a infecção sistêmica do hospedeiro.

No VM, a maioria dos begomovírus estão associado a DNAs satélites, incluindo os alfassatélites (família *Alphasatellitidae*; BRIDDON et al., 2018). Alfassatélites são moléculas de DNA circular de fita simples que replicam independente do vírus auxiliar (um begomovírus ou um membro da família *Nanoviridae*), contudo dependem deste para infecção sistêmica e transmissão pelo inseto vetor (ZHOU, 2013; BRIDDON et al., 2018). Recentemente, alfassatélites foram relatados em associação com begomovírus do NM, infectando plantas não-cultivadas (*Cleome affinis*, *Euphorbia heterophylla*, *Leonurus sibiricus* e *Sida* sp.) no Brasil e em Cuba (PAPROTKA et al., 2010; JESKE et al., 2014; FERRO et al., 2017; MAR et al., 2017) e em cultivos de melancia na Venezuela (ROMAY et al., 2010).

O efeito da presença de alfassatélites na infecção por begomovírus ainda não está esclarecido. Estudos realizados com alfassatélites e begomovírus no VM relataram que os alfassatélites não alteram os sintomas causados pelos begomovírus (SAUNDERS; STANLEY, 1999; SAUNDERS et al., 2000; SAUNDERS et al., 2002). Entretanto, os alfassatélites do NM parecem contribuir positivamente para a infecção, aumentando a severidade dos sintomas causados pelo begomovírus auxiliar, ou até mesmo sendo um determinante para a indução de sintomas (PAPROTKA et al., 2010; MAR et al., 2017). Nesse sentido, demonstrou-se que a proteína alpha-Rep codificada pelos alfassatélites pode atuar como supressora do silenciamento gênico transcricional (ABBAS et al., 2016), o que provavelmente favorece a patogenicidade.

Os fungos são microorganismos eucarióticos, e constituem um grupo extremamente vasto com grande diversidade (AGRIOS, 2005; DOEHLEMANN et al., 2017). Estima-se que dois terços das doenças de plantas sejam causadas por fungos. Todas as plantas cultivadas de importância econômica são atacadas por um ou mais fungos, e frequentemente muitos fungos diferentes podem causar doenças em uma mesma espécie de planta (PELCZAR et al., 2019).

Um gênero de fungos que se destaca por incluir várias espécies de grande importância é o gênero *Colletotrichum,* como gênero fúngico assexual, foi incluído na classificação morfológica de Ascomycota como gênero sexual *Glomerella* (filo Ascomycota, classe Sordariomycetes, ordem Glomerellales, Família Glomerellaceae) (CANNON et al., 2012; MYCOBANK, 2018). Causadores de doenças em uma ampla variedade de plantas lenhosas e herbáceas, estão principalmente distribuídos em regiões tropicais e subtropicais, embora existam algumas espécies que afetam culturas de clima temperado (CROUS et al., 2004; UDAYANGA et al., 2013). O gênero *Colletotrichum* passou por várias reclassificações, atualmente mais de 200 espécies são reconhecidas por causar doenças em plantas em todo o mundo (MARIN-FELIX et al., 2017; UDAYANGA et al., 2013). As espécies patogênicas de *Colletotrichum* estão entre os principais causadores de antracnose, e também incluem diversos agentes causais de podridões de fruta pré- e pós-colheita e *damping-off* em plântulas (SUTTON et al., 1992; DEAN et al., 2012).

A identificação das espécies de *Colletotrichum* é tradicionalmente baseada em características morfológicas, como tamanho, formato dos conídios, conformações da célula conidiogênica, coloração da colônia (matiz e intensidade) e o formato dos apressórios (SUTTON et al., 1992; MENEZES 2002). A caracterização das espécies de *Colletotrichum* baseada unicamente em critérios morfológicos podem levar a conclusões incorretas, devido à grande variabilidade destas características para algumas espécies deste gênero, principalmente quando os isolados em estudo são cultivados em meios artificiais. Para contornar esses problemas, a combinação de técnicas complementares como bioquímicas e moleculares têm sido cada vez mais utilizadas para assegurar a acurácia na identificação e classificação de novas espécies de *Colletotrichum* (CANNON et al., 2012).

Os métodos moleculares baseados em sequencias de DNA possibilitam uma análise mais precisa permitindo confirmar a identidade do agente causal em estudo com rapidez e confiabilidade. Em vários estudos a comparação de sequências que codifica o DNA ribossomal (rDNA), bem como as sequências da região dos espaçadores internos transcritos ribossomal nuclear (conhecido como: *Internal Transcribed Spacer*, ITS) foi considerado como principal "barcode" utilizado para o reino dos fungos (SCHOCH et al., 2012). A

comparação da sequências da região ITS entre os gêneros *Colletotrichum* levaram ao desenvolvimento de oligonucleotídeos iniciadores (primers) táxons-específicos para a diferenciação entre espécies deste gênero por PCR (reação em cadeia da polimerase) (MILLS et al., 1992). Atualmente, esses primers vem sendo utilizado para a confirmação da identidade dos isolados patogênicos a diversas espécies hospedeiras (ALFANADOR-KAFURI et al., 2003; LUBBE et al., 2004; MORIKAWI; SATO; TSUKIBOSHI, 2003; PILEGGI et al., 2009)

Embora as sequências de ITS auxiliem na identificação de espécies de *Colletotrichum*, não fornecem resoluções suficientes para diferenciar espécies, principalmente espécies intimamente relacionadas (CAI et al., 2009; CROUCH et al., 2009; YANG et al., 2009; PHOULIVONG et al., 2010), já que esse fragmento é evolutivamente conservado para distinguir os táxons (DU et al., 2005, CROUCH et al., 2009a, GAZIS et al., 2011), sendo necessário aliar o uso do marcador ITS juntamente a outros marcadores moleculares, tais como, o gene que codificam o gliceraldeído-3-fosfato desidrogenase (GAPDH) e a β-tubulina (TUB-2), com isso, conseguindo distinguir espécies em *Colletotrichum* (DAMM et al., 2012; WEIR et al., 2012).

Pitaia (*Hylocereus* sp.) é o nome dado aos frutos de diversas espécies de cactos nativos das florestas tropicais da América Central e do Sul e Ásia, (NERD; MIZRAHI, 1999), é uma espécie exótica no Brasil. O cultivo da pitaia no Brasil é recente, seu cultivo se expandiu e atualmente existem áreas comerciais em Minas Gerais, Paraná, Santa Catarina, Mato Grosso do Sul, Rio Grande do Norte, Ceará e Pernambuco (SILVA, 2014).

A região Sudeste do Brasil é a principal produtora do país, onde a cultura da pitaia se aclimatou muito bem, com produção de frutos nos meses de dezembro a maio, e produtividade média anual de 14 toneladas de frutos por hectare (BASTOS et al. 2006).

Como toda cultura que entra em processo de aumento de produção, a pitaia vem sendo acometida por diferentes patógenos, os quais acarretam uma diminuição no rendimento com perdas de até 44% (VALENCÍA-BOTÍN et al., 2003). Doenças causadas por fungos são predominantes, sendo a principal doença o olho de peixe causada por *Botryosphaeria dothidea,* caracterizada por sintomas iniciais de pontos cloróticos no caule (MASYAHIT et al., 2009).

No Brasil, ainda são poucos estudos a respeito das doenças que ocorrem em pitaia, em 2008 foi pela primeira vez relatado no Brasil no Estado de São Paulo em Botucatu plantas de pitaia sendo acometidas por antracnose causadas por *Colletotrichum gloeosporioides* (TAKAHASHI et al., 2008). A antracnose, causada por *Colletotrichum gloesporioides* e *Colletotrichum truncatum,* afeta não só os frutos, mas também o caule e a característica marcante da doença é a presença de lesões marrom-avermelhadas coalescidas com halos cloróticos (KIM et al., 2000; GUO et al., 2014).

Assim, os objetivos desta tese foram: i) verificar a capacidade de interação do alfassatélite *Leonurus yellow spot alphasatellite* (LeYSA) com três begomovírus (*Euphorbia yellow mosaic virus*, EuYMV; *Tomato yellow spot virus*, ToYSV; e *Tomato severe rugose virus*, ToSRV), investigando os efeitos da presença do alfassatélites na infectividade, sintomas, acúmulo de DNA viral em três hospedeiros (*Leonurus sibiricus*, *Nicotiana benthamiana* e tomateiro), além de verificar se os alfassatélites interferem na transmissão de ToYSV e ToSRV pelo inseto vetor (*Bemisia tabacci*); ii) identificar o agente causal da mancha de antracnose em folhas e frutos de pitaia.

Artigo 1

ASPECTS OF THE ASSOCIATION BETWEEN *Leonurus yellow spot alphasatellite* AND BIPARTITE BEGOMOVIRUSES: EFFECTS ON INFECTION AND TRANSMISSION BY *Bemisia tabaci* Middle East-Asia Minor 1

Nascimento MB, Nogueira AM, Barbosa TMC, Quadros AFF, Barros, DR, Zerbini FM. Aspects of the association between *Leonurus yellow spot alphasatellite* and bipartite begomoviruses: Effects on infection and transmission by *Bemisia tabaci* Middle East-Asia Minor 1 **Archives of Virology**, *in preparation*.

2 Artigo 1 - Aspects of the association between *Leonurus yellow spot alphasatellite* and bipartite begomoviruses: Effects on infection and transmission by *Bemisia tabaci* Middle East-Asia Minor 1

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Abstract

The genus *Begomovirus* (family *Geminiviridae*) includes plant viruses with circular, single-stranded DNA (ssDNA) genomes which are transmitted by the withefly *Bemisia tabaci*. Begomoviruses in the New World can be found in association with alphassatellites, which are circular, ssDNA molecules, capable of autonomous replication but dependent on the helper begomovirus for encapsidation, systemic infection and insect transmission. The impact of the interaction between alphassatellites and begomoviruses is unknown. The objective of this work was to verify the effect of *Leonurus yellow spot alphasatellite* (LeYSA) in the infection induced by *Tomato yellow spot alphasatellite* (ToYSV), *Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV) in three hosts, *Leonurus sibiricus, Nicotiana benthamiana* and tomato. The plants were inoculated with each virus in the presence or absence of the alphasatellite. Percentage of infectivity and symptom development for each begomovirus alone or in the presence of LeYSA were evaluated, and viral DNA accumulation was quantified for each virus alone or in the presence of LeYSA. The association of LeYSA with ToYSV was less efficient in tomato that in *L. sibiricus* and *N. benthamiana*, as measured by a lower percentage of plants in which the presence of the alphasatellite was detected. The association between ToSRV and LeYSA was similar in both tomato and *N. benthamiana*. The association between EuYMV and LeYSA in tomato was the least efficient, and in *N. benthamiana* the presence of the alphasatellite was not detected in any of the plants infected with EuYMV. Together, these results indicate distinct levels of interaction between the alphasatellite and different

begomoviruses. Quantification of ToYSV and ToSRV DNA-A accumulation indicated that LeYSA does not intefere in the DNA accumulation of these begomoviruses. However, symptoms were more severe in the presence of LeYSA for both viruses and in all hosts. The accumulation of LeYSA varied in relation to the host and the associated begomovirus. Transmission assays with the whitefly indicated that LeYSA negatively affects the transmission of ToSRV. Together with previous experimental studies, these results further emphasize the potential risk of alphasatellites in cultivated and non-cultivated plants.

Keywords: alphasatellite, begomovirus, cultivated and non-cultivated hosts, whitefly.

Introduction

The family *Geminiviridae* is comprised of non-enveloped plant viruses with one or two circular, single-stranded (ss) DNA genomic components encapsidated by a single structural protein into twinned, quasi-icosahedral particles. The family includes nine genera (*Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* and *Turncurtovirus*) defined according to the type of insect vector, host range, genomic organization and phylogenetic relationships (Brown *et al.*, 2012; Varsani *et al.*, 2014; Varsani *et al.*, 2017; Zerbini *et al.*, 2017). The genus *Begomovirus* includes the most economically important viruses within the family, causing serious damage to crops in all tropical and subtropical regions of the world. Begomoviruses are transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex (Hemiptera:Aleyrodidae) and infect dicot plant species (Rojas *et al.*, 2018).

Begomoviruses can be divided into two major groups: Old Word (OW; Europe, Africa, Asia and Oceania) and New World (NW; the Americas), based on phylogenetic relationships and genomic features (Harrison & Robinson, 1999; Rojas *et al.*, 2005). Most NW begomoviruses possess a bipartite genome comprised of similarly sized (approx. 2.6 kb) genomic components referred to as DNA-A and DNA-B. The DNA-A contains genes involved in replication, encapsidation of the viral progeny and suppression of host defenses, while the DNA-B contains genes required for intra- and intercellular movement in the plant, host range determination and suppression of host defenses (Rojas *et al.*, 2005; Mahajan *et al.*, 2011; Hanley-Bowdoin *et al.*, 2013). The majority of the begomoviruses that occur in the OW are monopartite, with a genomic

organisation similar to the DNA-A of the bipartite viruses (Padidam *et al.*, 1996; Mansoor *et al.*, 2003).

Monopartite OW begomoviruses are frequently found in association with additional circular, ssDNA satellite molecules. Three types of DNA sattellites have been described: alphasatellites (previously known as DNA-1), betasatellites (previsouly known as DNA-β), and deltasatellites (Zhou, 2013; Lozano *et al.*, 2016). In the NW, only alphasatellites and deltasatellites have been detected so far, always in association with bipartite begomoviruses (Paprotka *et al.*, 2010; Romay *et al.*, 2010; Fiallo-Olivé *et al.*, 2016). These DNA satellites require a helper begomovirus to complete one or more steps of their infection cycle (Zhou, 2013; Lozano *et al.*, 2016).

Betasatellite genomes contain a region that is conserved among all betasatellites, named satellite conserved region (SCR), an adenine-rich region and a single open reading frame (ORF) in the complementary-sense strand encoding for the betaC1 protein. The presence of betasatellites is usually a determinant of symptoms due to an increase in the accumulation of their helper begomoviruses, probably due to the RNA silencing suppressor activity of the betaC1 protein (Briddon *et al.*, 2003; Cui *et al.*, 2005; Saeed *et al.*, 2005; Briddon *et al.*, 2008). Betasatellites have never been reported in association with NW begomoviruses.

Alphasatellite genomes are approximately 1.3 kb long (half the size of begomovirus components), and contain a stem-loop structure with a conserved nonanucleotide sequence (5'-TAGTATTAC-3') comprising the origin of replication, an A-rich region, and a single ORF in the virion-sense strand, encoding a replication-associated protein named alpha-Rep. The alpha-Rep

protein has significant sequence identity with the master-Rep protein encoded by the DNA-R component of nanoviruses (family *Nanoviridae*). Thus, alphasatellites are capable of independent replication in host plants but require a helper begomovirus for movement within the plant as well as for transmission by the whitefly vector (Saunders *et al.*, 2000; Saunders *et al.*, 2002; Briddon *et al.*, 2018).

Although alphasatellites were discovered more than a decade ago, little is known about the effects of their presence on the infectivity, accumulation of viral DNA and symptoms induced by the helper begomovirus. They are generally described as having no obvious effects on the induction of symptoms by begomoviruses or by begomovirus/betasatellite complexes (Zhou, 2013). An early report that the alpha-Rep protein may act as a suppressor of post-transcriptional gene silencing (Nawaz-Ul-Rehman *et al.*, 2010) has not been confirmed by other groups, and a more recent report indicated that alphaRep may act as a suppressor of transcriptional gene silencing (Abbas *et al.*, 2016). In contrast with reports of alpha-Rep as a suppressor of host defenses, an unusual alphasatellite (DNA-2-type) from Oman caused attenuation of the symptoms induced by a begomovirus/betasatellite complex by reducing accumulation of the betasatellite (Idris *et al.*, 2011).

Recently, two distintc alphasatellites have been found in association with NW bipartite begomoviruses infecting cultivated and non-cultivaded plants in Brazil, Cuba and Venezuela (Paprotka *et al.*, 2010; Romay *et al.*, 2010; Jeske *et al.*, 2014). These NW alphasatellites are more closely related to DNA-2-type alphasatellites than to the initially characterized, DNA-1-type alphasatellites, and

were classified as a third phylogenetically distinct group (DNA-3-type) (Rosario *et al.*, 2016).

Interestingly, the DNA-3-type alphasatellites found in Brazil were reported to increase the severity of symptoms induced by the helper begomovirus (Paprotka *et al.*, 2010; Mar *et al.*, 2017a). Symptoms induced by *Euphorbia yellow mosaic virus* (EuYMV) were more severe when it was inoculated in combination with *Euphorbia yellow mosaic alphasatellite* (EuYMA) in *N. benthamiana* and *Euphorbia heterophylla*, and the presence of the alphasatellite was required for symptom development in *Arabidopsis thaliana* (Mar *et al.*, 2017a). Moreover, these alphasatellites seem to display a wide range of hosts and flexibility in their association with begomoviruses: *Leonurus yellow spot alphasatellite* (LeYSA) was detected in association with *Tomato yellow spot virus* (ToYSV) infecting plants of *Leonurus sibiricus* (fam. Lamiaceae); EuYMA was detected in *Euphorbia heterophylla* (Euphorbiaceae) plants in association with *Euphorbia* yellow mosaic virus (EuYMV) and also in *Sida* spp. (Malvaceae) associated with *Sida micrantha mosaic virus*; *Cleome leaf crumple alphasatellite* (ClLCrA) was detected in association with *Cleome leaf crumple virus* (ClLCrV) in plants of *Cleome affinis* (Cleomaceae) (Paprotka *et al.*, 2010; Ferro *et al.*, 2017; Mar *et al.*, 2017a).

In this context, and considering the enormous diversity of begomoviruses infecting cultivated and non-cultivated plants in Brazil, it is important to better understand the dynamics of the interaction between begomoviruses and DNA-3-type alphasatellites, including any effects on vector transmission. Mar *et al.* (2017a) reported a decrease in the transmission efficiency of EuYMV by *Bemisia tabaci* MEAM1 when EuYMA was present. It is important to determine whether

this negative effect is restricted to this particular combination of begomovirus and alphasatellite, or is a general effect of the presence of DNA-3-type alphasatellites in plants infected by NW, bipartite begomoviruses.

The objective of this study was to verify the interaction capacity of the alphasatellite LeYSA with three begomoviruses: EuYMV, ToYSV and *Tomato severe rugose virus* (ToSRV; the most common begomovirus found in tomatoes in southern, southeastern and central Brazil). We investigated the effects of the presence of the alphasatellite on infectivity, symptom modulation, viral DNA accumulation and transmission by the whitefly vector in three hosts (*L. sibiricus*, *Nicotiana benthamiana* and tomato). An increase in the severity of symptoms induced by ToYSV and ToSRV in the presence of LeYSA was observed in all three hosts. Transmission assays with the whitefly indicated that LeYSA negatively affects the transmission of ToSRV.

Material and Methods

Construction of an infectious clone of *Leonurus yellow spot alphasatellite* (LeYSA)

An infectious clone of *Leonurus yellow spot alphasatellite* (LeYSA) was constructed using the full-length clone obtained by Ferro *et al.* (2017) from sample CF1095 of *Leonurus sibiricus*. This clone (BR:Dou1095.1:11; GenBank access number KX348228) was cleaved with *Eco*RI and *Pst*I, releasing a 400 nt fragment of the alphasatellite genome containing the common region. This fragment was cloned into the pBluescript KS+ plasmid vector (Stratagene). Then, the complete copy of the alphasatellite genome, linearized with *Eco*RI, was

inserted into the "0.3mer" clone generating constructs corresponding to 1.3 copies of the genome and containing two origins of replication in the same orientation. To confirm that the LeYSA-[BR:Dou1095.1:11] "1.3mer" clone was infectious, a biolistic inoculation test (Aragão *et al.*, 1996) was performed in which the clone was inoculated in plants of *L. sibiricus* together with an infectious clone of the virus with which it was originally detected (ToYSV; Ferro *et al.*, 2017). Infection by the two agents was assayed by polymerase chain reaction (PCR)-based amplification of genomic fragments of ToYSV and LeYSA using virus- and alphasatellite-specific primers (see below), and also by rolling-circle amplification (RCA; Inoue-Nagata *et al.*, 2004).

Infectivity/host range assay

To study the effects of the alphasatellite on infectivity and symptoms induced by begomoviruses, an infectivity and host range assay was conducted using biolistic inoculation. Ten micrograms of each genomic component (DNA-A and DNA-B) of the isolates ToYSV-[BR:Bic2:99] (Andrade *et al.*, 2006), ToSRV-[BR:Pir1:05] (Lima, 2007) and EuYMV-[BR:Cha510:10] (Mar *et al.*, 2017b), alone or in combination with LeYSA-[BR:Dou1095.1:11], were inoculated onto 30 *L. sibiricus* plants, also popularly known as “erva-de-macaé” or “marijuana” (15 plants in each of two experiments), 40 tomato plants cv. Santa Clara (10 plants in the first experiment and 15 plants each in the second and third experiments) and 25 *Nicotiana benthamiana* plants (10 in the first experiment and 15 in the second experiment). For the negative control, healthy plants of each species were inoculated with tungsten particles without DNA. Following the inoculations, the plants were kept in a greenhouse for visual evaluation of symptoms. The

youngest leaves of each plant were collected at 14 and 28 days post-inoculation (dpi). Total DNA was extracted (Doyle & Doyle, 1986) from these leaves and used as a template for conventional and quantitative (real-time) PCR detection of the viruses and the alphasatellite, using specific primers for each agent (Table 1).

Detection and quantification of begomovirus and alphasatellite genomic components

The presence of each begomovirus and of the alphasatellite were confirmed in biolistically-inoculated plants using conventional PCR. Amplifications were performed in a total volume of 10 µL, with 1 µL of template DNA, 0.4 µM of each primer (Table 1), 5X Green GoTaq Reaction Buffer (Promega), 0.2 mM of each dNTP and 0.5 U of GoTaq DNA polymerase (Promega). The amplification program used to detect ToYSV genomic fragments consisted of an initial denaturation of 95°C for 2 min followed by 34 cycles of 95°C for 1min, 66°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 5 min. The program for ToSRV and LeYSA consisted of an initial denaturation of 95°C for 2 min followed by 34 cycles of 95°C for 1min, 66°C for 1min and 72°C for 30s, with a final extension at 72°C for 5 min. The amplification program for EuYMV consisted of an initial denaturation of 95°C for 2 min followed by 34 cycles of 95°C for 1min, 55°C for 1min and 72°C for 1 min 30 s, with a final extension at 72°C for 5 min. PCR products were separated by 1% agarose gel electrophoresis and stained with ethidium bromide.

Accumulation of begomovirus and alphassatellite DNA was determined by quantitative, real-time PCR (qPCR). DNA samples were quantified using the NanoDrop 2000c spectrophotometer (Thermo Scientific). Standard curves were

obtained by serial dilutions (10^2 to 10^7) of previously quantified plasmid minipreps containing a copy of the corresponding genomic component.

The primers (1 µM of each primer) used for qPCR of begomovirus and alphasatellite are listed in Table 1. Reactions were prepared in a final volume of 10 µL using SYBR Green PCR Master Mix (Bio-Rad) in a StepOne Plus Real-Time PCR System (Applied Biosystems). The cycling conditions for the three begomoviruses consisted of an initial denaturing step of 95°C for 3 min followed by 40 cycles of 95°C for 15 s and 60ºC for 1 min. For the alphasatellite, cycling conditions consisted of an initial denaturing step of 95°C for 3 min followed by 40 cycles of 95°C for 15 s and 64ºC for 1 min, with a final dissociation step to verify the specificity of amplification.

Each sample was analyzed in triplicate by the amplification of 10 ng of total DNA. Viral accumulation was determined by interpolation of the Ct values of each tested sample within the standard curve. The data were submitted to analysis of variance and the differentiation of the means was performed using Student's *t* test imlemented with the R package**.**

Whithefly transmission assay

Whitefly transmission assays were performed to evaluate the transmission efficiency of ToYSV and ToSRV by *Bemisia tabaci* Middle East-Asia Minor 1 (BtMEAM1) from source plants with or without the alphasatellite. A population of BtMEAM1 was obtained from colonies kept in cabbage plants (*Brassica oleracea* var. *capitata*; a non-host for both ToYSV and ToSRV) and was maintained inside whitefly-proof screened cages in a growth chamber, with a temperature of 25°C and photoperiod of 14 h of light and 10 h of darkness. Fifteen plants (at 15 days

post-germination) were used per treatment. Inoculum source plants were obtained from the biolistic inoculation with ToYSV and ToSRV with or without the alphasatellite. To confirm the infection, total DNA of all plants was extracted as described above and the presence of the two agents was confirmed by conventional PCR with specific primers for ToYSV DNA-A, ToSRV DNA-A and LeYSA (Table 1).

Inoculum source plants were used for acquisition by BtMEAM1. Three or four plants were placed in separate screened cages with about 1000 aviruliferous whiteflies for an acquisition access period (AAP) of 48 h. After the AAP, whiteflies were transferred to plastic cups containing healthy plants (30 adult insects/plant) using a mouth aspirator for an inoculation access period (IAP) of 48 h. After the IAP, whiteflies were eliminated mechanically and by the application of acetamiprid (80 mg A.I./L). The plants were kept in a greenhouse and the appearance of symptoms was evaluated up to 28 dpi. As negative controls, aviruliferous whiteflies were transferred to healthy plants for AAP and IAP of 48 h.

To confirm the presence of the virus and alphasatellite in each plant, total DNA was extracted at 14 and 28 dpi and used as a template for conventional PCR as described above. A qualitative evaluation of the plants was performed (PCR-positive or PCR-negative) to calculate the transmission rate.

Results and Discussion

The influence of LeYSA on the infectivity, symptoms and DNA accumulation of ToYSV, ToSRV and EuYMV was evaluated in three hosts: *(i) L.*

sibiricus, the host from which LeYSA was originally isolated in association with ToYSV; *(ii)* tomato, a known host of both ToYSV and ToSRV; *(iii) N. benthamiana*, a commonly used laboratory host of begomoviruses, and known to be infected by the three viruses used.

No symptoms were observed in plants of *L. sibiricus* inoculated with ToSVR and EuYMV, up to 28 dpi. PCR analysis at 14 and 28 dpi confirmed that none of the inoculated plants were infected with these viruses (Table 2). When *L. sibiricus* plants were inoculated with ToYSV alone, symptoms were observed in 19 out of 28 plants (68%) at 28 dpi. When ToYSV was inoculated together with LeYSA, symptoms were observed in 21 out of 30 plants (70%), with the presence of LeYSA being detected by PCR in 17 out of the 21 virus-infected plants (81%) (Figure 1; Table 2). Statistical analysis indicated that ToYSV infectivity in *L. sibiricus* was the same in the presence or absence of the alphasatellite LeYSA at both 14 and 28 dpi (Figure 1).

In contrast to studies reporting that the presence of DNA-2-type OW alphasatellites attenuate symptoms (Wu & Zhou, 2005;Idris *et al.*, 2011), the presence of the NW, DNA-3-type LeYSA increased the severity of symptoms in *L. sibiricus* plants, with the development of severe mosaic, leaf distortion and blistering (Figure 2). The first symptoms started to appear at 6 dpi, with a mosaic that was of equivalent severity in plants inoculated with ToYSV alone or in combination with LeYSA. However, at 14 dpi it was already possible to observe the difference between plants that only had the presence of ToYSV and plants inoculated with ToYSV and LeYSA. The latter displayed mosaic and leaf deformation symptoms, while the former displayed yellow spots. The mosaic symptoms in plants infected with the virus and the alphasatellite evolved to

severe mosaic, leaf distortion and blistering by 28 dpi, while the plants infected with the virus alone showed the same yellow spot symptoms at this time point (Figure 2). Increased symptom severity in the presence of a NW DNA-3-type alphasatellite was also observed by Mar *et al.* (2017a).

When *N. benthamiana* plants were inoculated with ToYSV or ToSRV alone, symptoms were observed in 95% and 81% of the plants, respectively, at 28 dpi (Figure 1; Table 2). When the plants were inoculated with the two begomoviruses plus LeYSA, symptoms were observed in 100% and 70% of the plants at 28 dpi for ToYSV and ToSRV, respectively (Figure 1; Table 2). Statistical analysis confirmed that, as observed for ToYSV in *L. sibiricus*, the infectivity of the two viruses in *N. benthamiana* was the same in the presence or absence of LeYSA (Figure 1).

The first symptoms observed in *N. benthamiana* infected with ToYSV alone appeared at 5 dpi, and included mosaic and mild foliar distortion. At 14 dpi all plants displayed mosaic and severe leaf distortion. Also, from a total of 25 *N. benthamina* plants inoculated with ToYSV plus the alphasatellite, LeYSA was detected in 68% of the plants. Symptoms of severe mosaic, leaf deformation, severe curling and dwarfism were observed in all plants infected with ToYSV plus LeYSA at 28 dpi. Thus, the severity of symptoms increased in the presence of the alphasatellite (Figure 3).

Upon inoculation with ToSRV alone, *N. benthamiana* plants displayed mild chlorosis. The first symptoms were observed at 9 dpi, and 81% of the inoculated plants became infected plants by 28 dpi (Figure 1; Table 2). When ToSRV was inoculated together with LeYSA, 70% of the inoculated plants were infected at 28 dpi, and 86% had the presence of LeYSA (Table 2). The symptoms observed in

plants with the alphasatellite were mosaic, chlorosis, and a severe reduction in leaf development. However, these symptoms were only slightly more severe than those observed in the absence of LeYSA (Figure 3).

Regarding infection with EuYMV, *N. benthamiana* plants infected with the virus alone showed a light vein chlorosis, the same symptoms observed by Mar *et al.* (2017a). The symptoms appeared at 9 dpi, in both the plants inoculated with EuYMV alone and in those inoculated with the virus plus LeYSA. Also in both cases 54% of the plants were infected with EuYMV at 28 dpi. In plants inoculated wth the virus plus LeYSA, the alphasatellite was detected in 10 out of the 13 (77%) plants which were positive for the virus (Figure 1; Table 2).

All tomato plants infected with ToYSV and ToSRV were symptomatic. The symptoms in plants inoculated with ToSRV and ToYSV, both in the presence or absence of LeYSA, began to appear at 7 dpi and consisted of a yellow mosaic on ToSRV-infected plants and severe mosaic and leaf curling on ToYSV-infected plants. The severity of symptoms induced by ToYSV increased in the presence of LeYSA. Plants infected with ToYSV and LeYSA showed more severe mosaic and leaf curling (Figure 4). Plants infected with ToYSV alone were 64% of the inoculated ones at 28 dpi, while plants infected with ToYSV together with LeYSA were 63% of the inoculated plants, with the presence of LeYSA confirmed in 60% of the virus-infected plants (Figure 1; Table 2). Symptoms in plants infected with ToSRV and LeYSA were of the same nature compared to plants infected with ToSRV alone, with no difference in severity between the two treatments (Figure 4). However, there was a statistical difference in the infectivity of ToSRV alone and in the presence of LeYSA at both 14 and 28 dpi (Figure 1). A total of 59% of the plants were infected at 28 dpi when ToSRV was inoculated alone, while 39%

were infected when ToSRV was inoculated together with LeYSA, with the presence of LeYSA confirmed in 50% of the virus-infected plants (Figure 1; Table 2).

As previously reported (Mar *et al.*, 2017a), the infection of tomato by EuYMV was mostly asymptomatic, with only a few plants showing faint chlorotic punctuations (Figure 4). There was a statistical difference in the infectivity of EuYMV in the presence of LeYSA between 14 dpi and 28 dpi. About 28% of the plants were infected with EuYMV plus LeYSA at 14 dpi, while 69% of the plants were infected at 28 dpi. The presence of LeYSA was confirmed in 100% and 96% of the virus-infected plants at 14 and 28 dpi, respectively (Figure 1; Table 2). EuYMV alone infected 55% of the plants at 28 dpi.

Of the three hosts analyzed in this study, the one that had the highest infectivity rate for all three viruses, alone or in the presence of LeYSA, was *N. benthamiana*. This result suggests that, while the alphasatellite may be promiscuous in terms of its helper virus, the interaction with host factors may be more important as far as the success of the systemic infection is concerned. Thus, *N. benthamiana* would be a more suitable host for all viruses, regardless of the presence of the alphasatellite.

To evaluate whether the presence of LeYSA affects the accumulation of ToYSV, ToSRV and EuYMV, the DNA-A accumulation of each virus in the presence or absence of the alphasatellite was quantified at 14 and 28 dpi. Accumulation of the alphasatellite was also quantified (Figures 5, 6 and 7).

The accumulation of ToYSV DNA-A in *L. sibiricus*, tomato and *N. benthamiana*, as well as the accumulation of ToSRV and EuYMV DNA-A in tomato and *N. benthamiana*, did not present statistically significant differences in

the presence or absence of LeYSA (Figures 5, 6 and 7), indicating that this alphasatellite does not interfere with DNA accumulation of helper begomoviruses.

A higher level of LeYSA DNA accumulation compared to ToYSV DNA accumulation was observed in the upper leaves of *L. sibiricus* and *N. benthamiana* at both 14 and 28 dpi (Figures 5 and 7). In tomato, LeYSA DNA accumulated at a lower level than ToYSV DNA at 14 dpi, and the two agents reached similar levels of DNA accumulation at 28 dpi (Figure 6). ToYSV acccumulated at similar levels in all three hosts (Figures 5, 6 and 7). Together, these results indicate that, besides (or rather than) virus encoded factors, host-encoded factors are (also) involved in the accumulation of alphasatellite DNA, and suggests a higher adaptability of the alphasatellite to *L. sibiricus* and *N. benthamiana* compared to tomato. This is not entirely surprising, since alphasatellites encode their own Rep, and therefore are capable of replicating independently from the helper virus.

Nevertheless, the level of LeYSA DNA accumulation was also dependent on the helper begomovirus. Thus, the accumulation of LeYSA was significantly higher when associated with ToYSV in *L. sibiricus* and *N. benthamiana* compared to all other combinations of helper begomovirus and host, and was much lower when in association with EuYMV than with ToYSV or ToSRV in both *N. benthamiana* and tomato (Figures 5, 6 and 7).

Our results indicate that the outcome of the interaction between NW bipartite begomoviruses and DNA-3-type alphasatellites is similar, regardless of the helper begomovirus and the host. The presence of the alphasatellite did not cause an increase in the accumulation of ToYSV, ToSRV and EuYMV in either one of the three hosts, but increased symptom severity independently of host and

helper virus. However, one parameter of the interaction which varied depending on the host and on the helper virus was the accumulation of the alphasatellite itself. Based on this parameter, LeYSA is better adapted to *L. sibiricus* and *N. benthamiana* than to tomato, and the interaction between LeYSA and ToYSV was the most effective, followed by the interaction with ToSRV, and then with EuYMV (the least effective).

The transmission of ToYSV and ToSRV to tomato by BtMEAM1 was compared in the presence or absence of LeYSA. None of the 30 plants inoculated with ToYSV (15 plants inoculated with the virus alone, and 15 plants inoculated with the virus and the alphasatellite) displayed any symptoms. PCR analysis confirmed that none of the plants were infected (Table 3). The isolate used for the assay, BR:Bic2:99, has been maintained in plants by successive sap-inoculations for almost 20 years. Therefore, it is possible that it is no longer whitefly-transmitted.

A high efficiency of transmission (13 out of 15 plants, 86%) was observed for ToSRV alone (Table 3). This was higher than the efficiency of transmission using biolistics (22 out of 39 plants, 56%; Table 2), and confirms the results reported by other groups in terms of the high efficiency of BtMEAM1 as a vector for this begomovirus (Macedo *et al.*, 2015; De Marchi *et al.*, 2017). However, ToSRV was transmitted to only 6 out of 15 plants (40%) when inoculated together with LeYSA (Table 3). These plants displayed the same mild mosaic symptoms observed in the plants of the host range assay (Figure 4). These results suggest that the alphasatellite LeYSA negatively interferes in the transmission of the ToSRV by BtMEAM1. Similar results were reported by Mar *et al.* (2017a) when comparing the transmission efficiency of EuYMV by BtMEAM1 in the absence

and presence of *Euphorbia yellow mosaic alphasatellite*. Thus, the available evidence points towards a negative effect of DNA-3-type alphasatellites on whitefly transmission of NW begomoviruses. However, both the experiment reported here and the results reported by Mar *et al.* (2017a) were based on a small number of inoculated plants. Thus, additional transmission assays need to be performed.

It will be interesting to extend these studies to other DNA-3-type alphasatellites, such as *Cleome leaf crumple alphasatellite* (ClLCrA) and *Euphorbia yellow mosaic alphasatellite* (EuYMA). In the case of EuYMA, equivalent results (increase in the severity of symptoms without an increase in helper virus DNA accumulation; decrease in whitefly transmission efficiency) were obtained for its interaction with EuYMV, but this has not been extended to other begomoviruses. Such additional studies are necessary to fully understand the significance and the consequences of the interaction between NW bipartite begomoviruses and DNA-3-type alphasatellites.

Literature Cited

Abbas, Q.; Amin, I.; Mansoor, S.; Wassenegger, M.; Briddon, R.W. Geminivirus-associated alphasatellites suppress transcriptional not post-transcriptional gene silencing. **VirusDisease,** v. 27, p. 419, 2016.

Andrade, E.C.; Manhani, G.G.; Alfenas, P.F.; Calegario, R.F.; Fontes, E.P.B.; Zerbini, F.M. *Tomato yellow spot virus*, a tomato-infecting begomovirus from Brazil with a closer relationship to viruses from *Sida* sp., forms pseudorecombinants with begomoviruses from tomato but not from *Sida*. **Journal of General Virology,** v. 87, p. 3687-3696, 2006.

Aragão, F.J.L.; Barros, L.M.G.; Brasileiro, A.C.M.; Ribeiro, S.G.; Smith, F.D.; Sanford, J.C.; Faria, J.C.; Rech, E.L. Inheritance of foreign genes in transgenic bean (*Phaseolus vulgaris* L.) co-transformed via particle bombardment. **Theoretical and Applied Genetics,** v. 93, p. 142-150, 1996.

Briddon, R.W.; Brown, J.K.; Moriones, E.; Stanley, J.; Zerbini, M.; Zhou, X.; Fauquet, C.M. Recommendations for the classification and nomenclature of the DNA-β satellites of begomoviruses. **Archives of Virology,** v. 153, p. 763-781, 2008.

Briddon, R.W.; Bull, S.E.; Amin, I.; Idris, A.M.; Mansoor, S.; Bedford, I.D.; Dhawan, P.; Rishi, N.; Siwatch, S.S.; Abdel-Salam, A.M.; Brown, J.K.; Zafar, Y.; Markham, P.G. Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. **Virology,** v. 312, p. 106-121, 2003.

Briddon, R.W.; Martin, D.P.; Roumagnac, P.; Navas-Castillo, J.; Fiallo-Olive, E.; Moriones, E.; Lett, J.M.; Zerbini, F.M.; Varsani, A. *Alphasatellitidae*: a new family with two subfamilies for the classification of geminivirus- and nanovirus-associated alphasatellites. **Archives of Virology,** v. 163, p. 2587-2600, 2018.

Brown, J.K.; Fauquet, C.M.; Briddon, R.W.; Zerbini, F.M.; Moriones, E.; Navas-Castillo, J. Family *Geminiviridae*. In: King, A.M.Q.;Adams, M.J.;Carstens, E.B. e Lefkowitz, E.J. (Ed.). **Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses**. London, UK: Elsevier Academic Press, 2012. p.351-373.

Cui, X.F.; Li, G.X.; Wang, D.W.; Hu, D.W.; Zhou, X.P. A begomovirus DNA beta-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. **Journal of Virology,** v. 79, p. 10764-10775, 2005.

De Marchi, B.R.; Marubayashi, J.M.; Favara, G.M.; Yuki, V.A.; Watanabe, L.F.M.; Barbosa, L.F.; Pavan, M.A.; Krause-Sakate, R. Comparative transmission of five viruses by *Bemisia tabaci* NW2 and MEAM1. **Tropical Plant Pathology,** v. 42, p. 495-499, 2017.

Doyle, J.; Doyle, J. **A Rapid DNA Isolation Procedure from Small Quantities of Fresh Leaf Tissues**. 1986.

Ferro, C.G.; Silva, J.P.; Xavier, C.a.D.; Godinho, M.T.; Lima, A.T.M.; Mar, T.B.; Lau, D.; Zerbini, F.M. The ever increasing diversity of begomoviruses infecting non-cultivated hosts: new species from *Sida* spp. and *Leonurus sibiricus*, plus two New World alphasatellites. **Annals of Applied Biology,** v. 170, p. 204-218, 2017.

Fiallo-Olivé, E.; Tovar, R.; Navas-Castillo, J. Deciphering the biology of deltasatellites from the New World: maintenance by New World begomoviruses and whitefly transmission. **New Phytologist,** v. 212, p. 680-692, 2016.

Hanley-Bowdoin, L.; Bejarano, E.R.; Robertson, D.; Mansoor, S. Geminiviruses: Masters at redirecting and reprogramming plant processes. **Nature Reviews Microbiology,** v. 11, p. 777-88, 2013.

Harrison, B.D.; Robinson, D.J. Natural genomic and antigenic variation in whitefly transmitted geminiviruses (begomoviruses). **Annual Review of Phytopathology,** v. 39, p. 369-398, 1999.

Idris, A.M.; Shahid, M.S.; Briddon, R.W.; Khan, A.J.; Zhu, J.K.; Brown, J.K. An unusual alphasatellite associated with monopartite begomoviruses attenuates symptoms and reduces betasatellite accumulation. **Journal of General Virology,** v. 92, p. 706-717, 2011.

Inoue-Nagata, A.K.; Albuquerque, L.C.; Rocha, W.B.; Nagata, T. A simple method for cloning the complete begomovirus genome using the bacteriophage phi29 DNA polymerase. **Journal of Virological Methods,** v. 116, p. 209-211, 2004.

Jeske, H.; Kober, S.; Schäfer, B.; Strohmeier, S. Circomics of Cuban geminiviruses reveals the first alphasatellite DNA in the Caribbean. **Virus Genes,** v. 49, p. 312-324, 2014.

Lima, A.T.M. **Caracterização de dois begomovírus (*Tomato severe rugose virus* e *Tomato yellow vein streak virus*) que infectam tomateiro e obtenção de clones infecciosos**. 2007. 76 Tese M.S. Dep. de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG.

Lozano, G.; Trenado, H.P.; Fiallo-Olive, E.; Chirinos, D.; Geraud-Pouey, F.; Briddon, R.W.; Navas-Castillo, J. Characterization of non-coding DNA satellites associated with sweepoviruses (genus *Begomovirus*, *Geminiviridae*) - definition of a distinct class of begomovirus-associated satellites. **Frontiers in Microbiology,** v. 7, p. 162, 2016.

Macedo, M.A.; Michereff, M.; Navas-Castillo, J.; Inoue-Nagata, A.K. Host range and whitefly transmission efficiency of *Tomato severe rugose virus* and *Tomato golden vein virus* in tomato plants. **Tropical Plant Pathology,** v. 40, p. 405-409, 2015.

Mahajan, N.; Parameswari, C.; Veluthambi, K. Severe stunting in blackgram caused by the *Mungbean yellow mosaic virus* (MYMV) KA27 DNA B component is ameliorated by co-infection or post-infection with the KA22 DNA B: MYMV nuclear shuttle protein is the symptom determinant. **Virus Research,** v. 157, p. 25-34, 2011.

Mansoor, S.; Briddon, R.W.; Zafar, Y.; Stanley, J. Geminivirus disease complexes: An emerging threat. **Trends in Plant Science,** v. 8, p. 128-134, 2003.

Mar, T.B.; Mendes, I.R.; Lau, D.; Fiallo-Olive, E.; Navas-Castillo, J.; Alves, M.S.; Zerbini, F.M. Interaction between the New World begomovirus *Euphorbia yellow mosaic virus* and its associated alphasatellite: effects on infection and transmission by the whitefly *Bemisia tabaci*. **Journal of General Virology,** v. 98, p. 1552-1562, 2017a.

Mar, T.B.; Xavier, C.a.D.; Lima, A.T.M.; Nogueira, A.M.; Silva, J.C.F.; Ramos-Sobrinho, R.; Lau, D.; Zerbini, F.M. Genetic variability and population structure of the New World begomovirus *Euphorbia yellow mosaic virus*. **Journal of General Virology,** v. 98, p. 1537-1551, 2017b.

Nawaz-Ul-Rehman, M.S.; Nahid, N.; Mansoor, S.; Briddon, R.W.; Fauquet, C.M. Post-transcriptional gene silencing suppressor activity of two non-pathogenic alphasatellites associated with a begomovirus. **Virology,** v. 405, p. 300-308, 2010.

Padidam, M.; Beachy, R.N.; Fauquet, C.M. The role of AV2 ("precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. **Virology,** v. 224, p. 390-404, 1996.

Paprotka, T.; Metzler, V.; Jeske, H. The first DNA 1-like alphasatellites in association with New World begomoviruses in natural infections. **Virology,** v. 404, p. 148-157, 2010.

Rojas, M.R.; Hagen, C.; Lucas, W.J.; Gilbertson, R.L. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. **Annual Review of Phytopathology,** v. 43, p. 361-394, 2005.

Rojas, M.R.; Macedo, M.A.; Maliano, M.R.; Soto-Aguilar, M.; Souza, J.O.; Gilbertson, R.L.; Briddon, R.W.; Kenyon, L.A.; Bustamante, R.F.R.; Zerbini, F.M.; Adkins, S.; Legg, J.P.; Kvarnheden, A.; Wintermantel, W.M.; Sudarshana, M.R.; Peterschmitt, M.; Lapidot, M.; Martin, D.P.; Moriones, E.; Inoue-Nagata, A.K. World management of geminiviruses. **Annual Review of Phytopathology,** v. 56, p. 637-677, 2018.

Romay, G.; Chirinos, D.; Geraud-Pouey, F.; Desbiez, C. Association of an atypical alphasatellite with a bipartite New World begomovirus. **Archives of Virology,** v. 155, p. 1843-1847, 2010.

Rosario, K.; Marr, C.; Varsani, A.; Kraberger, S.; Stainton, D.; Moriones, E.; Polston, J.E.; Breitbart, M. Begomovirus-associated satellite DNA diversity captured through vector-enabled metagenomic (VEM) surveys using whiteflies (Aleyrodidae). **Viruses,** v. 8, p. 36, 2016.

Saeed, M.; Behjatnia, S.a.A.; Mansoor, S.; Zafar, Y.; Hasnain, S.; Rezaian, M.A. A single complementary-sense transcript of a geminiviral DNA beta satellite is determinant of pathogenicity. **Molecular Plant-Microbe Interactions,** v. 18, p. 7-14, 2005.

Saunders, K.; Bedford, I.D.; Briddon, R.W.; Markham, P.G.; Wong, S.M.; Stanley, J. A unique virus complex causes *Ageratum* yellow vein disease. **Proceedings of the National Academy of Sciences, USA,** v. 97, p. 6890-6895, 2000.

Saunders, K.; Bedford, I.D.; Stanley, J. Adaptation from whitefly to leafhopper transmission of an autonomously replicating nanovirus-like DNA component associated with ageratum yellow vein disease. **Journal of General Virology,** v. 83, p. 907-913, 2002.

Varsani, A.; Navas-Castillo, J.; Moriones, E.; Hernández-Zepeda, C.; Idris, A.; Brown, J.K.; Zerbini, F.M.; Martin, D.P. Establishment of three new genera in the family *Geminiviridae*: *Becurtovirus*, *Eragrovirus* and *Turncurtovirus*. **Archives of Virology,** v. 159, p. 2193-2203, 2014.

Varsani, A.; Roumagnac, P.; Fuchs, M.; Navas-Castillo, J.; Moriones, E.; Idris, A.; Briddon, R.W.; Rivera-Bustamante, R.F.; Zerbini, F.M.; Martin, D.P. *Capulavirus* and *Grablovirus*: two new genera in the family *Geminiviridae*. **Archives of Virology,** v. 162, p. 1819-1831, 2017.

Wu, P.J.; Zhou, X.P. Interaction between a nanovirus-like component and the *Tobacco curly shoot virus*/satellite complex. **Acta Biochimica et Biophysica Sinica**, v. 37, p. 25-31, 2005.

Zerbini, F.M.; Briddon, R.W.; Idris, A.; Martin, D.P.; Moriones, E.; Navas-Castillo, J.; Rivera-Bustamante, R.; Varsani, A.; Ictv Consortium. ICTV Virus Taxonomy Profile: *Geminiviridae*. **Journal of General Virology,** v. 98, p. 131-133, 2017.

Zhou, X. Advances in understanding begomovirus satellites. **Annual Review of Phytopathology,** v. 51, p. 357-381, 2013.

Table 1 Primers used for conventional and quantitative, real-time PCR

Primer name	**Sequence(5'-3')**	**(C/Q)***	**Detection**
LeYSA (rep-sat)$_{(For)}$	GCAAGGGAGCAACAAGAGGA	C	LeYSA Alpha-Rep
LeYSA (rep-sat)$_{(Rev)}$	AAGAAGGCAAGATGGTAGCCC	C	LeYSA Alpha-Rep
ToYSV-A$_{(For)}$	CACTTCGTTCGAGAAAACCTTTGAG	C	ToYSV DNA-A
ToYSV-A$_{(Rev)}$	CATGAGGGGACCATCAAGG	C	ToYSV DNA-A
ToSRV-A $_{(For)}$	CAGTAGTTGCCCTCAAATTGAAG	C	ToSRV DNA-A
ToSRV-A$_{(Rev)}$	CACGTGTAGCAATCTCCTTAAAGAG	C	ToSRV DNA-A
1978$_{(For)}$	GCATCTGCAGGCCCACATYGTCTTYCCNGT	C	EuYMV
496$_{(Rev)}$	GGCTTYCTRTACATRGG	C	EuYMV
qToYSV-A$_{(For)}$	CCCTCATGCTTGATTTTCTCC	Q	ToYSV-DNA-A
qToYSV-A$_{(Rev)}$	ACAATAAAACTTAGGGACCACG	Q	ToYSV-DNA-A
qToSRV-A$_{(For)}$[&]	AAAGTAAAGTGATTGTCTGTGG	Q	ToSRV-DNA-A
qToSRV-A $_{(Rev)}$[&]	GCCGTTCAACAAATTGGG	Q	ToSRV-DNA-A
qLeYSA$_{(For)}$	TGGGTGTATGGCTCTCAA	Q	LeYSA Alpha-Rep
qLeySA$_{(Rev)}$	CTTGATGTTCTCTCCCTTTCC	Q	LeYSA Alpha-Rep
qEuYMV-A$_{(For)}$[#]	AAGGCCTCTTCATGGGTGAA	Q	EuYMV-DNA-A
qEuYMV-A$_{(Rev)}$[#]	TTCGGTACATCTGGGCCTCTA	Q	EuYMV-DNA-A

* C, conventional PCR; Q, quantitative, real-time PCR

[&] From Silva *et al.* (2014)

[#] From Mar *et al.* (2017)

Table 2 Infectivity and symptoms induced by three bipartite begomoviruses (*Tomato yellow spot virus*, ToYSV; *Tomato severe rugose virus*, ToSRV; *Euphorbia yellow mosaic virus*, EuYMV), alone or in association with *Leonurus yellow spot alphasatellite* (LeYSA), in *Leonurus sibiricus*, tomato (*Solanum lycopersicum*) and *Nicotiana benthamiana*.

Leonurus sibiricus												
	ToYSV				ToSRV				EuYMV			
	14 dpi		28 dpi		14 dpi		28 dpi		14 dpi		28 dpi	
Alphasatellite	-	+	-	+	-	+	-	+	-	+	-	+
Symptoms[#]	M, Mc	M, Ld, Ba	M, Mc	M, Ld, Ba	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Virus detection[&]	18/28	20/30	19/28	21/30	0/24	0/25	0/24	0/25	0/23	0/29	0/23	0/29
Alphasatellite detection	-	15/20	-	17/21	-	-	-	-	-	-	-	-
***Solanum lycopersicum*[¥]**												
	ToYSV				ToSRV				EuYMV			
	14 dpi*		28 dpi		14 dpi		28 dpi		14 dpi		28 dpi	
Alphasatellite	-	+	-	+	-	+	-	+	-	+	-	+
Symptoms[#]	M	M,Ld	M, Mc	M, Ld, Cr	Mc	M, Mc	Mc	M,Mc	Ns	Ns, Cp	Ns, Cp	Ns, Cp
Virus detection[&]	25/40	22/39	25/40	25/39	23/39	13/36	23/39	14/36	15/40	11/40	22/40	27/39
Alphasatellite detection		08/22		15/25		06/13		07/14		11/11		26/27
Nicotiana benthamiana												
	ToYSV				ToSRV				EuYMV			
	14 dpi		28 dpi		14 dpi		28 dpi		14 dpi		28 dpi	
Alphasatellite	-	+	-	+	-	+	-	+	-	+	-	+
Symptoms[#]	Sc, Ld, Cr	Mc, Ld, Cr, N	M, Ld, Sc	Ld, Sc, Cr, N	Mc	M, Mc	Mc	M, Mc	Vc	Vc	Vc, Mc	Vc
Virus detection[&]	22/24	25/25	21/22	25/25	18/22	14/21	17/21	14/20	12/24	10/23	13/24	13/23
Alphasatellite detection		17/25		17/25		12/14		12/14		10/10		10/13

* Days post-biolistic inoculation;

[#] Ns: no symptoms; Ba: blister-like appearance; Cr: curling; Ld: leaf deformation; M: mosaic; Mc: mild chlorosis; N: Nanism; Sc: severe chrolosis; Cp: chlorotic punctuations; Vc: vein chlorosis.

[&] Number of PCR-positive plants/number of inoculated plants. The data is the sum of two independent experiments. **¥** Tomato was performed with three independent experiments

Table 3 Transmission of *Tomato yellow spot virus* (ToYSV) and *Tomato severe rugose virus* (ToSRV) to tomato (*Solanum lycopersicum*) plants, alone or in the presence of *Leonurus yellow spot alphasatellite* (LeYSA), by *Bemisia tabaci* Middle East-Asia Minor1.

	Number of infected plants/Number of inoculated plants (%)				
Treatments	ToSRV*	ToSRV and LeYSA#	ToYSV&	ToYSV and LeYSA£	Negative control¥
	13/15 (86)	6/15 (40)	0/15 (0)	0/15 (0)	0/3 (0)

* PCR detection of ToSRV DNA-A in plants inoculated with ToSRV alone;
# PCR detection of ToSRV DNA-A and LeYSA in plants inoculated with both ToSRV and LeYSA;
& PCR detection of ToYSV DNA-A in plants inoculated with ToYSV alone;
£ PCR detection of ToYSV DNA-A and LeYSA in plants inoculated with both ToYSV and LeYSA;
¥ Transmission with aviruliferous whiteflies.

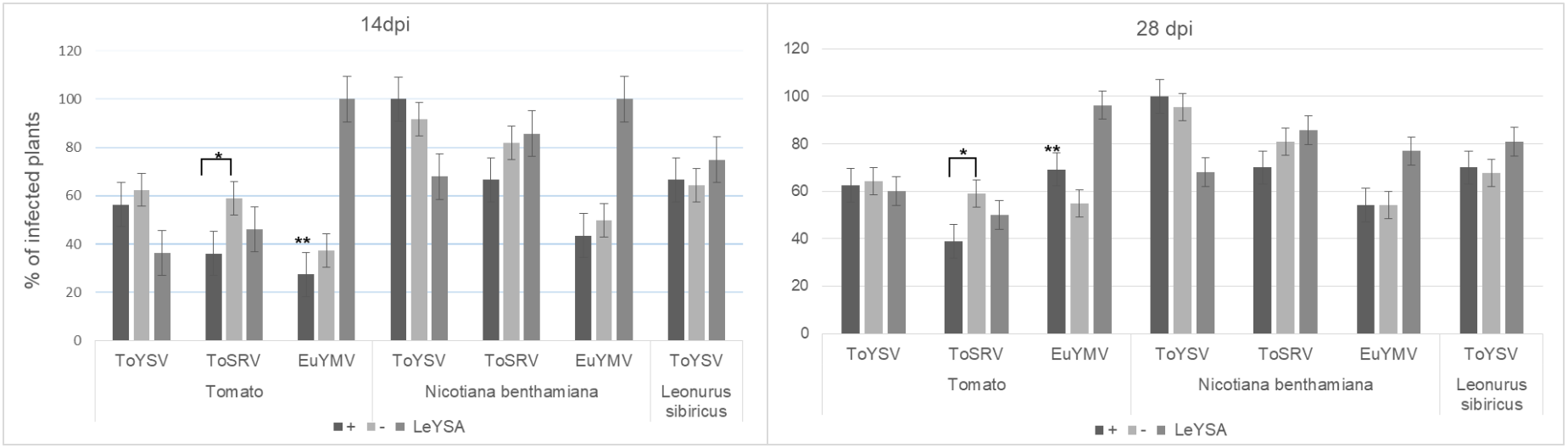

Figure 1 Percentage of detection of the begomoviruses *Tomato yellow spot virus* (ToYSV), *Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV) in the absence (-) or presence (+) of the alphasatellite *Leonurus yellow spot alphasatellite* (LeYSA), and percentage of detection of LeYSA, in systemic (non-inoculated) leaves of tomato, *Nicotiana benthamiana* and *Leonurus sibiricus* plants at 14 and 28 days post-inoculation (dpi). ToSRV and EuYMV were not detected when inoculated on *L. sibiricus* plants. Statistically significant diferences (Student's t test) are indicated with horizontal brackets (*, p≤0.01; **, p≤0.05).

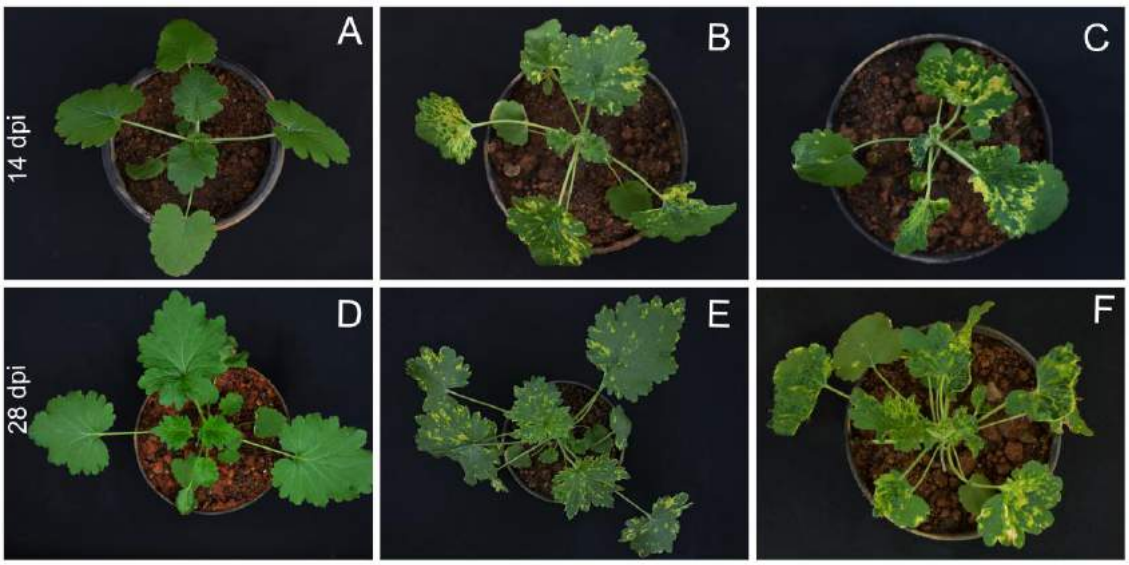

Figure 2 Symptoms in *L. sibiricus* plants inoculated with *Tomato yellow spot virus* (ToYSV), alone (**B, E**) or in combination with *Leonurus yellow spot alphasatellite* (LeYSA) (**C, F**) at 14 and 28 dpi. **A, D**: plants inoculated with tungsten particles without DNA.

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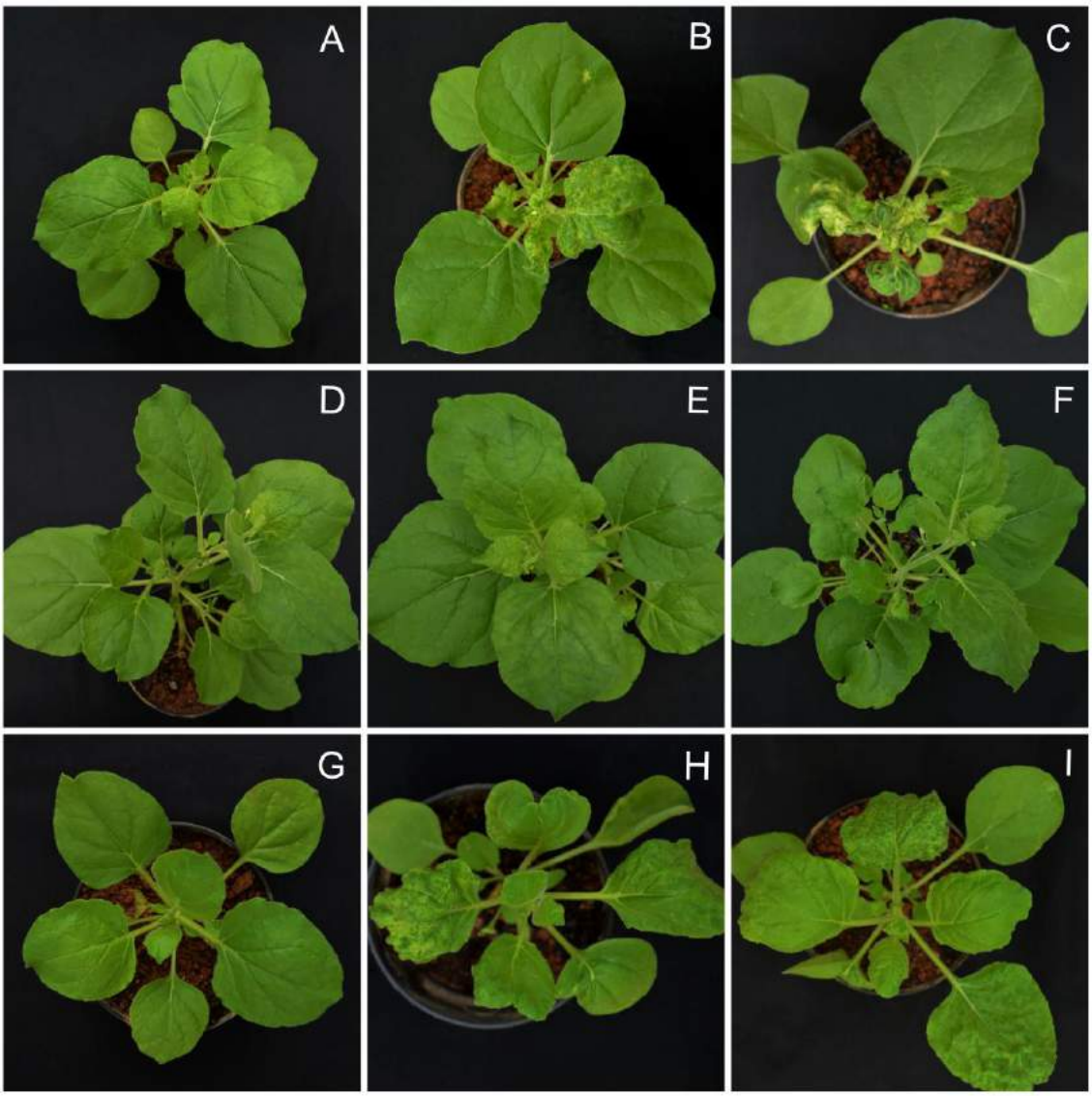

Figure 3 Symptoms in *Nicotiana benthamiana* plants inoculated with *Tomato yellow spot virus* (ToYSV), *Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV), alone (**B, E, H,** respectively) or in combination with *Leonurus yellow spot alphasatellite* (LeYSA) (**C**, **F**, **I**, respectively) at 14 dpi. **A**, **D**, **G:** plants inoculated with tungsten particles without DNA.

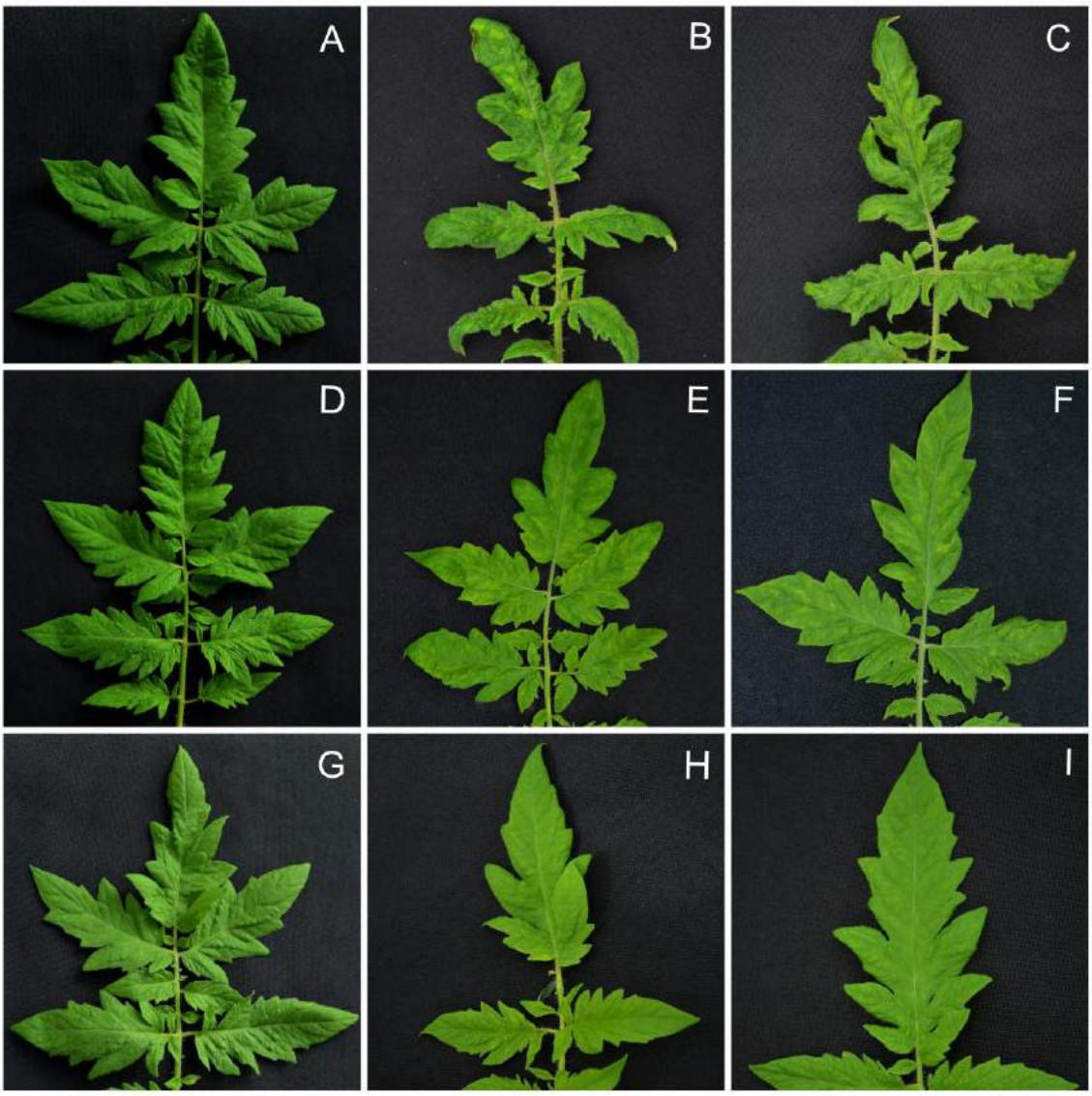

Figure 4 Symptoms in tomato plants inoculated with *Tomato yellow spot virus* (ToYSV), *Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV), alone (**B, E, H**, respectively) or in combination with *Leonurus yellow spot alphasatellite* (LeYSA) (**C, F**, **I**, respectively) at 14 dpi. **A**, **D**, **G:** plants inoculated with tungsten particles without DNA.

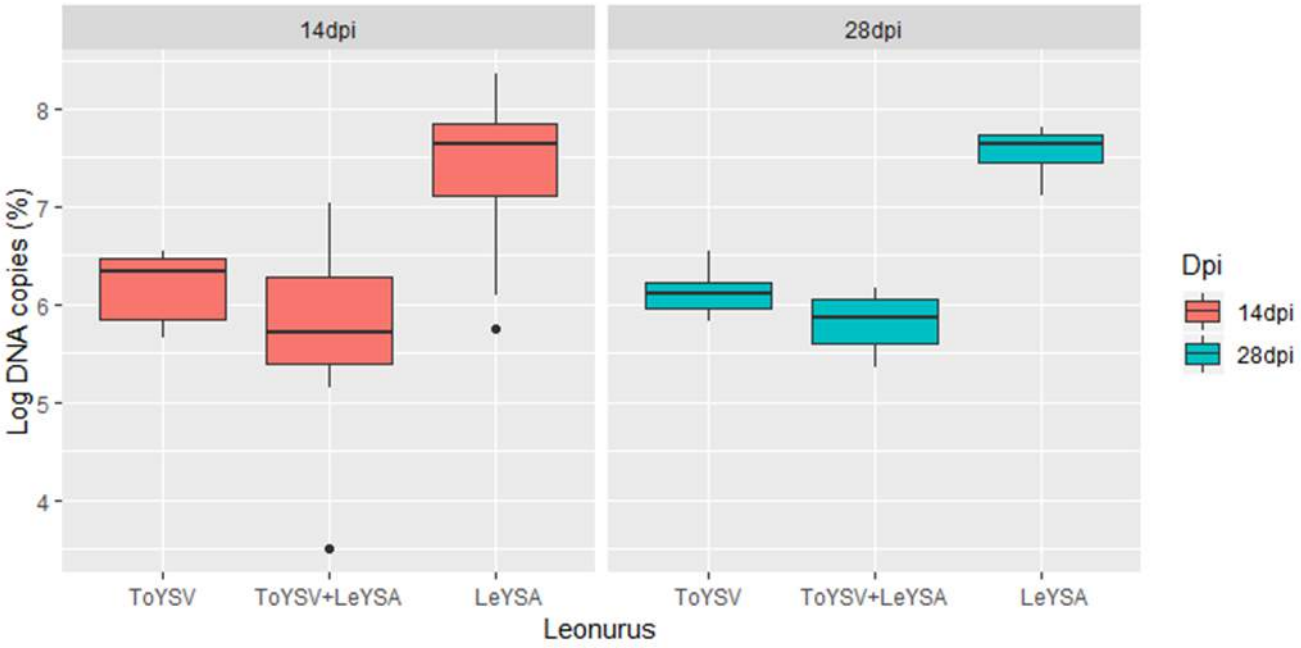

Figure 5 Absolute quantification by quantitative, real-time PCR of *Tomato yellow spot virus* (ToYSV) DNA-A in the absence or presence of *Leonurus yellow spot alphasatellite* (LeYSA), and accumulation of LeYSA in *Leonurus sibiricus* plants. Viral accumulation is presented as the log of the number of DNA molecules. Data refers to all plants of two independent replications, evaluated at 14 and 28 days pos-inoculation (dpi). Means were compared using Student's t test ($P< 0.05$).

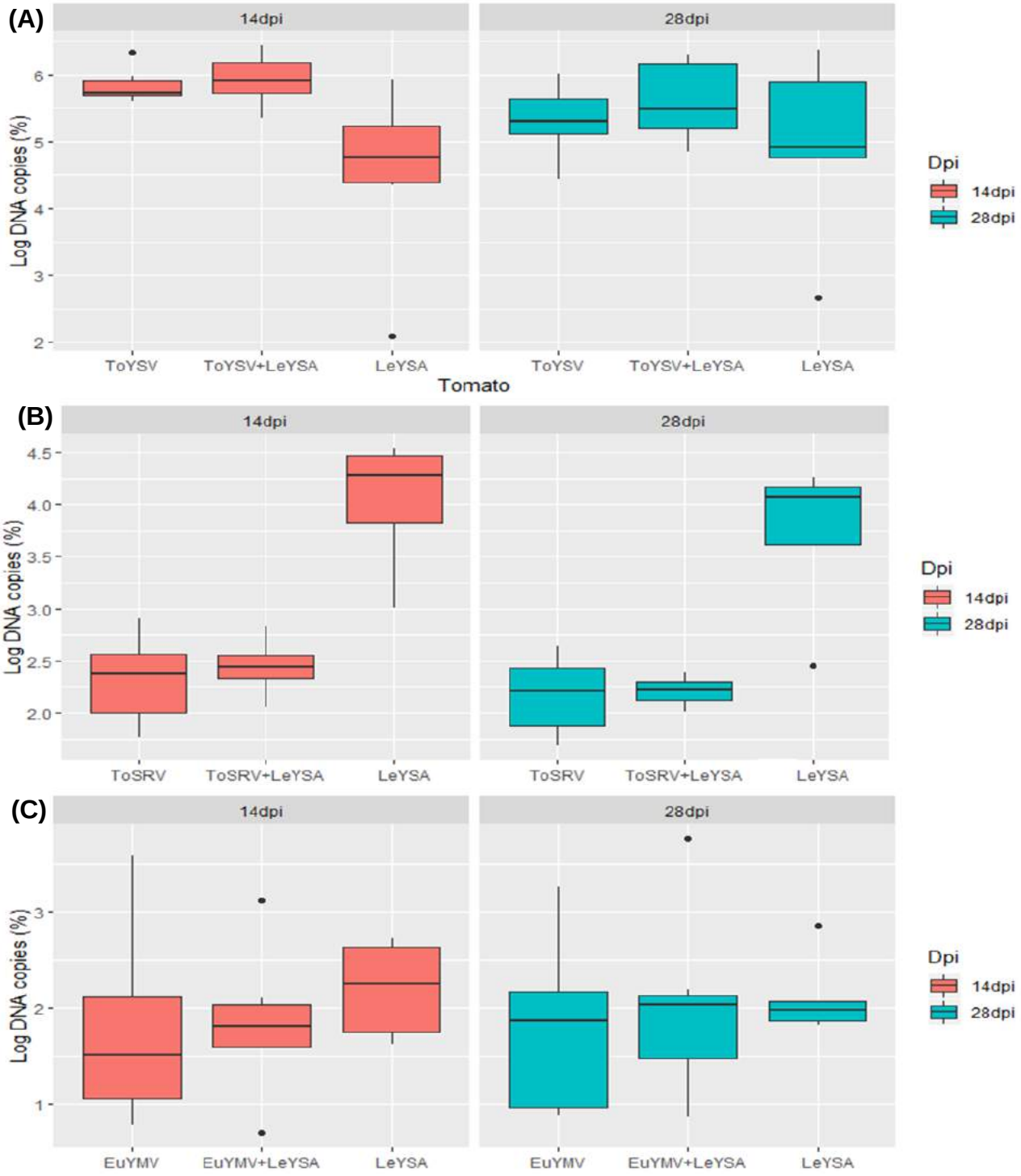

Figure 6 Absolute quantification by quantitative, real-time PCR of (**A**) *Tomato yellow spot virus* (ToYSV), (**B**) *Tomato severe rugose virus* (ToSRV) and (**C**) *Euphorbia yellow mosaic virus* (EuYMV) DNA-A in the absence or presence of *Leonurus yellow spot alphasatellite (LeYSA),* and accumulation of LeYSA in tomato plants. Viral accumulation is presented as the log of the number of DNA molecules. Data refers to all plants of three independent replications, evaluated at 14 and 28 days pos-inoculation (dpi). Means were compared using Student's t test *(p≤0.05).*

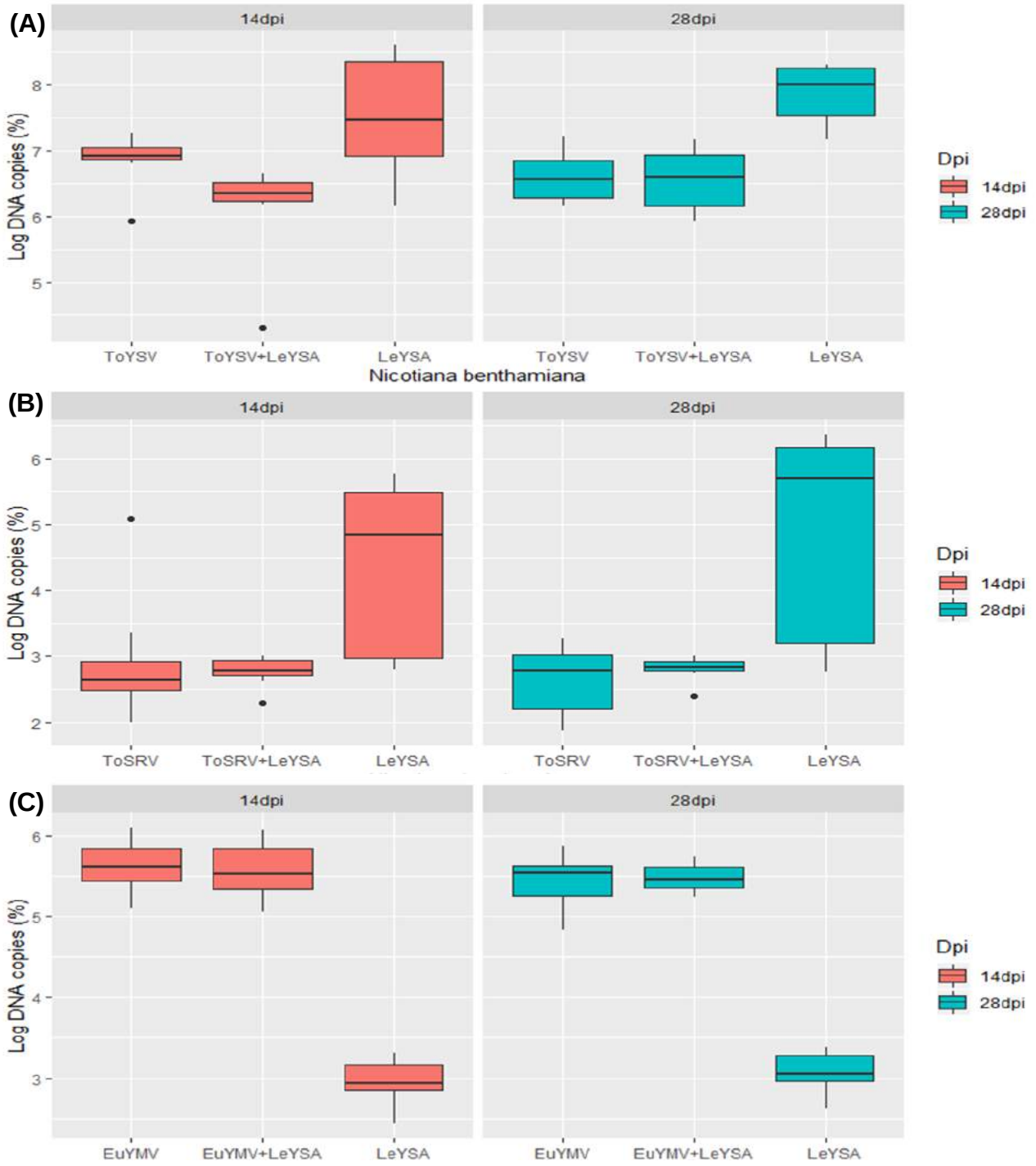

Figure 7 Absolute quantification by quantitative, real-time PCR of (**A**) *Tomato yellow spot virus (ToYSV*), (**B**) *Tomato severe rugose virus (ToSRV)* and (**C**) *Euphorbia yellow mosaic virus (EuYMV*) DNA-A in the absence or presence of *Leonurus yellow spot alphasatellite (LeYSA),* and accumulation of LeYSA in Nicotiana benthamiana plants. Viral accumulation is presented as the log of the number of DNA molecules. Data refers to all plants of two independent replications, evaluated at 14 and 28 days pos-inoculation (dpi). Means were compared using Student's t test ($p \leq 0.05$).

Artigo 2

FIRST REPORT OF *Colletotrichum Karstii* CAUSING ANTHRACNOSE SPOT ON PITAYA (*Hylocereus undatus*) IN BRAZIL.

Nascimento MB, Bellé C, Azambuja RHM, Paz Maich SLd, Neves CG, Souza Junior IT, Farias CRJ, Barros DRd. First Report of Colletotrichum karstii causing anthracnose spot on pitaya (Hylocereus undatus) in Brazil. Plant Disease, 2019. doi:10.1094/PDIS-02-19-0400-PDN.

3 Artigo 2 - First Report of *Colletotrichum karstii* causing anthracnose spot on pitaya (Hylocereus undatus) in Brazil.

M. B. Nascimento[1], C. Bellé[1 2], R. M. Azambuja[1], S. L. P. Maich[1], C. G. Neves[1], I. T. Souza Junior[1], C. R. F. Jacobsen[1] and D. R. Barros[1].

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M. B. Nascimento and C. Bellé contributed equally to this work.

First Report of Colletotrichum karstii causing anthracnose spot on pitaya (Hylocereus undatus) in Brazil

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M. B. Nascimento and C. Bellé contributed equally to this work.

Pitaya [(Hylocereus undatus (Haw.) Britt. & Rose] is a species of Cactaceae that has been planted as fruit crop in Brazil. In August 2017, infected plants of pitaya with symptoms of anthracnose were obtained from the plantations in the municipality of Pelotas, Rio Grande do Sul state, Brazil. Symptoms comprised round or irregularly shaped lesions that initially appeared as reddish-orange spots and commonly coalesced into larger, dark-brown lesions in their stems. The centers of the lesions were gray-white with purple-brown borders, surrounded by a chlorotic halo where black dots would appear later. The incidence of the disease was up to 35%, and the severity ranged from 30 to 40%. Lesions were isolated from stem samples and cultured on solid medium potato dextrose agar (PDA) that had been surface-disinfected (70% ethanol for 30 s and 1% NaClO for 1 min, followed by rinsing twice in sterile water and drying on sterilized filter paper). Colonies on PDA exhibited white aerial mycelia with an Orange conidial mass. The color of the colony on the reverse side was light orange. Conidia (n = 100) were 12.3–17.1 (length) × 3.9–6.5 (width) µm, mean ± SD = 13.2 ± 1.4 × 4.2 ± 0.4 µm, L/W ratio = 3.1, hyaline, aseptate, straight, cylindrical, and obtuse at the apex. Morphological features suggested that these isolates possessed the same characteristics as previously described for Colletotrichum spp. (Damm et al. 2012). Further diagnostic information was obtained by sequencing partial internal transcribed spacers (ITS), actin (ACT), beta-tubulin (TUB2), calmodulin (CAL),

glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chitin synthase 1 (CHS-1), and histone 3 (HIS3) genes of a representative isolate, using the method and primers described by Damm et al. (2012). Sequences of the studied DNA regions were submitted to GenBank (ITS: MG693784; ACT: MG916907; TUB2: MH817038; CAL: MH817039; GAPDH: MH817040; CHS-1: MH817041; HIS3: MH817042). BLAST searches showed 99%–100% identity with sequences of Colletotrichum karstii Yang et al. 2011 (ITS: JQ005776; GAPDH: JQ948677; CHS-1: JQ005797; HIS3: JQ005818; ACT: JQ005839; TUB2: JQ005860). The phylogram constructed using combined datasets showed that isolates clustered into six distinct clades with high posterior probabilities; the isolate from this study clustered with *C. karstii* with 94% posterior probabilities. Moreover, pathogenicity tests were performed by adding sterile water on PDA cultures of *C. karstii*, and the resulting conidial suspension, adjusted to a concentration of 1.0 × 105 conidia/ml, was sprayed on the stem surface of pitaya plants (six replicates). The same number of uninoculated plants were used as controls. The experiment was performed twice. After inoculation, the plants were placed in glass culture dishes and maintained at 25°C in an incubator with constant relative humidity of 80% and a 12-h photoperiod. One week after inoculation, all inoculated plants showed stem spot symptoms, which were similar to the symptoms previously observed in the field. The uninoculated plants remained symptomless. The fungus re-isolated from inoculated stems exhibited the same morphological and molecular traits as the initial isolate. *C. karstii* has a wide host range; it was previously reported on *Bombax aquaticum*, *Carica papaya*, *Eugenia uniflora*, *Malus domestica*, *Mangifera indica*, and *Vaccinium* spp. in Brazil (Farr and Rossman 2018). To the best of our knowledge, this is the first report on anthracnose caused by *C. karstii* in pitaya plants in Brazil or anywhere in the world. This finding is of great importance for Brazilian pitaya production because this pathogen can severely damage pitaya plants and become a major problem for the cultivation of this crop.

References:

Yang, Y. L., et al. 2011. Cryptogam. Mycol. 32:241.

Damm, U., et al. Stud. Mycol. 73:1, 2012.

Farr, D. F., and Rossman, A. Y. 2018. Fungal Databases. Syst. Mycol. Microbiol. Lab. Online publication. ARS, USDA. Retrieved 25 February 2018 from https://nt.ars-grin.gov/fungaldatabases/.

Ronquist F, and Huelsenbeck JP (2003) Mrbayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

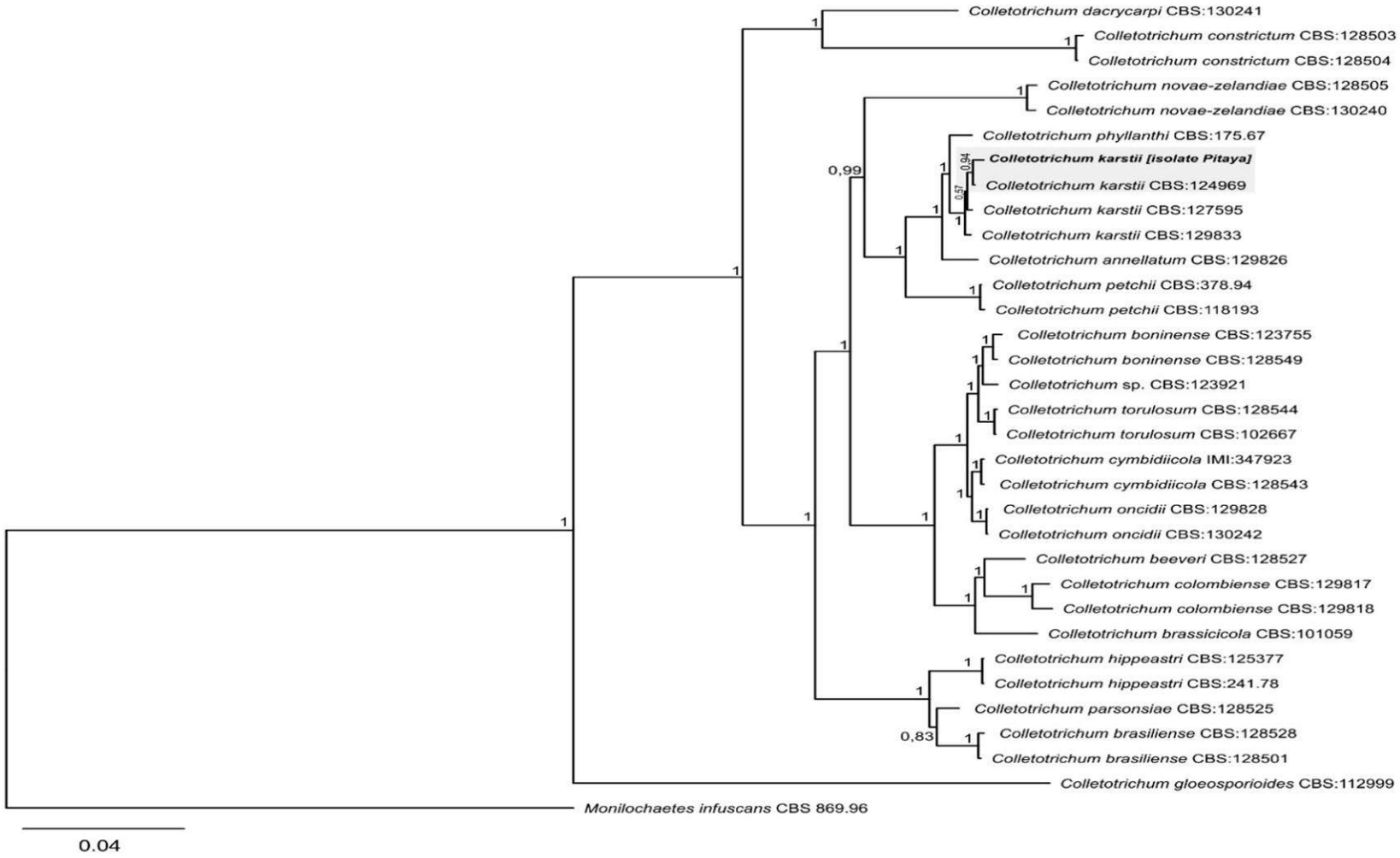


Figure 8 Phylogenetic tree derived from a Bayesian analysis of a partitioned, concatenated alignment of CHS-1, ACT, TUB2, GAPDH, HIS and ITS sequences, the colletotrichum described in this work (highlighted in bold), Monilochaetes infuscans was used as outgroup. Numbers as the nodes indicate Bayesian posterior probabilities.

4 Considerações finais

As interações entre begomovírus e DNAs satélites podem levar ao surgimento de associações prejudiciais às plantas, assim, é de grande importância o monitoramento de complexos begomovírus-satélites presentes em campos de cultivo e na vegetação nativa. Neste estudo, a associação de begomovírus com o alfassatélite LeYSA, forneceu novas informações que aumentaram a compreensão das interações entre vírus, DNA satélites, vetor e hospedeiro, que contribuíram para a adaptação e predominância de begomovírus em campo. Esta associação, também pode ocorrer em plantas cultivadas, como em tomateiro, representando uma ameaça aos sistemas de cultivo, já que no campo ainda não estão bem esclarecidas como essas interações ocorrem e quais são os prejuízos que poderiam trazer as culturas. Assim, há necessidade de estudos futuros para uma melhor compreensão da dinâmica das interações begomovírus-alfassatélites, bem como o entendimento do papel dos alfassatélites na patogenicidades de doenças.

Além disso, neste estudo, houve a diagnose da doença da antracnose em pitaia (*Colletotrichum karstii),* sendo esta, relatada a primeira vez no Brasil. Com a crescente produção de pitaia na região sul e com o aumento do trânsito de material propagativo advindo de vários locais, que favorece o risco de surgimento de novas doenças nessa cultura, é de grande importância a realização de uma diagnose correta, que se torne uma ferramenta fundamental para auxiliar os agricultores na escolha do manejo mais adequado para as doenças. Assim, é de extrema importância que os produtores tenham conhecimento de novos agentes causais de doenças na área de produção, para que sejam desenvolvidas novas formas de manejo e proteção da cultura.

Referências

ABBAS, Q.; AMIN, I.; MANSOOR, S.; WASSENEGGER, M.; BRIDDON, R.W. Geminivirus-associated alphasatellites suppress transcriptional not post-transcriptional gene silencing. **Virus Disease,** v. 27, p. 419, 2016.

AFANADOR-KAFURI, L., MINZ, D., MAYMON, M.; FREEMAN, S. Characterization of Colletotrichum isolates from tamarillo, passiflora, and mango in Colombia and identification of a unique species from the genus. **Phytopathology**, v. 93, p. 579-587, 2003.

AGRIOS, G. N. **Plant pathology**. Academic press,, 2005.

ANDRADE, E.C.; MANHANI, G.G.; ALFENAS, P.F.; CALEGARIO, R.F.; FONTES, E.P.B.; ZERBINI, F.M. *Tomato yellow spot virus*, a tomato-infecting begomovirus from Brazil with a closer relationship to viruses from *Sida* sp., forms pseudorecombinants with begomoviruses from tomato but not from *Sida*. **Journal of General Virology,** v. 87, p. 3687-3696, 2006.

ARAGÃO, F.J.L.; BARROS, L.M.G.; BRASILEIRO, A.C.M.; RIBEIRO, S.G.; SMITH, F.D.; SANFORD, J.C.; FARIA, J.C.; RECH, E.L. Inheritance of foreign genes in transgenic bean (*Phaseolus vulgaris* L.) co-transformed via particle bombardment. **Theoretical and Applied Genetics,** v. 93, p. 142-150, 1996.

BASTOS, D.C.; PIO, R.; SCARPARE FILHO, J.A.; LIBARDI, M.N.; ALMEIDA, L.D.; GALUCHI, T.P.D.; BAKKER, S.T. Propagação da pitaya 'vermelha' por estaquia. **Ciência e Agrotecnologia**, v. 30, p. 1106-1109, 2006.

BRIDDON, R.W.; BROWN, J.K.; MORIONES, E.; STANLEY, J.; ZERBINI, M.; ZHOU, X.; FAUQUET, C.M. Recommendations for the classification and nomenclature of the DNA-β satellites of begomoviruses. **Archives of Virology,** v. 153, p. 763-781, 2008.

BRIDDON, R.W.; BULL, S.E.; AMIN, I.; IDRIS, A.M.; MANSOOR, S.; BEDFORD, I.D.; DHAWAN, P.; RISHI, N.; SIWATCH, S.S.; ABDEL-SALAM, A.M.; BROWN, J.K.; ZAFAR, Y.; MARKHAM, P.G. Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. **Virology,** v. 312, p. 106-121, 2003.

BRIDDON, R.W.; MARTIN, D.P.; ROUMAGNAC, P.; NAVAS-CASTILLO, J.; FIALLO-OLIVE, E.; MORIONES, E.; LETT, J.M.; ZERBINI, F.M.; VARSANI, A. Alphasatellitidae: a new family with two subfamilies for the classification of geminivirus- and nanovirus-associated alphasatellites. **Archives of Virology**, v. 163, p. 2587-2600, 2018.

BROWN, J.K.; FAUQUET, C.M.; BRIDDON, R.W.; ZERBINI, F.M.; MORIONES, E.; NAVAS-CASTILLO, J. Family *Geminiviridae*. In: King, A.M.Q.;Adams, M.J.;Carstens, E.B. e Lefkowitz, E.J. (Ed.). **Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses**. London, UK: Elsevier Academic Press, 2012. p.351-373.

BROWN, J.K.; ZERBINI, F.M.; NAVAS-CASTILLO, J.; MORIONES, E.; RAMOS-SOBRINHO, R.; SILVA, J.C.; FIALLO-OLIVE, E.; BRIDDON, R.W.; HERNANDEZ-ZEPEDA, C.; IDRIS, A.; MALATHI, V.G.; MARTIN, D.P.; RIVERA-BUSTAMANTE, R.; UEDA, S.; VARSANI, A. Revision of Begomovirus taxonomy based on pairwise sequence comparisons. **Archives of Virology**, v. 160, p. 1593-619, 2015.

CAI, L.; HYDE, K.; TAYLOR, P.; WEIR, B.; WALLER, J.; ABANG, M.; ZHANG, J.; YANG, Y.; PHOULIVONG, S.; LIU, Z. A polyphasic approach for studying Colletotrichum. **Fungal Diversity,** v. 39, p. 183-204, 2009.

CANNON, P.F.; DAMM, U.; JOHNSTON, P.R.; WEIR, B.S. Colletotrichum - current status and future directions. **Studies in Mycology**, v. 73, p. 181-213, 2012.

CROUCH, J. A.; CLARKE, B. B.; HILLMAN, B. I. What is the value of ITS sequence data in Colletotrichum systematics and species diagnosis? A case study using the falcate-spored graminicolous Colletotrichum group. **Mycologia**, v. 101, p. 648-656, 2009.

CROUCH, J.A.; CLARKE, B.B.; WHITE JR, J.F.; HILLMAN, B.I. Systematic analysis of the falcate-spored graminicolous Colletotrichum and a description of six new species from warm-season grasses. **Mycologia**, v. 101, p. 717-732, 2009a.

CROUS, P.W.; GAMS, W.; STALPERS, J.A.; ROBERT, V.; STEGEHUIS, G. MycoBank: an online initiative to launch mycology into the 21st century. **Studies in Mycology**, v. 50, p. 19-22, 2004.

CUI, X.F.; LI, G.X.; WANG, D.W.; HU, D.W.; ZHOU, X.P. A begomovirus DNA beta-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. **Journal of Virology,** v. 79, p. 10764-10775, 2005.

DAMM, U.; CANNON, P.F.; WOUDENBERG, J.H.C.; JOHNSTON, P.R.; WEIR, B.S.; TAN, Y.P.;...; CROUS, P.W. The Colletotrichum boninense species complex. **Studies in mycology**, v. 73, p. 1-36, 2012.

DE MARCHI, B.R.; MARUBAYASHI, J.M.; FAVARA, G.M.; YUKI, V.A.; WATANABE, L.F.M.; BARBOSA, L.F.; PAVAN, M.A.; KRAUSE-SAKATE, R. Comparative transmission of five viruses by *Bemisia tabaci* NW2 and MEAM1. **Tropical Plant Pathology,** v. 42, p. 495-499, 2017.

DEAN, R.; VAN KAN, J.A.; PRETORIUS, Z.A.; HAMMOND-KOSACK, K.E.; DI PIETRO, A.; SPANU, P.D.; RUDD, J.J.; DICKMAN, M.; KAHMANN, R.; ELLIS, J. The Top 10 fungal pathogens in molecular plant pathology. **Molecular Plant Patholog**y, v. 13, p. 414-430, 2012.

DOEHLEMANN, G.; ÖKMEN, B., ZHU, W.; SHARON, A. Plant Pathogenic Fungi. **Microbiology spectrum**, v. 5, n. 1, 2017.

DOYLE, J.; DOYLE, J. **A Rapid DNA Isolation Procedure from Small Quantities of Fresh Leaf Tissues**. 1986.

DU, M.; SCHARDL, C.L.; NUCKLES, E.M.; VAILLANCOURT, L.J. Using mating-type gene sequences for improved phylogenetic resolution of Collectotrichum species complexes. **Mycologia**, v. 97, p. 641-658, 2005.

FARR, D. F.; ROSSMAN, A. Y. Fungal databases, US National Fungus Collections, ARS, USDA [Internet]. Beltsville: Systematic Mycology and Microbiology Laboratory; 2018 [cited 2018 Feb 5].

FERRO, C.G.; SILVA, J.P.; XAVIER, C.A.D.; GODINHO, M.T.; LIMA, A.T.M.; MAR, T.B.; LAU, D.; ZERBINI, F.M. The ever increasing diversity of begomoviruses infecting non-cultivated hosts: new species from Sida spp. and Leonurus sibiricus, plus two New World alphasatellites. **Annals of Applied Biology**, v. 170, p. 204-218, 2017.

FIALLO-OLIVÉ, E.; TOVAR, R.; NAVAS-CASTILLO, J. Deciphering the biology of deltasatellites from the New World: maintenance by New World begomoviruses and whitefly transmission. **New Phytologist,** v. 212, p. 680-692, 2016.

GAZIS, R.; REHNER, S.; CHAVERRI, P. Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. **Molecular ecology**, v. 20, p. 3001-3013, 2011.

GUO, L.W.; WU, Y.X.; HO, H.H.; SU, Y.Y.; MAO, Z.C.; HE, P.F.; HE, Y.Q. First report of dragon fruit (Hylocereus undatus) anthracnose caused by Colletotrichum truncatum in China. **Journal of Phytopathology**, v. 162, p. 272-275, 2014.

HANLEY-BOWDOIN, L.; BEJARANO, E.R.; ROBERTSON, D.; MANSOOR, S. Geminiviruses: Masters at redirecting and reprogramming plant processes. **Nature Reviews Microbiology,** v. 11, p. 777-88, 2013.

HARRISON, B.D.; ROBINSON, D.J. Natural genomic and antigenic variation in whitefly transmitted geminiviruses (begomoviruses). **Annual Review of Phytopathology,** v. 39, p. 369-398, 1999.

IDRIS, A.M.; SHAHID, M.S.; BRIDDON, R.W.; KHAN, A.J.; ZHU, J.K.; BROWN, J.K. An unusual alphasatellite associated with monopartite begomoviruses attenuates symptoms and reduces betasatellite accumulation. **Journal of General Virology,** v. 92, p. 706-717, 2011.

INOUE-NAGATA, A.K.; ALBUQUERQUE, L.C.; ROCHA, W.B.; NAGATA, T. A simple method for cloning the complete begomovirus genome using the bacteriophage phi29 DNA polymerase. **Journal of Virological Methods,** v. 116, p. 209-211, 2004.

JESKE, H.; KOBER, S.; SCHÄFER, B.; STROHMEIER, S. Circomics of Cuban geminiviruses reveals the first alphasatellite DNA in the Caribbean. **Virus Genes**, v. 49, p. 312-324, 2014.

KIM, Y.H.; JUN, O.K.; SUNG, M.J.; SHIN, J.S.; KIM, J.H.; JEONG, M.I. Occurrence of Colletotrichum stem rot caused by Glomerella cingulata on graft-cactus in Korea. **The Plant Pathology Journal**, v. 16, p. 242-245, 2000.

LAZAROWTIZ, S.G.; WU, L.C.; ROGERS, S.G.; ELMER, J.S. Sequence-specific interaction with the viral AL1 protein identifies a geminivirus DNA replication origin. **Plant Cell**, v. 4, p. 799-809, 1992.

LIMA, A.T.M. **Caracterização de dois begomovírus (*Tomato severe rugose virus* e *Tomato yellow vein streak virus*) que infectam tomateiro e obtenção de clones infecciosos**. 2007. 76 Tese M.S. Dep. de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG.

LOZANO, G.; TRENADO, H.P.; FIALLO-OLIVE, E.; CHIRINOS, D.; GERAUD-POUEY, F.; BRIDDON, R.W.; NAVAS-CASTILLO, J. Characterization of non-coding DNA satellites associated with sweepoviruses (genus *Begomovirus*, *Geminiviridae*) - definition of a distinct class of begomovirus-associated satellites. **Frontiers in Microbiology,** v. 7, p. 162, 2016.

LUBBE, C.M., DENMAN, S., CANNON, P.F., GROENEWALD, J.Z., LAMPRECHT, S.C., GROUS, P.W. Characterization of Colletotrichum species associated with diseases of Proteaceae. **The Mycological Society of America**, pp. 1268-1279. 2004.

MACEDO, M.A.; MICHEREFF, M.; NAVAS-CASTILLO, J.; INOUE-NAGATA, A.K. Host range and whitefly transmission efficiency of *Tomato severe rugose virus* and *Tomato golden vein virus* in tomato plants. **Tropical Plant Pathology,** v. 40, p. 405-409, 2015.

MAHAJAN, N.; PARAMESWARI, C.; VELUTHAMBI, K. Severe stunting in blackgram caused by the *Mungbean yellow mosaic virus* (MYMV) KA27 DNA B component is ameliorated by co-infection or post-infection with the KA22 DNA B: MYMV nuclear shuttle protein is the symptom determinant. **Virus Research,** v. 157, p. 25-34, 2011.

MANSOOR, S.; BRIDDON, R.W.; ZAFAR, Y.; STANLEY, J. Geminivirus disease complexes: An emerging threat. **Trends in Plant Science,** v. 8, p. 128-134, 2003.

MAR, T.B.; MENDES, I.R.; LAU, D.; FIALLO-OLIVE, E.; NAVAS-CASTILLO, J.; ALVES, M.S.; ZERBINI, F.M. Interaction between the New World begomovirus Euphorbia yellow mosaic virus and its associated alphasatellite: effects on infection and transmission by the whitefly Bemisia tabaci. **Journal of General Virology**, v. 98, p. 1552-1562, 2017a.

MAR, T.B.; XAVIER, C.A.D.; LIMA, A.T.M.; NOGUEIRA, A.M.; SILVA, J.C.F.; RAMOS-SOBRINHO, R.; LAU, D.; ZERBINI, F.M. Genetic variability and population structure of the New World begomovirus *Euphorbia yellow mosaic virus*. **Journal of General Virology,** v. 98, p. 1537-1551, 2017b.

MARIN-FELIX, Y. et al. Genera of phytopathogenic fungi: GOPHY 1. **Studies in mycology**, v. 86, p. 99-216, 2017.

MARTIN NETO, L.; GALERANI, P. R.; COSTA, JEFFERSON L. DA S. Research, development, and innovations in health risk assessment for Brazilian agriculture. **Pesquisa Agropecuária Brasileir**a, v. 51, n. 5, p. i-viii, 2016.

MARTINELLI, F.; SCALENGHE, R.; DAVINO, S.; PANNO, S.; SCUDERI, G.; RUISI, P.; VILLA, P.; STROPPIANA, D.; BOSCHETTI, M.; GOULART, L.R. Advanced methods of plant disease detection. A review. Agronomy for Sustainable Development, v. 35, p. 1-25, 2015.

MASYAHIT, M.; SIJAM, K.; AWANG, Y.; GHAZALI, M. First report on bacterial soft rot disease on dragon fruit (Hylocereus spp.) caused by Enterobacter cloacae in peninsular Malaysia. **International Journal of Agriculture and Biology**, v. 11, p. 659-666, 2009.

MENEZES, M. Aspectos biológicos e taxonômicos de espécies do gênero Colletotrichum. **Fitopatologia brasileira**, v.27 (supl.), p. 23-24, 2002.

MILLS, P.R.; SREENIVASAPRASAD, S.; BROWN, A.E. Detection and differenciation of Colletotrichum gloeosporioides using PCR. **FEMS Microbiology Letters**, v. 98, p.137-144, 1992.

MORIWAKI, J., SATO, T.; TSUKIBOSHI, T. Morphological and molecular characterization of Colletotrichum boninense sp. nov. from Japan. **Mycoscience**, v. 44, p. 47- 53, 2003.

MYCOBANK. **MycoBank.org.** Disponível em: http://www.mycobank.org/BioloMICS.aspx?TableKey=14682616000000067&Rec=33692&. Acesso em: 18 de set. 2019.

NAWAZ-UL-REHMAN, M.S.; NAHID, N.; MANSOOR, S.; BRIDDON, R.W.; FAUQUET, C.M. Post-transcriptional gene silencing suppressor activity of two non-pathogenic alphasatellites associated with a begomovirus. **Virology,** v. 405, p. 300-308, 2010.

NERD, A.; MIZRAHI, Y. The effect of ripening stage on fruit quality after storage of yellow pitaya. **Postharvest Biology and Technology**, v. 15, p. 99-105, 1999.

O'DONNELL, K.; GUEIDAN, C.; SINK, S.; JOHNSTON, P.R.; CROUS, P.W.; GLENN, A.; RILEY, R.; ZITOMER, N.C.; COLYER, P.; WAALWIJK, C. A two-locus DNA sequence database for typing plant and human pathogens within the Fusarium oxysporum species complex. **Fungal Genetics and Biology**, v. 46, p. 936-948, 2009.

ORTIZ-HERNÁNDEZ, Y.D.; CARRILLO-SALAZAR, J.A. Pitahaya (Hylocereus spp.): a short review. **Comunicata Scientiae**, v. 3, p. 220-237, 2012.

PADIDAM, M.; BEACHY, R.N.; FAUQUET, C.M. The role of AV2 ("precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. **Virology,** v. 224, p. 390-404, 1996.

PADIDAM, M.; SAWYER, S.; FAUQUET, C.M. Possible emergence of new geminiviruses by frequent recombination. **Virology,** v. 265, p. 218-224, 1999.

PAPROTKA, T.; METZLER, V.; JESKE, H. The first DNA 1-like alphasatellites in association with New World begomoviruses in natural infections. **Virology**, v. 404, p. 148-157, 2010.

PAXIMADIS, M.; IDRIS, A.M.; TORRES-JEREZ, I.; VILLARREAL, A.; REY, M.E.C.; BROWN, J.K. Characterization of tobacco geminiviruses in the Old and New world. **Archives of Virology**, v. 144, p. 703-717, 1999.

PELCZAR, M.J. *et al.* Plant disease. Encyclopedia Britannica. **Enclyclopedia Britannica**, **inc,** 10 maio. 2019. Disponível: https://www.britannica.com/science/plant-disease. Acesso em: 08 set. 2019.

PHOULIVONG, S.; CAI, L.; CHEN, H.; MCKENZIE, E.; ABD-ELSALAM, K.; CHUKEATIROTE, E.; HYDE, K. Colletotrichum gloeosporioides is not a common pathogen on tropical fruits. **Fungal Diversity**, v. 44, p. 33-43, 2010.

PILEGGI, S.A.V.; OLIVEIRA, S.F.V.; WACULICZ-ANDRADE, C.E.; VICENTE, V.A.; DALZOTO, P.R.; CRUZ, G.K.; GABARDO, J.; MASSOLA, N.Jr.; TORRES, H.J. Jr.; PILEGGI,M.; KAVA-CORDEIRO, V.; GALLI-TERESAWA, L.V.; PIMENTEL, I.C.; GLIENKE, C. Molecular and Morphological Identification of Colletotrichum gloeosporioides and Colletotrichum boninense isolated from Maytenus ilicifolia, **Canadian Journal of Microbiology,** 55: 1076-1088. 2009.

ROJAS, M.R.; HAGEN, C.; LUCAS, W.J.; GILBERTSON, R.L. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. **Annual Review of Phytopathology,** v. 43, p. 361-394, 2005.

ROJAS, M.R.; MACEDO, M.A.; MALIANO, M.R.; SOTO-AGUILAR, M.; SOUZA, J.O.; GILBERTSON, R.L.; BRIDDON, R.W.; KENYON, L.A.; BUSTAMANTE, R.F.R.; ZERBINI, F.M.; ADKINS, S.; LEGG, J.P.; KVARNHEDEN, A.; WINTERMANTEL, W.M.; SUDARSHANA, M.R.; PETERSCHMITT, M.; LAPIDOT, M.; MARTIN, D.P.; MORIONES, E.; INOUE-NAGATA, A.K. World management of geminiviruses. **Annual Review of Phytopathology**, v. 56, p. 637-677, 2018.

ROMAY, G.; CHIRINOS, D.; GERAUD-POUEY, F.; DESBIEZ, C. Association of an atypical alphasatellite with a bipartite New World begomovirus. **Archives of Virology,** v. 155, p. 1843-1847, 2010.

RONQUIST, Fredrik; HUELSENBECK, John P. MrBayes 3: Bayesian phylogenetic inference under mixed models. **Bioinformatics**, v. 19, n. 12, p. 1572-1574, 2003.

ROSARIO, K.; MARR, C.; VARSANI, A.; KRABERGER, S.; STAINTON, D.; MORIONES, E.; POLSTON, J.E.; BREITBART, M. Begomovirus-associated satellite DNA diversity captured through vector-enabled metagenomic (VEM) surveys using whiteflies (Aleyrodidae). **Viruses,** v. 8, p. 36, 2016.

RYBICKI, E.P. A phylogenetic and evolutionary justification for three genera of Geminiviridae. **Archives of Virolog**y, v. 139, p. 49-77, 1994.
SAEED, M.; BEHJATNIA, S.A.A.; MANSOOR, S.; ZAFAR, Y.; HASNAIN, S.; REZAIAN, M.A. A single complementary-sense transcript of a geminiviral DNA beta satellite is determinant of pathogenicity. **Molecular Plant-Microbe Interactions,** v. 18, p. 7-14, 2005.

SAUNDERS, K.; BEDFORD, I.D.; BRIDDON, R.W.; MARKHAM, P.G.; WONG, S.M.; STANLEY, J. A unique virus complex causes Ageratum yellow vein disease. **Proceedings of the National Academy of Sciences**, USA, v. 97, p. 6890-5, 2000.

SAUNDERS, K.; BEDFORD, I.D.; STANLEY, J. Adaptation from whitefly to leafhopper transmission of an autonomously replicating nanovirus-like DNA component associated with ageratum yellow vein disease. **Journal of General Virology**, v. 83, p. 907-13, 2002.

SAUNDERS, K.; STANLEY, J. A nanovirus-like DNA component associated with yellow vein disease of Ageratum conyzoides: Evidence for interfamilial recombination between plant DNA viruses. **Virology**, v. 264, p. 142-152, 1999.

SCHOCH, C.L.; SEIFERT, K.A.; HUHNDORF, S.; ROBERT, V.; SPOUGE, J.L.; LEVESQUE, C.A.; CHEN, W.; CONSORTIUM, F.B. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. **Proceedings of the National Academy of Sciences**, USA, v. 109, p. 6241-6246, 2012.

SHARMA, P. D. **Plant Pathology**. Rastogi Publications: India, 2013.

SILVA, A. Pitaya: melhoramento e produção de mudas. 2014. Tese de Doutorado. Tese (Doutorado em Agronomia)–Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal.

SUTTON, B.C.; BAILEY, J.A.; JEGER M.J. The genus Glomerella and its anamorph Colletotrichum. **The genus Glomerella and its anamorph Colletotrichum.** p. 1-26, 1992.

TAKAHASHI, L.M.; ROSA, D.D.; BASSETO, M.A.; DE SOUZA, H.G.; FURTADO, E.L. First report of Colletotrichum gloeosporioides on Hylocereus megalanthus in Brazil. **Australasian Plant Disease Notes**, v. 3, p. 96-97, 2008.

UDAYANGA, D.; MANAMGODA, D. S.; LIU, X.; CHUKEATIROTE, E.; HYDE, K. D. What are the common anthracnose pathogens of tropical fruits?. **Fungal Diversity**, v. 61, n. 1, p. 165-179, 2013.

VALENCIA-BOTÍN, A.J.; CRUZ HERNÁNDEZ, P.; RODRÍGUEZ CANTO, A. avances en la etiología y manejo de la pudrición blanda de tallos de pitahaya, Hylocereus undatus H.(CACTACEAE). **Fitosanidad,** v. 7, n. 2, 2003.

VARSANI, A.; NAVAS-CASTILLO, J.; MORIONES, E.; HERNÁNDEZ-ZEPEDA, C.; IDRIS, A.; BROWN, J.K.; ZERBINI, F.M.; MARTIN, D.P. Establishment of three new genera in the family Geminiviridae: Becurtovirus, Eragrovirus and Turncurtovirus. **Archives of Virology**, v. 159, p. 2193-2203, 2014.

VARSANI, A.; ROUMAGNAC, P.; FUCHS, M.; NAVAS-CASTILLO, J.; MORIONES, E.; IDRIS, A.; BRIDDON, R.W.; RIVERA-BUSTAMANTE, R.F.; ZERBINI, F.M.; MARTIN, D.P. Capulavirus and Grablovirus: two new genera in the family Geminiviridae. **Archives of Virology,** v. 162, p. 1819-1831, 2017.

WEIR, B.S.; JOHNSTON, P.R.; DAMM, U. The Colletotrichum gloeosporioides species complex. **Studies in mycology**, v. 73, p. 115-180, 2012.

WU, P.J.; ZHOU, X.P. Interaction between a nanovirus-like component and the *Tobacco curly shoot virus*/satellite complex. **Acta Biochimica et Biophysica Sinica**, v. 37, p. 25-31, 2005.

YANG, Y.L.; CAI, L.; YU, Z.; LIU, Z.; HYDE, K.D. Colletotrichum species on Orchidaceae in southwest China. **Cryptogamie, Mycologie**, v. 32, p. 229-254, 2011.

YANG, Y.L.; LIU, Z.Y.; CAI, L.; HYDE, K.D.; YU, Z.N.; MCKENZIE, E.H.C. Colletotrichum anthracnose of Amaryllidaceae. **Fungal Diversity**, v. 39, p. 123-146, 2009.

ZERBINI, F.M.; BRIDDON, R.W.; IDRIS, A.; MARTIN, D.P.; MORIONES, E.; NAVAS-CASTILLO, J.; RIVERA-BUSTAMANTE, R.; VARSANI, A.; ICTV Consortium. ICTV Virus Taxonomy Profile: Geminiviridae. **Journal of General Virology**, v. 98, p. 131-133, 2017.

ZHOU, X. Advances in understanding begomovirus satellites. **Annual Review of Phytopathology**, v. 51, p. 357-381, 2013.

Apêndices

Apêndice A – Results of the host range assay. Number of PCR-positive plants from 10 tomato plants in Experiment 1 and 15 tomato plants, *N. benthamiana* and *L. sibiricus* in the other experiments, inoculated at 14 and 28 days post-inoculation with *Tomato yellow spot virus* (ToYSV),*Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV) and its associated alphasatellite *Leonurus yellow spot alphasatellite* (LeYSA) in two independent experiments, except tomato with three independent experiments.

	ToYSV								ToYSV+LeYSA							
	14dpi				28dpi				14dpi				28dpi			
	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**
Solanum lycopersicum	7	8	10	**25** (40)	7	8	10	**25** (40)	10	2	10	**22** (39)	10	5	10	**25** (39)
Nicotiana benthamiana	8	14	-	**22** (24)	7	14	-	**21** (22)	10	15	-	**25** (25)	10	15	-	**25** (25)
Leonurus sibiricus	7	11	-	**18** (28)	8	11	-	**19** (28)	8	12	-	**20** (30)	8	13	-	**21** (30)

	ToSRV								ToSRV+LeYSA							
	14dpi				28dpi				14dpi				28dpi			
	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**
Solanum lycopersicum	6	10	7	**23** (39)	6	10	7	**23** (39)	7	3	3	**13** (36)	7	3	4	**14** (36)
Nicotiana benthamiana	7	11	-	**18** (22)	6	11	-	**17** (21)	5	9	-	**14** (21)	5	9	-	**14** (20)
Leonurus sibiricus	0	0	-	**0** (24)	0	0	-	**0** (24)	0	0	-	**0** (25)	0	0	-	**0** (25)

	EuYMV								EuYMV+LeYSA							
	14dpi				28dpi				14dpi				28dpi			
	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**	Exp 1	Exp 2	Exp 3	**Total**
Solanum lycopersicum	4	1	10	**15** (40)	4	5	13	**22** (40)	5	0	6	**11** (40)	8	8	11	**27** (39)
Nicotiana benthamiana	3	9	-	**12** (24)	4	9	-	**13** (24)	2	8	-	**10** (23)	5	8	-	**13** (23)
Leonurus sibiricus	0	0	-	**0** (23)	0	0	-	**0** (23)	0	0	-	**0** (29)	0	0	-	**0** (29)

Apêndice B – Symptoms in *Leonurus sibiricus* of *Tomato yellow spot virus* (ToYSV) in the presence or absence of *Leonurus yellow spot alphasatellite* (LeYSA) at 14 days post-inoculation. Images are coded as follows: Uppercase L: *Leonurus sibiricus*; First number: 1 to 14, ToYSV in the absence of LeYSA; 15 to 19, ToYSV in the presence of LeYSA; Second number: 1, first replication.

Apêndice C – Symptoms in tomato of *Tomato yellow spot virus* (ToYSV), in the presence or absence of *Leonurus yellow spot alphasatellite* (LeYSA) at 28 days post-inoculation. Images are coded as follows: Uppercase T: Tomato; First number: 1, ToYSV; Second number: 1 to 10, ToYSV in the absence of LeYSA; 11 to 20, ToYSV in the presence of LeYSA.

Apêndice D – Symptoms in tomato of *Tomato severe rugose virus* (ToSRV) in the presence or absence of *Leonurus yellow spot alphasatellite* (LeYSA) at 28 days post-inoculation. Images are coded as follows: Uppercase T: Tomato; First number: 2, ToSRV; Second number: 1 to 10, ToSRV in the absence of LeYSA; 11 to 19, ToSRV in the presence of LeYSA.

Apêndice E – Symptoms in tomato of *Euphorbia yellow mosaic virus* (EuYMV) in the presence or absence of *Leonurus yellow spot alphasatellite* (LeYSA) at 28 days post-inoculation. Images are coded as follows: Uppercase T: Tomato; First number: 3, EuYMV; Second number: 1 to 10, EuYMV in the absence of LeYSA; 11 to 19, EuYMV in the presence of LeYSA.

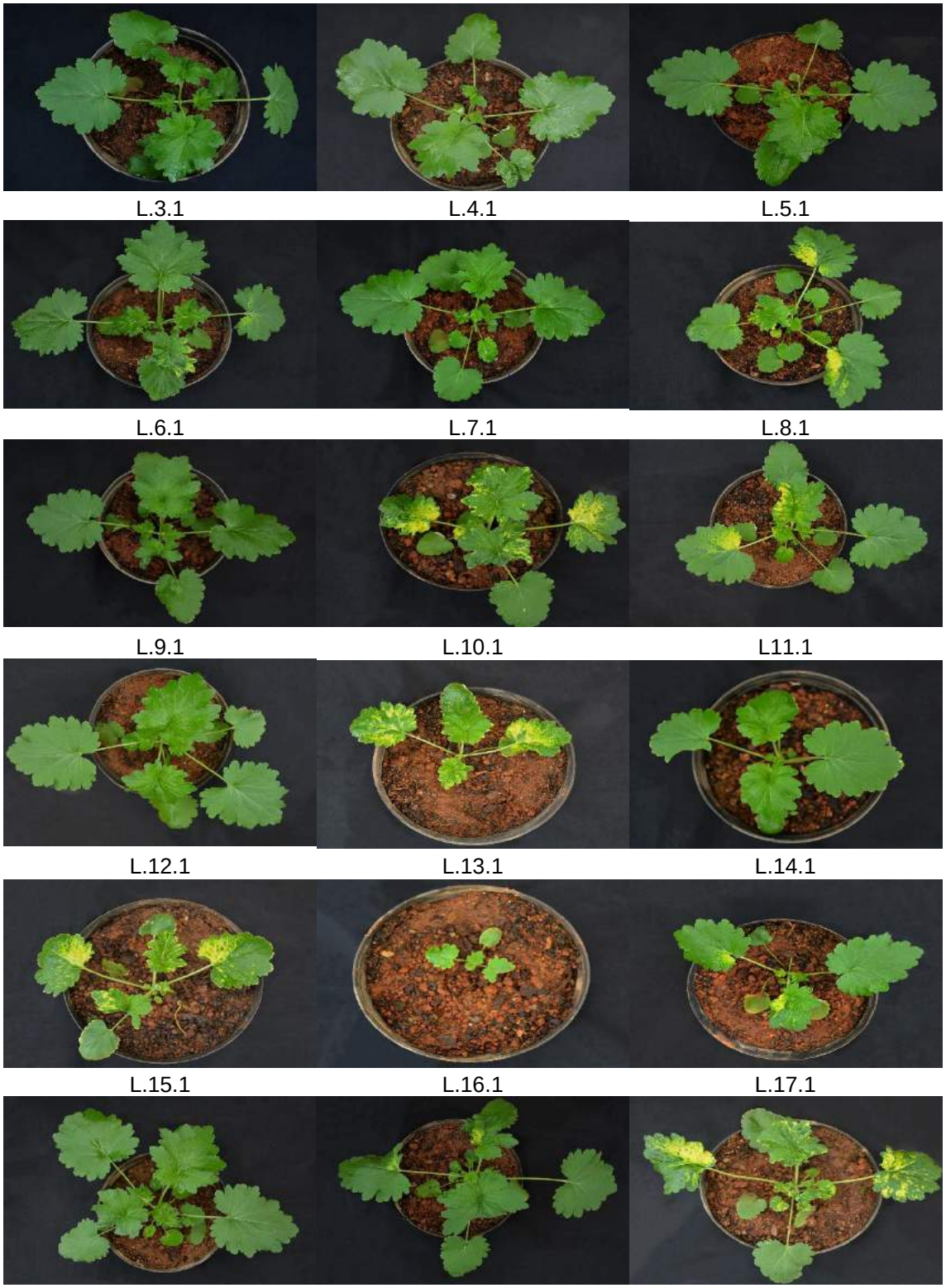
Apêndice F – Symptoms in *Nicotiana benthamiana* of *Tomato yellow spot virus* (ToYSV), in the presence or absence of *Leonurus yellow spot alphasatellite* (LeYSA) at 28 days post-inoculation. Images are coded as follows: Uppercase N: *Nicotiana benthamiana*; First number: 1, ToYSV; Second number: 1 to 8, ToYSV in the absence of LeYSA; 9 to 18, ToYSV in the presence of LeYSA.

Apêndice G – Symptoms in *Nicotiana benthamiana* of *Tomato severe rugose virus* (ToSRV) in the presence or absence of *Leonurus yellow spot alphasatellite* (LeYSA) at 28 days post-inoculation. Images are coded as follows: Uppercase N: *Nicotiana benthamiana*; First number: 2, ToSRV; Second number: 1 to 7, ToSRV in the absence of LeYSA; 8 to 15, ToSRV in the presence of LeYSA.

Apêndice H – Symptoms in *Nicotiana benthamiana* of *Euphorbia yellow mosaic virus* (EuYMV) in the presence or absence of *Leonurus yellow spot alphasatellite* (LeYSA) at 28 days post-inoculation. Images are coded as follows: Uppercase N: *Nicotiana benthamiana*; First number: 3, EuYMV; Second number: 1 to 10, EuYMV in the absence of LeYSA; 11 to 20, EuYMV in the presence of LeYSA.

Apêndice B

Leonurus sibiricus



Apêndice B (cont.)

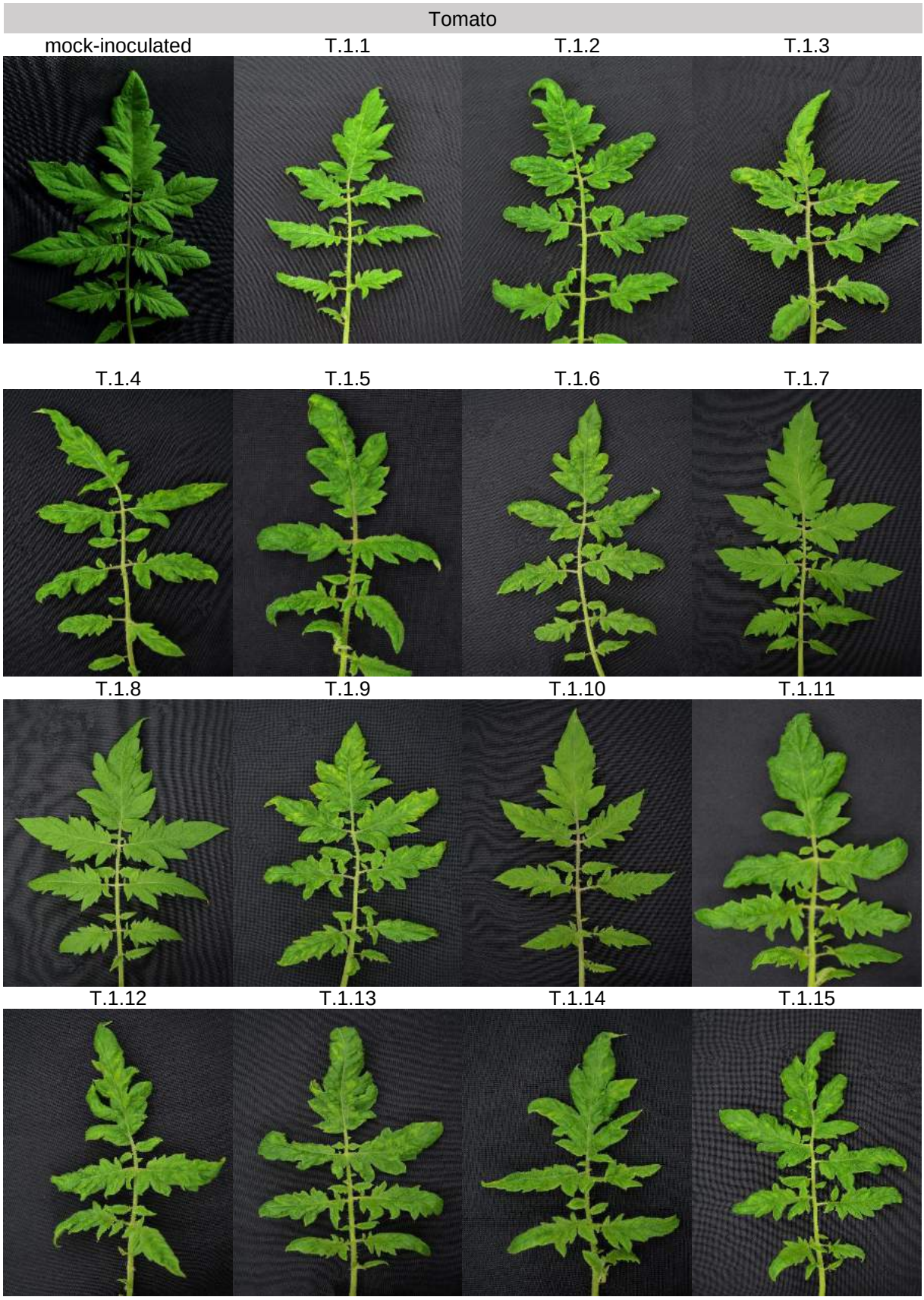
L.18.1 L.19.1 L.20.1

L.21.1 L.22.1 L.23.1

L.24.1 L.25.1 L.26.1

L.27.1 L.28.1 L.29.1

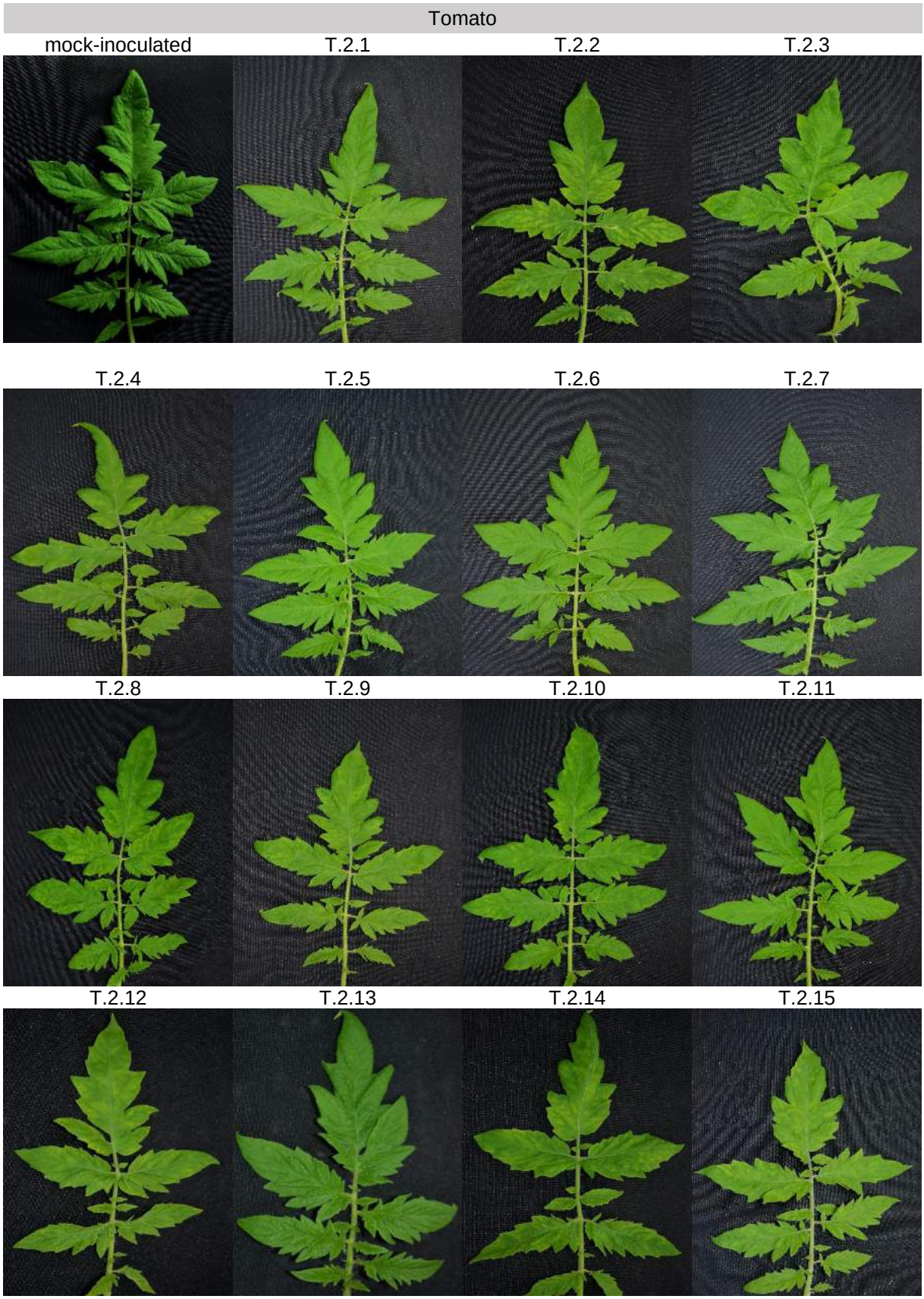
Apêndice C


Tomato
mock-inoculated
T.1.1
T.1.2
T.1.3
T.1.4
T.1.5
T.1.6
T.1.7
T.1.8
T.1.9
T.1.10
T.1.11
T.1.12
T.1.13
T.1.14
T.1.15

T.1.16 T.1.17 T.1.18 T.1.19

T.1.20

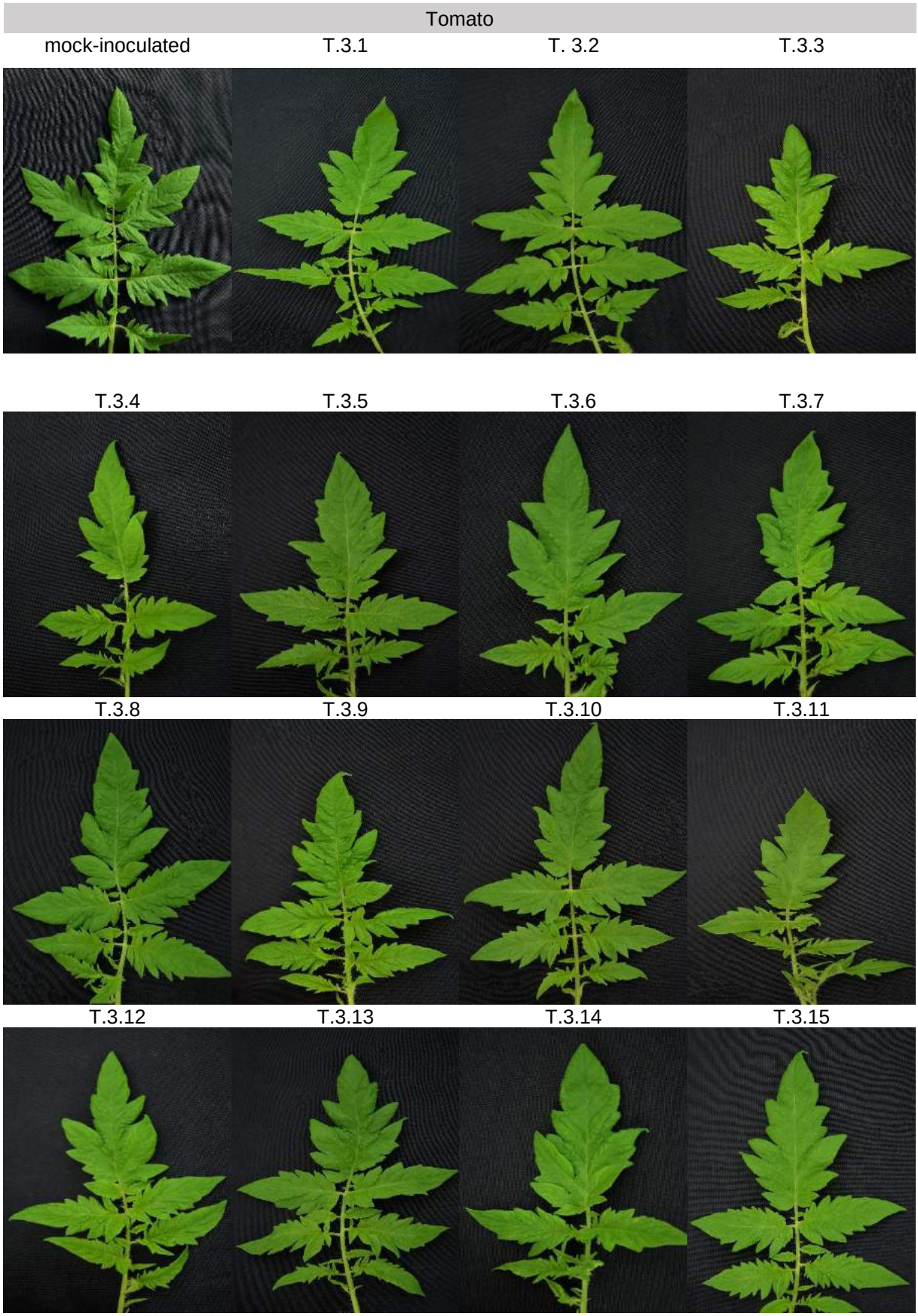
Apêndice C (cont.)


Tomato
mock-inoculated
T.2.1
T.2.2
T.2.3
T.2.4
T.2.5
T.2.6
T.2.7
T.2.8
T.2.9
T.2.10
T.2.11
T.2.12
T.2.13
T.2.14
T.2.15

Apêndice D

Apêndice D (cont.)

T.2.16 T.2.17 T.2.18 T.2.19


Tomato
mock-inoculated
T.3.1
T. 3.2
T.3.3
T.3.4
T.3.5
T.3.6
T.3.7
T.3.8
T.3.9
T.3.10
T.3.11
T.3.12
T.3.13
T.3.14
T.3.15

Apêndice E

Apêndice E (cont.)

T.3.16 T.3.17 T.3.18 T.3.19

Apêndice F

Nicotiana benthamiana

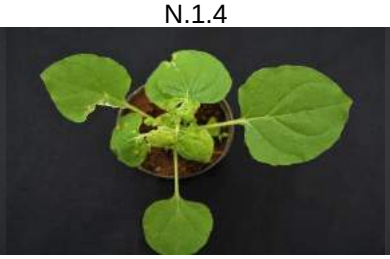
mock-inoculated

N.1.1

N.1.2

N.1.3

N.1.4

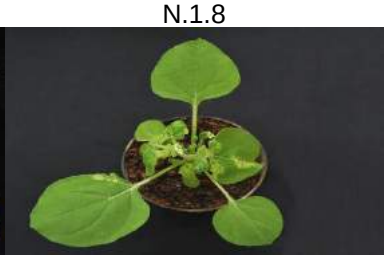


N.1.5

N.1.6

N.1.7

N.1.8



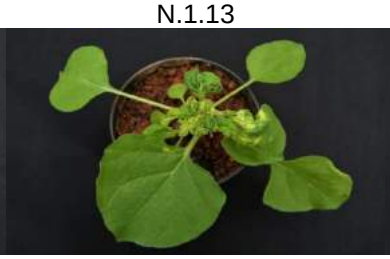
N.1.9

N.1.10

N.1.11

N.1.12

N.1.13



N.1.14

N.1.15

N.1.16

N.1.17

N.1.18

Apêndice G

Nicotiana benthamiana

mock-inoculated

N.2.1

N.2.2

N.2.3

N.2.4

N.2.5

N.2.6

N.2.7

N.2.8

N.2.9

N.2.10

N.2.11

N.2.12

N.2.13

N.2.14

N.2.15

Apêndice H

Nicotiana benthamiana

mock-inoculated	N.3.1	N.3.2
		
N.3.3	N.3.4	N.3.5
		
N.3.6	N.3.7	N.3.8
		
N.3.9	N.3.10	N.3.11
		
N.3.12	N.3.13	N.3.14
		
N.3.15	N.3.16	N.3.17
		
N.3.18	N.3.19	N.3.20
		

Apêndice I - Plantas de pitaia com sintomas típicos de antracnose, sintomas iniciais no caule, lesões arredondadas ou de formas irregulares com mancha laranja-avermelhadas (**A**); as lesões no caule coalesceram em grandes lesões marrom-escuras, no centro das lesões eram cinza-esbranquiçados com bordas marrom-púrpura circundadas por um halo clorótico onde pontos pretos aparecem mais tardiamente (**B**); crescimento micelial em meio de cultura BDA (Batata-Dextrose-Ágar), micélios aéreos brancos com massa conidial alaranjada (**C**); Estruturas reprodutivas de *C. karstii*: acérvulos (pontos negros) formados na superfície do tecido de caule de pitaia (**D**); e conídios (esporos) (**E**).