



ANTONIA THALYTA LOPES SILVEIRA

**Biological and molecular study, incidence, and spatial
distribution of viruses in pitaya (*Selenicereus* spp.)**

**LAVRAS – MG
2024**

ANTONIA THALYTA LOPES SILVEIRA

**Estudo biológico, molecular, incidência e distribuição espacial de vírus em pitaya
(*Selenicereus* spp.)**

**Biological and molecular study, incidence, and spatial distribution of viruses in pitaya
(*Selenicereus* spp.)**

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Agronomia/Fitopatologia, área de concentração em Virologia, para obtenção do título de Doutor.

Prof (a). Dr (a). Antonia dos Reis Figueira
Orientador (a)

**LAVRAS – MG
2024**

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca
Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Silveira, Antonia Thalyta Lopes.

Biological and molecular study, incidence, and spatial
distribution of viruses in pitaya (*Selenicereus* spp.) / Antonia
Thalyta Lopes Silveira. - 2023.

91 p. : il.

Orientador(a): Antonia dos Reis Figueira.

Tese (doutorado) - Universidade Federal de Lavras, 2023.
Bibliografia.

1. Agronomia. 2. Fitopatologia. 3. Virologia. I. Figueira,
Antonia dos Reis. II. Título.

ANTONIA THALYTA LOPES SILVEIRA

**ESTUDO BIOLÓGICO, MOLECULAR, INCIDÊNCIA E DISTRIBUIÇÃO
ESPACIAL DE VÍRUS EM PITAIA (*Selenicereus* spp.)**

**BIOLOGICAL AND MOLECULAR STUDY, INCIDENCE, AND SPATIAL
DISTRIBUTION OF VIRUS IN PITAYA (*Selenicereus* spp.)**

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Agronomia/Fitopatologia, área de concentração em Virologia, para obtenção do título de Doutor.

APROVADA em 10 de março de 2023.

Dr. João Eduardo Melo de Almeida AGRICHEM DO BRASIL S.A.

Dra. Leila Aparecida Salles Pio UFLA

Dra. Claudine Marcia Carvalho UFLA

Dra. Patrícia Ricardino Silveira UFLA

Prof (a). Dr (a). Antonia dos Reis Figueira
Orientador (a)

**LAVRAS – MG
2024**

Ao meu pai Célio Soares Lopes e a minha tia Maria Silveira (in memoria).

A minha mãe Maria do Carmo e a minha irmã Thacyla.

A minha família, Lopes e Silveira, aos que partiram e as futuras gerações.

Dedico

AGRADECIMENTOS

À Deus, senhor do universo, que ampara em todos os momentos;

À Universidade Federal de Lavras, especialmente ao Departamento de Fitopatologia, pelo ensino gratuito e de excelência.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de doutorado e custos no desenvolvimento da pesquisa.

À professora Dra. Antonia dos Reis Figueira, pela orientação, pela amizade, por compartilhar seus conhecimentos, pela paciência e disposição em ajudar, pelo acolhimento nos momentos desafiadores.

Aos professores da Universidade Federal de Lavras, em especial aos professores Dra. Leila Aparecida Salles Pio, Dr. Edson Ampélio Pozza e Dra. Claudine Marcia Carvalho por todas as orientações, pelo suporte e amizade.

A todos os funcionários do Departamento de Fitopatologia/UFLA, especialmente ao Carlos Roberto, Viviane, Ana Maria e Ariane, pela amizade, pelo respeito, por todo suporte e auxílio nos momentos de alegrias e de tristeza, sempre com palavras de carinho e ânimo.

Aos meus colegas de laboratório, especialmente a Sérgio, Jéssica, Daniele, Sandra, Marcos e Guilherme que me auxiliaram na bancada do laboratório, pela permuta de ideais e conhecimentos.

Aos meus pais, Célio (*in memoria*) e Maria do Carmo pelo amor e apoio incondicional, pelas palavras de carinho e incentivo, pelos conselhos, acolhimento e fortalecimento nos momentos difíceis e conflituosos.

À minha irmã Thacyla, por todo amor e apoio, sempre disponível em todos os momentos em que precisei.

À minha família, Lopes e Silveira, pelo apoio, pela força e por suas orações.

A todos os colegas de departamento, pois muitos se tornaram amigos, e alguns irmãos, em especial a Amanda e Adriano.

À Luciana, Liliana, Débora, Marielle e Marcela, pela amizade, apoio e carinho na convivência diária na república Camponesas.

À família Garcia Fernandez, pelo amor e carinho.

As minhas amigas, Luciana e Natália, pelo carinho, pelas risadas, pelo apoio e todas as conversas acolhedoras.

Ao Prof. Dr. Cleilson Uchôa por apresentar a fitopatologia, pelo incentivo na busca e luta dos meus sonhos, de ser uma fitopatologista, plantando esta semente em meu coração.

“Tudo tem o seu tempo determinado, e há tempo para todo o propósito debaixo do céu. Há tempo de nascer, e tempo de morrer; tempo de plantar, e tempo de arrancar o que se plantou.” (Eclesiastes 3).

RESUMO GERAL

O cultivo da pitiaia, fruta pertencente ao gênero *Selenicereus* spp., tem aumentado ininterruptamente nos últimos anos, devido às suas propriedades nutracêuticas e organolépticas. Além disso, tem se mostrado bastante rentável para os pequenos produtores no Brasil. Entretanto, devido à sua recente introdução no país, são raros os estudos envolvendo a ocorrência e controle das doenças e pragas, sendo a maioria deles focando nos seus compostos bioquímicos e bioativos. Entre as doenças que podem afetar a pitiaia, as viroses se destacam pela ausência de métodos de controle curativos e por serem subnotificadas, pois os sintomas podem ser confundidos com distúrbios nutricionais. Como a propagação vegetativa predomina nesta cultura e o seu cultivo envolve diversas operações manuais, a disseminação dos vírus é bastante favorecida, de modo que plantas com suspeita de viroses têm sido frequentemente relatadas pelos produtores. Neste trabalho foram coletadas 212 amostras de cladódios com sintomas suspeitos de viroses, em diferentes regiões do Brasil, com a finalidade de identificar e fazer a caracterização biológica dos vírus encontrados, bem como estudar a sua variabilidade molecular com base na sequência do gene da capa proteica (CP). Além disso, foi feito um levantamento em lavouras comerciais de cinco municípios localizados no sul de Minas para determinar a incidência e o padrão espacial de plantas com suspeita de viroses, empregando-se os modelos Run, Doublet e o índice de dispersão. As plantas de *Chenopodium quinoa* e *C. Amaranticolor* inoculadas mecanicamente reagiram com sintomas de lesões cloróticas e necróticas. Entre as 212 amostras analisadas, 181 se encontravam infectadas com uma ou mais das seguintes espécies do gênero *Potexvirus*: *Cactus virus* (CVX), *Pitaya virus X* (PiVX), *Zygocactus virus X* (ZyVX) e *Schlumbergera virus X* (SchVX), que se encontravam em infecções simples e mistas com dois a quatro vírus na mesma amostra. Na análise do gene CP, os isolados de CVX apresentaram dois grupos distintos, sendo que um deles apresentou uma deleção de nove nucleotídeos na região inicial do gene CP, na posição 42 a 50, na direção 5'→3'. No levantamento realizado, todos os pomares apresentaram altas incidências de potexvirus, variando entre 80 e 100%. A maioria dos pomares apresentou um padrão agregado nos testes Run e Doublet, enquanto que no índice de dispersão prevaleceu o padrão regular, sugerindo uma disseminação por práticas culturais a partir de mudas infectadas. Este foi o primeiro trabalho envolvendo a detecção e identificação de viroses em pitiaia, bem como a incidência e o padrão espacial em lavouras comerciais de pitiaia. Os resultados mostraram despreparo dos produtores para a adoção de medidas preventivas de viroses na cultura da pitiaia. Ficou também evidente que as mudas comercializadas devem ser as responsáveis pela introdução de vírus nos pomares, ressaltando a necessidade premente de um sistema de controle na produção e na comercialização de mudas o país.

Palavras-chave: fruta do dragão; diagnose; epidemiologia; capa proteica; mosaico; *Potexvirus*.

ABSTRACT

Raising pitaya, which belongs to the genus *Selenicereus* spp., has increased uninterruptedly in recent years, due to its nutraceutical and organoleptic properties. Furthermore, it has proven to be quite profitable for small producers in Brazil. However, due to its recent introduction in the country, studies involving the occurrence and control of diseases and pests are rare, with most of them focusing on their biochemical and bioactive compounds. Among the diseases that could affect pitaya, viruses stand out for the lack of curative control methods and for being underreported, as symptoms can be confused with nutritional disorders. Vegetative propagation and the need for several manual operations in pitaya crops have a high potential to increase the spread of viral diseases. Therefore, plants with symptoms of viruses in orchards is frequently reported by producers. In this work, 212 samples of cladodes with symptoms suspected of viruses were collected in different regions of Brazil, with the purpose of identifying and carrying out the biological characterization of the viruses found, as well as studying their molecular variability based on the sequence of the coat protein gene (CP). Furthermore, a survey was carried out on orchards in five municipalities located in the southern of the states of Minas Gerais to determine the incidence and spatial pattern of virus-infected plants, using the Run, Doublet and dispersion index models. The *Chenopodium quinoa* and *C. amaranticolor* plants that were mechanically inoculated reacted with symptoms of chlorotic and necrotic lesions. Among the 212 analyzed samples, 181 were infected with one or more of the following potexvirus species: *Cactus virus X* (CVX); *Pitaya virus X* (PiVX); *Zygocactus virus X* (ZyVX) and *Schlumbergera virus X* (SchVX). The mixed infections were found with two to four viruses in the same sample. The analysis of the virus CP gene showed the CVX isolates present in two distinct groups, one of which presented a deletion of nine nucleotides in the initial region of the CP gene, in position 42 to 50, in the 5'→3' direction. All the orchards inspected showed high incidences of potexvirus, varying between 80 and 100%. Most orchards showed an aggregated pattern when analyzed by Run and Doublet models, while the dispersion index prevailed with a regular pattern, suggesting a spread through cultural practices from infected seedlings. This is the first work involving the detection and identification, as well as the incidence and spatial pattern of viruses in commercial orchards of pitaya. The results revealed that Brazilian pitaya producers do not have the necessary know-how to adopt preventive measures to control viruses in pitaya orchards. It was also evident that the commercialized seedlings must be responsible for the introduction of viruses into orchards, which shows the urging need for a system to control the production and commercialization of seedlings in the country.

Keywords: *dragon fruit*; diagnosis; epidemiology; protein coat; mosaic; *Potexvirus*.

INDICADORES DE IMPACTOS

A pitáia é considerada uma fruta exótica, com uma crescente demanda de mercado devido à procura por alimentos com propriedades nutracêuticas, refletindo as mudanças nos hábitos alimentares da população brasileira e mundial. Por ser uma fruta que pode ser cultivada em ambientes com baixa precipitação pluviométrica, tem sido considerada uma cultura adequada para regiões do semiárido, tornando-se uma opção rentável para os produtores familiares que podem ter um ganho extra em regiões com poucas alternativas de desenvolvimento sustentável. Entretanto, são escassas as informações sobre as doenças que podem comprometer o cultivo da pitáia e, conseqüentemente, a sua lucratividade. Desde o final do ano de 2020, tem sido detectada nos pomares de pitáia doenças de etiologia viral desconhecidas, reduzindo o crescimento das plantas e a produção de frutos em diferentes regiões do Brasil. O presente estudo mostrou que todos os pomares de pitáia inspecionados apresentaram plantas com infecção simples e mistas, com até quatro espécies do gênero *Potexvirus*. Estudos epidemiológicos indicaram que havia alta incidência de viroses em 80% a 100%, dos pomares, e que a disseminação prevalente foi do tipo agregado, sugerindo uma introdução no pomar por mudas infectadas e disseminação por práticas culturais. Esses dados geraram os impactos nas áreas temáticas da extensão de comunicação, educação, meio ambiente, tecnologia e produção, listados a seguir. a) Sociais e econômicos: 1-disseminação de conhecimentos para os produtores, possibilitando a eles a oportunidade de eliminar os prejuízos causados pelos potexvirus, pela adoção de métodos preventivos de controle; 2-garantia de ganhos reais aos produtores familiares que cultivam a pitáia; 3-fixação de produtores em regiões com clima semiárido, tornando a sua subsistência menos sofrida; b) tecnológicos: 1-identificação das espécies de potexvirus que se encontram presentes em infecções simples e mistas nas plantas de pitáia; 2- caracterização molecular dos potexvirus, mostrando que existem dois variantes moleculares do *Cactus virus X* (CVX) infectando as plantas de pitáia; 3-esclarecimento sobre o fato de que os potexvirus só são transmitidos mecanicamente, ou seja, não possuem vetor na natureza, portanto são eficientemente controláveis por medidas de controle preventivas; 4-evidências de que as mudas são as responsáveis pela introdução dos vírus na cultura, indicando a necessidade de um controle na produção e comercialização de mudas de pitáia; c) culturais: 1-alerta aos produtores de que não se trata de uma doença que possui controle químico, evitando gastos desnecessários com produtos ineficientes e contribuindo para a preservação do meio ambiente; 2-mudança de comportamento em relação à aquisição de mudas de origem desconhecida; 3- adoção de medidas preventivas de controle envolvendo o cuidado com as operações culturais e o uso de mudas sadias. Todos esses impactos somados, contribuirão efetivamente para a sustentabilidade da cultura da pitáia tornando-a socialmente justa, economicamente rentável e ambientalmente correta.

SUMÁRIO

PRIMEIRA PARTE	12
1. INTRODUÇÃO	13
2. REVISÃO BIBLIOGRÁFICA	15
2.1. A família <i>Cactaceae</i>	15
2.2. Pitaia, dragon fruit ou pitahaya	16
2.3. Características botânicas da pitaia	18
2.4 Colheita e Pós-colheita dos frutos	21
2.5 Usos e Aplicações da Pitaia nas Indústrias Alimentícias e Farmacêutica	21
2.6 Doenças	23
REFERÊNCIAS	36
SEGUNDA PARTE - ARTIGOS	48
ARTIGO 1 - Detection, identification and molecular analysis of viruses present in pitaya plants in Brazil	49
ARTIGO 2 - Incidence and spatial pattern of viruses in commercial dragon fruit orchards in southern Minas Gerais, Brazil	72
CONSIDERAÇÕES FINAIS	91

PRIMEIRA PARTE

1. INTRODUÇÃO

A fruticultura é uma das principais atividades agrícolas no Brasil, que é o terceiro maior produtor mundial de frutas, perdendo apenas para a China e a Índia (Abrafrutas, 2023). Somente no ano de 2021, foram exportadas 1,24 milhões de toneladas de frutas como manga, melão, uva, limas e limões, proporcionando uma receita de US\$ 1,21 bilhão (Agrostat, 2022). Em 2022, o país produziu 32 milhões de toneladas com faturamento de R\$ 97 bilhões, sendo que a banana, a laranja, a maçã, o mamão e a melancia foram as principais frutas comercializadas (Conab, 2022). A área de produção no país chega a 2,3 milhões de hectares, gerando mais de 4,6 milhões de empregos (Embrapa, 2020).

Devido às suas dimensões continentais e diferentes condições edafoclimáticas, o Brasil tem potencial para produção de uma grande diversidade de frutas tropicais, subtropicais, temperadas e de semiárido. Dentre elas, as consideradas como frutas exóticas geralmente apresentam variações no formato, na cor e no sabor, o que as tornam únicas, excêntricas e diferentes das demais frutas disponíveis no mercado. Nesta categoria estão incluídas as espécies de plantas de outras regiões do mundo, as que são produzidas em pequenas quantidades ou ainda as que são desconhecidas ou pouco conhecidas pelo consumidor. O mercado de frutas exóticas é um nicho com grande potencial que agrega altos valores aos produtos, exigindo a aplicação constante de tecnologias e inovações na produção.

A expansão do mercado frutícola, em especial das frutas exóticas, está atrelada na mudança de hábitos da população brasileira e mundial nos últimos anos (Santos *et al.*, 2022). Ao buscar alternativas saudáveis e que proporcionem bem-estar, tornou-se crescente o consumo de frutas frescas e seus derivados na dieta, enriquecendo-a com vitaminas, minerais e antioxidantes, presentes em maiores proporções, nestas espécies de plantas (Chang; Alasalvar; Shahidi, 2018).

Dentre as espécies de plantas frutíferas que são classificadas como exóticas, destaca-se a pitáia (*Selenicereus spp.* sin. *Hylocereus spp.*) (Korotkova; Borsch; Arias, 2017), uma espécie pertencente à família *Cactaceae* e que possui metabolismo adaptado para ambientes com déficit hídrico e com altas temperaturas. Com seu centro de origem nas Américas, as principais espécies conhecidas mundialmente são *Selenicereus undatus* (frutos com casca vermelha e polpa branca), *Selenicereus costaricensis* (frutos com casca vermelha e polpa vermelha), *Selenicereus megalanthus* e *Selenicereus setaceus* (frutos com casca vermelha com espinhos e polpa branca).

A produção comercial da cultura da pitiaia iniciou-se em Vietnã, na década de 80, atraindo novos consumidores e se expandindo para todo o mercado Asiático e Europeu (Mercado-Silva, 2018). Apesar da grande diversidade de espécies nativas encontradas no Brasil, a maioria das cultivares comercializadas atualmente são oriundas da China e de Israel (Faleiro *et al.*, 2021; Santos *et al.*, 2022). O início do cultivo da cultura no Brasil se deu pelo Estado de São Paulo, com pomares implantados inicialmente por colecionadores da cultura, no início dos anos 2000 (Nunes *et al.*, 2015).

Devido ao fato de requerer uma menor quantidade de tratamentos culturais comparados a outras frutas e ao seu alto preço de comercialização, a pitiaia tem atraído cada vez mais fruticultores. No ano de 2022, foram produzidos cerca de 3 milhões de quilos da fruta no Brasil, gerando um faturamento de R\$ 44 milhões (Conab, 2022). Esse crescimento no mercado das frutas gera uma demanda de conhecimentos sobre o manejo da cultura, conhecer as doenças e pragas que impactam na produção e outras informações essenciais, de forma a extrair todo o potencial genético da planta. Essas informações, que são imprescindíveis para o aumento da qualidade e quantidade da produção, são bastante escassas na literatura brasileira, devido ao pouco tempo em que esta foi introduzida no país.

Com o desenvolvimento da agricultura, as doenças infecciosas das plantas tornaram-se um fator cada vez mais significativo que pode afetar diretamente o rendimento das culturas (Nazarov *et al.*, 2020). Dentre os fitopatógenos, os vírus apresentam um risco substancial para a produção agrícola, sendo responsável pela metade das doenças emergentes entre as diferentes espécies de plantas cultivadas, reduzindo a qualidade e a quantidade da produção (Mehetre *et al.*, 2021; Takahashi *et al.*, 2019). Atualmente, quatro espécies de vírus que pertencem a família *Alphaflexiviridae*, gênero *Potexvirus* já foram relatadas infectando espécies de pitiaia do gênero *Selenicereus*: *Cactus virus X* (CVX), *Pitaya virus X* (PiVX), *Schlumbergera virus X* (SchVX) e *Zygocactus virus X* (ZyVX) (Duarte *et al.*, 2008; Evallo *et al.*, 2021; Koenig *et al.*, 2004; Park *et al.*, 2018) causando redução no crescimento da planta e na produção de frutos, as plantas infectadas podem apresentar sintomas como mosaico ou serem assintomáticas.

Neste contexto, este trabalho teve como objetivo analisar cladódios de pitiaia provenientes dos Estados de São Paulo, Minas Gerais, Rio Grande do Sul, Distrito Federal e Pará, visando a identificação e caracterização molecular dos vírus presentes. Foram também realizados um levantamento da incidência e determinação do padrão espacial de plantas com sintomas suspeitos de vírus em pomares de cinco municípios do Sul de Minas Gerais. Os resultados obtidos são aqui apresentados e discutidos.

2. REVISÃO BIBLIOGRÁFICA

2.1. A família *Cactaceae*

O período das navegações foi marcado pela procura de novas terras, como a descoberta do continente Americano. Neste novo ambiente, a diversidade de animais e plantas atraíram a atenção dos exploradores marítimos e posteriormente de estudiosos e admiradores até os dias atuais. Dentre as espécies de plantas que foram introduzidas nos outros continentes, temos as cactáceas, com centro de origem nas Américas. Por suas características excêntricas e sua diversidade de formatos e tamanhos ganharam espaços em jardins e coleções de admiradores em diversos países (Novoa *et al*, 2015).

As cactáceas possuem características morfológicas e fisiológicas que permitem diferencia-las das demais espécies de angiospermas. A filogenia do grupo se define como monofilético, com classificação botânica pertencente à divisão *Magnoliophyta*, classe *Magnoliopsida*, ordem *Caryophyllales*, família *Cactaceae* com quatro subfamílias: *Cactoideae*, *Maihuenioideae*, *Opuntioideae* e *Pereskioideae*. Atualmente, são cerca de 130 gêneros e aproximadamente 1600 espécies que são encontradas em diversas condições ambientais, desde o Canadá até a Argentina, com uma maior diversidade de espécies no México (Chandra *et al*, 2019; Das *et al*, 2020; Goettsch *et al*, 2018; Guerrero *et al*, 2019;).

Para se adaptar em ambiente xérico, em que a indisponibilidade de água é a principal característica, apresentam células morfológicamente adaptadas com vacúolos especializados no armazenamento de água e mucilagem que proporcionam retenção de água, tolerância a longos períodos de seca e o aspecto suculento da maioria das espécies. O caule realiza fotossíntese, com baixa densidade estomática e uma espessa camada de epiderme e cutícula que reduzem a transpiração d'água (Guerrero *et al*, 2019; Santos-Día; Camarena-Rangel, 2019;).

A principal característica desta família é a presença de auréolas que é composto por tecido merismático onde são formados os espinhos, flores e frutos. Os espinhos são folhas modificadas que proporcionam a defesa da planta contra predadores e reduzem a perda de água, conferindo a característica de planta xerófita. As sementes possuem dormência evitando a germinação em períodos de escassez hídrica. Fisiologicamente as cactáceas apresentam metabolismo ácido das crassuláceas (CAM) que fixam CO₂ durante a noite, reduzindo a perda de água causada pela abertura dos estômatos, e produzem diversos metabolitos secundários

como alcaloides, compostos fenólicos e terpenos para defesa da planta contra estresses bióticos e abióticos (Chandra *et al.*, 2019; Das *et al.*, 2020; Ventura-Aguilar *et al.*, 2017).

A família *Cactaceae* possui uma riqueza que extrapola a diversidade ao nível de espécies e sua importância etnobotânica, para os povos que convivem com essas plantas, demonstram diferentes usos e adaptações. Estudos históricos e sociológicos demonstram a utilização destas plantas no tratamento a diversas doenças, na alimentação humana e de animais e na decoração de ambientes e jardins (De Araújo *et al.*, 2021). Nos últimos anos, as pesquisas nas áreas metabólicas e de genética ressaltam o potencial de diversas espécies como biocombustíveis, como matéria prima para as indústrias alimentícia, farmacêutica, estética e na bioengenharia para inserção de genes visando adaptação de plantas a ambientes adversos como seca, calor e desertificação (Inglese *et al.* 2017; Yang *et al.* 2015;).

As espécies com potencial para alimentação humana pertencem aos gêneros *Cereus*, *Hylocereus*, *Selenicereus*, *Nopalea*, *Opuntia*, *Pereskia* e *Rhipsalis*, estando presentes nos hábitos alimentares de pessoas residentes em diferentes regiões do planeta (Mizhari, 2014; 2015; Qin *et al.*, 2021). Dentre estas espécies, as frutas conhecidas como pitaia vêm ganhando popularidade no mercado nacional e internacional.

2.2. Pitaia, dragon fruit ou pitahaya

Com centro de origem nas florestas tropicais e subtropicais da América Central e do Sul, a pitaia vem se destacando e ganhando a preferência no mercado hortícola nacional e internacional nos últimos anos como fruta exótica. A palavra pitaia é originária do idioma taíno, pertencente à família linguística arahuaca que significa “fruta escamosa” devido a presença de brácteas nos frutos da planta. É conhecida por diferentes nomes, dependendo da região do planeta como: pitaia, fruta-do-dragão, *pitaya*, *pitahaya*, *tuna*, *dragon fruit*, *strawberry pear*, *night-blooming cereus*, *belle of the night*, *Thanh Long*, *vine-cacti* (Luu *et al.*, 2021; Mercado-Silva, 2018; Miszhari, 2014, 2015; Ortiz-Fernandes; Carrillo-Salazar, 2012;).

Vinte e cinco anos atrás, a pitaia era praticamente uma planta desconhecida, porém atualmente ela é uma fruta popular no mercado global, principalmente no mercado europeu. Cultivada em diferentes países e continentes, na Oceania (Austrália e Nova Zelândia), Ásia (Malásia, Indonésia, Filipinas, Vietnã, Camboja, Tailândia, China, Coreia, Taiwan, Japão, Israel e Índia), Europa (Espanha, Itália e Portugal), América do Norte (Estados Unidos e México), América Central (Guatemala, El Salvador, Nicarágua, Costa Rica e Panamá) e

América do Sul (Colômbia, Venezuela, Equador, Peru, Uruguai e Brasil) (Luu *et al.*, 2021; Mercado-Silva, 2018; Ortiz; Takashi, 2020; Trivellini *et al.*, 2020), sendo o Vietnã o maior produtor e exportador da fruta e a União Europeia como maior importador (Luu *et al.*, 2021; Mercado-Silva, 2018).

Os frutos da pitáia podem ser encontrados em quatro gêneros distintos da família Cactaceae: *Stenocereus*, *Cereus*, *Hylocereus* e *Selenicereus* (Le Bellec; Vaillant, 2006). Introduzida por padres franceses em 1860, foi no Vietnã que a planta melhor se adaptou, o que os levou a iniciar o cultivo visando a produção comercial, que se expandiu para outros países asiáticos. Isso sugeriu a ilusão de que a sua origem poderia estar ligada a essas regiões (Miszhari, 2014, 2015; Ortiz-Fernandes; Carrillo-Salazar, 2012).

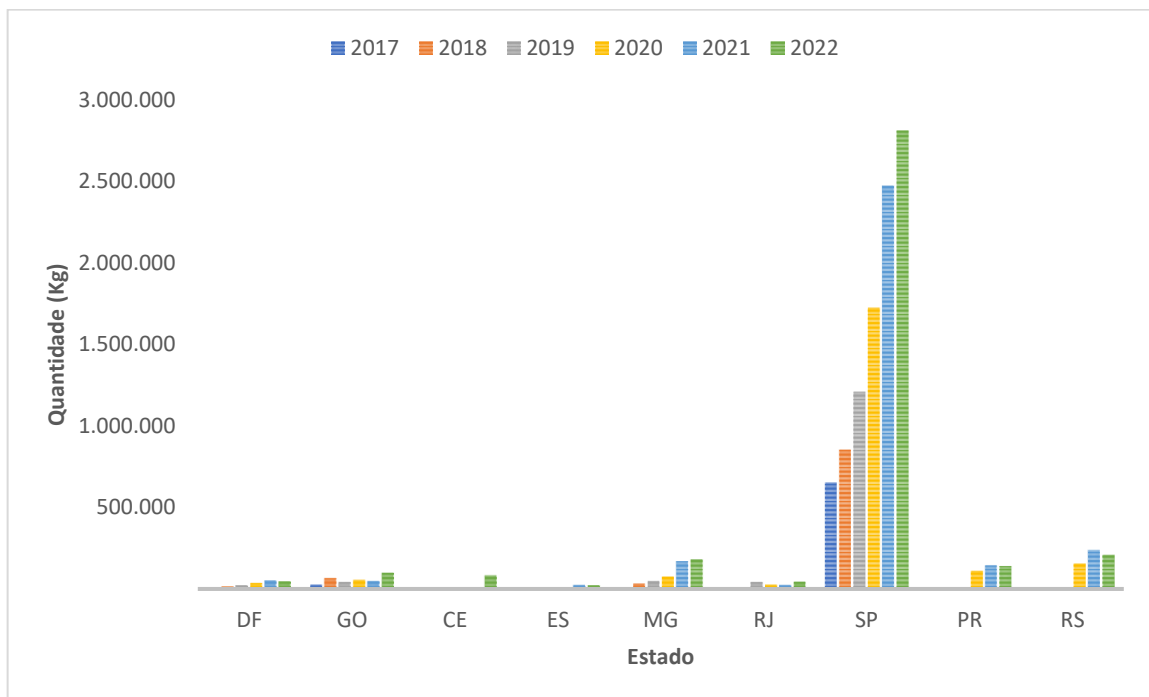
As principais espécies comercializadas no momento pertencem ao gênero *Selenicereus*, destacando-se *Selenicereus undatus* (Haw.) D. R. Hunt sin. *Hylocereus undatus* (Haw.) Britton e Rose (frutos com casca vermelha e polpa branca), *Selenicereus costaricensis* (F. A. C. Weber) S. Arias e N. Korotkova ex Hammel sin. *Hylocereus costaricensis* Britton e Rose (frutos com casca vermelha e polpa vermelha), *Selenicereus megalanthus* (K. Schum. ex Vaupel) Moran (frutos com casca amarela com espinhos e polpa branca) e *Selenicereus setaceus* (Salm-Dyck ex DC.) A. Berger ex Werderm (frutos com casca vermelha com espinhos e polpa branca) (Faleiro *et al.*, 2021; Korotkova *et al.*, 2021; Korotkova, Borsch; Arias, 2017).

No Brasil, a pitáia é considerada uma fruta exótica pelo fato de ser pouco conhecida, exuberante e comercializada com alto valor. Destaca-se das demais frutas encontradas no mercado por atrelar as propriedades nutracêuticas, terapêuticas e funcionais que conferem qualidades e características únicas ao fruto (Attar *et al.*, 2022; Das *et al.*, 2020). Possuem características organolépticas que facilmente são detectadas pelos sentidos humanos e pelas análises fitoquímicas, possuindo compostos que ajudam a prevenir e tratar doenças (Hossain *et al.*, 2021).

O aumento do interesse pela fruta se deve ao fato de a cultura ter vislumbrado novos nichos de mercado, atraindo produtores pelo alto valor agregado ao quilo da fruta e pela rusticidade da planta. Isso estimulou a Embrapa Cerrado a desenvolver cultivares adaptadas às condições edafoclimáticas do Brasil: BRS Luz do Cerrado (frutos alongados e vermelhos de polpa branca), BRS Lua do Cerrado (frutos redondos e vermelhos de polpa branca), BRS Granada do Cerrado (frutos vermelhos de polpa roxa), BRS Âmbar do Cerrado (frutos amarelos de polpa branca) e BRS Minipitáia do Cerrado (frutos vermelhos com espinhos e polpa branca) (Faleiro; Junqueira, 2022; Faleiro; Oliveira, 2021).

Segundo o IBGE (2017), a fruta é cultivada em todas as regiões do país, com uma produção de aproximadamente 1,5 ton/ano. Segundo os dados da Conab (2022), a demanda do mercado interno tem sido crescente nos últimos anos, tendo sido comercializados nos CEASAS de todo o Brasil mais de 3,6 milhões de toneladas somente no ano de 2022 (Figura 1). Os principais estados produtores foram São Paulo, Rio Grande do Sul, Paraná, Minas Gerais e Goiás com uma produtividade média de 10 a 40 toneladas por hectare (Santos *et al.*, 2022).

Figura 1 - Quantidade de frutos em quilograma comercializados nos centros de abastecimentos (Ceasas) do Brasil e dos principais estados produtores nos últimos seis anos.



Fonte: Adaptado de Conab (2022).

2.3. Características botânicas da pitáia

A pitáia é uma planta perene, terrestres ou rupícolas, de hábito trepador, epífita ou hemiepífita, capaz de frutificar por 15 a 25 anos, resistente ao estresse hídrico e adaptada às altas temperaturas (Crane *et al.*, 2017; Korotkova *et al.*, 2017, 2021; Luu *et al.*, 2021; Nunes *et al.*, 2014; Ortiz-Fernandes; Carrillo-Salazar, 2012). Possui sistema radicular raso e superficial, que alcança profundidade no solo entre 40 a 60 cm, com raízes adventícias aéreas para auxiliar na fixação do caule em superfícies verticais e também para obter de nutrientes (Mizrahi, 2014).

O caule é suculento e classificado morfologicamente como cladódio, com formato triangular, podendo apresentar de três a cinco costelas, com margens que variam entre côncava, plana ou convexa (Faleiro *et al.* 2021). A cor do cladódio pode variar entre as espécies, desde

a cor verde até a presença da coloração avermelhada, característica presente em cladódios jovens. Com a maturidade da planta, o cladódio velho apresenta a coloração acinzentada (Faleiro *et al.* 2021). A textura da superfície do cladódio pode ser lisa, média ou rugosa. Nas costelas são encontradas as auréolas, pontos onde estão localizadas as gemas axilares, protegidas por pequenos espinhos. A cor da auréola, o número dos espinhos, o tamanho dos espinhos e a distância entre as auréolas também pode variar com a espécie (Faleiro *et al.* 2021; Rezende *et al.* 2017; Silva, 2014).

As flores são hermafroditas e aromáticas, produzidas a partir da diferenciação celular nas auréolas e os botões florais podem apresentar diferentes formatos e cores (Faleiro *et al.* 2021). Possuem formato de sino, com tamanho grande, medindo cerca de 20 a 30 cm de largura, com pétalas de coloração que varia entre branca, creme, amarela e verde amarelada e sépalas que variam entre branca, verde e vermelha. Apresentam o estigma mais elevado que as anteras, impedindo a autopolinização. Algumas espécies e cultivares de pitiaia são auto incompatíveis, ou seja, se forem fertilizadas com o seu próprio pólen são incapazes de formar sementes (Li *et al.*, 2022). Com antese noturna e única, a flor abre entre as 19 h e 21 h e permanece aberta por cerca de 9 a 14 h seguidas, fechando-se entre as 7 h e 9 h da manhã. Caracterizam-se, também por serem solitárias, ou seja, com formação de somente uma flor por extremidade de cladódio (Faleiro *et al.*, 2021; Rezende *et al.*, 2017; Santos *et al.*, 2022; Silva, 2014).

O fruto é formado a partir do desenvolvimento do ovário que será a polpa e a casca do receptáculo que o circunda, sendo do tipo baga e com tamanho variando de globoso a subgloboso. A coloração varia de tons vermelho ao rosa ou amarelas, cobertos por brácteas (escamas) com ou sem presença de espinhos. A cor da polpa varia de translúcida, branca, cinza-claro, tons do rosa ao vermelho e arroxeada, dependendo da espécie. As sementes apresentam coloração pretas, obovadas, de 2-3 mm de largura, em grande quantidade e com elevada capacidade de germinação (Faleiro *et al.*, 2021; Rezende *et al.*, 2017; Silva, 2014). Na figura 2, são apresentadas algumas características que foram descritas no texto.

Figura 2 - Principais características botânicas da planta, da flor e dos frutos de *Selenicereus* spp.



Legenda: A: Muda recém-implantada no pomar; B: Planta em crescimento vegetativo; C: Raízes adventícias; D: Auréolas com espinhos; E: Início da formação do botão floral nas auréolas; F: Botão floral; G: Flor aberta; H: Flores fechadas após polinização; I: Frutos em diferentes estádios fenológicos; J, K: Fruto maduro; L: Sementes após remoção da polpa do fruto com presença de mucilagem; M: Sementes sem mucilagem. Fonte: Autor (2023).

2.4 Colheita e Pós-colheita dos frutos

Os frutos da pitaita são classificados como não climatéricos, portanto, uma definição do momento ideal para colheita dos frutos interfere na qualidade e no sabor final do produto para o consumidor (Ortiz; Takashi, 2020). De maneira prática, a colheita pode ser embasada pela coloração da casca do fruto que ao maturar modifica-se para as cores vermelho-rosa ou amarelo a depender da cultivar implantada no pomar. De forma empírica, induz a colheita de frutos com baixo teor de sólidos solúveis (Mercado-Silva, 2018; Ortiz; Takashi, 2020). A segunda maneira é a contagem de dias após a antese (DAA), que deve ser entre 25 a 32 DAA (Figura 5), quando os frutos se encontram com maturação e sabor agradável ao paladar do consumidor e com tempo de prateleira de sete dias (Lone *et al.*, 2020; Mercado-Silva, 2018; Ortiz; Takashi, 2020).

Para remoção do fruto na planta, recomenda-se o corte no início do cladódio que produziu o fruto e cuidados no manuseio do fruto, para evitar quedas e possíveis ferimentos que resultaram em danos por patógenos de pós-colheita ou na liberação de gás etileno que acelera a degradação do fruto (Castro *et al.*, 2017; Rezende *et al.*, 2017). Ao armazenar a temperatura ambiente, em torno de 25°C a 30°C, o fruto permanece íntegro por 6 a 8 dias (Santos *et al.*, 2022). Diminuindo a temperaturas para 8°C a 10°C, com umidade relativa de 90% o tempo de armazenamento se prolonga para 30 dias, mas a exposição a esta temperatura resulta no murchamento das brácteas no fruto, prejudicando a sua comercialização (Mizhari, 2014; Ortiz; Takashi, 2020). Para resolver este problema, Ba *et al.* (2022) investigaram o efeito da melatonina no retardamento da senescência e obtiveram resultados preliminares promissores. Porém maiores estudos necessitam ser desenvolvidos para delinear um protocolo que possa ser realizado na rotina dos produtores.

2.5 Usos e Aplicações da Pitaita nas Indústrias Alimentícias e Farmacêutica

Nos últimos anos trabalhos de revisão de literatura ressaltam a qualidade e os altos níveis de compostos fitoquímicos bioativos presentes na pitaita, que são benéficos à saúde e com grande potencial para as indústrias de alimentação, farmacêutica e estética (Cheok *et al.*, 2020; De Araújo *et al.*, 2021; Huang *et al.*, 2021; Jimenes-Garcia *et al.*, 2022).

As frutas *in natura* são nutritivas, ricas em ácidos orgânicos (como ascórbico, cítrico, isocítrico e málico), carboidratos, aminoácidos (prolina), alguns pigmentos naturais (incluindo betacianina e compostos fenólicos), minerais (principalmente potássio e magnésio) e com alto

teor de ácidos graxos saturados (ácidos mirístico, palmítico e esteárico) e insaturados (ácidos oléico, linoléico e linolênico) (De Araújo *et al.*, 2021). Segundo Choek *et al.* (2020) o consumo diário da fruta promoveu um aumento nos níveis do colesterol HDL, uma diminuição nos níveis de glicose, de colesterol LDL e de triglicerídeos no sangue, além de redução na pressão arterial sistólica e na diastólica.

Além da polpa da fruta, os mesmos nutrientes podem ser encontrados na casca da fruta e nos cladódios (Jimenez-Garcia *et al.*, 2022). Diferentes trabalhos são encontrados na literatura com a finalidade de reaproveitamento dos resíduos da pitiaia para substituir ou incrementar os alimentos processados. A polpa da fruta pode ser utilizada como corante natural, com características adequadas para aplicação em iogurtes e vinhos (Huang *et al.*, 2021) e outras matrizes, como balas e refrigerantes (Utpott *et al.*, 2020a). A farinha da casca da fruta poder ser usada para substituir ou complementar a farinha de trigo em pães e bolos (Utpott *et al.*, 2018).

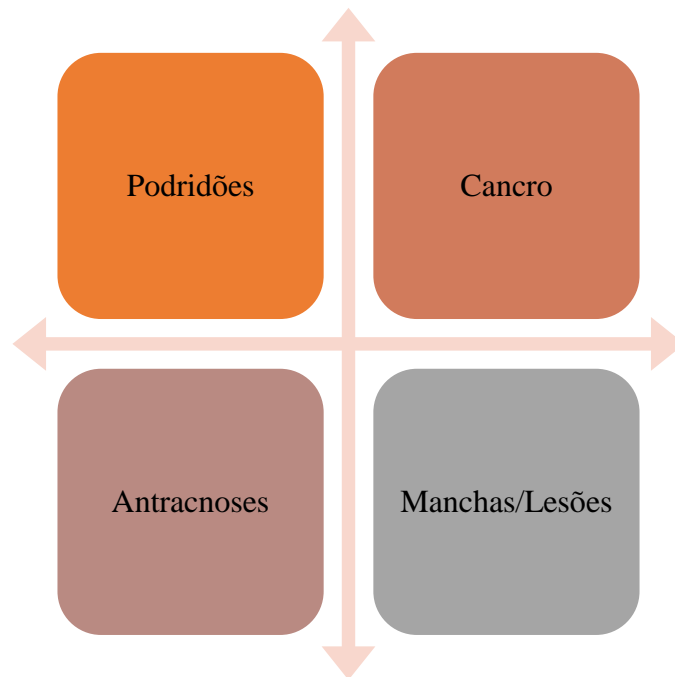
Outras possibilidades de uso da pitiaia são: na fabricação de sorvete reduzindo o teor de gordura e como substituto do corante sintético E-162 (Gengatharan; Dykes; Choo, 2021; Utpott *et al.*, 2020b); como fonte de pectinas e como agente dispersante, emulsificante e gelificante em produtos como geleias, bebidas e laticínios (Zaid *et al.* 2019) e na fabricação de biofilmes (Qin *et al.*, 2020). Finalmente, as propriedades antioxidantes e das betacianinas são aproveitadas em produtos na indústria de cosméticos, como em cremes para o rosto e o corpo antienvelhecimento, prolongação da hidratação da pele e em batons como pigmento natural (De Araújo *et al.*, 2021). Sua riqueza em vitamina B3 faz com que a pitiaia seja um bom princípio ativo para pomadas utilizadas em peles queimadas pelo sol (Verma *et al.*, 2017). A presença de ácido linoleico e linolênico ajudam no balanço do metabolismo epitelial, controlando o fluxo de óleos e nutrindo o colágeno que estrutura a pele (Kamairudin *et al.*, 2014).

2.6 Doenças

Nos últimos anos o número de patógenos relatados afetando o rendimento da cultura da pitaia aumentaram. Nos anos 2000, período que iniciou o cultivo de pomares comerciais pelo mundo, 14 patógenos foram relatados por Valencia-Botín *et al.* (2013). Em uma nova revisão realizada por Balendres e Bengoa (2019) este número aumentou para 25 espécies patogênicas encontradas na cultura. Atualmente já foram relatados 49 patógenos ao redor do mundo onde a pitaia é cultivada, causando doenças em diferentes tecidos da planta e gerando perdas na produtividade e rentabilidade da cultura (Figura 3).

O maior número de patógenos que causam doenças em pitaia é de etiologia fúngica, sendo que 37 espécies em 17 gêneros foram detectadas gerando perdas na produção. Quatro espécies de vírus pertencentes ao gênero *Potexvirus*, que podem ocorrer em infecções simples ou mistas, têm sido detectadas praticamente em todas as lavouras, agravadas pelo método de propagação vegetativa da pitaia. As doenças de etiologia bacteriana que já foram relatadas têm sido causadas por cinco espécies distribuídas em quatro gêneros de fitobactérias. Por fim, os nematóides começaram a ser relatados nos últimos anos, principalmente no Brasil causando galhas nas raízes. Na tabela 1 estão discriminados os patógenos já descritos na literatura como causadores de doenças em pitaia.

Figura 3 - Principais sintomas relatados em pitaia causados por diferentes fitopatógenos.



Fonte: Autor (2023).

Tabela 1 - Descrição das doenças relatadas ocorrendo em pitaiá em diferentes regiões produtoras no mundo, os tecidos que são afetados e as espécies de *Selenicereus* spp. (continua...)

Doença	Espécie	Tecido afetado	<i>Selenicereus</i> sp.	Local	Referência
Antracnose	<i>Colletotrichum</i> spp.	F	<i>S. undatus</i> , <i>S. polyrhizus</i> e <i>S. costaricensis</i>	Taiwan	Lin <i>et al.</i> , 2017
		C	<i>Selenicereus</i> sp.	Indonésia Brasil Sul da Florida, EUA	Masnilah <i>et al.</i> , 2018 Almeida, 2018 Hong <i>et al.</i> , 2020
	<i>Colletotrichum gloeosporioides</i>	C	<i>S. undatus</i>	Florida	Palmateer <i>et al.</i> , 2007
		C	<i>S. undatus</i>	Brasil	Takahashi <i>et al.</i> , 2008
		C + F	<i>Selenicereus</i> sp.	Malásia	Masyahit <i>et al.</i> , 2009; Mohd <i>et al.</i> , 2015
		C	<i>S. undatus</i>	China	Ma <i>et al.</i> , 2014
			<i>S. undatus</i>	Tailândia	Meetum <i>et al.</i> , 2015; Athipunyakom <i>et al.</i> , 2015
		C + F	<i>S. undatus</i>	Tailândia	Meetum <i>et al.</i> , 2015
	<i>Colletotrichum siamense</i>	C	<i>S. polyrhizus</i>	China	Zhao <i>et al.</i> , 2018
		C	<i>S. undatus</i>	Índia	Abirammi <i>et al.</i> , 2019
		C + F	<i>S. undatus</i>	Brasil	Moura, 2020
		F	<i>S. undatus</i>	China	Guo <i>et al.</i> , 2014
	<i>Colletotrichum truncatum</i>	C	<i>S. polyrhizus</i>	Malásia	Iskandar Vijaya <i>et al.</i> , 2015; Mohd <i>et al.</i> , 2015
		C + F	<i>S. undatus</i>	Tailândia	Meetum <i>et al.</i> , 2015; Athipunyakom <i>et al.</i> , 2015
		C + F	<i>Selenicereus</i> sp.	Índia	Salunkhe <i>et al.</i> , 2023a
	<i>Colletotrichum karstii</i>	C + F	<i>S. undatus</i>	Brasil	Nascimento <i>et al.</i> , 2019
<i>Colletotrichum fructicola</i>	C	<i>S. undatus</i>	Filipinas	Evallo <i>et al.</i> , 2021b	
<i>Colletotrichum aenigma</i>	C + F	<i>S. undatus</i>	Tailândia	Meetum <i>et al.</i> , 2015	
<i>Colletotrichum tropicale</i>	F	<i>S. costaricensis</i>	México	Nuñez-García <i>et al.</i> , 2023	

Legenda: F: fruto, C: cladódio. Fonte: Autor (2023).

Tabela 1 - Continuação...

Doença	Espécie	Tecido afetado	<i>Selenicereus</i> sp.	Local	Referência
Cancro da haste e do fruto, Podridão negra interna	<i>Neoscytalidium dimidiatum</i>	C	<i>S. undatus</i>	China	Lan <i>et al.</i> , 2012
		FL + F	<i>S. undatus</i>	Israel	Ezra <i>et al.</i> , 2013
		C	<i>S. undatus</i> e <i>S. polyrhizus</i>	Taiwan	Chuang <i>et al.</i> , 2012; Yi <i>et al.</i> , 2015
		C	<i>S. undatus</i>	Tailândia	Athipunyakom <i>et al.</i> , 2015
		C	<i>S. polyrhizus</i>	Malásia	Masratul Hawa <i>et al.</i> , 2013a; Mohd <i>et al.</i> , 2015
		C + F	<i>Selenicereus</i> sp.	Florida, EUA	Sanahuja <i>et al.</i> , 2016; Hong <i>et al.</i> , 2020
		C	<i>S. costaricensis</i>	Costa Rica	Retana-Sanchez <i>et al.</i> , 2019a
		C	<i>Selenicereus</i> sp.	Porto Rico	Serrato-Diaz e Goenaga, 2021
		C	<i>S. undatus</i> e <i>S. polyrhizus</i>	Índia	Salunkhe <i>et al.</i> , 2023b
		C + F	<i>S. megalantus</i>	Equador	Espinoza-Lozano <i>et al.</i> , 2023
Podridões	<i>Botryosphaeria dothidea</i> (anamorfa: <i>Fusicoccum</i>)	C	<i>S. undatus</i>	Brasil	Andrade <i>et al.</i> , 2019
		C + F	<i>S. polyrhizus</i>	Bangladesh	Briste <i>et al.</i> , 2019
		C	<i>S. monocanthus</i>	Filipinas	Evallo <i>et al.</i> , 2022a
		C	<i>Selenicereus</i> sp.	Brasil	de Mello <i>et al.</i> , 2022
		FL + F	<i>S. undatus</i>	Florida, EUA	Tarnowski <i>et al.</i> , 2010
		C + F	<i>S. undatus</i>	Israel	Ben-Ze'ev <i>et al.</i> , 2011
Podridões	<i>Bipolaris cactivora</i>	F	<i>S. undatus</i>	Vietnã	He <i>et al.</i> , 2012
		FL + F	<i>S. undatus</i>	Tailândia	Athipunyakom <i>et al.</i> , 2015; Oeurn <i>et al.</i> , 2015
		FL	<i>S. costaricensis</i>	China	Qiu <i>et al.</i> , 2021
		C + F	<i>S. undatus</i>	Brasil	Pierangeli, 2019; Moura, 2020
		FL	<i>S. undatus</i>	China	Li <i>et al.</i> , 2023
Podridões	<i>Mucor irregulares</i>	FL	<i>S. undatus</i>	China	Li <i>et al.</i> , 2023

Legenda: C: cladódio, FL: flores e F: fruto. Fonte: Autor (2023).

Tabela 1 - Continuação...

Doença	Espécie	Tecido afetado	<i>Selenicereus</i> sp.	Local	Referência
Podridão do colo e em frutos	<i>Rhizopus</i> sp.	C + F	<i>S. undatus</i> e <i>S. megalanthus</i>	Brasil	Maia <i>et al.</i> , 2019; Lone <i>et al.</i> , 2020
Podridão basal	<i>Fusarium oxysporum</i>	C	<i>S. undatus</i>	Argentina	Wright <i>et al.</i> , 2007
			<i>S. megalanthus</i>	Colômbia	Salazar-González <i>et al.</i> , 2016
			<i>S. polyrhizus</i>	Malásia	Mohd Hafifi <i>et al.</i> , 2019
			<i>S. polyrhizus</i>	Bangladesh	Mahmud <i>et al.</i> , 2021
Podridão mole dos frutos	<i>Fusarium oxysporum</i> e <i>F. dimerum</i>	F	<i>S. undatus</i>	China	Zhi-Jing <i>et al.</i> , 2011
			<i>S. costaricensis</i>	China	Guo <i>et al.</i> , 2012
	<i>Gilbertella persicaria</i>	F	<i>S. ocamponis</i> , <i>S. undatus</i> e <i>S. costaricensis</i>	México	Carrillo-Fasio <i>et al.</i> , 2022
Podridão da haste	<i>Fusarium proliferatum</i>	C	<i>S. polyrhizus</i>	Malásia	Masratul Hawa <i>et al.</i> , 2013b; Mohd <i>et al.</i> , 2015
	<i>Fusarium solani</i>	C	<i>S. undatus</i> e <i>S. polyrhizus</i>	Indonésia	Sholinah <i>et al.</i> , 2019; Rita <i>et al.</i> , 2013
	<i>Fusarium fujikuroi</i>	C	<i>S. polyrhizus</i>	Malásia	Masratul Hawa <i>et al.</i> , 2017
	<i>Fusarium verticillioides</i>	C	<i>S. undatus</i>	Brasil	Pierangeli, 2019
	<i>Neocosmospora rubicola</i>	C	<i>S. costaricensis</i>	China	Zheng <i>et al.</i> , 2018b
Fumagina	<i>Cladosporium tenuissimum</i>	C + F	<i>S. costaricensis</i>	China	Xu <i>et al.</i> , 2019
Mancha de Alternaria	<i>Alternaria</i> sp.	C	<i>S. undatus</i>	Florida, EUA	Patel e Zhang, 2017; Hong <i>et al.</i> , 2020
	<i>Alternaria alternata</i> e <i>A. gossypina</i>	C + F	<i>S. undatus</i>	Brasil	Moura, 2020
Mofo cinzento	<i>Sclerotium rolfsii</i>	C	<i>S. undatus</i>	China	Zheng <i>et al.</i> , 2018a
Mancha	<i>Epicoccum sorghinum</i>	C	<i>S. undatus</i> e <i>S. monacanthus</i>	Filipinas	Taguian <i>et al.</i> , 2020

Legenda: F: fruto, C: cladódio. Fonte: Autor (2023).

Tabela 1 - Continuação...

Doença	Espécie	Tecido afetado	<i>Selenicereus</i> sp.	Local	Referência
Podridão e mancha cinza	<i>Diaporthe ueckerae</i>	C	<i>S. undatus</i> e <i>S. polyrhizus</i>	Taiwan	Wang <i>et al.</i> , 2022
	<i>Diaporthe phaseolorum</i>	C	<i>S. undatus</i>	Bangladesh	Karim <i>et al.</i> , 2019
	<i>Diaporthe arecae</i> , <i>D. eugeniae</i> , <i>D. hongkongensis</i> , <i>D. phaseolorum</i> e <i>D. tectonendophytica</i>	C	<i>S. polyrhizus</i>	Malásia	Huda-Shakirah <i>et al.</i> , 2021
Podridão pós-colheita	<i>Alternaria alternata</i>	F	<i>S. undatus</i>	Brasil	Castro <i>et al.</i> , 2017; Pierangeli, 2019
Lesões	<i>Aureobasidium</i> spp.	C + F	<i>S. undatu</i>	Brasil	Almeida, 2018
	<i>Aureobasidium pullulans</i>	C + F	<i>S. undatus</i> e <i>S. polyrhizus</i>	China	Wu <i>et al.</i> , 2017
Lesão escura	<i>Nigrospora sphaerica</i>	C + F	<i>S. undatus</i>	China	Liu <i>et al.</i> , 2016
Lesões	<i>Nigrospora lacticolonia</i> e <i>N. sphaerica</i>	C	<i>S. polyrhizus</i>	Malásia	Kee <i>et al.</i> , 2019
	<i>Curvalaria</i> spp.	C	<i>S. undatus</i>	Brasil	Almeida, 2018
	<i>Curvularia lunata</i>	C	<i>S. polyrhizus</i>	Malásia	Masratul Hawa <i>et al.</i> , 2009
Mosaico/Mosqueado	<i>Cactus virus X</i>	C	<i>S. undatus</i>	Taiwan	Liou <i>et al.</i> , 2001
		C	<i>S. polyrhizus</i>	China	Peng <i>et al.</i> , 2016
		C	<i>S. undatus</i>	Coreia do Sul	Kim <i>et al.</i> , 2016
		C	<i>S. undatus</i>	Florida, EUA	Gazis <i>et al.</i> , 2018
		C + F	<i>Selenicereus</i> sp.	Malásia	Masanto <i>et al.</i> , 2018
		C	<i>Selenicereus</i> sp.	Índia	Parameswari <i>et al.</i> , 2022
	C	<i>S. monacanthus</i>	Filipinas	Evallo <i>et al.</i> , 2022b	
	<i>Zygocactus virus X</i> e <i>Schlumbergera virus X</i>	C	<i>S. undatus</i>	Brasil	Duarte <i>et al.</i> , 2008

Legenda: F: fruto, C: cladódio. Fonte: Autor (2023).

Tabela 1 - Continuação...

Doença	Espécie	Tecido afetado	<i>Selenicereus</i> sp.	Local	Referência
Mosaico/Mosqueado	<i>Zygocactus virus X</i>	C	<i>Selenicereus</i> sp.	Índia	Parameswari <i>et al.</i> , 2023
	<i>Schlumbergera virus X</i>	C	<i>S. undatus</i> , <i>S. hybridum</i> , <i>S. costaricensis</i> e <i>S. purpusii</i>	Espanha	Janssen <i>et al.</i> , 2022
	<i>Pitaya virus X</i> e <i>Zygocactus virus X</i>	C	<i>S. undatus</i>	Coreia do Sul	Bae; Park, 2022
	<i>Pitaya virus X</i> e <i>Cactus virus X</i>	C	<i>S. undatus</i>	Coreia do Sul	Kim <i>et al.</i> , 2023
Podridão mole bacteriana	<i>Enterobacter cloacae</i>	C	<i>S. polyrhizus</i> , <i>S. undatus</i> e <i>S. megalanthus</i>	Malásia	Masyahit <i>et al.</i> , 2009
	<i>Enterobacter hormaechei</i>	C	<i>S. undatus</i>	Peru	Soto <i>et al.</i> , 2019
	<i>Enterobacter hormaechei</i>	C	<i>S. costaricensis</i>	Costa Rica	Retana-Sanchez <i>et al.</i> , 2019b
	<i>Paenibacillus polymyxa</i>	C	<i>S. undulates</i>	China	Zhang <i>et al.</i> , 2017
	<i>Pectobacterium carotovorum</i>	C	<i>Selenicereus</i> sp.	Indonésia	Masnilah <i>et al.</i> , 2018
	<i>Pseudomonas syringae</i> pv. <i>syringae</i>	C	<i>S. undatus</i>	China	Wang, 2022
“Stunt Nematode”	<i>Tylenchorhynchus agri</i>	R + C + F	<i>S. undatus</i> e <i>S. polyrhizus</i>	China	Zhang <i>et al.</i> , 2018
Galhas	<i>Meloidogyne javanica</i>	R	<i>S. megalanthus</i>	Israel	Mizhari, 2015
	<i>Meloidogyne incógnita</i>	R + C	<i>S. megalanthus</i> <i>S. undatus</i>	Brasil	Nascimento <i>et al.</i> , 2020 Souza <i>et al.</i> , 2022
	<i>Meloidogyne enterolobii</i> e <i>M. javanica</i>	R + C	<i>Selenicereus</i> sp.	China	Long <i>et al.</i> , 2022

Legenda: F: fruto, C: cladódio e R: raiz. Fonte: Autor (2023).

2.6.1 Doenças causadas por fungos

Desde a última revisão de literatura realizada por Balendres e Bengoa (2019), dezesseis novos patógenos fúngicos foram relatados causando doenças em pitaia no mundo. A antracnose continua sendo a doença mais frequente, causando danos em cladódios e frutos de *S. undatus*, *S. polyrhizus* e *S. costaricensis*. Foram relatadas as espécies *Colletotrichum aenigma*, *C. gloeosporioides*, *C. karstii*, *C. siamense* e *C. truncatum* com perdas entre 5 a 50% na produção (Guo *et al.*, 2014; Iskandar Vijaya *et al.*, 2014; Lin *et al.*, 2017; Masnilah *et al.*, 2018; Masyahit *et al.*, 2009; Meetum *et al.*, 2015; Nascimento *et al.*, 2019; Yang *et al.*, 2014; Zhao *et al.*, 2018). A Índia relatou recentemente a presença de *C. siamense* e *C. truncatum* reduzindo a produção com incidência de 30% nos pomares (Abirammi *et al.*, 2019; Salunkhe *et al.*, 2023a;). Nas Filipinas foi relatado pela primeira vez *C. fructicola* (Evallo *et al.*, 2021b). No Brasil, a antracnose foi relatada em pomares nos estados de São Paulo, Ceará, Rio Grande do Sul e Minas Gerais (Almeida, 2018; Moura, 2020; Nascimento *et al.*, 2019; Takahashi *et al.*, 2008). Os sintomas são caracterizados por lesões necróticas de coloração marrom, que com o progresso da doença adquirem um halo amarelado causando podridão nos cladódios e frutos. Em alguns casos foi possível visualizar acérvulos negros no centro das lesões (Figura 4).

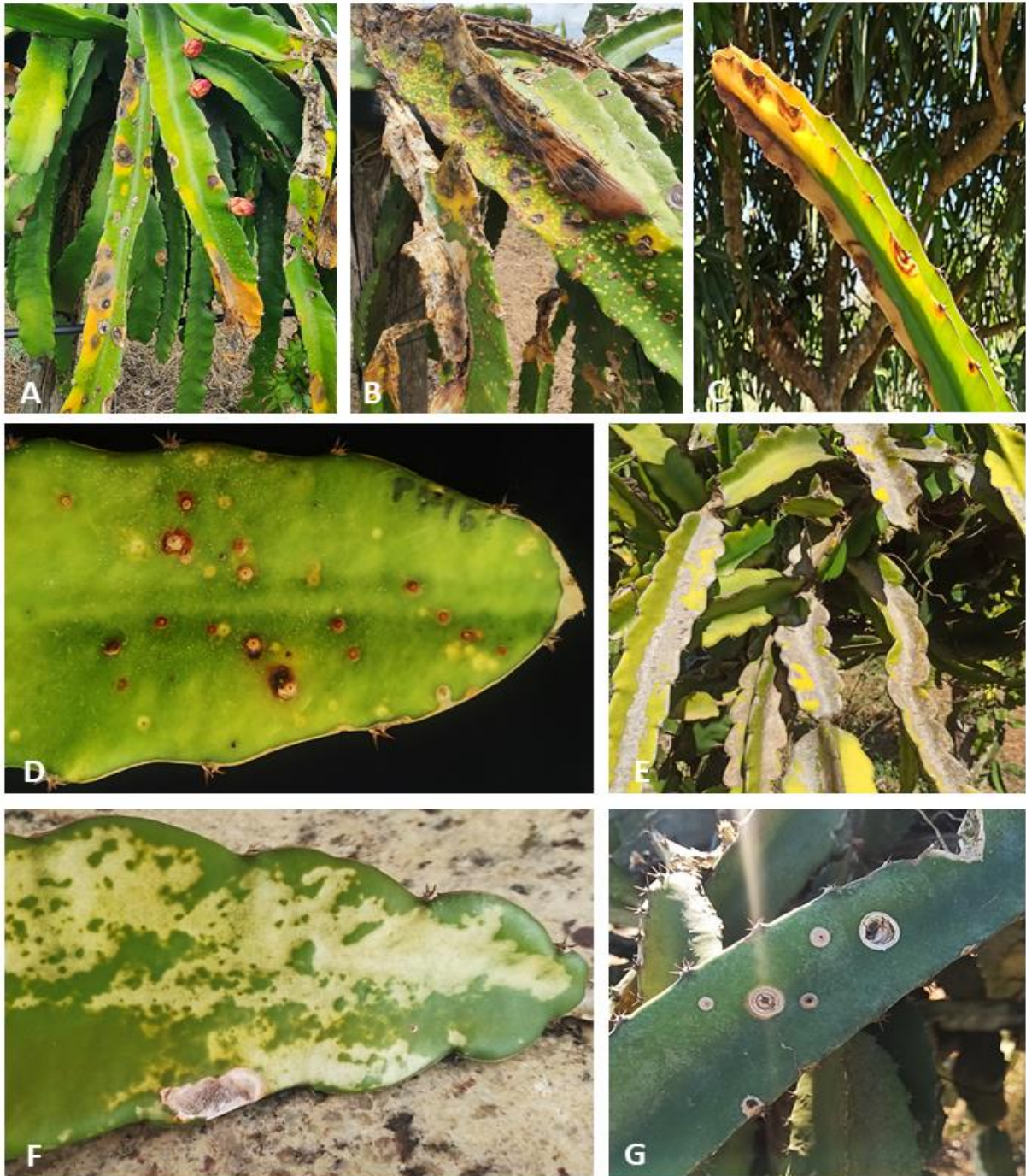
Apesar de a antracnose ser a doença mais relatada na literatura, a doença do cancro no cladódio vem aumentando nos pomares comerciais, causando grandes danos, com incidência de 40 a 80% em países como Porto Rico, Índia e Sul da Flórida (Hong *et al.*, 2020; Salunkhe *et al.*, 2023b; Serrato-Diaz; Goenaga, 2021). O principal patógeno relatado em diferentes países causando a doença é o fungo *Neoscytalidium dimidiatum* (Chuang *et al.*, 2012; Masratul Hawa *et al.*, 2013a; Sanahuja *et al.*, 2016; Yi *et al.*, 2015). Em 2019, os fungos *Lasiodiplodia theobromae* e *Botryosphaeria dothidea*, foram relatados causando os mesmos sintomas em Bangladesh e no Brasil, respectivamente (Andrade, 2019; Briste *et al.*, 2019). O cancro em cladódios apresenta como sintomas iniciais pequenas machas cloróticas e deprimidas. Com o avanço da doença as machas mudam para uma coloração escura de formato circular e elevação no local da lesão, tornando-se posteriormente necróticas, levando ao amarelecimento e podridão dos cladódios e frutos (Figura 4). Outro sintoma de podridão interna, com coloração escura em cladódios, flores e frutos, foi diagnosticado como sendo causados por *N. dimidiatum* na Malásia e em Israel (Ezra *et al.*, 2013; Mohd *et al.*, 2015).

As podridões causadas por *Fusarium* spp. são o terceiro grupo de doenças mais relatadas em pitaia (Figura 4). As espécies *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. fujikuroi* e *F. verticillioides* causam podridão em cladódios de *S. undatus*, *S. polirhisuz* e *S. megalantus*

(Mahmud *et al.*, 2020; Masratul Hawa *et al.*, 2017, 2013; Mohd *et al.*, 2015; Mohd Hafifi *et al.*, 2019; Moura, 2020; Pierangeli, 2019; Salazar-González *et al.*, 2016; Wright *et al.*, 2007). As espécies *F. oxysporum* e *F. dimerum* foram relatadas causando podridão em flores e frutos (Zhi-Jing *et al.*, 2011). Outras espécies foram relatadas causando os mesmos sintomas como *Gilbertella persicaria* no México e na China (Carrillo-Fasio *et al.*, 2022; Guo *et al.*, 2012) e *Neocosmospora rubicola* em cladódios de *S. costaricensis* na China (Zheng *et al.*, 2018b). Alguns países como Israel, Vietnã, Tailândia, China, Brasil e EUA (na Florida) relataram a presença de *Bipolaris cactivora* causando podridão em cladódios e frutos de *Selenicereus* spp. (Athipunyakom *et al.*, 2015; Ben-ze'ev *et al.*, 2011; He *et al.*, 2012; Moura, 2020; Oeurn *et al.*, 2015; Pierangeli, 2019; Qiu *et al.*, 2021; Tarnowski *et al.*, 2010).

Recentemente, espécies do gênero *Diaporthe* spp. foram relatadas na Malásia causando lesões cloróticas de coloração cinza com crescimento irregular em cladódios de *S. polyrhizus* (Huda-Shakirah *et al.*, 2021) e em Taiwan causando podridão nos cladódios de *S. undatus*, *S. polyrhizus* e *S. costaricensis* (Lin *et al.*, 2017). A fumagina foi relatada na China, sendo que o fungo *Cladosporium tenuissimum* cresceu sobre os cladódios e frutos *S. costaricensis* (Xu *et al.*, 2019). No Brasil, a espécie *Alternaria alternata* foi relatada como patógeno de podridão na pós-colheita de frutos (Castro *et al.*, 2017; Pierangeli, 2019). Ainda, o gênero *Alternaria* spp. foi relatado causando lesões em cladódios e frutos no Sul da Florida, EUA e no Brasil (Hong *et al.*, 2020; Moura, 2020; Patel; Zhang, 2017). Diversas espécies fúngicas foram encontradas causando lesões e manchas nos cladódios de *S. undatus* e *S. polyrhizus* na China, Malásia e Filipinas (Almeida, 2018; Liu *et al.*, 2016; Kee *et al.*, 2019; Masratul Hawa *et al.*, 2009; Taguiam *et al.*, 2020; Wu *et al.*, 2017). No Brasil, o gênero *Rhizopus* spp. causam podridões no colo dos cladódios e em frutos de *S. undatus* e *S. megalantus* (Lone *et al.*, 2020; Maia *et al.*, 2019).

Figura 4 - Principais doenças fúngicas na pitiaia e seus sintomas característicos.



Legenda: A-B: Antracnose; C: Podridões por *Fusarium* sp.; D: Cancro em cladódios; E: Lesões por *Diaporthe* spp.; F: Lesões de *Curvalaria* spp., G: Lesões por *Aureobasidium* spp.

Fonte: Autor (2023).

2.6.3. Doenças causadas por Vírus

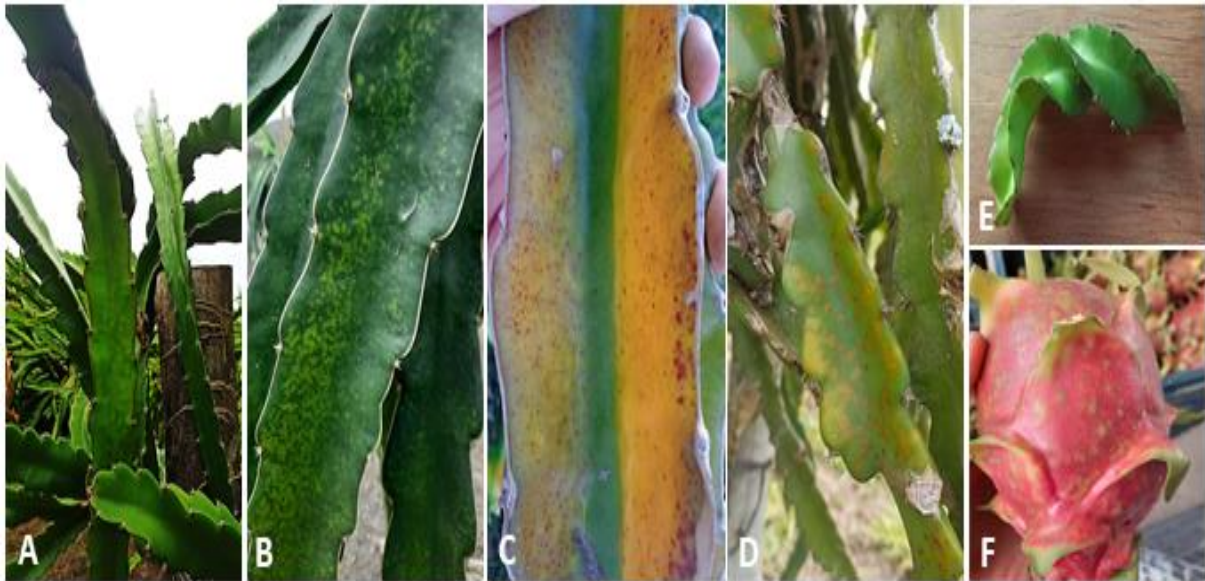
Atualmente, quatro espécies de vírus que pertencem a família *Alphaflexiviridae*, gênero *Potexvirus* já foram detectadas infectando espécies de pitiaia do gênero *Selenicereus* (Tabela 1). As espécies deste gênero possuem como característica partículas filamentosas e flexuosas, com

tamanho de 470-580 nm e diâmetro de 13 nm, contendo como ácido nucleico o RNA de fita simples senso positivo (ssRNA+) com 5,9 a 7 kb. Seu genoma codifica cinco proteínas: ORF1 está associada a replicação; as ORF2, ORF3 e ORF4 ou chamada de *triple gene block* (TGB) que estão envolvidas na movimentação célula a célula e a proteína da capa (Kreuze *et al.*, 2020).

A primeira espécie identificada foi o *Cactus virus X* (CVX) infectando cladódios de *S. undatus* em Taiwan. Atualmente é a espécie mais comum que infecta cactáceas no mundo (Evallo *et al.*, 2021a). O CVX foi encontrado com uma incidência de 44% em pomares da China (Peng *et al.*, 2016) e de 90% em pomares da Florida, EUA e na Malásia (Gazis *et al.*, 2018; Masanto *et al.*, 2018). Em 2008, Duarte *et al.* relatou a presença de duas espécies em *S. undatus*, o *Zygocactus virus X* (ZyVX) e *Schlumbergera virus X* (SchVX) em infecção mista no Brasil. Recentemente, ZyVX foi relatado em pomares da Índia (Parameswari *et al.*, 2023) e SchVX na Espanha (Janssen *et al.*, 2022). Em 2011, Mao *et al.* identificou uma nova espécie nos pomares de pitáia em Taiwan nomeando como *Pitaya virus X* (PiVX). Uma infecção mista entre as espécies PiVX e ZyVX foi relatada recentemente nos pomares comerciais da Korea (Bae; Park, 2022).

Geralmente as infecções por vírus em cactáceas ocorrem de maneira assintomática, dificultando sua diagnose através dos sintomas. Quando apresentam sintomas (Figura 6), podem-se visualizar manchas sistêmicas variando de leves a fortes, na forma de mosaico e mosqueado nos cladódios e nos frutos, além disso, a planta reduz seu crescimento vegetativo e diminui a produção dos frutos. Outros sintomas como enrolamento dos cladódios, lesões necróticas e cloróticas, anéis e halos cloróticos irregulares e manchas ou pontos de coloração marrom-avermelhadas podem também ser visualizados nas plantas infectadas com potexvirus como nos frutos (Bae; Park, 2022; Evallo *et al.*, 2021a; Masanto *et al.*, 2018; Peng *et al.*, 2016). Os potexvirus não possuem vetores na natureza, de modo que a sua disseminação se dá por contato mecânico e por propagação de cladódios coletados a partir de plantas infectadas, bem como pelas ferramentas utilizadas para as podas de condução.

Figura 6 - Principais sintomas de vírus em cladódios e no fruto de pitiaia.



Legenda: A: mosaico, B: mosqueado; C: manchas de coloração marrons, D: anéis cloróticos, E: manchas nos frutos e F: enrolamento nos cladódios.

Fonte: Autor (2023).

2.6.2. Doenças causadas por Bactérias

A podridão mole bacteriana foi a única doença causada por bactéria já detectada em pitiaia no mundo (Tabela 1). Inicialmente, os primeiros relatos da doença foram atribuídos a espécie *Enterobacter cloacae* causando podridão em cladódio de *S. undatos*, *S. polirhizus* e *S. megalantus* na Malásia (Masyahit *et al.*, 2009) e *S. undatos* no Peru (Soto *et al.*, 2019). Atualmente, sintomas semelhantes foram detectados em algumas regiões produtoras da fruta, porém a doença foi causada por diferentes espécies de bactérias como *E. hormaechei* na Costa Rica (Ratana-Sanchez *et al.*, 2019b), *Pectobacterium carotovorum* na Indonésia (Masnilah *et al.*, 2018). As espécies *Pseudomonas syringae* pv. *Syringae* e *Paenibacillus polymyxa* foram detectadas na China, causando sintomas iniciais de lesões nos cladódios seguidas por podridão dos tecidos, com incidência de 40% e 100% nos pomares, respectivamente (Wang, 2022; Zhang *et al.*, 2017). No Brasil, sintomas semelhantes foram relatados em pomares comerciais por Lone *et al.* (2020) e por Santos *et al.* (2022), porém os agentes causais não foram identificados (Figura 5).

Figura 5. Sintoma da podridão mole bacteriana em cladódios de pitaiá.



Fonte: Autor (2023).

2.6.4. Doenças Causadas por Nematóides

O primeiro relato de nematóides infectando pitaiá ocorreu na China, onde foi detectada a espécie *Tylenchorhynchus agri*, um ectoparasita migratório de estomatoestilete relativamente curto. A presença do patógeno inibiu o crescimento das plantas e resultou na queda dos frutos (Zhang *et al.*, 2018). Recentemente, as espécies de *Meloidogyne javanica* e *M. incógnita* foram detectadas em pomares de *S. megalantus* e *S. undatus* no estado de São Paulo (De Souza *et al.*, 2022; Nascimento *et al.*, 2020) e as espécies *M. enterolobii* e *M. javanica* foram registradas na China (Long *et al.*, 2022). As espécies de *Meloidogyne* são endoparasitas sedentários, que se fixam nas raízes das plantas e formam as galhas, que afetam o crescimento da planta resultando em nanismo e amarelecimento dos cladódios.

2.6.5 Métodos de controle de doenças

Como a cultura da pitaiá em regime comercial é relativamente recente, não existem na literatura muitos estudos com recomendações de métodos para o controle das pragas e patógenos que ocorrem na cultura. Entretanto, pelo tipo de cultivo, que usa basicamente fragmentos dos cladódios, em um método de propagação vegetativa, as doenças podem ser propagadas a partir da planta mãe, caso esteja infectada. Portanto, alguns métodos de controle podem ser recomendados.

Para evitar a ocorrência e disseminação de doenças, os métodos de controle devem adotar medidas preventivas, entre as quais a aquisição do material vegetal com sanidade aprovada é a mais importante. Outra medida que se aplica a fungos e bactérias é a remoção de tecidos infectados (poda de limpeza) da planta e do pomar, a remoção de resíduos florais e de frutos, a limpeza e desinfecção dos equipamentos utilizados no processo de condução do pomar com álcool 70%, hipoclorito de sódio, iodo, 40% cloreto de benzalcônio, detergentes, dentre outros (Lone *et al.*, 2020; Queiroga *et al.*, 2021; Riaño *et al.*, 2013; Santos *et al.*, 2022). Após a remoção dos tecidos afetados, recomenda-se a aplicação de calda bordalesa na região de corte da poda e a aplicação de caldas a base de extratos vegetais de *Azadirachta indica* que possui propriedades fungicida, bactericida e nematocida que podem ser aplicados como uma alternativa na tentativa de controlar ou reduzir as doenças no campo (Queiroga *et al.*, 2021). Nos países em que os agroquímicos são liberados, recomendam-se a aplicação de fungicidas a base dos Ditiocarbamatos, Benzimidazóis e Imidazóis (Riaño *et al.*, 2013). Estudos *in vitro* obtiveram resultados promissores no uso da bactéria *Bacillus amyloliquefaciens*, *Bacillus vezenensis* e *Bacillus atrophaeus* como uma opção de controle biológico ao fungo *N. dimidiatum* (An *et al.*, 2020) e para *A. alternata* (Trung *et al.*, 2021).

No caso de viroses, como os vírus não possuem controle curativo e se espalham sistemicamente pela planta, os métodos de controle preventivos são de extrema importância para a cultura. Como os potexvirus não possuem vetores, as medidas de controle devem ser direcionadas a três alvos principais: o primeiro é a utilização de material comprovadamente livre de vírus, ou seja, mudas certificadas, para o estabelecimento da cultura; o segundo é a eliminação de fontes de inóculo nas redondezas, evitando estabelecer pomares novos nas imediações de plantações mais velhas; o terceiro consiste no cuidado com as operações culturais, como podas de condução e colheita. As ferramentas utilizadas para poda de condução e para a colheita dos frutos devem ser desinfetadas e limpas entre uma planta e outra, para evitar possíveis transmissões de plantas infectadas para plantas sadias (Kreuze *et al.*, 2020).

Fazendo-se a propagação de material sadio e a eliminação da fonte de inóculo, aliada ao cuidado com as ferramentas, a cultura têm uma grande chance de evitar perdas pela ocorrência de doenças nos campos de produção.

REFERÊNCIAS

- ABIRAMI, K. *et al.* Occurrence of anthracnose disease caused by *Colletotrichum siamense* on dragon fruit (*Hylocereus undatus*) in Andaman Islands, India. **Plant Disease**, v. 103, p. 768-768, 2019. <https://doi.org/10.1094/PDIS-09-18-1489-PDN>
- AGROFIT - Sistema de Agrotóxicos Fitossanitários. Disponível em <agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acesso: Dez/2022.
- AGROSTAT – Estatística do comercio exterior do agronegócio do Brasil. Disponível em <<https://indicadores.agricultura.gov.br/agrostat/index.htm>>. Acesso: Dez/2022.
- ALMEIDA, Mirla Maria Mesquita. **Doenças fúngicas em pitaia (*Hylocereus sp.*) no estado do Ceará**. Trabalho de Conclusão de Curso (Graduação em Agronomia) – Universidade Federal do Ceará, Centro de Ciências Agrárias, Fortaleza, 2018.
- AN, N. N. *et al.* Isolation, identification and characterization of bacterial antagonists of the dragon fruit fungal pathogen *Neoscytalidium dimidiatum*. **Journal of Science and Technology-IUH**, v. 44. 2020. <https://doi.org/10.46242/jst-ih.v44i02.1036>
- ANDRADE, C. C. L.; DALBOSCO, M.; DUARTE, V. *Botryosphaeria dothidea* on Dragon Fruit (Pitaya). *Agriporticus*. 2019. Disponível em: <http://www.agronomicabr.com.br/agriporticus/detalhe.aspx?id=847>. Acesso em: Dez/2022.
- ATTAR, S. H. *et al.* Nutritional analysis of red-purple and white-fleshed pitaya (*Hylocereus*) species. **Molecules**, v. 27, p. 808, 2022. <https://doi.org/10.3390/molecules27030808>
- ATHIPUNYAKOM, P.; SEEMADUA, S.; DOUNGSA-ARD, C. Dragon fruit (pitaya) diseases in Thailand: Incidence and management strategies. **Management of pests and diseases**, 1. 2015. Disponível em: <<https://dfnet.fftc.org.tw/Page/ArticleDetail.aspx?ArticleID=zSts%2BA4AJG4%3D&PI=YnhAMaZeMZU%3D&Co=ztBzV%2F10sco%3D&Ca=Lu54OrWCb54LgS5un8dVJo5p%2FDkXW8oeta6ywGjwATrc0LtIP5dkXL9R%2FSoU9jcJ%2BU3iRG6FeDfbPLA8HKV1Xd7wa93BxeBcQRAbQIjebL%2BiCMFXW7dnNFXXk9KQ%2BwOENweA2iLMdiUTJZCYm2hQAgBJWwMTK8GpPh9Nr4BJ7oBAtqLxBjK%2BGuGMPhtocfE94IQfEVCiBQNpSBOhM80yMLs8vJOBEMSbGcqZbTyvYk%3D&Ws=ztBzV%2F10sco%3D&Keyword=ztBzV%2F10sco%3D>>. Acesso em: Out/2022.
- BA, L. *et al.* Exogenous melatonin treatment in the postharvest storage of pitaya fruits delays senescence and regulates reactive oxygen species metabolism. **Food Science and Technology**, v.42, e15221, 2022. <https://doi.org/10.1590/fst.15221>
- BAE, M.; PARK, M. First report of pitaya virus X and zygocactus virus X infection of dragon fruit (*Selenicereus undatus*) in Korea. **Journal of Plant Pathology**, v. 104, n. 4, p. 1581-1581, 2022. <https://doi.org/10.1007/s42161-022-01206-y>
- BALENDRES, M. A.; BENGGOA, J. C. Diseases of dragon fruit (*Hylocereus* species): Etiology and current management options. **Crop protection**, v. 126, p. 104920, 2019. <https://doi.org/10.1016/j.cropro.2019.104920>

BEN-ZE'EV, I. S. *et al.* First report of *Bipolaris cactivora* causing fruit blotch and stem rot of dragon fruit (pitaya) in Israel. **Phytoparasitica**, v. 39, p. 195-197, 2011.
<https://doi.org/10.1007/s12600-011-0143-y>

BRISTE, P. S. *et al.* First report of dragon fruit stem canker caused by *Lasiodiplodia theobromae* in Bangladesh. **Plant Disease**, v. 103, n. 10, p. 2686, 2019.
<https://doi.org/10.1094/PDIS-03-19-0619-PDN>

CARRILLO-FASIO, J. A. *et al.* First Report of Soft Rot of Pitahaya Fruit Caused by *Gilbertella persicaria* in Mexico. **Plant Disease**, v. 106, n. 12, p. 3218, 2022.
<https://doi.org/10.1094/PDIS-01-22-0142-PDN>

CASTRO, J. C. *et al.* Bioactivity of essential oils in the control of *Alternaria alternata* in dragon fruit (*Hylocereus undatus* Haw.). **Industrial Crops and Products**, v. 97, p. 101-109, 2017. <http://dx.doi.org/10.1016/j.indcrop.2016.12.007>

CHANDRA, R. *et al.* Health benefits of cactus. **Annals of Phytomedicine**, v. 8, n. 2, p. 179-185, 2019. <http://dx.doi.org/10.21276/ap.2019.8.2.23>

CHANG, S. K.; ALASALVAR, C.; SHAHIDI, F. Superfruits: Phytochemicals, antioxidant efficacies, and health effects—A comprehensive review. **Critical Reviews in Food Science and Nutrition**, v. 59, n. 10, p. 1580-1604, 2019.
<https://doi.org/10.1080/10408398.2017.1422111>

CHEOK, A. *et al.* The effects of betalain-rich cacti (dragon fruit and cactus pear) on endothelial and vascular function: A systematic review of animal and human studies. **Food & function**, v. 11, n. 8, p. 6807-6817, 2020. <https://doi.org/10.1039/d0fo00537a>

CHUANG, M. F. *et al.* First report of stem canker disease of Pitaya (*Hylocereus undatus* and *H. polyrhizus*) caused by *Neoscytalidium dimidiatum* in Taiwan. **Plant disease**, v. 96, n. 6, p. 906-906, 2012. <https://doi.org/10.1094/PDIS-08-11-0689-PDN>

CONAB – Companhia Nacional de Abastecimento. Disponível em: <
<http://dw.ceasa.gov.br/>>. Acesso em: Dez/2022.

CRANE, J. H.; BALERDI, C. F. **Pitaya (Dragonfruit) Growing in the Florida Home Landscape**. Institute of Food and Agricultural Sciences Extension Service, University of Florida. HS1068. 6p. Disponível em:< <https://edis.ifas.ufl.edu/publication/HS303>>. Acesso em: Out/2022.

DAS, G. *et al.* Cactus: Chemical, nutraceutical composition and potential bio-pharmacological properties. **Phytotherapy Research**, v. 35, n. 3, p. 1248-1283, 2021.
<https://doi.org/10.1002/ptr.6889>

DE ARAUJO, F. F. *et al.* Underutilized plants of the Cactaceae family: Nutritional aspects and technological applications. **Food chemistry**, v. 362, p. 130196, 2021.
<https://doi.org/10.1016/j.foodchem.2021.130196>

DE MELLO, J. F. *et al.* First report of *Lasiodiplodia pseudotheobromae* causing cladode rot in *Hylocereus* sp. in Brazil. **Journal of Plant Pathology**, v. 104, n. 2, p. 899-899, 2022. <https://doi.org/10.1007/s42161-022-01118-x>

DUARTE, L. M. L. *et al.* Potexvirus diversity in Cactaceae from Sao Paulo state in Brazil. **Journal of Plant Pathology**, p. 545-551, 2008. <https://www.jstor.org/stable/41998550>

EMBRAPA – Empresa Brasileira de Pesquisa. **Reunião da câmara setorial de fruticultura**. Disponível em <https://www.gov.br/agricultura/pt-br/assuntos/camaras-setoriais-tematicas/documentos/camaras-setoriais/fruticultura/2022/66-ro/apresentacao-embrapa-compactado.pdf>. Acesso: Dez/ 2022

ESPINOZA-LOZANO, L. *et al.* First report of *Neoscytalidium dimidiatum* causing stem canker on yellow dragon fruit (*Hylocereus megalantus*) in Ecuador. **Plant Disease**, p. PDIS-06-22-1403-PDN, 2023. <https://doi.org/10.1094/PDIS-09-22-2054-PDN>

EVALLO, E. *et al.* First report of Cactus virus X in *Selenicereus monacanthus* in the Philippines. **Indian Phytopathology**, v. 75, n. 4, p. 1237-1239, 2022b. <https://doi.org/10.1007/s42360-022-00555-z>

EVALLO, E. *et al.* First report of *Lasiodiplodia theobromae* causing stem canker of *Selenicereus monacanthus* in the Philippines. **Journal of Plant Pathology**, v. 104, n. 2, p. 831-832, 2022a. <https://doi.org/10.1007/s42161-022-01042-0>

EVALLO, E. *et al.* First report of *Colletotrichum fructicola*, causing anthracnose of *Hylocereus* plants, in the Philippines. **Czech Mycology**, v. 73, n. 1, p. 79–90, 2021b. <https://doi.org/10.33585/cmy.73106>

EVALLO, E.; TAGUIAM, J. D.; BALENDRES, M. A. A brief review of plant diseases caused by Cactus virus X. **Crop Protection**, v. 143, p. 105566, 2021a. <https://doi.org/10.1016/j.cropro.2021.105566>

EZRA, D. *et al.* First report of internal black rot caused by *Neoscytalidium dimidiatum* on *Hylocereus undatus* (Pitahaya) fruit in Israel. **Plant disease**, v. 97, n. 11, p. 1513-1513, 2013. <https://doi.org/10.1094/PDIS-05-13-0535-PDN>

FALEIRO, F.G., JUNQUEIRA, N.T.V. **Espécies, variedades e cultivares**. In: Pitaya: uma alternativa frutífera / Editores técnicos Dalílhia Nazaré dos Santos, Leila Aparecida Salles Pio, Fábio Gelape Faleiro. – Brasília: ProImpress, 2022.

GAZIS, R. *et al.* First Report of Cactus Virus X in *Hylocereus Undatus* (Dragon Fruit) in Florida. **Plant Disease**, v. 102, n. 12, p. 2666, 2018. <https://doi.org/10.1094/PDIS-05-18-0725-PDN>

GENGATHARAN, A.; DYKES, G.; CHOO, W. S. Betacyanins from *Hylocereus polyrhizus*: Pectinase-assisted extraction and application as a natural food colourant in ice cream. **Journal of Food Science and Technology**, v. 58, p. 1401-1410, 2021. <https://doi.org/10.1007/s13197-020-04651-8>

GOETTSCHE, B.; DURÁN, A. P.; GASTON, K. J. Global gap analysis of cactus species and priority sites for their conservation. **Conservation Biology**, v. 33, n. 2, p. 369-376, 2019. <https://doi.org/10.1111/cobi.13196>

GUERRERO, P. C. *et al.* Phylogenetic relationships and evolutionary trends in the cactus family. **Journal of Heredity**, v. 110, n. 1, p. 4-21, 2019. <https://doi.org/10.1093/jhered/esy064>

GUO, L. W. *et al.* First report of dragon fruit (*Hylocereus undatus*) anthracnose caused by *Colletotrichum truncatum* in China. **Journal of Phytopathology**, v. 162, n. 4, p. 272-275, 2014. <https://doi.org/10.1111/jph.12183>

GUO, L. W. *et al.* Storage Rot of Dragon Fruit Caused by *Gilbertella persicaria*. **Plant disease**, v. 96, n. 12, p. 1826-1826, 2012. <https://doi.org/10.1094/PDIS-07-12-0635-PDN>

HE, P. F. *et al.* *Bipolaris cactivora* causing fruit rot of dragon fruit imported from Vietnam. **Plant Pathology & Quarantine**, v. 2, n. 1, p. 31-35, 2012. <https://doi.org/10.5943/ppq/2/1/5>

HONG, C. F. *et al.* Prevalence and epidemics of *Neoscytalidium* stem and fruit canker on pitahaya (*Hylocereus* spp.) in South Florida. **Plant disease**, v. 104, n. 5, p. 1433-1438, 2020. <https://doi.org/10.1094/PDIS-10-19-2158-RE>

HOSSAIN, F. M.; NUMAN, S. M.; AKHTAR, S. Cultivation, nutritional value, and health benefits of dragon fruit (*Hylocereus* spp.): a review. **Journal of Horticultural Science and Technology**, v. 8, n. 3, p. 259-269, 2021. <https://dx.doi.org/10.22059/ijhst.2021.311550.400>

HUANG, Y. *et al.* Maturation Process, Nutritional Profile, Bioactivities and Utilisation in Food Products of Red Pitaya Fruits: A Review. **Foods**, v. 10, n. 11, p. 2862, 2021. <https://doi.org/10.3390/foods10112862>

HUDA-SHAKIRAH, A. R. *et al.* Diaporthe species causing stem gray blight of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. **Scientific reports**, v. 11, n. 1, p. 3907, 2021. <https://doi.org/10.1038/s41598-021-83551-z>

IBGE – Instituto Brasileiro de Geografia e Estatística. Produção de Pitaia. Disponível em: <<https://www.ibge.gov.br/explica/producao-agropecuaria/pitaia/br>>. Acesso em: Dez/2022.

ISKANDAR VIJAYA, S.; MOHD ANUAR, I. S.; ZAKARIA, L. Characterization and Pathogenicity of *Colletotrichum truncatum* Causing Stem Anthracnose of Red-Fleshed Dragon Fruit (*Hylocereus polyrhizus*) in Malaysia. **Journal of Phytopathology**, v. 163, n. 1, p. 67-71, 2015. <https://doi.org/10.1111/jph.12261>

JANSSEN, D.; GARCÍA, C.; RUIZ, L. First Report of *Schlumbergera* Virus X in Dragon Fruit (*Hylocereus* spp.) in Spain. **Plant Disease**, v. 106, n. 7, p. 2004, 2022. <https://doi.org/10.1094/PDIS-09-21-1879-PDN>

JIMENEZ-GARCIA, S. N. *et al.* Pitahaya Peel: A By-Product with Great Phytochemical Potential, Biological Activity, and Functional Application. **Molecules**, v. 27, n. 16, p. 5339, 2022. <https://doi.org/10.3390/molecules27165339>

- KAMAIRUDIN, N. *et al.* Optimization of natural lipstick formulation based on pitaya (*Hylocereus polyrhizus*) seed oil using D-optimal mixture experimental design. **Molecules**, v. 19, n. 10, p. 16672-16683, 2014. <https://doi.org/10.3390/molecules191016672>
- KARIM, M. M. *et al.* Occurrence of stem rot disease of *Hylocereus undatus* in Bangladesh. **Indian Phytopathology**, v. 72, p. 545-549, 2019. <https://doi.org/10.1007/s42360-019-00166-1>
- KEE, Y. J. *et al.* First report of reddish brown spot disease of red-fleshed dragon fruit (*Hylocereus polyrhizus*) caused by *Nigrospora laticolonia* and *Nigrospora sphaerica* in Malaysia. **Crop Protection**, v. 122, p. 165-170, 2019. <https://doi.org/10.1016/j.cropro.2019.05.006>
- KIM, J. S. *et al.* First report of Cactus virus X infecting *Hylocereus undatus* in Korea. **Plant Disease**, v. 100, n. 12, p. 2544-2544, 2016. <https://doi.org/10.1094/PDIS-06-16-0889-PDN>
- KIM, N. Y. *et al.* First report of pitaya virus X infecting dragon fruit (*Selenicereus undatus*) in Korea. **Journal of Plant Pathology**, p. 1-1, 2023. <https://doi.org/10.1007/s42161-023-01335-y>
- KREUZE, J. F. *et al.* ICTV Virus Taxonomy Profile: Alphaflexiviridae. **Journal of General Virology**, v. 101, n. 7, p. 699-700, 2020. <https://doi.org/10.1099/jgv.0.001436>
- KOROTKOVA, N. *et al.* Cactaceae at Caryophyllales.org – a dynamic online species-level taxonomic backbone for the family. **Willdenowia**, v. 51, n. 2, p. 251-270, 2021. <https://doi.org/10.3372/wi.51.51208>
- KOROTKOVA, N.; BORSCH, T.; ARIAS, S. A phylogenetic framework for the Hylocereeae (Cactaceae) and implications for the circumscription of the genera. **Phytotaxa**, v. 327, n. 1, p. 1-46, 2017. <https://doi.org/10.11646/phytotaxa.327.1.1>
- LAN, G. B. *et al.* First report of brown spot disease caused by *Neoscytalidium dimidiatum* on *Hylocereus undatus* in Guangdong, Chinese Mainland. **Plant disease**, v. 96, n. 11, p. 1702-1702, 2012. <https://doi.org/10.1094/PDIS-07-12-0632-PDN>
- LE BELLEC, F.; VAILLANT, F.; IMBERT, E. Pitahaya (*Hylocereus* spp.): a new fruit crop, a market with a future. **Fruits**, v. 61, n. 4, p. 237-250, 2006. <http://dx.doi.org/10.1051/fruits:2006021>
- LI, H.; LIU, C. Y.; LIU, Y. L. First Report of *Mucor irregularis* Causing Flower Rot on *Selenicereus undatus* in China. **Plant Disease**, n. ja, 2023. <https://doi.org/10.1094/PDIS-01-23-0117-PDN>
- LI, J. C.; WANG, Y.; DAI, H. F.; SUN, Q. Global transcriptome dissection of pollen–pistil interactions induced self-incompatibility in dragon fruit (*Selenicereus* spp.). **PeerJ**, v. 10, p. e14165, 2022. <https://doi.org/10.7717/peerj.14165>
- LIN, C. P. *et al.* Anthracnose of pitaya (*Hylocereus* spp.) caused by *Colletotrichum* spp., a new postharvest disease in Taiwan. **J. Taiwan Agric. Res.**, v. 66, p. 171–183, 2017. <https://doi.org/10.6156/JTAR/2017.06603.01>

LIU, M. R.; HUNG, C. L.; LIU, R. F. First report of Cactus virus X on *Hylocereus undatus* (Cactaceae) in taiwan. **Plant Disease**, v. 85, n. 2, p. 229-229, 2001. <https://doi.org/10.1094/PDIS.2001.85.2.229A>

LIU, F.; WU, J. B.; ZHAN, R. L.; OU, X. C. First report of reddish-brown spot disease on pitaya caused by *Nigrospora sphaerica* in China. **Plant Disease**, v. 100, n. 8, p. 1792, 2016. <https://doi.org/10.1094/PDIS-01-16-0063-PDN>

LONE, A. B. *et al.* **Cultivo de Pitaia**. Florianópolis, p. 44, 2020. Epagri. Boletim Técnico, 196.

LONG, H. *et al.* Occurrence and Identification of Root-Knot Nematodes on Red Dragon Fruit (*Hylocereus polyrhizus*) in Hainan, China. **Agronomy**, v. 12, n. 5, p. 1064, 2022. <https://doi.org/10.3390/agronomy12051064>

LUU, T. T. H. *et al.* Dragon fruit: A review of health benefits and nutrients and its sustainable development under climate changes in Vietnam. **Czech Journal of Food Sciences**, v. 39, n. 2, p. 71-94, 2021. <https://doi.org/10.17221/139/2020-CJFS>

MA, W. J. *et al.* First report of anthracnose disease on young stems of bawanghua (*Hylocereus undatus*) caused by *Colletotrichum gloeosporioides* in China. **Plant disease**, v. 98, n. 7, p. 991-991, 2014. <https://doi.org/10.1094/PDIS-06-13-0609-PDN>

MAHMUD, N. U. *et al.* Report of basal rot of dragon fruit caused by *Fusarium oxysporum* in Bangladesh. **Plant Dis**, v. 105, n. 1, p. 218, 2021. <https://doi.org/10.1094/PDIS-01-20-0005-PDN>

MAIA, J. P. *et al.* Agente etiológico e sintomas da podridão do colo em cladódios de pitaias. **Scientia Agraria Paranaensis**, v. 18, p. 93–96. Disponível em: <<https://e-revista.unioeste.br/index.php/scientiaagraria/article/view/20772>>. Acesso em: 8 nov. 2022.

MASANTO, M. *et al.* First report of necrotic spot disease caused by Cactus Virus X on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. **Jurnal Perlindungan Tanaman Indonesia**, v. 22, n. 1, p. 1-12, 2018. <https://doi.org/10.22146/jpti.23763>

MASNILAH, R.; NURCAHYANTI, S. D.; WAFI, A. Disease Pathogens of Dragon Fruits (*Hylocereus* Sp.) in Sukamade, Banyuwangi District East Java Indonesia. In: **Proceeding International Conference Sustainable Agriculture and Natural Resources Management (ICoSAaNRM)**. 2018.

MASRATUL HAWA, M. *et al.* *Fusarium fujikuroi* associated with stem rot of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. **Annals of Applied Biology**, v. 170, n. 3, p. 434-446, 2017. <https://doi.org/10.1111/aab.12348>

MASRATUL HAWA, M.; SALLEH, B.; LATIFFAH, Z. Characterization and pathogenicity of *Fusarium proliferatum* causing stem rot of *Hylocereus polyrhizus* in Malaysia. **Annals of applied Biology**, v. 163, n. 2, p. 269-280, 2013b. <https://doi.org/10.1111/aab.12057>

MASRATUL HAWA, M.; SALLEH, B.; LATIFFAH, Z. Identification and molecular characterizations of *Neoscytalidium dimidiatum* causing stem canker of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. **Journal of Phytopathology**, v. 161, n. 11-12, p. 841-849, 2013a. <https://doi.org/10.1111/jph.12146>

MASRATUL HAWA, M.; SALLEH, B.; LATIFFAH, Z. First report of *Curvularia lunata* on red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. **Plant disease**, v. 93, n. 9, p. 971-971, 2009. <https://doi.org/10.1094/PDIS-93-9-0971C>

MASYAHIT, M. *et al.* First report on bacterial soft rot disease on dragon fruit (*Hylocereus* spp.) caused by *Enterobacter cloacae* in peninsular Malaysia. **Int. J. Agric. Biol**, v. 11, p. 659-666, 2009. ISSN: 1814-9596.

MEETUM, P.; LEKSOMBOON, C.; KANJANAMANEESATHIAN, M. First report of *Colletotrichum aenigma* and *C. siamense*, the causal agents of anthracnose disease of dragon fruit in Thailand. **Journal of Plant Pathology**, v. 97, n. 2, p. 402, 2015. <https://dx.doi.org/10.4454/JPP.V97I2.048>

MEHETRE, G. T. *et al.* Current developments and challenges in plant viral diagnostics: A systematic review. **Viruses**, v. 13, n. 3, p. 412, 2021. <https://doi.org/10.3390/v13030412>

MERCADO-SILVA E. M. **Pitaya - *Hylocereus undatus* (Haw)**. In: RODRIGUES S.; DE OLIVEIRA SILVA E.; DE BRITO E.S. (Eds): *Exotic Fruits Reference Guide*. 1st Ed. Academic Press: p. 339-349. 2018.

MIZRAHI, Y. Thirty-one years of research and development in the vine cacti pitaya in Israel. **Improving Pitaya Production and Marketing**, p. 1-18. 2015. Disponível em: <<https://growables.com/information/TropicalFruit/documents/PitayaIsrael.pdf>>. Acesso em: Set/2022.

MIZRAHI, Y. Vine-cacti pitayas-the new crops of the world. **Revista Brasileira de Fruticultura**, v. 36, p. 124-138, 2014. <https://doi.org/10.1590/0100-2945-452/13>

MOHD HAFIFI, A. B.; KEE, Y. J.; MASRATU HAWA, M. First report of *Fusarium oxysporum* as a causal agent of stem blight of red-Fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. **Plant Disease**, v. 103, n. 5, p. 1040-1040, 2019. <https://doi.org/10.1094/PDIS-07-18-1249-PDN>

MOHD, M. H.; SALLEH, B.; ZAKARIA, L. An overview of fungal diseases of pitaya in Malaysia. **Management pests and diseases**, 882. 2015. Disponível em: <<https://dfnet.ffc.org.tw/Page/ArticleDetail.aspx?ArticleID=P3vM4piLYmw%3D&PI=1%2Fo%2Bf4QLuNA%3D&Co=ztBzV%2F10sco%3D&Ca=p9XulnOECFqvVf7RXaAxvJ%2Bn%2F2h14wo0jAQK7xtHmPlyN4UGPEgQIw%3D%3D&Ws=ztBzV%2F10sco%3D&Keyword=ztBzV%2F10sco%3D>>. Acesso em: Set/2022.

MOURA, Nevenka de Matos. 2020. **Doenças fúngicas da cultura da pitaiia**. Dissertação (Mestrado acadêmico em Agronomia/Fitopatologia) - Universidade Federal de Lavras, Lavras, 2020.

NASCIMENTO, D. D. *et al.* First Report of *Meloidogyne javanica* Infecting *Hylocereus megalanthus* in Brazil. **Plant Disease**, v. 104, n. 9, p. 2526, 2020.
<https://doi.org/10.1094/PDIS-02-20-0437-PDN>

NASCIMENTO, M. B. *et al.* First report of *Colletotrichum karstii* causing anthracnose spot on pitaya (*Hylocereus undatus*) in Brazil. **Plant Disease**, v. 103, n. 8, p. 2137, 2019.
<https://doi.org/10.1094/PDIS-02-19-0400-PDN>.

NOVOA, A. *et al.* Introduced and invasive cactus species: a global review. **AoB Plants**, v. 7, p. plu078, 2015. <https://doi.org/10.1093/aobpla/plu078>

NUNES, E. N. *et al.* Pitaia (*Hylocereus* sp.): uma revisão para o Brasil. **Gaia Scientia**, v. 8, n. 1, p. 90-98, 2014. <http://periodicos.ufpb.br/ojs2/index.php/gaia/index>

NUÑEZ-GARCÍA, P. R. *et al.* First Report of *Colletotrichum tropicale* Causing Anthracnose on Pitahaya Fruit in Mexico. **Plant Disease**, p. PDIS-09-22-2054-PDN, 2023.
<https://doi.org/10.1094/PDIS-09-22-2054-PDN>

OEURN, S.; JITJAK, W.; SANOAMUANG, N. Fungi on dragon fruit in Loei Province, Thailand and the ability of *Bipolaris cactivora* to cause post-harvest fruit rot. **Asia-Pacific Journal of Science and Technology**, v. 20, n. 4, p. 405-418, 2015.
<http://dx.doi.org/10.14456/kkurj.2015.34>

OLIVEIRA, J. S.; FALEIRO, F. G. **As pitayas e o processo de registro e proteção de cultivares**. In: Aplicação de descritores morfoagronômicos utilizados em ensaios de DHE de cultivares de pitayas: manual prático / editores técnicos, Fábio Gelape Faleiro, Jamile da Silva Oliveira, Nilton Tadeu Vilela Junqueira. – Brasília, DF: Embrapa, 2021.

ORTIZ-HERNÁNDEZ, Y. D.; CARRILLO-SALAZAR, J. Á. Pitahaya (*Hylocereus* spp.): a short review. **Comunicata Scientiae**, v. 3, n. 4, p. 220-237, 2012.
<https://doi.org/10.14295/cs.v3i4.334>

ORTIZ, T. A.; TAKAHASHI, L. S. A. Pitaya fruit quality (*Hylocereus undatus* [Haworth] Britton & Rose) according to physiological maturity. A review. **Colombiana de Ciencias Hortícolas**, v. 14, n. 1, p. 63-75, 2020. <https://doi.org/10.17584/rcch.2020v14i1.8422>

PALMATEER, A. J. *et al.* First occurrence of anthracnose caused by *Colletotrichum gloeosporioides* on pitahaya. **Plant Disease**, v. 91, n. 5, p. 631-631, 2007.
<https://doi.org/10.1094/PDIS-91-5-0631A>

PARAMESWARI, B. *et al.* First report of the association of *Zygocactus virus X* with dragon fruit (*Hylocereus* spp.) plants from Telangana, India. **Plant Disease**, v. 107, n. 4, p. 1249, 2023. <https://doi.org/10.1094/PDIS-05-22-1242-PDN>

PARAMESWARI, B. *et al.* First record of *Cactus virus X* in Dragon Fruit (*Hylocereus* spp.) in India. **Indian Phytopathology**, v. 75, n. 1, p. 297-299, 2022.
<https://doi.org/10.1007/s42360-021-00421-4>

PATEL, J. S.; ZHANG, S. First Report of Alternaria Blight of Pitahaya (*Hylocereus undatus*) Caused by *Alternaria* sp. in South Florida of the United States. **Plant Disease**, v. 101, n. 6, p. 1046-1046, 2017. <https://doi.org/10.1094/PDIS-11-16-1607-PDN>

PENG, C. *et al.* Molecular identification of Cactus virus X infecting *Hylocereus polyrhizus* (Cactaceae) in Hainan island, China. **Plant Disease**, v. 100, n. 9, p. 1956, 2016. <https://doi.org/10.1094/PDIS-01-16-0048-PDN>

PIERANGELI, Elaine Cristina Galvão. **Espécies de fungos e bactérias associados à cultura da pitáia e avaliação de estádios de maturação na qualidade do fruto**. Tese (Doutorado em Agronomia/Fitotecnia) - Universidade Federal de Lavras, 2019.

QIN, Q. *et al.* The complete plastomes of red fleshed pitaya (*Selenicereus monacanthus*) and three related *Selenicereus* species: insights into gene losses, IR expansions and phylogenomic implications. **Physiology and Molecular Biology of Plants**, v. 28, n. 1, p. 123-137, 2022. <https://doi.org/10.21203/rs.3.rs-373541/v1>

QIN, Y.; LIU, Y.; ZHANG, X.; LIU, J. Development of active and intelligent packaging by incorporating betalains from red pitaya (*Hylocereus polyrhizus*) peel into starch/polyvinyl alcohol films. **Food Hydrocolloids**, v. 100, p. 105410, 2020. <https://doi.org/10.1016/j.foodhyd.2019.105410>.

QIU, F. *et al.* First Report of *Bipolaris cactivora* Causing Flower Rot of Pitaya (*Hylocereus costaricensis*) in China. **Plant Disease**, v. 105, n. 4, p. 1205-1205, 2021. <https://doi.org/10.1094/PDIS-09-20-2055-PDN>.

QUEIROGA, V.P. **Pitahaya (*Hylocereus* spp.): Sistema produtivo de cactos trepadeiras**. 1ed. / Organizadores, Vicente de Paula Queiroga, Ênio Giuliano Girão, Josivanda Palmeira Gomes, Salles Pio, Fábio Gelape Faleiro. – Brasília: ProImpress, 2022.

RETANA-SÁNCHEZ, K. *et al.* Etiología de las pudriciones en el tallo de *Hylocereus costaricensis*, provocadas por *Enterobacter hormaechei*, en Costa Rica. **Agronomía Costarricense**, v. 43, n. 2, p. 61-74, 2019b. <http://dx.doi.org/10.15517/rac.v43i2.37949>

RETANA-SÁNCHEZ, K.; BLANCO-MENESES, M.; CASTRO-ZÚÑIGA, O. Etiology of stem canker caused by *Neoscytalidium dimidiatum* (Penz) on *Hylocereus costaricensis*, in Costa Rica. **Agronomía Costarricense**, v. 43, n. 1, p. 21-33, 2019a. <http://dx.doi.org/10.15517/rac.v43i1.35646>

RIAÑO, N. M. *et al.* **Enfermedades limitantes en el cultivo de pitaya amarilla**. In: Manual técnico: Tecnología para el manejo de pitaya amarilla *Selenicereus megalanthus* (K. Schum. Ex Vaupel) Moran en Colombia. Valle del Cauca (Colombia): CORPOICA, 2013. 96 p.

RITA, W. S. *et al.* First report on *Fusarium solani*, a pathogenic fungus causing stem rot disease on dragon fruits (*Hylocereus* sp.) in Bali. **Journal of Biology, Agriculture and Healthcare**, v. 3, n. 17, p. 93-99, 2013. ISSN 2225-093X. Disponível em: <<https://repositori.unud.ac.id/protected/storage/upload/penelitianSimdos/e339d177a5f848181a84857c5873c145.pdf>>. Acesso em: Out/2022.

SALAZAR-GONZÁLEZ, C.; SERNA-COCK, L.; GÓMEZ-LÓPEZ, E. Caracterización molecular de Fusarium asociado a pudrición basal del fruto en pitahaya (*Selenicereus megalanthus*). **Agronomía Mesoamericana**, p. 277-285, 2016. <https://doi.org/10.15517/am.v27i2.21269>

SALUNKHE, V. N. *et al.* First Report of *Neoscytalidium dimidiatum* Causing Stem Canker of Dragon fruit (*Hylocereus* spp.) in India. **Plant Disease**, v. 107, n. 4, p. 1222, 2023b. <https://doi.org/10.1094/PDIS-04-22-0909-PDN>

SALUNKHE, V. N. *et al.* First report of *Colletotrichum truncatum* causing anthracnose of dragon fruit (*Hylocereus* spp.) in India. **Plant Disease**, v. 107, n. 3, p. 945, 2023a. <https://doi.org/10.1094/PDIS-04-22-0809-PDN>

SANAHUJA, G.; LOPEZ, P.; PALMATEER, A.J. First report of *Neoscytalidium dimidiatum* causing stem and fruit canker of *Hylocereus undatus* in Florida. **Plant Disease**, v. 100, n. 7, p. 1499-1499, 2016. <https://doi.org/10.1094/PDIS-11-15-1319-PDN>

SANTOS, D. N. *et al.* **Sistema de produção da pitaya**. In: Pitaya: uma alternativa frutífera / Editores técnicos Dalíhia Nazaré dos Santos, Leila Aparecida Salles Pio, Fábio Gelape Faleiro. – Brasília: ProImpress, 2022.

SANTOS-DÍAZ, M. D. S.; CAMARENA-RANGEL, N. G. Cacti for production of metabolites: current state and perspectives. **Applied Microbiology and Biotechnology**, v. 103, p. 8657-8667, 2019. <https://doi.org/10.1007/s00253-019-10125-5>

SERRATO-DIAZ, L.M., GOENAGA, R. 2021. First Report of *Neoscytalidium dimidiatum* Causing Stem Canker on Dragon fruit (*Hylocereus* spp.) in Puerto Rico. **Plant disease**, v. 105, n. 9, p. 2728, 2021. <https://doi.org/10.1094/PDIS-10-20-2265-PDN>

SHOLIHAN, R. I.; SRITAMIN, M. A. D. E.; WIJAYA, I. N. Identifikasi Jamur *Fusarium solani* yang Berasosiasi dengan Penyakit Busuk Batang pada Tanaman Buah Naga (*Hylocereus* sp.) Di Kecamatan Bangorejo, Kabupaten Banyuwangi. **Kabupaten Banyuwangi Agroekoteknologi Trop**, v. 8, p. 91-102, 2019. <https://ojs.unud.ac.id/index.php/JAT>

SILVA, Adriana de Castro Correia da. 2014. **Pitaya: Melhoramento e produção**. Tese (Doutorado em Agronomia) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias. 2014.

SOTO, J. *et al.* First report of *Enterobacter cloacae* as a causative agent of soft rot disease in dragon fruit (*Hylocereus undatus*) stems in Peru. **Peruvian Journal of Agronomy**, v. 3, n. 3, p. 144–152, 2019. <http://dx.doi.org/10.21704/pja.v3i3.1367>

SOUZA, V. H. M. *et al.* First report of *Meloidogyne incognita* infecting white Pitahaya plants. **Revista Brasileira de Fruticultura**, v. 44, p. e-822, 2022. <https://doi.org/10.1590/0100-29452022822>

TAGUIAM, J. D.; EVALLO, E.; BENGGOA, J.; MAGHIRANG, R.; BALENDRES, M. A. Pathogenicity of *Epicoccum sorghinum* towards dragon fruits (*Hylocereus* species) and in

- vitro evaluation of chemicals with antifungal activity. **Journal of Phytopathology**, v. 168, n. 6, p. 303-310, 2020. <https://doi.org/10.1111/jph.12893>
- TAKAHASHI, H.; FUKUHARA, T.; KITAZAWA, H.; KORMELINK, R. Virus latency and the impact on plants. **Frontiers in Microbiology**, v. 10, p. 485724, 2019. <https://doi.org/10.3389/fmicb.2019.02764>
- TAKAHASHI L. M. *et al.* First report of Colletotrichum gloeosporioides on Hylocereus megalanthus in Brazil. **Australasian Plant Disease Notes**, v. 3, p. 96-97, 2008. <https://doi.org/10.1071/DN08038>
- TARNOWSKI, T.; PALMATEER, A.; CRANE, J. First report of fruit rot on Hylocereus undatus caused by Bipolaris cactivora in South Florida. **Plant Disease**, v. 94, n. 12, p. 1506-1506, 2010. <https://doi.org/10.1094/PDIS-06-10-0406>
- TRIVELLINI, A. *et al.* Pitaya, an attractive alternative crop for mediterranean region. **Agronomy**, v. 10, n. 8, p. 1065, 2020. doi:10.3390/agronomy10081065
- TRUNG, D. Q. *et al.* Endophytic bacteria isolated from a weed plant as a potential biocontrol agent against stem end rot pathogen of pitaya in Vietnam. **Egyptian Journal of Biological Pest Control**, v. 31, p. 1-7, 2021. <https://doi.org/10.1186/s41938-021-00451-0>
- UTPOTT, M. *et al.* Utilização da farinha da casca de pitaya vermelha (Hylocereus polyrhizus) como substituto de gordura em pães de forma. In: **6º Simpósio de Segurança Alimentar**, p. 1-6, 2019. http://www.schenautomacao.com.br/ssa/envio/files/116_arqnov.pdf.
- UTPOTT, M. *et al.* Evaluation of the use of industrial wastes on the encapsulation of betalains extracted from red pitaya pulp (Hylocereus polyrhizus) by spray drying: Powder stability and application. **Food and Bioprocess Technology**, v. 13, p. 1940-1953, 2020b. <https://doi.org/10.1007/s11947-020-02529-3>
- UTPOTT, M. *et al.* Characterization and application of red pitaya (Hylocereus polyrhizus) peel powder as a fat replacer in ice cream. **Journal of Food Processing and Preservation**, v. 44, n. 5, p. e14420, 2020a. <https://doi.org/10.1111/jfpp.14420>
- VALENCIA-BOTÍN, A. J.; KOKUNU, H.; ORTÍZ-HERNÁNDEZ, Y. D. A brief overview on pitahaya (Hylocereus spp.) diseases. **Australasian Plant Pathology**, v. 42, p. 437-440, 2013. <https://doi.org/10.1007/s13313-012-0193-8>
- VENTURA-AGUILAR, R. I. *et al.* Cactus stem (Opuntia ficus-indica Mill): Anatomy, physiology and chemical composition with emphasis on its biofunctional properties. **Journal of the Science of Food and Agriculture**, v. 97, n. 15, p. 5065-5073, 2017. <https://doi.org/10.1002/jsfa.8493>
- WANG, M. First report of stem rot on dragon fruit (Hylocereus undatus) caused by Pseudomonas syringae pv. syringae in China. **Journal of Plant Pathology**, v. 104, n. 3, p. 1185-1186, 2022. <https://doi.org/10.1007/s42161-022-01156-5>

WANG, Y. C. *et al.* First report of dragon fruit (*Hylocereus undatus*) stem rot caused by *Diaporthe ueckerae* in Taiwan. **Plant Disease**, v. 106, n. 5, p. 1527, 2022. <https://doi.org/10.1094/PDIS-09-21-1902-PDN>

WRIGHT, E. *et al.* Basal rot of *Hylocereus undatus* caused by *Fusarium oxysporum* in Buenos Aires, Argentina. **Plant Disease**, v. 91, n. 3, p. 323-323, 2007. <https://doi.org/10.1094/PDIS-91-3-0323A>

WU, J.; ZHAN, R.; LIU, F.; CANG, J. First report of a stem and fruit spot of pitaya caused by *Aureobasidium pullulans* in China. **Plant Disease**, v. 101, n. 1, p. 249, 2017. <https://doi.org/10.1094/PDIS-05-16-0702-PDN>

XU, G. *et al.* First Report of Sooty Mold Caused by *Cladosporium tenuissimum* on Pitaya in China. **Plant Disease**, v. 104, n. 1, p. 290, 2020. <https://doi.org/10.1094/PDIS-06-19-1218-PDN>

YANG, X. *et al.* A roadmap for research on crassulacean acid metabolism (CAM) to enhance sustainable food and bioenergy production in a hotter, drier world. **New Phytologist**, v. 207, n. 3, p. 491-504, 2015. <https://doi.org/10.1111/nph.13393>

YI, R. H.; MO, J. J., WU, F. F., CHEN, J. Fruit internal brown rot caused by *Neoscytalidium dimidiatum* on pitahaya in Guangdong province, China. **Australasian Plant Disease Notes**, v. 10, p. 1-4, 2015. <https://doi.org/10.1007/s13314-015-0166-1>

ZAID, R. M. *et al.* *Hylocereus polyrhizus* peel's high-methoxyl pectin: A potential source of hypolipidemic agent. **International journal of biological macromolecules**, v. 134, p. 361-367, 2019. <https://doi.org/10.1016/j.ijbiomac.2019.03.143>.

ZHANG, R. Y. *et al.* First Report of Bacterial Stem Rot Disease Caused by *Paenibacillus polymyxa* on *Hylocereus undulatus* in China. **Plant Disease**, v. 101, n. 6, p. 1031-1031, 2017. <https://doi.org/10.1094/PDIS-11-16-1577-PDN>

ZHANG, Y.; LU, X.; HUANG, J.; LIU, Z. First report of a stunt nematode (*Tylenchorhynchus agri*) from pitaya (*Hylocereus polyrhizus*) in Guangxi Province of China. **Plant disease**, v. 102, n. 12, p. 2662, 2018. <https://doi.org/10.1094/PDIS-02-18-0343-PDN>

ZHAO, H. J. *et al.* First report of red dragon fruit (*Hylocereus polyrhizus*) anthracnose caused by *Colletotrichum siamense* in China. **Plant Disease**, v. 102, n. 6, p. 1175-1175, 2018. <https://doi.org/10.1094/PDIS-08-17-1193-PDN>

ZHENG, F.; XU, G.; ZHENG, F.; DING, F.; XIE, C. *Neocosmospora rubicola* causing stem rot of pitaya (*Hylocereus costaricensis*) in China. **Plant Disease**, v. 102, n. 12, p. 2653-2653, 2018b. <https://doi.org/10.1094/PDIS-09-17-1469-PDN>

ZHENG, F. *et al.* First report of southern blight in pitaya (*Hylocereus undatus*) caused by *Sclerotium rolfsii* in China. **Plant Disease**, v. 102, n. 2, p. 441-441, 2018a. <https://doi.org/10.1094/PDIS-06-17-0869-PDN>

ZHI-JING, C.; YI-WEN, W.; YUE, Y.; LING, X. Pathogens analysis of soft rot disease of imported pitaya in Shanghai. **J. Microbiology China**, v. 38, n. 10, p. 1499-1506, 2011.

SEGUNDA PARTE - ARTIGOS

ARTIGO 1 - Detection, identification and molecular analysis of viruses present in pitaya plants in Brazil

Manuscript prepared for submission to Crop Protection

Antonia Thalyta Lopes Silveira^{a*}, Antonia dos Reis Figueira^{a*}, Sérgio Bruno Fernandes^a, Marcos Levi Medeiros^a, Sandra Carvalho Ismael Mussa Barros^a, Claudine Marcia Carvalho^a, Leila Aparecida Salles Pio^a

^aUniversidade Federal de Lavras, Trevo Rotatório Professor Edmir Sá Santos, s/n, CEP 37203-202, Lavras, MG, Brazil.

*Corresponding author: Universidade Federal de Lavras, Trevo Rotatório Professor Edmir Sá Santos, s/n, CEP 37203-202, Lavras, MG, Brazil. E-mail address: thalytasilveira@gmail.com (A.T.L. Silveira); antonia@ufla.br (A.R. Figueira).

ABSTRACT

The cultivation of pitaya has been recently established in several regions of the world, so there is a lack of information on its management, especially regarding the control of diseases and pests. In places where pitaya is grown around the world, symptoms such as mosaic and mottling caused by simple or mixed viral infections have been reported. In Brazil, similar symptoms have also been observed, but the etiology of the disease has often not been investigated. This study aimed to detect and identify the virus species that infect pitaya in Brazil by RT-PCR and molecular analysis of the coat protein gene (CP) of the viruses. A total of 212 samples of cladodes with suspected symptoms of viral disease were collected from different production regions of Brazil and analyzed. Four species of Potexvirus were detected, *Cactus virus X* (CVX), *Pitaya virus X* (PiVX), *Zygocactus virus X* (ZyVX) and *Schlumbergera virus X* (SchVX), which appeared in single infections (35%) and mixed infections (65%). In the cases of mixed infection, two and/or three species of potexvirus were identified in the same plant. The CVX isolates were divided into two groups due to the deletion of 9 nucleotides at position 41 (5' → 3') of the CP gene in one of them. This is the first study conducted on the viruses that infect pitaya in Brazil, showing the need to develop control measures that ensure productivity and profitability of pitaya crops in the country.

KEYWORDS: *Selenicereus* spp., *Hylocereus* spp., potexvirus, dragon fruit.

1. INTRODUCTION

Dragon fruit (pitaya), an exotic fruit of the genus *Selenicereus* spp. syn. *Hylocereus* spp. (Korotkova et al., 2017), has unique characteristics ranging from its shape, color and flavor to its exceptional nutraceutical properties. These attributes, together with the financial return of the crop, have won over Brazilian consumers and fruit growers in recent years. In 2022 alone, Brazil produced approximately 3 million kilograms of the fruit, with a domestic market that generated sales of more than 44 million reais (Conab, 2023).

Although dragon fruit is considered a “rustic” plant, recent reports of diseases in commercial orchards have grown in several regions of the world (Valencia-Botín et al., 2013; Balendres and Bengoa, 2019). Many viruses have been reported in cacti, resulting from infection by several species of viruses belonging to different genera, such as *Orthospovirus tomatomaculae* (TSWV), *Orthospovirus tomatoflavi* (TCSV) and *Orthospovirus impatiensnecromaculae* (INSV) of the genus *Orthospovirus* (Baker and Adkins, 2015; Park et al., 2018); *Opuntia chlorotic ringspot virus* (SOV), *Cactus mild mottle virus* (CMMoV) and *Rattail cactus necrosis-associated virus* (RCNaV) of the genus *Tobamovirus* (Park et al., 2018); and *Cactus virus X* (CVX), *Opuntia virus X* (OpVX), *Pitaya virus X* (PiVX), *Schlumbergera virus X* (SchVX) and *Zygocactus virus X* (ZyVX) of the genus *Potexvirus* (Evallo et al., 2021; Park et al., 2018; Duarte et al., 2008; Koenig et al., 2004).

Tospoviruses are transmitted by several species of thrips, but tobamoviruses and potexviruses do not have vectors in nature. However, in crops such as dragon fruit, which in addition to being vegetatively propagated are subjected to frequent agricultural operations such as pruning and fruit harvesting, the spread of viruses throughout the life cycle of the plant is greatly favored.

Viral infections can occur in the form of simple or mixed infections and have been detected occurring naturally in the field, both with species of the same genus and with species of different genera (Moreno and López-Moya, 2020). Although mixed infections can be classified as a coinfection, i.e., cases in which different virus species infect the plant simultaneously or in a short time interval, and as a superinfection, when different virus species infect the plant at different times (Singhal et al., 2021; Moreno and López-Moya, 2020), the exact period in which the plant was infected could not be determined.

The virus species that belong to the genus *Potexvirus*, subgenus *Mandarivirus*, and family *Alphaflexiviridae* are the most common in this crop and can be moderately pathogenic depending on the strain, concentration of virus in the plant, and environmental conditions and because they can be found in the form of simple or mixed infections (Kreuze et al., 2020).

Recently, several authors reported the presence of simple and mixed infections with potexvirus species in dragon fruit in India, Spain, Thailand and China (Parameswari et al., 2023, 2022; Janssen et al., 2022; Evallo et al., 2022; Bae and Park, 2022). The symptoms described include chlorotic spots distributed in mosaics or mottled form and, in some cases, spots with a reddish-brown color. In Brazil, little is known about the occurrence of the virus in dragon fruit. The only report of the virus available in the literature was by Duarte et al. (2008), who detected the presence of ZyVX and SchVX in *Selenicereus undatus* (Haw.) DR Hunt syn. *Hylocereus undatus* (Haw.) Britton and Rose in the State of São Paulo.

This is probably because the cultivation of dragon fruit is still relatively new in the country, associated with the lack of encouragement to study this crop among Brazilian researchers. In addition to the scarce information on these and other pathogens in dragon fruit orchards, the erroneous identification of the disease, which in most cases is based on visual identification, hinders the development of preventive control methods. Field inspections performed by the authors of this study revealed a high incidence of dragon fruit plants with symptoms of viruses in several fields located in Minas Gerais. This study aimed to detect and identify the viruses present in pitaya and to sequence and analyze the coat protein genes (CP) of isolates representative of the regions where they were collected.

2. MATERIAL AND METHODS

2.1. Collection and analysis of samples

The samples of *Selenicereus* spp. studied here were collected from commercial orchards in different regions of Brazil, as described in Figure 1, between March 2021 and September 2022, totaling 212 samples of cladodes with the suspected symptoms of viral infection reported in the literature. These samples were inoculated into indicator plants, and their RNA was extracted and subjected to diagnostic tests by reverse transcription polymerase chain reaction (RT-PCR). Next, the CP gene of the 49 chosen viruses isolates were amplified, sequenced and analyzed.

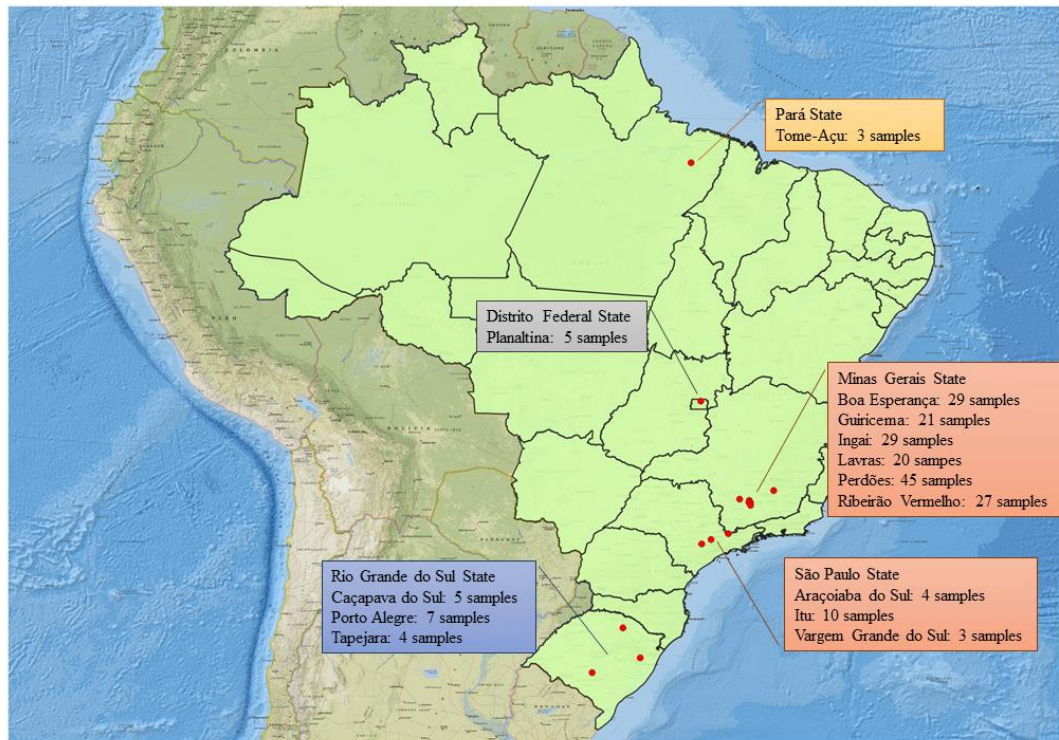


Figure 1. Map of sample collection locations in the different regions of Brazil analyzed in this study. Red points represent the geographic coordinates of the local where collected samples.

2.2. Mechanical inoculation into indicator plants

Plant extract was obtained by maceration of cladodes in 0.01 M phosphate buffer solution, pH 7, containing 0.01 M sodium sulfite, at a proportion of 1 g/5 mL. Mechanical inoculation was performed by rubbing the extract on leaves previously sprayed with carborundum (CSi) of the following indicator plants: *Chenopodium quinoa*, *Chenopodium amaranticolor*, *Datura stramonium*, *Nicotiana benthamiana* and *Nicotiana tabacum* cv. TNN. The plants were kept in a greenhouse throughout the experiment and observed daily for symptoms.

2.3. Detection and identification by RT-PCR

The total RNA of the cladodes was extracted with the lithium chloride method (Chang et al., 1993). Synthesis of the complementary strand of viral RNA (RT) was performed using M-MLV reverse transcriptase (Sigma-Aldrich Co., Saint Louis, MO, USA) following the manufacturer's instructions. The reaction was performed in a total volume of 20 μ l as follows: an initial step with 1 μ l of random hexamers (10 μ M), 1 μ l of dNTPs (10 mM), 6 μ l of ultrapure water and 2 μ l of total RNA (500 ng/ μ l). After incubation for 10 minutes at 70°C, each microtube was immediately transferred to a container with ice. In the last step, 2 μ l of enzyme

buffer was added to the microtubes (10X) together with 7 μ l of ultrapure water and 1 μ l of the M-MLV enzyme, and the mixtures were incubated for 50 minutes at 37°C and 10 minutes at 85°C.

In the PCR, specific primer pairs were used to detect of several viral species, as described in Table 2. The PCR was conducted in a total volume of 50 μ l consisting of 5 U of Taq High Fidelity Pol (Cellco Biotec do Brasil Ltda, São Carlos, SP, BRA), 5 μ l of 10X Taq DNA polymerase enzyme buffer (Cellco Biotec do Brasil Ltda, São Carlos, SP, BRA), 1 μ l of dNTPs (10 mM), 1 μ l each of the species-specific forward and reverse primers (10 μ M), 1 μ l of the cDNA obtained in the previous step (approximate concentration of 30 ng/ μ l) and enough ultrapure water to reach the total reaction volume. Amplification was performed with initial denaturation at 94°C for 3 minutes followed by 35 cycles as follows: 94°C for 45 seconds, annealing temperature of the specific primer pair for each virus for 30 seconds (Table 1), and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The PCR product was analyzed in a 0.7% agarose gel stained with Red Nucleic Acid Gel Stain (Biotium®) and observed with a MiniBis Pro photodocumenter (DNR Bio-Imaging Systems®).

Table 1. List of specific primer pairs used to detect of viral species reported in cacti.

Primer	Sequence (5'-3')	Temp (°C)	Size (bp)	Gene	Reference
CVX 1*	GTCTACTACTGGAGTCCAG	55	455	CP	Zhang <i>et al.</i> , 2016
	AAGAAGTCGAAGCAGC	52			
CVX 2**	CGGCACTTAATAACCGCT	53	784	CP	This study
	TAGGCACAGTTGGAACCC	55			
PiVX	ATGGCTACTCAAACAGCACAA	54	678	CP	Park <i>et al.</i> , 2018
	CTACTCTGGGGAGGGAAAG	57			
ZyVX	ATGTCTAACACTGCAGGAGT	53	681	CP	Park <i>et al.</i> , 2018
	TCATTCGGGACCCGGTAGGA	60			
SchVX	ATGTCGACCACTCCATCTTC	55	678	CP	Park <i>et al.</i> , 2018
	TTATTCAGGGGATGGTAGTA	51			
OpVX	ATGGCTTCCACTCCACAGAC	57	690	CP	Park <i>et al.</i> , 2018
	TTATTCAGGACCTGGTAGGA	53			
CMMoV	ATGGCGGGTTCTTACACCAA	55	486	CP	Park <i>et al.</i> , 2018
	CTAGGTGTGGCACCTAAGGT	57			
RCNaV	ATGCCTTACATCAACGTACA	51	513	CP	Park <i>et al.</i> , 2018
	TTAAGTACGACTGTCACCTTG	51			
Orthospovirus	AGAGCAATTGTGTCA	40	453	N	Eiras <i>et al.</i> , 2001
	ATCAAGCCTTCTGAAAGTTCAT	50			

* primer pair used for virus diagnosis, ** primer pair used for genomic analysis. The remaining primers were used for both diagnosis and genomic analysis.

2.4. Phylogenetic analysis

After the identification of the viruses in all the samples, 49 isolates representing the different collection sites were chosen for genomic analysis (Table 2).

Table 2. Potexvirus isolates chosen for sequencing and analysis with their respective symptoms

Isolate	Geographic origin	Virus species	Symptoms*	GenBank accession number
MG_Per01	Perdões, BR	CVX	MO, MOT	OR941727
MG_Per02	Perdões, BR		MO, MOT	OR941728
MG_Rib01	Ribeirão Vermelho, BR		MO, MOT	OR941729
MG_Rib02	Ribeirão Vermelho, BR		MO, MOT	OR941730
MG_Ing01	Ingaí, BR		MO, MOT	OR941731
MG_Ing02	Ingaí, BR		MO, MOT, CR	OR941732
MG_Ing03	Ingaí, BR		MO, MOT	OR941733
MG_Ing04	Ingaí, BR		MO, MOT	OR941734
MG_Ing05	Ingaí, BR		MO, MOT	OR941735
MG_Boa01	Boa Esperança, BR		MO, MOT, CR	OR941736
MG_Boa02	Boa Esperança, BR		MO, MOT, CR	OR941737
MG_Gui06	Guiricema, BR		MOT, CR	OR941742
MG_Gui07	Guiricema, BR		MOT, CR	OR941743
MG_Lav03	Lavras, BR		MO, CR, SS	OR941745
RS_Tap01	Tapejara, BR		MO, CR, SS	OR941738
RS_Porto01	Porto Alegre, BR		MOT, CR, SS	OR941744
SP_Itu01	Itu, BR		MO, MOT, SS	OR941739
DF_Pla01	Planaltina, BR		MO, MOT	OR941740
PA_Tome01	Tomé-Açu, BR		MO, CR	OR941741
MG_Rib04	Ribeirão Vermelho, BR	PiVX	MO, MOT	OR941746
MG_Rib05	Ribeirão Vermelho, BR		MO, MOT, CR	OR941747
MG_Rib06	Ribeirão Vermelho, BR		MO, MOT	OR941748
MG_Ing06	Ingaí, BR		MO, MOT	OR941749
MG_Ing07	Ingaí, BR		MO, MOT, CR	OR941750
MG_Ing08	Ingaí, BR		MO, MOT	OR941751
MG_Boa03	Boa Esperança, BR		MO, MOT	OR941752
MG_Gui01	Guiricema, BR		MOT, CR	OR941756
RS_Tap02	Tapejara, BR		MO, CR, SS	OR941754
SP_Itu02	Itu, BR		MO, MOT, SS	OR941757
SP_Var01	Vargem Grande do Sul, BR		MO, MOT, CR, SS	OR941755
PA_Tome02	Tomé-Açu, BR		MO, CR	OR941753
MG_Per03	Perdões, BR	ZyVX	MO, MOT	OR941758
MG_Rib07	Ribeirão Vermelho, BR		MO, MOT	OR941759
MG_Rib08	Ribeirão Vermelho, BR		MO, MOT, CR	OR941760
MG_Ing09	Ingaí, BR		MO, MOT	OR941761
MG_Ing10	Ingaí, BR		MO, MOT, CR	OR941762
MG_Lav01	Lavras, BR		MO, CR, SS	OR941763
MG_Lav02	Lavras, BR		MO, CR, SS	OR941764
MG_Boa04	Boa Esperança, BR		MO, MOT, CR	OR941765
MG_Boa05	Boa Esperança, BR		MO, MOT, CR	OR941766
MG_Boa06	Boa Esperança, BR		MO, MOT, CR	OR941767
MG_Gui02	Guiricema, BR		MOT, CR	OR941769
RS_Tap03	Tapejara, BR		MO, CR, SS	OR941768
MG_Gui03	Guiricema, BR	SchVX	MO, CR	OR795702
MG_Gui04	Guiricema, BR		MOT, CR	OR795703
MG_Gui05	Guiricema, BR		MOT, CR	OR795704
SP_Var02	Vargem Grande do Sul, BR		MO, MOT, CR, SS	OR795706
SP_Itu03	Itu, BR		MO, MOT, SS	OR795707
RS_Tap04	Tapejara, BR		MO, CR, SS	OR795705

*MO: mosaic, MOT: mottling, CR: chlorotic rings and SS: small brownish-red spots.

Alignment of the nucleotide and amino acid sequences was performed using the Clustal W program on the server (<https://www.genome.jp/tools-bin/clustalw>), and the sequence analysis was performed using NCBI Blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The isolates used for comparison are listed in Table 3. The phylogenetic relationships were studied

using the neighbor-joining algorithm for nucleotides and amino acids, using bootstrapping with 2,000 repeats in the Mega 11 program (Tamura et al., 2021).

Table 3. Information about isolates used in the phylogenetic analysis of this study.

GenBank accession number	Virus species	Geographic origin	Reference	
NC_002815.2	CVX	China	Liou <i>et al.</i> , 2004	
LC128411.1		South Korea	Unpublished	
JF937699.1		China	Unpublished	
KU497494.1		China	Unpublished	
OM912831.1		China	Unpublished	
BK011045.1		EUA	Jiang <i>et al.</i> , 2019	
MW051787.1		Germany	Unpublished	
OP037852.1		Spain	Janssen <i>et al.</i> , 2022	
NC_024458.1		PiVX	China	Unpublished
MN982523.1			China	Unpublished
MN982522.1	China		Unpublished	
LC654699.2	South Korea		Unpublished	
KY581589.1	South Korea		Park <i>et al.</i> , 2018	
NC_006059.1	ZyVX	Germany	Koenig <i>et al.</i> , 2004	
OL584352.1		Germany	Unpublished	
JF930326.1		China	Unpublished	
KM288844.1		China	Liao <i>et al.</i> , 2016	
KY581590.1		South Korea	Park <i>et al.</i> , 2018	
NC_011659.1	SchVX	Germany	Koenig <i>et al.</i> , 2004	
KP090203.1		Brazil	Unpublished	
KU854929.1		Mexico	Unpublished	
KY581588.1	OpVX	South Korea	Park <i>et al.</i> , 2018	
NC_006060.1		Germany	Koenig <i>et al.</i> , 2004	
D00344.1		<i>Potato virus X</i> (PVX) Out group	Netherlands	Huisman <i>et al.</i> , 1988

2.5. Analysis of selection pressure

To identify codons under selection with synonymous (dN) and nonsynonymous (dS) mutations, the nucleotide sequences were aligned using Muscle implemented in Mega 11 (Tamura et al., 2021) and analyzed by models implemented in Mega 11 and the HyPhy package, the latter of which is available on the Datamonkey server (<http://www.datamonkey.org/>). In this analysis, when the dN/dS ratio is >1, positive selection is occurring; when dN/dS <1, negative selection is occurring; and when dN/dS =1, neutral selection is occurring. In the maximum likelihood (ML) analyses, the programs Fixed Effects Likelihood (FEL) (Kosakovsky Pond and Frost, 2005) and Mixed Effects Model of Evolution (MEME) (Murrell et al., 2012) were used. In the Bayesian analysis, the Fast, Unconstrained Bayesian Approximation (FUBAR) program was used (Murrell et al., 2013).

3. RESULTS

3.1. Biological assays and symptoms in cladodes

No symptoms were observed in *D. stramonium* and in the two species of *Nicotiana*. The symptoms observed in *C. quinoa* and *C. amaranticolor* plants inoculated with the cladode extract of *Selenicereus* spp. plants collected in the field are shown in Figure 2. There was no difference in the symptoms caused by the four species of viruses, which included local chlorotic lesions that progressed to necrosis over time. There was no difference in plant reaction when the extract of plants with single or mixed infections was inoculated.

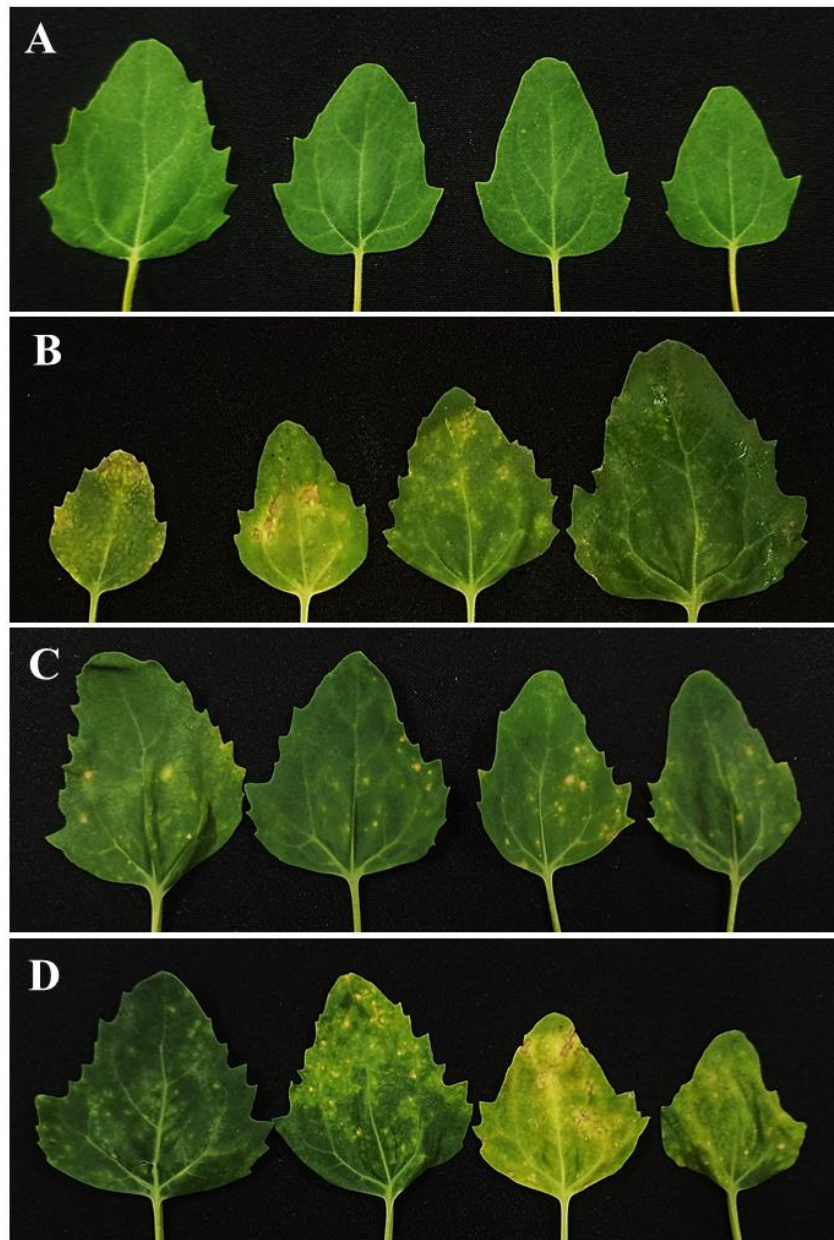


Figure 2. Symptoms of chlorotic and necrotic lesions observed in the indicator plants *C. quinoa* and *C. amaranticolor* 20 days after mechanical inoculation. A: healthy; B: CVX; C: CVX+PiVX; D: CVX+PiVX+ZyVX.

3.2. Detection and identification of viruses

Based on RT-PCR analysis, no infected samples for the genera *Tobamovirus* and *Orthospovirus* were detected (Table 4). Most samples, i.e., 181 (84.9%) of the total, were infected with one or more virus species belonging to the genus *Potexvirus*, while the remaining 32 (15.1%) were negative for viruses (Table 4). Among the positive samples, the species CVX, PiVX, ZyVX and SchVX were identified, with 63 samples (35.0%) showing simple infections, 81 samples (44.7%) with two species of virus, 30 samples (16.5%) with three virus species and 7 samples (3.8%) with four Potexvirus species. In cases of simple infections, CVX occurred in 34 of the 63 infected samples (18.9%), ZyVX in 24 (13.3%), PiVX in 2 (1.1%) and SchVX in 3 (1.7%).

Table 4. Detection and identification of different viruses using RT-PCR reactions in *Selenicereus spp.* samples, with simple and mixed infections.

City	Infection simple				Infections Mixed					Neg
	CVX	ZyVX	PiVX	SchVX	CVX+PiVX	CVX+ZyVX	PiVX+ZyVX	CVX+PiVX+ZyVX	CVX+PiVX+ZyVX+SchVX	
Lavras	4				2		4	5		5
Ribeirão Vermelho	2	14			7	11	3	1		7
Ingaí	7				12	1		7		
Perdões		7				14		6		2
Boa Esperança	11				2	7		7		2
Guiricema	5	2		1	3	2			2	7
Itu					4		1	1	2	
Vargem Grande do Sul	1					1			1	
Araçoiaba do Sul		1						1		2
Planaltina	1				2					2
Tomé Açu					3					
Tapejara	1							1	2	
Caçapava do Sul	1		1	1			1			2
Porto Alegre	1		1	1			1	1		3
TOTAL (%)*	34(18.8%)	24(13.2%)	2(1.1%)	3(1.6%)	35(19.3%)	36(19.9%)	10(5.5%)	30(16.5%)	7 (3.8%)	32

*Percentage in relation to the 181 infected samples.

The species CVX was identified most often at all sampled sites, while the species ZyVX had the highest incidence in the state of Minas Gerais and the species SchVX and PiVX had the highest incidence in the state of Rio Grande do Sul. In samples with mixed infections, identification of the combination CVX+PiVX was higher in the states of Minas Gerais, Brasília and Pará, while the combination CVX+PiVX and PiVX+ZyVX prevailed in Minas Gerais, São Paulo and Rio Grande do Sul. Finally, infections with all four species of potexvirus occurred in specific locations in the states of Minas Gerais, São Paulo and Rio Grande do Sul.

The symptoms observed in the cladodes are shown in Figure 3. The plants infected with CVX alone showed chlorotic ring spots and necrotic lesions. In the samples with mixed infections of two and three species, the symptoms were stronger, with more intense chlorosis due to the coalescence of the rings, distributed in the form of mosaics and/or mottling on the cladodes. In cladodes infected with the four viral species CVX, PiVX, ZyVX and SchVX, small brownish-red spots appeared, in addition to spots and chlorotic rings. All plants with simple or mixed infections exhibited cladode folding, mosaic and mottling, both in younger and in older cladodes.

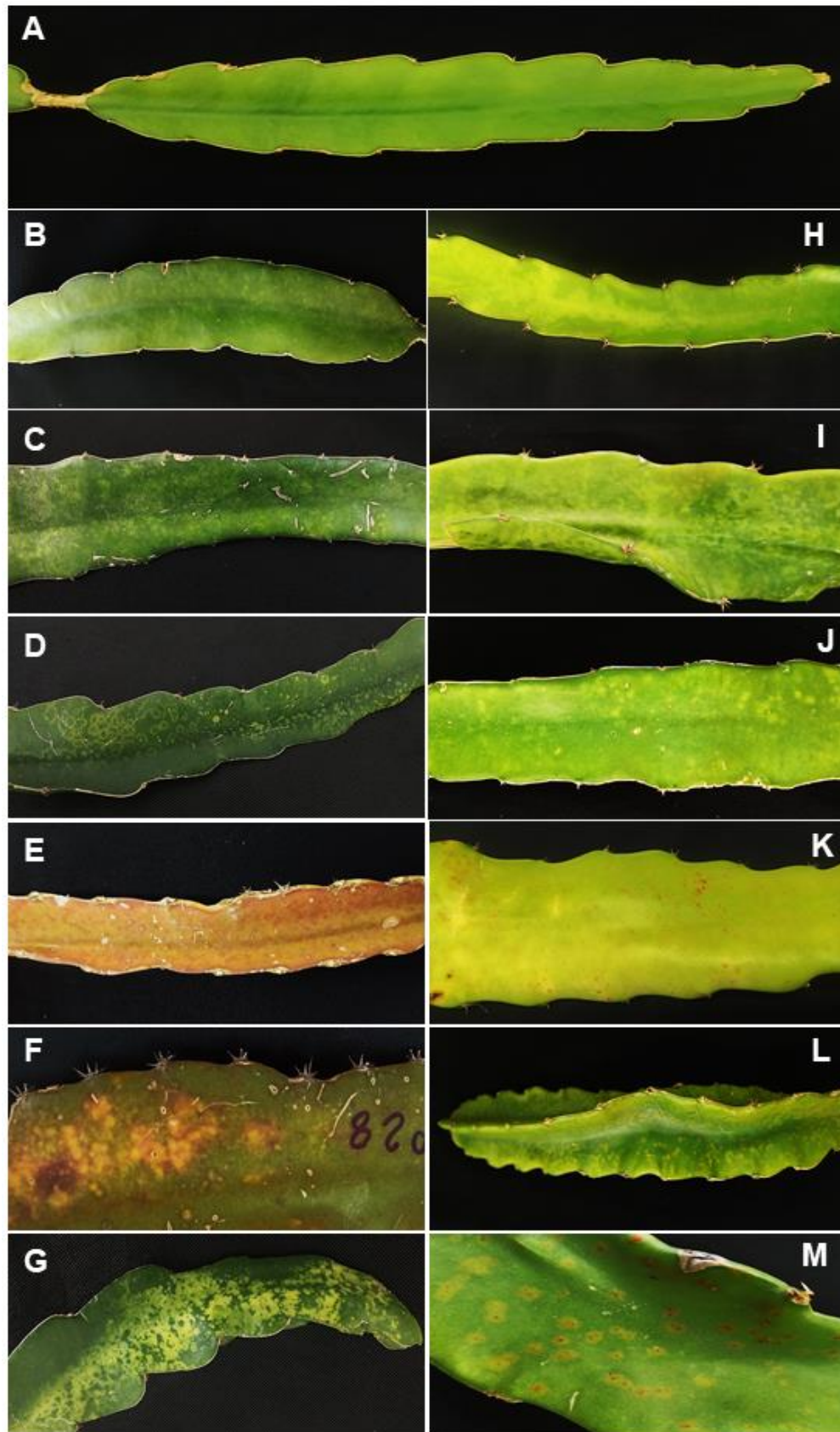


Figure 3. Symptoms observed in pitaya cladodes caused by viruses with simple and mixed infections of potexvirus species. A: healthy; B, C and D: CVX; E and F: CVX+PiVX; G: CVX+ZyVX; H, I and J: CVX+PiVX+ZyVX; K, L and M: CVX+PiVX+ZyVX+SchVX.

3.3. Phylogenetic analysis

Based on the results obtained, the 49 isolates selected to represent the potexvirus species from the different regions sampled in this study as shown earlier in Table 2. When the nucleotide sequences of the CVX isolates were compared, they were clearly subdivided into two groups (Figure 4): the first group consisted of the isolates MG_ING01, MG_ING02, MG_ING03, MG_ING04, MG_ING05, MG_GUI07, RS_TAP01, RS_PORTO01, SP_ITU01, DF_PLA01 and PA_TOME01 and also the GenBank isolates used for comparison (NC_002815 and KU497494 from China, BK011045 from the United States, LC128411 from South Korea, MW051787 from Germany and OP037852 from Spain). The second group consisted of isolates MG_PER01, MG_PER02, MG_RIB01, MG_RIB02, MG_BOA01, MG_BOA02, MG_LAV03 and MG_GUI06, which in turn were similar to isolates JF937699 and OM912831 from China. Notably, both groups of CVX isolates were also found in China.

The main difference between the sequences of the two groups was a deletion of 9 nucleotides in group 1: the deletion occurred at positions 42 to 50 in the initial region of the CP gene, in the 5' → 3' direction (Figure 4). This region, which encodes the three 'SSA' amino acids, is highly conserved among the isolates that contain it.



Figure 4. Alignment of nucleotides at the 5' terminal of isolates from both groups showing the deletion of 9 nucleotides at position 42 to 50 of the CP gene.

When the isolates from group 1 were compared with each other and with the similar isolates from GenBank cited above, they showed high shared nucleotide identity between 96%

and 99%. The same was observed for the second group, which presented shared nucleotide identities between 94% and 99% among themselves and 93% and 98% with the GenBank isolates. The shared identities between the isolates from group 1 and group 2 were between 74 and 76%.

The amino acid identities of the Brazilian isolates from group 1 ranged from 97 to 100% compared to each other and from 83% to 99% compared to the GenBank isolates. The amino acid identities of the Brazilian isolates from group 2 ranged from 96% to 100% compared to each other and from 94% to 100% compared to the GenBank isolates. The identities between the isolates from group 1 and group 2 ranged from 87 to 88%.

Considering the ICTV criteria, which establish a minimum of 72% nucleotide identity and a minimum of 80% amino acid identity for species to be considered equal within the genus *Potexvirus* (Kreuze et al., 2020), although the second group presented smaller identities, they were all considered to belong to the CVX species.

The nucleotide and amino acid identities of the isolates of PiVX, ZyVX and SchVX were above 90%, both when they were compared to each other and when they were compared to the isolates of the respective species available in GenBank.

The cladogram constructed based on the nucleotide sequences (Figure 5A) showed clear clustering between isolates of the same species with a possible common ancestor. The first clade, which grouped the CVX isolates, was separated into two subclades, which corroborates the findings of the nucleotide sequence analysis. The clades including the isolates of ZyVX and PiVX showed lower variability than the others. The cladogram constructed based on the amino acid sequences (Figure 5B) showed the same trend observed in the previous cladogram, with few variations, especially among the SchVX isolates.

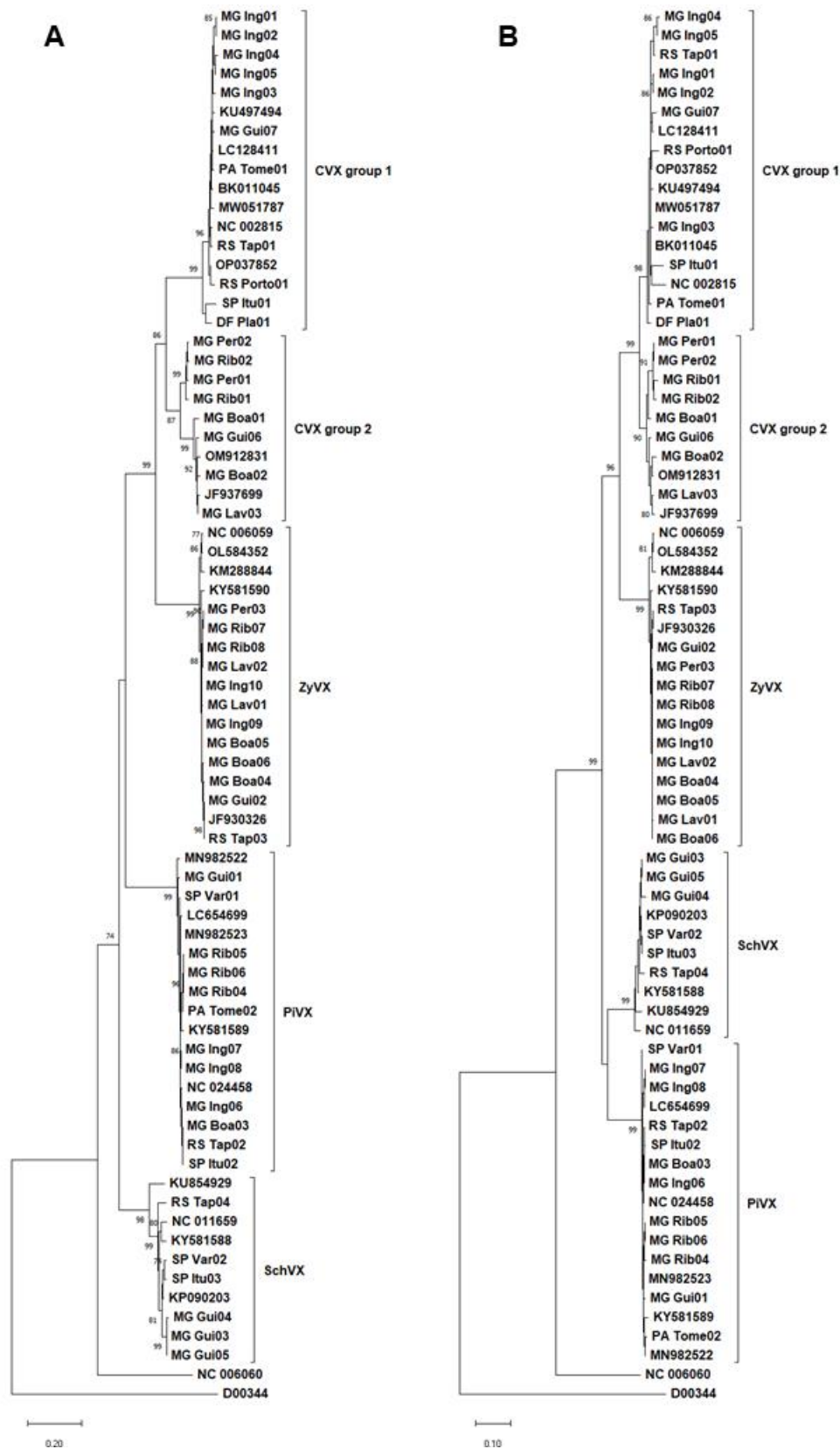


Figure 5. Phylogenetic tree constructed based on the nucleotide (A) and amino acid (B) sequence of the CP gene of the isolates obtained in this study and the isolates available in GenBank. Bootstrap values were obtained using the Mega 11 and Neighbor-Joining program with 2.000 repetitions.

3.4. Selection pressure

Sequence analysis region of the CP gene of the studied potexvirus isolates showed that the dN/dS ratio was negative, that is, less than 1, which indicates that they are under a process of purifying selection (Table 6). When comparing the regions under selection pressure using the FEL and FUBAR methods, selection pressure was observed for the same region at 82 sites in CVX, 6 sites in PiVX, 16 sites in ZyVX and 36 sites in SchVX.

Table 6. Selective pressures on the codons of the CP gene of isolates from Brazil, belonging to the species CVX, PiVX, ZyVX and SchVX.

Method	Virus species	Codons	Pressure Selection*		
			Positive	Negative	Neutral
FEL	CVX	229		102	
	PiVX	225		8	
	ZyVX	226		24	
	SchVX	225		49	
FUBAR	CVX	229		113	
	PiVX	225	2	6	
	ZyVX	226		16	
	SchVX	225		39	
MEME	CVX	229	6		
	PiVX	225			
	ZyVX	226	1		
	SchVX	225			

*P < 0,01.

4. DISCUSSION

The high infection rate of dragon fruit cladodes, detected by RT-PCR of samples collected in several commercial fields in Brazil, indicated that this is a crop whose management has been neglected, preventing the full expression of its genetic potential in the field. This type of study had not yet been performed in Brazil and showed the presence of four species of potexvirus, CVX, PiVX, ZyVX and SchVX, in both simple and mixed infections, aggravating the overall health status of the plant.

The symptoms observed in the indicator plants of *C. quinoa* and *C. amaranticolor* were similar to those reported by several authors when they inoculated the extract of infected cacti into cultivated and experimental plants: *Opuntia ficus* by Berrera et al. (2013), *Opuntia tuna*, *Hylocereus undatus* and *Schlumbergera truncata* by Duarte et al. (2008) and *Schlumbergera bridgesii*, *Zygocactus* sp. and *Opuntia* sp. by Koenig et al. (2004). The symptoms observed in

pitaya cladodes were also similar to those reported by authors who detected the presence of at least one potexvirus in different parts of the world where the plant is grown (Parameswari et al., 2023, 2022; Bae and Park, 2022; Janssen et al., 2022; Evallo et al., 2021a; Gazis et al., 2018; Masanto et al., 2018; Peng et al., 2016).

In recent years, reports of plants with mixed infections in other countries have suggested that they tend to occur more frequently in nature and are more complex than simple infections (Xu et al., 2022; Singhal et al., 2021; Moreno and López-Moya, 2020). According to Xu et al. (2022), in nature, mixed infections with two or more viruses are frequent in plants, interacting in multiple and intricate ways, as could be observed in the present study. Similar results have also been observed in other pathosystems, where mixed infections with the presence of two, three, four and up to eight virus species per sample were detected: tomato plants in China (Xu et al., 2017), bean plants in Mexico (Chiquito-Almanza et al., 2021), vines in Canada (Xiao et al., 2018) and Slovenia (Miljanic et al., 2022), and wheat in the USA (Tatineni et al., 2022).

In general, mixed infections tend to be more severe when the viruses are spread by vectors. However, although potexviruses do not have a vector in nature, they are easily transmissible by mechanical means, especially during cultivation (Kreuze et al., 2020). In addition to being subjected to routine pruning and fruit harvesting with sharp objects, dragon fruit also undergoes vegetative propagation, conditions that facilitate viral transmission.

The lack of information on the occurrence of the virus has led to a lack of planning and control of viruses in dragon fruit in Brazil. This may be because the production of the fruit in the country is relatively recent and established by admirers of ornamental plants, and it is common for them to freely exchange propagation material to obtain the diversities of shapes and colors that the cacti have. This exchange of genetic material results in the spread of the virus over long distances, facilitating the introduction of the virus into newly planted crops and its subsequent spread by cultural practices (Hossain et al., 2021; Kreuze et al., 2020; Mercado-Silva, 2018).

The phylogenetic analyses revealed that the potexvirus isolates from different regions of Brazil showed high identity with the isolates from different regions of the world available in GenBank, suggesting that the species detected in this study entered the country through propagation material contaminated with virus. The viruses were then distributed to different regions through infected seedlings. This proposed route is supported by the presence of CVX isolates belonging to two distinct groups in the same region and in most of the sampled regions.

The results obtained in this study emphasize the importance of taking actions to promote the dissemination of knowledge about specific measures to control dragonflies, aimed at dragon

fruit producers. In Brazil, the vast majority of these producers are family farmers with small planted areas and with little knowledge about disease and pest control. In addition, it is necessary to establish inspection measures in nurseries producing seedlings, which are responsible for the initial introduction of viruses into orchards.

5. CONCLUSION

The high occurrence of four potexvirus species (CVX, PiVX, ZyVX and SchVX) in every sampled Brazilian orchards for commercial production of dragon fruit demand additional studies, aiming to evaluate the incidence of viruses over the country. The distribution of potexvirus species among the producing areas indicates an intensive movement of infected seedlings from grower to grower, showing a lack of sanitary control, probably due to their unpreparedness to live with viral diseases. Efficient control measures must be taken to allow the pitaya cultivation in Brazil to be successful and promotes its sustainable cultivation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Knowledgements

The authors would like to thank Brazilian Institutions: National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), Foundation for Research Support of the State of Minas Gerais (FAPEMIG) for the resource funds to support the current work.

Data availability

Data will be made available on request.

REFERENCES

- Alcaide, C., Rabadán, M.P., Moreno-Perez, M. G., Gómez, P. 2020. Implications of mixed viral infections on plant disease ecology and evolution. *Advances in virus research*, 106, 145-169. <https://doi.org/10.1016/bs.aivir.2020.02.001>.
- Bae, M., Park, M. 2022. First report of pitaya virus X and zygocactus virus X infection of dragon fruit (*Selenicereus undatus*) in Korea. *Journal of Plant Pathology*, 104, 1581. <https://doi.org/10.1007/s42161-022-01206-y>.
- Baker, C.A., Adkins, S. 2015. First report of Tomato chlorotic spot virus in *Hoya wayetii* and *Schlumbergera truncata*. *Plant Health Progress*, 16, 29-30. <https://doi.org/10.1094/PHP-BR-14-0043>.
- Balendres, M.A., Bengoa, J.C. 2019. Diseases of dragon fruit (*Hylocereus* species): Etiology and current management options. *Crop Protection* 126, 104920. <https://doi.org/10.1016/j.cropro.2019.104920>.
- Chang, S., Puryear, J., Cairney, J. 1993. A simple and efficient method for isolating RNA from pine trees. *Plant molecular biology reporter*, 11, 113-116.
- Chiquito-Almanza, E., Caballero-Pérez, J., Acosta-Gallegos, J.A., Montero-Tavera, V., Mariscal-Amaro, L.A., Anaya-López, J.L. 2021. Diversity and distribution of viruses infecting wild and domesticated *Phaseolus* spp. in the mesoamerican center of domestication. *Viruses*, 13, 1153. <https://doi.org/10.3390/v13061153>.
- Conab – Companhia Nacional de Abastecimento. <http://dw.ceasa.gov.br/> (accessed 10 March 2023).
- Eiras, M., Resende, R.O., Missiaggia, A.A., ÁVILA, A.C. 2001. RT-PCR and dot blot hybridization methods for a universal detection of tospoviruses. *Fitopatologia Brasileira*, 26, 170-175. <https://doi.org/10.1590/S0100-41582001000200009>.
- Evallo, E., Taguam, J.D., Tumolva, J.A., Bengoa, J., Maghirang, R., Balendres, M.A. 2022. First report of Cactus virus X in *Selenicereus monacanthus* in the Philippines. *Indian Phytopathology*, 75, 1237-1239. <https://doi.org/10.1007/s42360-022-00555-z>.
- Evallo, E., Taguam, J.D., Balendres, M.A. 2021. A brief review of plant diseases caused by Cactus virus X. *Crop Protection* 143, 105566. <https://doi.org/10.1016/j.cropro.2021.105566>.
- Hai, D., Li, J., Lan, S., Wu, T., Li, Y., Cheng, J., Fu, Y., Lin, Y., Jiang, D., Wang, M., Xie, J. 2022. Discovery and Evolution of Six Positive-Sense RNA Viruses Co-infecting the Hypovirulent Strain SCH733 of *Sclerotinia sclerotiorum*. *Phytopathology®*, 112, 2449-2461. <https://doi.org/10.1094/PHYTO-05-22-0148-R>.
- Hossain, F.M., Numan, S. M., Akhtar, S. 2021. Cultivation, nutritional value, and health benefits of dragon fruit (*Hylocereus* spp.): a review. *International Journal of Horticultural Science and Technology*, 8, 259-269. <https://dx.doi.org/10.22059/ijhst.2021.311550.400>.

- Huisman, M.J., Linthorst, H.J., Bol, J.F., Cornelissen, J.C. 1988 The complete nucleotide sequence of potato virus X and its homologies at the amino acid level with various plus stranded RNA viruses. *J. Gen. Virol.* 69, 1789-1798. DOI: 10.1099/0022-1317-69-8-1789.
- Jacobson, A.L., Duffy, S., Sseruwagi, P. 2018. Whitefly-transmitted viruses threatening cassava production in Africa. *Current opinion in virology*, 33, 167-176. <https://doi.org/10.1016/j.coviro.2018.08.016>.
- Janssen, D., García, C., Ruiz, L. 2022. First Report of Schlumbergera Virus X in Dragon Fruit (*Hylocereus* spp.) in Spain. *Plant Dis.* 106, 2004. <https://doi.org/10.1094/PDIS-09-21-1879-PDN>.
- Jiang, P., Shao, J., Nemchinov, L.G. 2019. Identification of emerging viral genomes in transcriptomic datasets of alfalfa (*Medicago sativa* L.). *Virology journal*, 16, 1-12. <https://doi.org/10.1186/s12985-019-1257-y>.
- Koenig, R., Pleij, C.W., Loss, S., Burgermeister, W., Aust, H., Schiemann, J. 2004. Molecular characterisation of potexviruses isolated from three different genera in the family Cactaceae. *Arch. Virol.* 149, 903-914. <https://doi.org/10.1007/s00705-003-0268-5>.
- Korotkova, N., Borsch, T., Arias, S. 2017. A phylogenetic framework for the Hylocereeae (Cactaceae) and implications for the circumscription of the genera. *Phytotaxa*, 327, 1-46. <https://doi.org/10.11646/phytotaxa.327.1.1>.
- Kosakovsky Pond, S.L., Frost, S.D. 2005. Not so different after all: a comparison of methods for detecting amino acid sites under selection. *Molecular biology and evolution*, 22, 1208-1222. <https://doi.org/10.1093/molbev/msi105>.
- Kreuze, J.F., Vaira, A.M., Menzel, W., Candresse, T., Zavriev, S.K., Hammond, J., Hyun Ryu, K., Consortium, I.R. 2020. ICTV Virus Taxonomy Profile: Alphaflexiviridae. *The Journal of General Virology*, 101, 699-700. <https://doi.org/10.1099/jgv.0.001436>.
- Liou, M.R., Chen, Y.R., Liou, R.F. 2004. Complete nucleotide sequence and genome organization of a Cactus virus X strain from *Hylocereus undatus* (Cactaceae). *Arch. Virol.* 149, 1037-1043. <https://doi.org/10.1007/s00705-003-0251-1>.
- Martin, D.P., Varsani, A., Roumagnac, P., Botha, G., Maslamoney, S., Schwab, T., Kelz, Z., Kumar, V., Murrell, B. 2021. RDP5: A computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. *Virus Evol.* 7, veaa087. <https://doi.org/10.1093/ve/veaa087>.
- Masanto, M., Sijam, K., Awang, Y., Satar, M.G.M. 2018. First report of necrotic spot disease caused by Cactus Virus X on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. *Jurnal Perlindungan Tanaman Indonesia* 22, 1–12. <https://doi.org/10.22146/jpti.23763>.
- Mercado-Silva E. M. 2018. Pitaya - *Hylocereus undatus* (Haw). In: Rodrigues S., de Oliveira Silva E., de Brito E.S. (Eds): *Exotic Fruits Reference Guide*. 1st Ed. Academic Press: 339–349.

- Miljanić, V., Jakše, J., Kunej, U., Rusjan, D., Škvarč, A., Štajner, N. 2022. Virome status of preclonal candidates of grapevine varieties (*Vitis vinifera* L.) from the slovenian wine-growing region primorska as determined by high-throughput sequencing. *Frontiers in microbiology*, 13, 830866. <https://doi.org/10.3389/fmicb.2022.830866>.
- Moreno, A.B., López-Moya, J.J. 2020. When viruses play team sports: mixed infections in plants. *Phytopathology*, 110, 29-48. <https://doi.org/10.1094/PHYTO-07-19-0250-FI>.
- Muhire, B.M., Varsani, A., Martin, D.P. 2014. SDT: A Virus Classification Tool Based on Pairwise Sequence Alignment and Identity Calculation. *PLoS ONE*, 9, e108277. <https://doi.org/10.1371/journal.pone.0108277>.
- Murrell, B., Wertheim, J. O., Moola, S., Weighill, T., Scheffler, K., Kosakovsky Pond, S. L. 2012. Detecting individual sites subject to episodic diversifying selection. *PLoS genetics*, 8, e1002764. <https://doi.org/10.1371/journal.pgen.1002764>.
- Murrell, B., Moola, S., Mabona, A., Weighill, T., Sheward, D., Kosakovsky Pond, S. L., Scheffler, K. 2013. FUBAR: a fast, unconstrained bayesian approximation for inferring selection. *Molecular biology and evolution*, 30, 1196-1205. <https://doi.org/10.1093/molbev/mst030>.
- Naidu, R.A., Maree, H.J., Burger, J.T. 2015. Grapevine leafroll disease and associated viruses: a unique pathosystem. *Annu Rev Phytopathol.* 53, 613-634. <https://doi.org/10.1146/annurev-phyto-102313-045946>.
- Naseem, M.T., Ashfaq, M., Khan, A.M., Kiss, Z., Akhtar, K.P., Mansoor, S. 2016. Transmission of viruses associated with carrot motley dwarf by *Myzus persicae*. *Journal of Plant Pathology*, 581-585. <https://www.jstor.org/stable/44280504>.
- Parameswari, B., Bajar, B., Karthikaiselvi, L., Sivaraj, N., Mangrauthia, S. K., Nagalakshmi, S., Holajjer, P., Srinivas, M., Celia Chalam, V., Anitha, K. 2023. First report of the association of *Zygocactus virus X* with dragon fruit (*Hylocereus* spp.) plants from Telangana, India. *Plant Disease.* 107, 1249. <https://doi.org/10.1094/PDIS-05-22-1242-PDN>.
- Parameswari, B., Bajar, B., Sivaraj, N., Mangrauthia, S. K., Nagalakshmi, S., Baranwal, V. K., Prasanna Holajjer, Srinivas, M., Anitha, K. 2022. First record of *Cactus virus X* in Dragon Fruit (*Hylocereus* spp.) in India. *Indian Phytopathology*, 75, 297–299. <https://doi.org/10.1007/s42360-021-00421-4>.
- Park, C.H., Song, E.G., Ryu, K.H. 2021. A multiplex PCR assay for the simultaneous detection of five potexviruses infecting cactus plants using dual-priming oligonucleotides (DPOs) primers. *Journal of Virological Methods*, 298, 114280. <https://doi.org/10.1016/j.jviromet.2021.114280>.
- Park, C.H., Song, E.G., Ryu, K.H. 2018. Detection of co-infection of *Notocactus leninghausii* f. *cristatus* with six virus species in South Korea. *Plant Pathol. J.* 34, 65. <https://doi.org/10.5423/PPJ.NT.08.2017.0187>.

- Rey, C., Vanderschuren, H. 2017. Cassava mosaic and brown streak diseases: current perspectives and beyond. *Annual Review of Virology*, 4, 429-452. <https://doi.org/10.1146/annurev-virology-101416-041913>.
- Singhal, P., Nabi, S.U., Yadav, M.K., Dubey, A. 2021. Mixed infection of plant viruses: diagnostics, interactions and impact on host. *Journal of Plant Diseases and Protection*, 128, 353-368. <https://doi.org/10.1007/s41348-020-00384-0>.
- Tamura, K., Stecher, G., Kumar, S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, 38, 3022-3027. <https://doi.org/10.1093/molbev/msab120>.
- Tatineni, S., Alexander, J., Qu, F. 2022. Differential Synergistic Interactions Among Four Different Wheat-Infecting Viruses. *Front. Microbiol.* 12, 800318. <https://doi.org/10.3389/fmicb.2021.800318>.
- Untiveros, M., Fuentes, S., Salazar, L.F. 2007. Synergistic interaction of Sweet potato chlorotic stunt virus (Crinivirus) with carla-, cucumo-, ipomo-, and potyvirus infecting sweet potato. *Plant Disease*, 91, 669-676. <https://doi.org/10.1094/PDIS-91-6-0669>.
- Valencia-Botín, A.J., Kokunu, H., Ortíz-Hernández, Y.D. 2013. A brief overview on pitahaya (*Hylocereus* spp.) diseases. *Australasian Plant Pathol.* 42, 437-440. <https://doi.org/10.1007/s13313-012-0193-8>.
- Xiao, H., Shabanian, M., Moore, C., Li, C., Meng, B. 2018. Survey for major viruses in commercial *Vitis vinifera* wine grapes in Ontario. *Virology journal*, 15, 1-11. <https://doi.org/10.1186/s12985-018-1036-1>.
- Xu, C., Sun, X., Taylor, A., Jiao, C., Xu, Y., Cai, X., Wang, X., Ge, C., Pan, G., Wang, Q., Fei, Z., Wang, Q. 2017. Diversity, distribution, and evolution of tomato viruses in China uncovered by small RNA sequencing. *J Virol.* 91, e00173-17. <https://doi.org/10.1128/JVI.00173-17>.
- Xu, Y., Ghanim, M., Liu, Y. 2022. Editorial: Mixed Infections of Plant Viruses in Nature and the Impact on Agriculture. *Front. Microbiol.* 13, 922607. <https://doi.org/10.3389/fmicb.2022.922607>.
- Zhang, Y., Liu, Z., Huang, Q., Luo, Y., Pennerman, K.K., Lai, D., Bai, H., Lin, Y., Yin, G. 2016. Rapid detection of cactus virus X in pitaya by efficient reverse transcription loop-mediated isothermal amplification. *Austin J Biotechnol Bioeng.* 3, 1055.

ARTIGO 2 - Incidence and spatial pattern of viruses in commercial dragon fruit orchards in southern Minas Gerais, Brazil

Manuscript prepared for submission to European Journal of plant pathology

Antonia Thalyta Lopes Silveira, Antonia dos Reis Figueira*, Gabriela Ribeiro Gontijo, Viviane de Fatima Silva Pessoa, Leila Aparecida Salles Pio, Edson Ampélio Pozza

A. T. L. Silveira, Department of Phytopathology, Federal University of Lavras (UFLA), Lavras, MG 37203-202, Brazil e-mail: thalytasilveira@gmail.br <https://orcid.org/0000-0001-9044-7284>

*A. R. Figueira, Department of Phytopathology, Federal University of Lavras (UFLA), Lavras, MG 37203-202, Brazil e-mail: antonia@ufla.com <https://orcid.org/0000-0002-2589-1394>

G. R. Gontijo, Department of Agriculture, Federal University of Lavras (UFLA), Lavras, MG 37203-202, Brazil e-mail: gabriela.gontijo2@estudante.ufla.br <https://orcid.org/0009-0002-7413-2025>

V. F. S. Pessoa, Department of Phytopathology, Federal University of Lavras (UFLA), Lavras, MG 37203-202, Brazil e-mail: viviane.pessoa@ufla.br <https://orcid.org/0000-0003-0956-1923>

L. A. S. Pio, Department of Agriculture, Federal University of Lavras (UFLA), Lavras, MG 37203-202, Brazil e-mail: leila.pio@ufla.br <https://orcid.org/0000-0002-0450-0227>

E. A. Pozza, Department of Phytopathology, Federal University of Lavras (UFLA), Lavras, MG 37203-202, Brazil e-mail: edsonpozza@gmail.com <https://orcid.org/0000-0003-2813-584X>

ABSTRACT

Dragon fruit (*Selenicereus* spp. syn. *Hylocereus* spp.) cultivation has grown significantly in recent years both in Brazil and throughout the world. This growth has demanded basic information on diseases caused by pathogens that can affect the crop. Among them, diseases of viral etiology stand out for the lack of curative control methods and for being underreported because the symptoms induced by viruses can be confounded with nutritional disorders. Previously, four viral species of the genus *Potexvirus* were detected in samples from different regions of Brazil. The objective of this study was to determine the incidence and spatial distribution of potexvirus in 12 commercial dragon fruit orchards in southern Minas Gerais. The number of plants evaluated for incidence determination was calculated based on the size of each orchard, and the spatial distribution was investigated throughout the area. High incidences of potexvirus were detected, ranging from 80% to 100%. The spatial distribution of diseased plants showed an aggregated pattern in planting rows and a regular pattern in plots, indicating dissemination through cultural operations and infected seedlings as a source of inoculum. This was the first study on the epidemiology of viruses in dragon fruit orchards, and the data obtained indicate that producers are unaware of control measures required to live with the disease. In addition, the results showed the need for control seedling commercialization, aiming at the propagation of healthy material, along with the use of appropriate management practices to decrease the spread of viruses within orchards.

Keywords: dragon fruit, epidemiology, Potexvirus, CVX, PiVX, ZyVX, SchVX.

1. INTRODUCTION

Fruit growing is one of the main agricultural activities in Brazil, which is the third largest fruit producer in the world, behind only China and India (ABRAFRUTAS, 2022). Due to its continental dimensions and varied soil and climate conditions, the country has the potential to produce a wide variety of tropical, subtropical, temperate and semiarid fruits.

The fruit market has experienced a recent global expansion, especially of exotic fruits, such as pitaya or Dragon fruit, reflecting the changing habits of the world population (SANTOS et al., 2022). Pitaya belongs to the family Cactaceae, genus *Selenicereus* syn. *Hylocereus* (KOROTKOVA et al., 2017), and the four main commercial species are *Selenicereus undatus*, which produces large fruits with red skin and white pulp; *Selenicereus costaricensis*, which produces medium-sized fruits with red skin and red pulp; *Selenicereus megalanthus*, which produces medium-sized fruits with yellow skin with thorns and white pulp; and *Selenicereus setaceus*, which produces small fruits with red skin, thorns and/or white pulp. The species *S. undatus* and *S. megalanthus* are the most cultivated species in the world (MERCADO-SILVA, 2018).

Despite the great diversity of native cultivars found in Brazil, most dragon fruit cultivars planted today come from Taiwan and Israel (FALEIRO et al., 2021; SANTOS et al., 2022). The wide temperature range in which dragon fruit can be grown (from 4 to 38 °C) allows its cultivation in the regions with different soil and climate conditions. In addition, dragon fruit is highly hardy because it is a CAM plant, i.e., it utilizes crassulacean acid metabolism, a special photosynthesis pathway observed in plants that grow in environments with hot and dry climates. As such, the dragon fruit plant closes its stomata during the day to decrease water loss through transpiration, making it highly efficient in water use.

These characteristics make the dragon fruit a perfect candidate to be cultivated in semiarid regions, covering nine Brazilian states and the northeast and north of the state of Minas Gerais. In 2022, approximately 3 million kg of the fruit were produced in Brazil, generating a revenue of R\$ 44 million (CONAB, 2023). This strong market has created a great demand for knowledge about the crop because in addition to knowledge on the phenological cycle and nutritional requirements of dragon fruit, understanding the diseases and pests that affect production is also indispensable. Therefore, investigate their etiology and epidemiology is crucial for sustainable management, and the tools developed in the field of epidemiology are essential to understand how diseases in general occur in a plant population.

Among the diseases that affect cultivated plants, viruses are economically important and, in general, increase proportionally with agricultural intensification, being responsible for

approximately 47% of emerging and reemerging diseases (JANSSEN & RUIZ, 2021; JONES, 2021). The unavailability of curative control measures and the ease of dissemination of viruses in the field, whether through vectors or cultural operations, mean that these diseases have great potential to cause considerable damage to cultivated plants.

Technological innovations have allowed considerable advances, especially in the understanding of the epidemiology of viral diseases (JONES, 2021). At the macro scale, examples include the implementation of information systems, modeling capacity, decision support and forecasting of disease incidence, analysis of spatiotemporal propagation patterns and general understanding of the factors responsible for the progress rates of epidemics. In contrast, at the micro level, molecular epidemiological approaches and evolutionary studies, together with the most recent procedures for disease detection, are additional innovations capable of significantly contributing to the understanding of disease dynamics (JONES, 2013).

Recently, three viral species belonging to the genus *Potexvirus* were detected and identified in commercial dragon fruit orchards in municipalities in the southern of the state of Minas Gerais: *Cactus virus X* (CVX), *Pitaya virus X* (PiVX) and *Zygocactus virus X* (ZyVX). The infected plants presented symptoms such as chlorotic lesions, progressing to mosaic and/or mottled and curled cladodes, which reduced vigor and fruit yield. However, there is a complete lack of knowledge about the different factors related to the etiology and epidemiology of the virus in the field. The objective of this study was to diagnose the infected plants present in these orchards and to determine the incidence and spatial distribution of disease to provide information for the sustainable management of viruses in the dragon fruit crop.

2. MATERIALS AND METHODS

Sample collection

The sample collections were performed in 12 dragon fruit orchards in five municipalities in the southern of the state of Minas Gerais (Table 1) from April to September 2022.

Table 1 Description, geographic coordinates and the cultivation system of the orchards evaluated

City	Orchard	Latitude	Longitude	N° of plants	Planting density	Type of cultivation
Boa Esperança	A	21° 0' 59" S	45° 34' 14" W	722	2x2	Organic
	B	21° 0' 57" S	45° 34' 11" W	765		
Ingai	C	21° 21' 30" S	44° 58' 3" W	1012	2x0.6	Conventional
	D	21° 21' 28" S	44° 58' 2" W	72	2x2	
	E	21° 21' 27" S	44° 58' 2" W	117	2x0.6	
Ribeirão Vermelho	F	21° 11' 46" S	45° 3' 35" W	617	2x0.6	Conventional
	G	21° 11' 47" S	45° 3' 34" W	211		
	H	21° 11' 48" S	45° 3' 35" W	1098		
Lavras ^a	I	21° 11' 50" S	45° 3' 36" W	351	2x2	Experimental
	J	21° 13' 45" S	44° 58' 53" W	198		
Perdões	K	21° 5' 7" S	45° 2' 8" W	223	2x2	Conventional
	L	21° 4' 24" S	45° 2' 19" W	305		

^aFederal University of Lavras, UFLA

Disease incidence

A piece of cladode approximately 20 cm long was used as the sampling unit and collected randomly in each the orchard. These cladode samples were sent for diagnosis of potexvirus by RT–PCR. The minimum number of samples collected for determination of viral incidence was calculated based on the total number of plants in each orchard (Table 1). For this, the equation proposed by Cooke (1998) was used $n = (Z^2 pqN) / d^2 (N-1) + Z^2 pq$, where n = number of samples to be collected; Z = tabulated value referring to the abscissa of the standard normal curve, with a 90% confidence level, that is, 1.65; p = 0.6, an estimate of the probability of finding virus in the sample, according to tests previously performed at the Molecular Virology Laboratory of the Federal University of Lavras; q = 1-p; N = total number of plants in the orchard and d= sampling error, that is, error attributed to the probability of finding or not virus in the collected sample.

The disease incidence (DI) was determined using the technique of Campbell and Madden (1990), i.e., by multiplying the ratio between the number of infected plants (PI) and the total number of plants sampled (PA). The distribution of normality was calculated using the Shapiro–Wilk test ($P < 0.05$), and the significance of differences was tested using the Kruskal–Wallis test for nonparametric data, which allows for the comparison of three or more groups in independent samples and is used in cases where the ANOVA prerequisites were not met. The p value was adjusted using Duncan's test, using the *rstat*, *dplyr* and *ggplot2* packages of the R statistical program (R CORE TEAM, 2022).

RT–PCR

The total RNA of the cladodes was extracted by the lithium chloride method (CHANG et al., 1993). These cladode samples were sent for diagnosis of potexvirus and CVX, PiVX, ZyVX and SchVX species by RT–PCR. The reverse transcriptase (RT) reaction was performed using M-MLV Reverse Transcriptase (Sigma–Aldrich Co., Saint Louis, MO, USA) following the manufacturer's instructions. The reaction was prepared to a final volume of 20 μ l, initially containing 1 μ l of 10 mM random primers, 1 μ l of 10 mM dNTPs, 6 μ l of ultrapure water and 2 μ l of total RNA (500 ng/ μ l). After heating at 70°C for 10 minutes, the tubes were immediately transferred to a container with ice, and then 2 μ l of enzyme buffer (10X), 1 μ l of enzyme and 7 μ l of ultrapure water were added, and the reaction was incubated again at 37°C for 50 minutes and at 85°C for another 10 minutes.

Recombinant Taq DNA Polymerase (Thermo Fisher Scientific Inc., Carlsbad, California, USA), degenerate primers for the genus *Potexvirus* and species-specific primers were used for PCR amplification: Potex 1RC 5' TCAGTRTTDGCRTCRAARGT 3' and Potex 2RC 5' AGCATRGCNSCRTCYTG 3' (VAN DER VLUGT & BERENDSEN, 2002), CVX 678 UP 5' ATGTCTACTACTGGAGTCCA 3' and CVX 678 DN 5' CTACTCAGGGCCTGGGAGAA 3', PiVX 678 UP 5' ATGGCTACTCAAACAGCACAA 3' and PiVX 678 DN 5' CTACTCTGGGGAGGGAAG 3', ZyVX 681 UP 5' ATGTCTAACACTGCAGGAGT 3' and ZyVX 681 DN 5' TCATTCGGGACCCGTAGGA 3', and SchVX 678 UP 5' ATGTCGACCACTCCATCTTC 3' and SchVX 678 DN 5' TTATTCAGGGGATGGTAGTA 3' (PARK et al., 2018). The amplification conditions were as follows: initial heating at 94°C for 3 minutes followed by 35 cycles of 94°C for 45 seconds, annealing temperature of primer pair for 30 seconds, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR product was analyzed on a 0.7% agarose gel counterstained with Red Nucleic Acid Gel Stain (Biotium®).

Spatial pattern of disease

The spatial pattern of the disease was evaluated in the planting rows using the doublet test and the run test (MADDEN et al., 2007; CAMPBELL & MADDEN, 1990; MADDEN et al., 1982). For studies in plots, the dispersion index was considered (UPTON & FINGLETON, 1985). For analysis, visual inspections were performed in each plant present in these orchards, recording the presence and/or absence of viral symptoms in the plants. The symptoms of mosaic and chlorotic rings were considered references to detect the presence and absence of viruses in the field (Figure 1). Data analyses were performed using Microsoft Office® Excel.

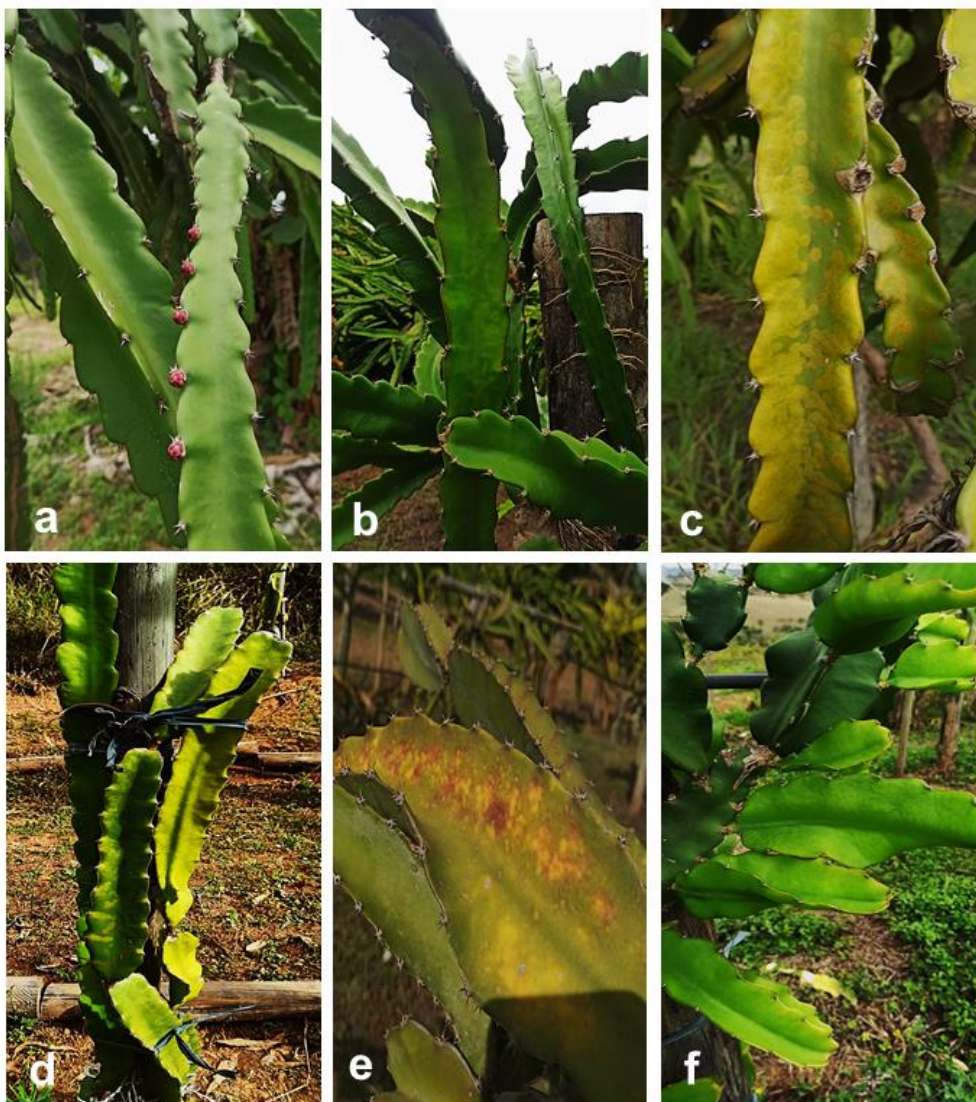


Fig. 1 Potexvirus symptoms in dragon fruit, used as a reference in data collection to determine the presence or absence of the virus in the plant. A: healthy; B: mosaic; C, D & E: chlorotic rings; F: cladode coiling

For the doublet test, the numbers of healthy (-) and diseased (+) plants in the planting rows were used. The expected number of doublets ($E_{(D)}$) (MADDEN et al., 1982) was calculated considering $E_{(D)} = m(m-1)/N$, where m = number of diseased plants and N = number of plants in the row. The calculation of the doublet variance was performed using the equation $\text{variance}_{(D)}^2 = [m(m-1)[N(N-1)+(2N(m-2)+N(m-2)(m-3)-(N-1)m(m-1)]/N^2(N-1)]$. The standardized value ($Z_{(D)}$), based on the normal distribution, was obtained using the equation: $Z_{(D)} = [(D+0.5-E_{(D)})/\sigma_{(D)}]$, where D = number of doublets (two adjacent diseased plants) and $\sigma_{(D)}$ = standard deviation. The distribution is considered to follow an aggregate pattern when $Z_{(D)} > 1.64$ ($P = 0.05$), and when $Z_{(D)} < -1.64$ ($P = 0.05$), it follows a random pattern. For orchards in which the presence of symptoms was observed in all plants, the variance formula was adjusted to $\text{variance}_{(D)}^2 = [m(m-1)[N(N-1)+(2N(m-2)+N(m-2)(m-3)-(N-1)m(m-1)]/N^2(N-1)]$.

The expected number of runs ($E_{(R)}$) (MADDEN et al., 1982) was obtained by the run test $E_{(R)} = 1+2m(Nm)/N$ and its variance: $\text{variance}_{(R)}^2 = 2m(Nm)[2m(Nm)-N]/[N^2(N-1)]$. The standardized value ($Z_{(R)}$) = $[R+0.5-E_{(R)})/s_{(R)}$ was calculated based on the normal distribution, where R : number of runs; m : number of diseased plants; N : number of plants in the row; $s_{(R)}$: standard deviation. $Z_{(R)} > -1.64$ ($P = 0.05$) indicates a random pattern, and $Z_{(R)} < -1.64$ ($P = 0.05$) indicates an aggregate pattern. For the orchards in which the presence of symptoms was observed in all plants, the variance formula was adjusted to $\text{variance}_{(R)}^2 = 1+2m(Nm)[2m(Nm)-N]/[N^2(N-1)]$.

The dispersion index (D) was obtained from the values of the mean (X), according to the equation $(X_1+X_2...)/\Sigma t$, where: $X_1+X_2...$ = the sum of the number of symptomatic plants in the entire area, Σt : total plants in the orchard. The variance (s^2), is calculated by $(s^2) = \Sigma[(X_1-\mu)^2/n-1]$, where: $X_1...$ = the number of infected plants, μ = arithmetic mean mentioned above, n = total number of plants. Based on the calculated values, the dispersion index (D) is the ratio between s^2/X . When $D < 1$, it indicates a regular spatial pattern, when $D = 1$, a random pattern, and when $D > 1$, an aggregate pattern (CAMPBELL & MADDEN, 1990; UPTON & FINGLETON, 1985).

3. RESULTS

Incidence of potexvirus

All samples that showed symptoms were positive, but plants without symptoms were not always negative for the presence of potexvirus, indicating a probable recent infection. The average incidences observed in each orchard are georeferenced in Figure 2 and detailed in Table

2. All orchards sampled had high incidences of potexvirus, with the majority between 80% and 100%.

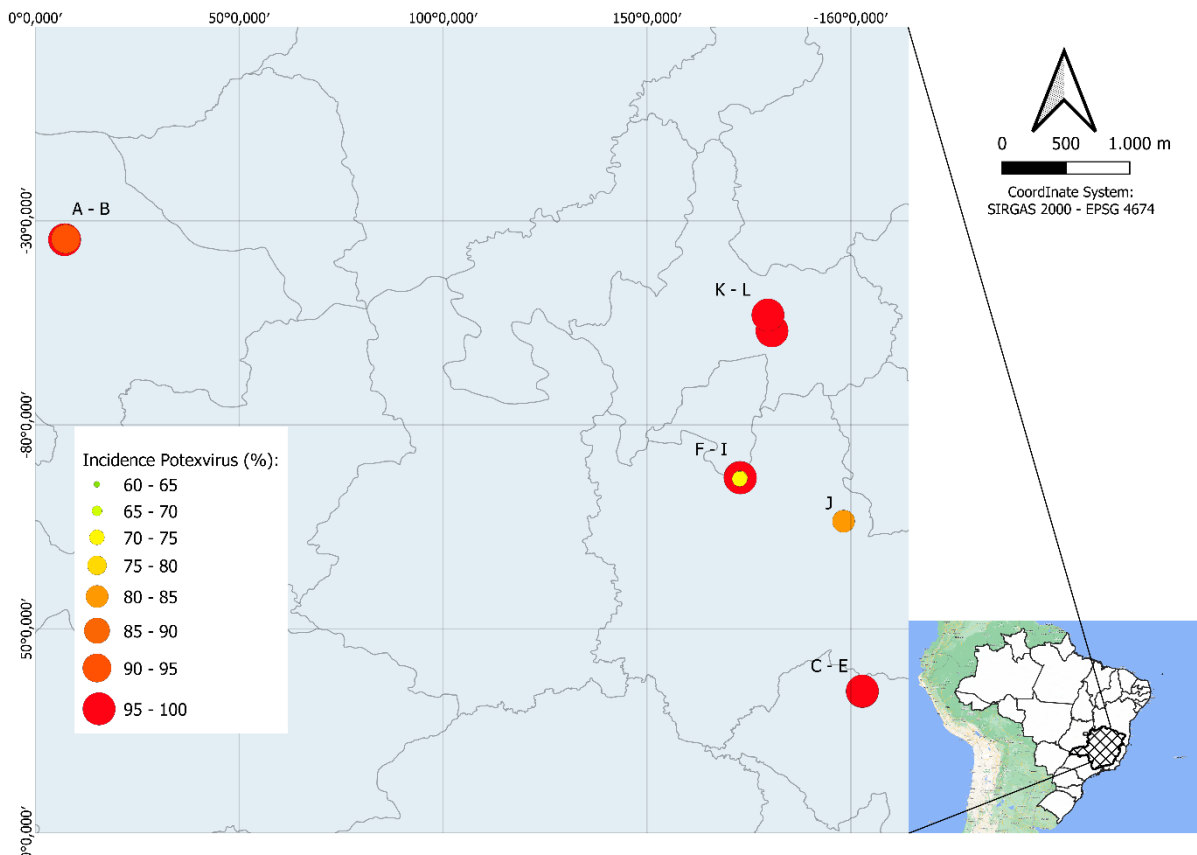


Fig. 2 Viruses incidence map in dragon fruit orchards in the southern region of the State of Minas Gerais, Brazil

Table 2 Incidence of viruses in dragon fruit orchards in the southern region of Minas Gerais

City	Orchard	RT-PCR result		Incidence (%)				
		Positive	Negative	<i>Potexvirus</i>	CVX	PiVX	ZyVX	SchVX
Boa Esperança	A	11		100	63.6	9	81.8	
	B	10	1	90.9	63.6	18.2	63.6	
	C	19	3	86.4	86.4	54.5	50	
Ingaí	D	4		100	100	50		
	E	4		100	100	50	25	
	F	11		100	100	45.5	90.9	
Ribeirão	G	6		100	33.3	16.6	83.3	
Vermelho	H	21	2	73.9	43.5	4.3	56.5	
	I	6	2	75	37.5	12.5	87.5	
Lavras	J	5	1	83.3	16.6	83.3	83.3	
Perdões	K	6		100	100		100	
	L	8		100	100	75	100	

The lowest incidence was observed in crop I (75%), and the highest incidence, with all plants infected, was observed in Boa Esperança, in orchard A; in Ingaí, in orchards D and E; in both Perdões orchards (K and L); and in orchards F and G of Ribeirão Vermelho. The test for nonparametric data included the assumptions that the incidence data by collection site were independent (Kruskal–Wallis test $P = 0.443$) and that the differences were not significant when the collection sites were compared (Table 3). Among the species analyzed, only SchVX was not detected. The CVX species was detected in all municipalities, with high incidences in Ingaí and Perdões. The ZyVX species had the highest incidence in Ingaí, with 100%; Ribeirão Vermelho in orchards F, G and I; Lavras; and Boa Esperança in the orchard in orchard A. For PiVX, the species was detected in most municipalities but only in Lavras it showed a high incidence.

Table 3 Probability of comparisons between orchards analyzed by municipalities using molecular detection by RT-PCR for potexvirus

City		Valor-p ^a
Boa Esperança	Ingaí	0.397 ^{ns}
Boa Esperança	Ribeirão Vermelho	0.452 ^{ns}
Boa Esperança	Perdões	0.562 ^{ns}
Boa Esperança	Lavras	0.237 ^{ns}
Ingaí	Ribeirão Vermelho	0.874 ^{ns}
Ingaí	Perdões	0.139 ^{ns}
Ingaí	Lavras	0.558 ^{ns}
Ribeirão Vermelho	Perdões	0.155 ^{ns}
Ribeirão Vermelho	Lavras	0.476 ^{ns}
Perdões	Lavras	0.097 ^{ns}

^a $P < 0.05$ ^{ns} não significativo

Spatial pattern of disease

The maps of the spatial disposition of the disease in the orchards are shown in Figure 3, where the red squares represent infected plants and the green squares represent healthy plants. They reflect the results that were obtained when random sampling was performed for viral diagnosis in plants. In general, visual observation indicated a slightly lower incidence than that observed in incidence tests based on diagnosis by RT–PCR. While the diagnostic tests indicated an incidence ranging from 61.5% to 100% in random collections, visual inspection showed a frequency of infected plants of 69.7% to 98.5%. Considering that in the diagnostic tests some plants that did not show symptoms were positive for potexvirus, it is likely that some of the plants evaluated as negative in the visual inspection were infected.

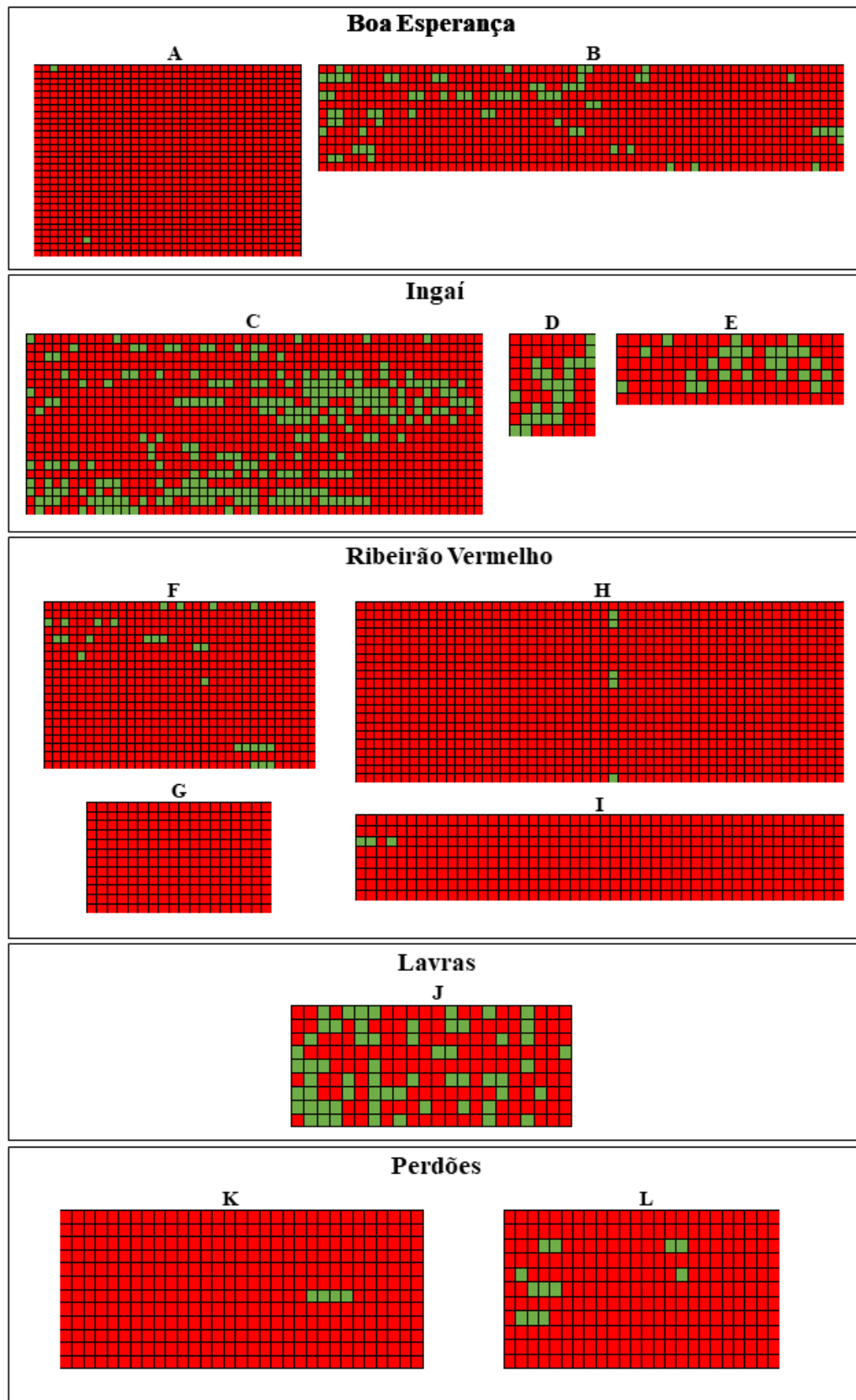


Fig. 3 Maps of the spatial position of symptomatic and asymptomatic potexvirus plants in dragon fruit orchards in the southern of the state of Minas Gerais. Red: plants with virus symptoms, Green: plants without virus symptoms.

The spatial distribution of viruses between rows, analyzed by the run and doublet tests, showed an aggregate pattern for most of the orchards analyzed (Table 4). The occurrence of the aggregate pattern was observed in all municipalities, in 9 orchards by the run test (75%) and in 8 orchards by the doublet test (66.6%), indicating the occurrence of inoculum dispersion via seedlings and cultural operations. This finding was confirmed in the spatial distribution in plots by the dispersion index, which showed a regular pattern in 11 orchards (91.6%). The exception was one orchard in the municipality of Ingaí (C), which showed the aggregate pattern.

Table 4 Result of the analyzes of run, doublet and dispersion index spatial patterns with $p < 0.05$

City	Orchard	Run				Doublet				D	
		E _(R)	σ^2 (R)	Z _(R)	P	E _(D)	σ^2 (D)	Z _(D)	P	VM	P
Boa	A	4.99	0.01	4.55	RD	956.01	0.01	6.25	A	0.002	R
Esperança	B	126.79	20.15	-10.54	A	647.19	5.14	11.16	A	0.26	R
	C	365.69	125.24	-11.72	A	642.88	31.49	11.72	A	1.49	A
Ingaí	D	32.31	13.36	-1.31	A	32.67	3.53	1.51	RD	0.23	R
	E	41.73	13.60	-0.61	A	72.85	3.58	0.87	RD	0.48	R
	F	50.95	3.71	-9.06	A	608.06	0.98	9.53	A	0.12	R
Ribeirão	G	1.00	1.00	0.50	A	215.00	21.50	0.11	RD	0.00	R
Vermelho	H	10.96	0.08	1.94	RD	1123.03	0.03	2.92	A	0.00	R
	I	6.95	0.08	16.39	RD	377.03	0.03	8.37	A	0.02	R
Lavras	J	84.64	35.08	-0.19	A	95.48	8.96	-0.33	RD	0.13	R
Perdões	K	8.91	0.15	1.53	A	363.05	0.05	1.95	A	0.01	R
	L	23.91	1.91	-7.53	A	293.59	0.54	8.05	A	0.11	R

E_(R): expected value of R, E_(D): expected value for D, σ^2 (R): variance of R, σ^2 (D): variance of D, Z_(R): standardized value for runs, Z_(D): standardized value for doublets, D: dispersion index, VM: relationship between variance and mean, P: standard, A: aggregate, RD: random, R: regular

4. DISCUSSION

This study demonstrated the vulnerability of dragon fruit to viral species belonging to the genus *Potexvirus*, which is corroborated by the high incidence rates recorded in all sampled fields. In addition to this crop being propagated vegetatively, it is subjected to several pruning and fruit harvesting operations with cutting tools. Therefore, the fact that these viruses do not have a vector in nature does not prevent their spread from easily occurring. Potexvirus are known to be transmitted by contaminated equipment, pollen, seeds and leaves (VERCHOT, 2021; BERCKES, 1949). As dragon fruit is not propagated by seeds in Brazil, transmission must

have occurred via cultural operations, without ruling out the possibility of transmission via pollen, which has not yet been studied in this crop (EVALLO et al., 2021).

The introduction of inoculum into the inspected orchards probably occurred via infected seedlings, since no other crop was detected near the visited fields. Once present in the crop, manual operations were responsible for its rapid dissemination to the other plants in the orchard. Several studies have warned of viruses being introduced into dragon fruit crops by cuttings (TAITANO et al., 2021; MERCADO-SILVA, 2018; MIZHARI, 2014, 2015). According to Alonso Barrera et al. (2015) and Masanto et al. (2018), the absence of tool disinfection protocols for during pruning and harvesting is the main route of disease transmission after the arrival of the inoculum in the orchard.

Studies that investigated the occurrence of potexvirus in dragon fruit in other countries indicated that after the viral particle enters the tissue, the disease develops, leading to systemic invasion of the plant. Thereafter, dissemination occurs rapidly, culminating in high incidences. Liao et al. (2003) reported incidences between 50% and 90% in Taiwan. Similar results were reported in Malaysia, where Masanto et al. (2018) reported incidences of 40% to 100% and Gazis et al. (2018) found incidences between 55% and 90% in the United States. According to Masanto et al. (2018), when correlating incidence with severity, it was shown that the higher the incidence of infected plants, the greater the severity of the disease in the orchard.

Our findings demonstrate the need for greater care in the production and acquisition of seedlings with high phytosanitary quality, which would have a greater chance of being achieved with the multiplication of healthy matrices *in vitro* (LEE & CHANG, 2022; EVALLO et al., 2021; CORREIA et al., 2017). In addition to these practices, tissue health must be confirmed by more sensitive diagnostic techniques, such as RT-PCR with degenerate primers for potexvirus (VAN DER VLUGT et al., 2002) or species-specific primers for potexvirus and other viruses that have been reported in cacti (PARK et al., 2018; ZHANG et al., 2016; BAKER & ADKINS, 2015). Simultaneous detection by multiplex PCR and RT-qPCR are also techniques that have the necessary sensitivity for the detection of the genera and/or species of viruses present (HOFFMEISTEROVÁ et al., 2022; PARK et al., 2021).

In Brazil, dragon fruit cultivation was introduced in the late 1990s (NUNES et al., 2014), so it is a relatively recent phenomenon that still needs to be studied in the context of the edaphic and climatic conditions of the country. Seedling production is usually performed by noncertified nurseries that do not apply any type of diagnostic method to verify seedling health. In addition, there are no large commercial orchards, with most production undertaken by family

farmers in low-technology operations, who acquire seedlings from dubious sources, including online, given the lack of options.

The aggregated spatial pattern of the disease observed in the evaluated orchards pointed to a clear viral spread between neighboring plants that certainly occurred through cultural operations. Part of the fields presented a regular spatial distribution pattern, also indicating the possible presence of the inoculum in the seedlings used for crop establishment. In field C of Ingaí, which presented a random distribution pattern, most healthy plants, according to the producers, were replaced by plants that showed high disease severity or that had a drop in vigor, which certainly contributed to the change in the spatial pattern.

Interestingly, in Boa Esperança, field A was established before field B and therefore the plants had longer exposure in the field, which indicates disease spread throughout the crop cycle, allowing an incidence of disease in plants of practically 100%. The same pattern was observed in the Perdões fields, where the K field was established before the L field.

Alonso Barrera et al. (2015) investigated the spatial pattern of viral spread in dragon fruit and observed an aggregate spatial pattern, with an evident direction along the row, suggesting spread through pruning and harvesting operations. Similar results were found by studies on other vegetatively propagated crops: Rice et al. (2019) determined the spatial pattern of spread of the sugarcane mosaic and concluded that spread must have occurred from infected propagative material; Arnold et al. (2017) and Cieniewicz et al. (2017) observed a high degree of spatial aggregation in grapevine, with spread dependent on the existence of inoculum. For infections caused by viruses, the aggregate pattern has been the most common due to the vector mode of transmission or because of spread via mechanical action through wounds caused by tools (JANSSEN & RUIZ, 2021; JEGER, 2020).

For an improvement in dragon fruit production in Brazil, official control agencies must regulate the production and commercialization of seedlings and provide training for farmers to enable them to content with the occurrence of viral diseases through roguing and special care in pruning and fruit harvesting operations.

5. CONCLUSION

- All orchards established in municipalities in southern Minas Gerais have a high incidence of potexvirus.
- The spatial distribution of diseased plants shows an aggregated pattern in most orchards, indicating spread via cultural operations and infected seedlings as an inoculum source.

Acknowledgements

The authors would like to thank the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), Foundation for Research Support of the State of Minas Gerais (FAPEMIG) for their support in this project.

Authors' contributions

All authors contributed to the study conception, design, data acquisition, data analysis and interpretation, as well as the drafting of the work. All authors read and approved the final manuscript.

Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflicts of interest/competing interests

The authors declare no competing interests.

Ethics approval/declarations

This article does not contain any studies with human participants or animals.

Consent to participate Not applicable.

Consent for publication Not applicable.

REFERENCES

- ABBAFRUTAS (2022). Associação Brasileira dos Produtores Exportadores de Frutas e Derivados. Estatística de exportações de frutas. Available at: <https://abrafrutas.org/dados-estatisticos/>. Accessed 20 Apr 2023.
- Alonso Barrera, B., Mora Aguilera, G., Valdovinos Ponce, G., Ochoa Martínez, D. L., Rodríguez Leyva, E., Tlapal Bolaños, B., & Torre-Almaraz, R. D. L. (2015). Asociación de un Potexvirus como Agente Causal de Manchas Cloróticas en *Opuntia ficus-indica*. *Mexican Society of Phytopathology*, 33(1), 75-86. Available at: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S018533092015000100075&lng=es&nrm=iso. Accessed 01 Mar 2023.
- Arnold, K., Golino, D. A., & McRoberts, N. (2017). A synoptic analysis of the temporal and spatial aspects of grapevine leafroll disease in a historic Napa vineyard and experimental vine blocks. *Phytopathology*, 107(4), 418-426. <https://doi.org/10.1094/PHYTO-06-16-0235-R>
- Baker, C. A., & Adkins, S. (2015). First report of Tomato chlorotic spot virus in *Hoya wayetii* and *Schlumbergera truncata*. *Plant Health Progress*, 16(1), 29-30. <https://doi.org/10.1094/PHP-BR-14-0043>
- Bercks, R. (1949). Potato virus X. *Nature*, 163, 502– 503.
- Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. John Wiley & Sons.
- Cieniewicz, E. J., Pethybridge, S. J., Gorny, A., Madden, L. V., McLane, H., Perry, K. L., & Fuchs, M. (2017). Spatiotemporal spread of grapevine red blotch-associated virus in a California vineyard. *Virus Research*, 241(2017), 156-162. <https://doi.org/10.1016/j.virusres.2017.03.020>
- Chang, S., Puryear, J., & Cairney, J. (1993). A simple and efficient method for isolating RNA from pine trees. *Plant molecular biology reporter*, 11, 113-116.
- Cooke, B.M. (1998). Disease assessment and yield loss. In Jones, D.G. (Eds.), *The Epidemiology of Plant Diseases* (1th ed., pp. 42–72). Springer, Dordrecht. https://doi.org/10.1007/978-94-017-3302-1_3
- CONAB (2023). Companhia Nacional de Abastecimento. Available at: <http://dw.ceasa.gov.br/>. Accessed 16 Jul 2023.
- Correia, D., Nascimento, E., Morais, J., Gomes Filho, A. A. H., & Silva, M. (2017). Germinação de sementes e tipos de explante na propagação in vitro da *Pitaya vermelha* (*Hylocereus polyrhizus*). *Boletim de Pesquisa e Desenvolvimento* 147. Embrapa Agroindústria Tropical, 19 p.
- Evallo, E., Taguam, J. D., & Balendres, M. A. (2021). A brief review of plant diseases caused by Cactus virus X. *Crop Protection* 143, 105566. <https://doi.org/10.1016/j.cropro.2021.105566>

- Faleiro, F. G., & Junqueira, N. T. V. (2022). Espécies, variedades e cultivares. In D. N. Santos, L. A. S. Pio, F. G. Faleiro (Eds.) *Pitaya: uma alternativa frutífera* (1th ed., pp. 9–16). ProImpress.
- Gazis, R., Poudel, K., Dey, K., Zhang, S., Palmateer, A., Campoverte, E., Baker, C., & Adkins, A. (2018). First Report of Cactus Virus X in *Hylocereus Undatus* (Dragon Fruit) in Florida. *Plant Dis.* 102, 2666. <https://doi.org/10.1094/PDIS-05-18-0725-PDN>
- Hoffmeisterová, H., Kratochvílová, K., Čeřovská, N., Slavíková, L., Dušek, J., Muller, K., Fousek, J., Plchová, H., Navrátil, O., Kundu, J. K., & Moravec, T. (2022). One-Enzyme RTX-PCR for the Detection of RNA Viruses from Multiple Virus Genera and Crop Plants. *Viruses*, 14(2), 298. <https://doi.org/10.3390/v14020298>
- Janssen, D., & Ruiz, L. (2021). Special Issue: “Plant Virus Epidemiology”. *Plants*, 10, 1188. <https://doi.org/10.3390/plants10061188>
- Jeger, M. J. (2020). The epidemiology of plant virus disease: Towards a new synthesis. *Plants*, 9(12), 1768. <https://doi.org/10.3390/plants9121768>
- Jones, R. A. C. (2021). Global Plant Virus Disease Pandemics and Epidemics. *Plants*, 10, 233. <https://doi.org/10.3390/plants10020233>
- Jones, R. A. C. (2013) Trends in plant virus epidemiology: Opportunities from new or improved technologies. *Virus Res.* <http://dx.doi.org/10.1016/j.virusres.2013.11.003>
- Korotkova, N., Borsch, T., & Arias, S. (2017). A phylogenetic framework for the Hylocereeae (Cactaceae) and implications for the circumscription of the genera. *Phytotaxa*, 327, 1-46. <https://doi.org/10.11646/phytotaxa.327.1.1>
- Kruskal, W. H., Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American statistical Association*, 47(260), 583-621. Issue 260.
- Lee, Y.-C., & Chang, J.-C. (2022). Development of an Improved Micropropagation Protocol for Red-Fleshed Pitaya ‘Da Hong’ with and without Activated Charcoal and Plant Growth Regulator Combinations. *Horticulturae*, 8, 104. <https://doi.org/10.3390/horticulturae8020104>
- Liao, J. Y., Chang, C. A., Yan, C. R., Chen, Y. C., & Deng, T. C. (2003). Detection and incidence of Cactus virus X in pitaya in Taiwan. *Plant Pathology Bulletin*, 12(4), 225-234. Available at: <https://www.cabdirect.org/cabdirect/abstract/20043051394>. Accessed 20 Feb 2023.
- Madden, L. V., Hughes, G., & Van Den Bosch, F. (2007). The study of plant disease epidemics. The American Phytopathological Society.
- Madden, L. V., Louie, R. A. J. J., JJ, A., & JK, K. (1982). Evaluation of tests for randomness of infected plants.
- Masanto, M., Sijam, K., Awang, Y., & Satar, M. G. M. 2018. First report of necrotic spot disease caused by Cactus Virus X on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. *Jurnal Perlindungan Tanaman Indonesia*. 22, 1–12. <https://doi.org/10.22146/jpti.23763>

Mercado-Silva E. M. (2018). Pitaya - *Hylocereus undatus* (Haw). In Rodrigues S., de Oliveira Silva E., de Brito E.S. (Eds.), *Exotic Fruits Reference Guide*. (1st ed., pp 339–349). Academic Press.

Mizrahi, Y. (2015). Thirty-one years of research and development in the vine cacti pitaya in Israel. *Improving Pitaya Production and Marketing*, 1-18 Available at: <https://growables.com/information/TropicalFruit/documents/PitayaIsrael.pdf>. Accessed 19 Sep 2022.

Mizrahi, Y. (2014). Vine-cacti pitayas-the new crops of the world. *Rev. Bras. Frutic.* 36, 124-138. <https://doi.org/10.1590/0100-2945-452/13>

Nunes, E. N., de Sousa, A. S. B., de Lucena, C. M., Silva, S. D. M., de Lucena, R. F. P., Alves, C. A. B., & Alves, R.E. (2014). Pitaia (*Hylocereus* sp.): uma revisão para o Brasil. *Gaia Scientia*, 8, 90-98. Retrieved Aug 5, 2022, from <http://periodicos.ufpb.br/ojs2/index.php/gaia/index>

Park, C. H., Song, E. G., & Ryu, K. H. (2021). A multiplex PCR assay for the simultaneous detection of five potexviruses infecting cactus plants using dual-priming oligonucleotides (DPOs) primers. *Journal of Virological Methods*, 298, 114280. <https://doi.org/10.1016/j.jviromet.2021.114280>

Park, C. H., Song, E. G., & Ryu, K. H. (2018). Detection of co-infection of *Notocactus leninghausii* f. *cristatus* with six virus species in South Korea. *The plant pathology journal*, 34, 65. <https://doi.org/10.5423/PPJ.NT.08.2017.0187>

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Rice, J. L., Hoy, J. W., & Grisham, M. P. (2019). Sugarcane mosaic distribution, incidence, increase, and spatial pattern in Louisiana. *Plant disease*, 103(8), 2051-2056. <https://doi.org/10.1094/PDIS-01-19-0099-RE>

Santos, D. N., Müller, D. H., Procópio, W., & Silva, W. M. (2022). Sistema de produção da pitaya. In D. N. Santos, L. A. S. Pio, F. G. Faleiro (Eds.) *Pitaya: uma alternativa frutífera* (1th ed., pp. 9–16). ProImpress.

Taitano, C., Ilai, A. J., Johnny, S., Acosta, M., Tuquero, J. (2021). *Dragon Fruit*. Cooperative Extension Outreach. FS 5 – 21. Available at: https://www.uog.edu/_resources/files/extension/publications/Dragon_Fruit.pdf. Accessed 23 Apr 2022.

Upton, G., & Fingleton, B. (1985). *Spatial data analysis by example*. Volume 1: Point pattern and quantitative data. John Wiley & Sons Ltd.

Van der Vlugt, R. A. A., & Berendsen, M. (2002). Development of a general potexvirus cDNA and PCR primer set. *European Journal of Plant Pathology*, 108(4), 367-371. <https://doi.org/10.1023/A:1015644409484>

Verchot, J. (2022). Potato virus X: A global potato-infecting virus and type member of the Potexvirus genus. *Molecular Plant Pathology*, 23(3), 315-320.
<https://doi.org/10.1111/mpp.13163>

Zhang, Y., Liu, Z., Huang, Q., Luo, Y., Pennerman, K. K., Lai, D., Bai, H., Lin, Y., & Yin, G. (2016). Rapid Detection of Cactus virus X in Pitaya by Efficient Reverse Transcription Loop-Mediated Isothermal Amplification. *Austin J Biotechnol Bioeng*. 3(1), 1055. ISSN: 2378-3036

CONSIDERAÇÕES FINAIS

Considerando o crescimento da produção dessa fruta exótica na agricultura e o consumo na alimentação global, as doenças podem impactar diretamente o rendimento da cultura. A quantidade de fitopatógenos relatados afetando o cladódio como o fruto estão aumentando ano após ano. Dentre os fitopatógenos, as viroses causam prejuízos ao reduzir o crescimento da planta e a produção de frutos. Detectamos e identificamos a presença de espécies do gênero *Potexvirus*: CVX, PiVX, ZyVX e SchVX em amostras de diferentes regiões do Brasil com infecções simples ou mistas, com duas ou mais espécies. A planta infectada pode ou não apresentar sintomas, quando apresenta sintomas, são observados mosaico e mosqueado, anéis cloróticos e pontuações a manchas de coloração marrom-avermelhado no cladódio e no fruto, reduzindo o crescimento da planta e a produção de frutos. Ao analisarmos a incidência e o padrão espacial da doença, altas incidências foram detectadas (80 a 100%) na maioria dos pomares e um padrão espacial agregado, demonstrando a vulnerabilidade da cultura da pitaia às espécies de vírus pertencentes ao gênero *Potexvirus*. Com inóculo inicial partindo de mudas infectadas e disseminando no pomar pelo uso de ferramentas de poda. Outra via de disseminação da doença é a permuta de mudas como material genético entre os produtores, o que ressalta a necessidade de material genético com fitossanidade comprovada. Diante desses resultados, podemos iniciar uma compreensão de como este patossistema ocorre na natureza. Demais estudos são fundamentais para um maior entendimento em como o vírus afeta a está cactácea gerando perdas direta na produção.