Microbiological Research 226 (2019) 1–9

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

RNA interference and CRISPR: Promising approaches to better understand and control citrus pathogens

Eduardo Henrique Goulin[a,*,1], Diogo Manzano Galdeano[a,1], Laís Moreira Granato[a], Emilyn Emy Matsumura[b], Ronaldo José Durigan Dalio[a], Marcos Antonio Machado[a]

[a] Centro de Citricultura Sylvio Moreira/IAC, Rodovia Anhanguera, Km 158, Cordeiropolis, SP, Brazil
[b] Department of Plant Pathology, University of California, Davis, CA, 95616, USA

ARTICLE INFO

Keywords:
RNA interference
CRISPR
Pathogens
Citrus crops

ABSTRACT

Citrus crops have great economic importance worldwide. However, citrus production faces many diseases caused by different pathogens, such as bacteria, oomycetes, fungi and viruses. To overcome important plant diseases in general, new technologies have been developed and applied to crop protection, including RNA interference (RNAi) and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) systems. RNAi has been demonstrated to be a powerful tool for application in plant defence mechanisms against different pathogens as well as their respective vectors, and CRISPR/Cas system has become widely used in gene editing or reprogramming or knocking out any chosen DNA/RNA sequence. In this article, we provide an overview of the use of RNAi and CRISPR/Cas technologies in management strategies to control several plants diseases, and we discuss how these strategies can be potentially used against citrus pathogens.

1. Introduction

Citrus is one of the most important crops worldwide (Gmitter et al., 2012) and Brazil ranks as the world's largest producer of sweet oranges, followed by the United States, China and India (FAO, 2016). However, several pathogens cause diseases in citrus (Singh and Rajam, 2009), affecting plant development and reducing yield and fruit quality. The main citrus diseases are the following: Huanglongbing (HLB) (Bove et al., 2006; Coletta-filho et al., 2004), citrus leprosis, citrus tristeza, citrus sudden death (Maccheroni et al., 2005), alternaria brown spot, postbloom fruit drop, citrus black spot (Laranjeira et al., 2005), citrus canker (Brunings and Gabriel, 2003), citrus variegated chlorosis (CVC) (Laranjeira et al., 2008), gummosis and root rot (Muniz et al., 2004).

Overall, the control of citrus diseases has been mainly conducted by chemical application (Alejandra et al., 2017; Camargos et al., 2016; Graham and Feichtenberger, 2015; Leeuwen et al., 2015; Mendonça et al., 2017; Wang et al., 2017), which has greatly contributed to the emerging of resistant vectors and/or pathogens (Boina and Bloomquist, 2015; Chen et al., 2017; Gray et al., 2018; Sánchez-torres and Tuset, 2011). Thus, new biotechnological approaches are needed in order to reduce the high production costs and support the citrus disease management. RNA interference (RNAi) and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-based technologies have emerged as potential and promising strategies for improving plant resistance to different pathogens (Mann et al., 2008).

RNAi is an internal cell process and a prominent strategy for regulating the gene expression, which allows further gene function investigations. This mechanism does not seem to modify the genomic structure of a target gene (Cheng et al., 2015), but it is able to down-regulate gene expression, leading to a specific phenotype (Nakayashiki and Nguyen, 2008). RNAi has emerged as a strategy to target genes in fungi (Dang et al., 2011), viruses (Qu, 2010), bacteria (Escobar et al., 2001), and plant disease vectors (Yu et al., 2013), and also it has allowed studies on the function(s) of several genes (Baulcombe, 2004). On the other hand, CRISPR-based approaches are able to change genomic structure (Gaj et al., 2013) and it has broadened the agricultural research area by bringing new methods to develop novel plant varieties with deletion of detrimental traits or addition of significant characters (Arora and Narula, 2017). Citrus breeding program is challenging due to multiple limitations, including polyploidy, polyembryony, extended juvenility, and long crossing cycles. However, CRISPR has the potential to shorten varietal development for some traits, including disease resistance (Jia et al., 2017).

Thus, the application of both RNAi and CRISPR technologies in crop

* Corresponding author.
E-mail address: eduardo@ccsm.br (E.H. Goulin).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.micres.2019.03.006
Received 3 October 2018; Received in revised form 16 February 2019; Accepted 16 March 2019
Available online 27 April 2019
0944-5013/

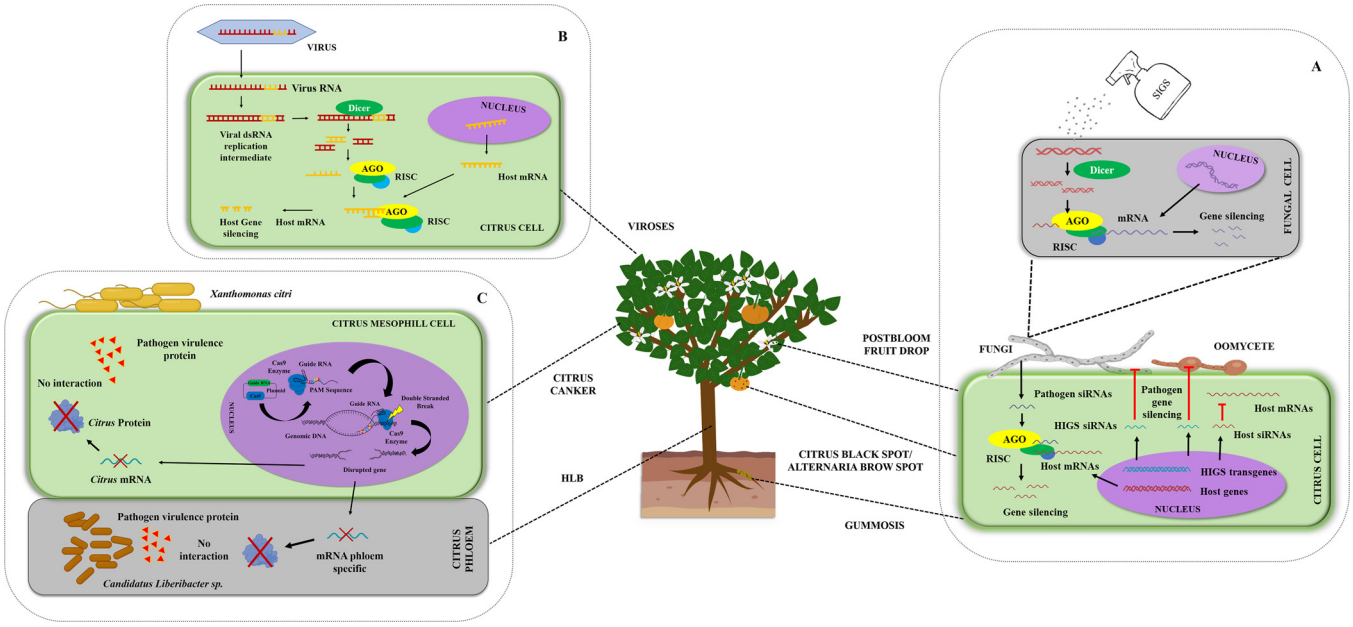

Fig. 1. schematic interaction of pathogens and the citrus plant, indicating the RNA interference pathways that contribute to the gene silencing in the plant and pathogens, and also new technologies applied to control. A) Fungi pathogens interaction, RNA interference induced inside the plant cell and the processing and gene silencing induced by fungal cell siRNA secretion; on the top, description of RNAi processing inside the fungal cell induced by nucleus hairpin transcription, plant cell transference, and double-strand RNA spray. B) Plant RNAi machinery inducing gene silencing after viral RNA infection. C) Crispr/Cas9 edition inducing plant gene deletion that result in non-interaction with pathogenic bacteria, consequently, no symptoms.

improvement has been strongly considered, specially in disease control. In this review, we summarize used strategies of RNAi and CRISPR for plant disease control and we discuss how these biotechnological tools can be applied in citrus disease managementsin future studies.

2. RNAi mechanism

RNAi is a conserved mechanism in eukaryotes, including plants, that involves knockdown of gene expression mediated by small interfering RNAs (siRNAs). The siRNAs are derived from two different sources: microRNAs (miRNAs), which are processed from endogenous gene transcripts and play a role in regulation of gene expression; and exogenous double-stranded RNA (dsRNA) molecules, which are also related to gene knockdown and mediate defense against viruses and transposable elements. The gene silencing mechanism by siRNAs is predominantly performed by cleavage of the target mRNA in a sequence-specific manner (Andrew and Craig, 2006; Fire et al., 1998; Sunkar and Zhu, 2004).

Once inside the cell, dsRNAs are cleaved by the RNase III Dicer into short nucleotide sequences (20–25 bp) with two-base overhang at the 3' end. These sequences are incorporated into the multi-protein RNA-induced silencing complex (RISC), where one of the two strands (passenger strand) is eliminated, while the other strand (guide strand) is retained (Andrew and Craig, 2006; Wynant et al., 2014). The regulation mechanism by this complex occurs when the catalytic component of the RISC, the RNase H-like domain of an Argonaute protein, cleaves single-stranded RNA molecules that have nucleotide sequences complementary to the guide strand RNA (Mlotshwa et al., 2002).

One of the main challenges in RNAi approaches is identifying the best sequence for dsRNA or siRNA preparation, especially deciding its length and its similarity to the target transcript (Scott et al., 2013). Another challenge is the development of a suitable and reliable method of dsRNAs or siRNAs delivery in plants. Previous works have had success in delivering these molecules via genetically modified (GM) plants (Beyene et al., 2017; Khatoon et al., 2016; Prins et al., 2008) and non-transformative strategies, such as symbionts, plant viruses, trunk injections, root soaking, and transplastomic plants (Joga et al., 2016) to target plant pathogens and/or its vectors (Zotti et al., 2017).

Studies have been shown the efficacy of RNAi as a functional genomics tool, assessing the function of genes involved in citrus defense against pathogens. One example is the silencing of *callose synthase 1* gene in *Citrus limon* by transient infiltration by *Agrobacterium tumefaciens* carrying dsRNAs expression vectors, encoding hairpin RNAs, which showed that plant cell wall-associated defense is the principal barrier against *Xanthomonas citri* subsp. *citri* (*Xcc*) infection in citrus plants (Enrique et al. 2011). However, few approaches have been reported about delivery methods of dsRNAs or siRNAs against citrus pathogens. To overcome this challenge, RNAi-based studies in other crops may bring knowledge to choose and apply novel strategies to control citrus disease.

3. RNA silencing-mediated resistance against fungi

Compared to other kingdoms, the fungi RNAi studies are distinguished, especially due to the diversity of the non-canonical RNA silencing pathways (Trieu et al., 2015). Moreover, some fungal species have partially or completely lost the RNAi machinery (Billmyre et al., 2013). On the other hand, recent studies have indicated the pontential application of RNAi technology to control fungi plant pathogens (Wang et al., 2016). The biotechnological use of fungal RNAi pathways includes the expression of a hairpin RNA derived by vector transformation, dsRNA generated by convergent transcription, and direct absorption of siRNA or dsRNA by the fungal cells (Dang et al., 2011). The hairpin RNA expression has been used for functional genomic studies in *Colletotrichum graminicola* (Münch et al., 2011; Oliveira-Garcia and Deising, 2013) and *Rosellinia necatrix* (Shimizu et al., 2014), which could be applied to gene function studies in the citrus pathogen species, including *Colletotrichum abscissum, Colletotrichum gloeosporioides* and *Phyllosticta citricarpa*, since they have well-established transformation protocols (Figueiredo et al., 2010).

The RNAi have a significant importance to supress the function of multi copies genes, or gene clusters. For example, the production of ACTT toxin in *Alternaria alternata* (tangerine pathotype) is the resulted of a biosynthetic chain. The genes that encode these proteins are

clustered in a small extra chromosome. Thus, the deletion or disruption of these genes by homologous recombination is ineffective. For this reason, hairpin expression vectors have been used in *A. alternata* to knockdown the *actt2* resulting in no toxin production and consequently loss of pathogenicity(Miyamoto et al., 2008).

Another strategy to control fungal diseases is the host-induced gene silencing (HIGS) that have been used in transgenic plants to knockdown genes of several plant fungal pathogens (Fig. 1A) such as *Blumeria graminis* (Nowara et al., 2010), *Fusarium* spp. (Cheng et al., 2015), *Sclerotinia sclerotiorum* (Andrade et al., 2016), *Puccinia striiformis f.* sp. *tritici* (Zhu et al., 2017), *Aspergillus spp*. (Thakare et al., 2017) and *Magnaporthe oryzae* (Zhu et al., 2017). However, HIGS is limited due to the public concern in releasing genetically modified organisms (GMOs), and the instability of engineered RNA silencing traits. To overcome this, novel strategies to control fungi diseases have been reported such as filamentous-induced gene silencing (FIGS) and spray-induced gene silencing – SIGS – (Fig. 1A). FIGS is a mechanism which small RNAs produced by the fungus induce gene silencing of host transcripts (Weiberg et al., 2014). Therefore, this silencing mechanism could be anotherstrategy for plant gene modulation using endophytes fungi to deliver the siRNA. Furthermore, SIGS is a strategy of disease control potentially sustainable and environmental friendly that use long dsRNA, specifically designed for the gene target, that is sprayed over the fungus pathogens leading to knockdown of the target gene (Koch et al., 2016). This approach for gene silencing is a potential alternative or complemental strategy for chemical fungicide control. Additionally, the resistance development will decrease, once major of chemical resistance related to plant pathogens fungi are due a single-point mutation (Hobbelen et al., 2014; Mallik et al., 2014; Wang and Jin, 2017).

All the fungal RNAi approaches reported here have been successfully applied for fungi phylogenetically close to citrus pathogens, which indicates a strong potential to extend this method in controlling fungi citrus disease, and also for gene function studies.

4. RNA silencing-mediated resistance against Oomycetes/ *Phytophthora*

Among the oomycetes, the *Phytophthora* genus stands out, with more than a hundred species of devastating plant pathogens. Notable species are *Phytophthora infestans*, a pathogen that caused the Irish famine, *P. ramorum*, responsible for sudden oak death in the USA, and *P. parasitica*, with an extremely broad host range capable of infecting more than 215 plant families and which is mainly responsible for *Phytophthora* diseases in citrus worldwide (Kamoun et al., 2015).

Studies in *Phytophthora* reported evidence of functional post-transcriptional and transcriptional gene silencing pathways. After a pioneer work in which the authors silenced genes in *P. infestans*, other studies showed a directed transgene and endogenous gene silencing by sense (van West et al., 1999v), anti-sense (Gaulin et al., 2002), and promoterless constructs (Blanco and Judelson, 2005). Subsequently, another approach was explored using a hairpin construct to induce silencing of an endogenous gene (Judelson and Tani, 2007).

The small RNAs (sRNAs) in *Phytophthora* are commonly placed between 21 and 25 nt (Fahlgren et al., 2013). Interestingly, it was found that the 21-nt sRNAs are derived from genes encoding effectors, such as CRNs, suggesting that these genes might be directly controlled by sRNAs (Åsman et al., 2016). Whereas gene silencing in oomycetes is most well characterized in *P. infestans*, most of its features and what has been achieved regarding this pathogen can potentially be extended to other *Phytophthora* species, such as *P. parasitica.* In *P. parasitica*, the main pathogen affecting citrus orchards worldwide, gene silencing has proven to be feasible. Through transgenic expression of dsRNAs, the cellulose binding elicitor lectin (CBEL) was suppressed in transgenic strains of *P. parasitica*, severely affecting adhesion of the pathogen to cellophane membranes, differentiation of lobed structures in contact with cellophane, and formation of branched aggregating hyphae (Gaulin et al., 2002). In another study, Narayan et al. (2010) showed that RNAi-mediated silencing of PnDLC1 (a dynein light chain 1 protein with activity in sporulating hyphae) resulted in transformants with zoospores that lack flagella and movement.

Successful RNAi-mediated silencing in *P. parasitica* opened the possibility of studying HIGS approaches (Fig. 1A). For instance, Zhang et al. (2011) developed stable transgenic *Arabidopsis* using HIGS strategy to study the interaction between plant and *P. parasitica*. The method that was performed targeted the gene PnPMA1, a plasma membrane H^{+-}ATPase, which is present only in oomycetes and would severely affect zoospore motility after its silencing. However, the authors reported that HIGS was not possible in the *Arabidopsis–P. parasitica* interaction, claiming that *P. parasitica* lacks the proper machinery required for the uptake of silencing signals. Koch and Kogel (2014) argued that the findings showed by Zhang et al. (2011) are contrary to what is currently available for oomycete research and affirmed that all oomycetes contain the machinery required for uptake of silencing molecules. Thus, further experiments are needed to shed light on this controversial issue and open new possibilities for studying HIGS in the citrus-*P. parasitica* interaction.

5. RNA silencing-mediated resistance against virus

Plant viruses have also been reported as the causal agents of many plant diseases and have caused significant negative impact on crop production worldwide (Tabassum et al., 2013), including citrus crop. The best example is citrus tristeza disease, which is caused by Citrus tristeza virus (CTV), a member of the family *Closteroviridae,* responsible for the loss of over 100 million citrus trees in the past years (Moreno et al., 2008). Conveniently, most of the plant viruses have RNA genomes and therefore produce dsRNAs molecules during their replication process, which are known to be potent inducers of RNAi-mediated gene silencing in plants (Lindbo and Dougherty, 2005). Thus, viral RNA itself may be a trigger for resistance against viruses through a natural antiviral defense mechanism (Qu, 2010; Sasaya et al., 2013) (Fig. 1B).

By taking advantage of the plant natural antiviral defense, plants can be engineered in attempt to express transgene containing homologous viral sequence that can trigger the HIGS by producing siRNA and targeting the respective viral genome (Duan et al., 2012; Kumar and Sarin, 2013). Many approaches have been used/developed for engineering a virus resistant transgenic plants and they are mostly based on different precursor RNA for siRNA production, including sense/antisense RNA, small/long hairpin RNA (hpRNA) and artificial miRNA precursors (Duan et al., 2012). All mentioned approaches have been recently reviewed in detail (Khalid et al., 2017).

The RNAi gene silencing technology has been successfully used to target several economically important plant viruses, such as barley yellow dwarf virus (BYDV) (Wang et al., 2000), banana bract mosaic virus (BBrMV) (Rodoni et al., 1997), bean golden mosaic virus (BGMV) (Bonfim et al., 2007), potato virus Y (PVY), sugarcane mosaic virus (SCMV) (Tabassum et al., 2013), cucumber green mottle mosaic virus (CGMMV) (Kamachi et al., 2007), tomato yellow leaf curl virus (TYLCV) (Yang et al., 2004), and rice tungro bacilliform virus (Tyagi et al., 2008), and, as far as we know, seven virus resistant transgenic crops have been approved for cultivation in the world (Khalid et al., 2017). Although most of the previous works have reported RNAi silencing-mediated resistance only against plant RNA viruses, it is worth to mention here that previous experiments have shown that this strategy is also effective in engineering resistance to DNA viruses (Duan et al., 2012).

Conversely, RNAi-mediated resistance against plant viruses has shown some disadvantages, including the occurrence of viral suppressors of RNA silencing (VSR) coded by some viruses. These suppressors are able to disrupt the RNAi-mediated silencing process by targeting key components of RNAi pathways (Qu, 2010; Tabassum et al., 2013). CTV, for example, is known to produce proteins associated

with silencing suppression activities in response to the defense systems of host citrus plant (Qu, 2010). To overcome this problem, Mexican lime plants were transformed with an intron-hairpin vector engineered with three silencing suppressors (*p25*, *p20* and *p23*) from CTV strain T36 to silence these genes in CTV-infected cells. Three transgenic lines showed viral resistance and did not develop any symptoms (Soler et al., 2012). Another problem we have faced is that mutation greater than 10–20% in the homologous viruses could contribute to break the plant resistance (Kumar and Sarin, 2013) and, in this case, the RNAi-mediated resistance could be ineffective (Tabassum et al., 2013). For this latter, the construction of multiple hpRNAs targeting different regions of the viral genome might be an alternative to overcome the problem (Kumar and Sarin, 2013).

Additionally to CTV, citrus plants have accumulated a large number of other important viruses, such as citrus yellow mosaic virus (CYMV) (Singh and Rajam, 2009), Satsuma dwarf virus (Singh and Rajam, 2009), citrus leprosis-associated viruses (*Cilevirus*, *Higrevirus*, and *Dichorhavirus*) (Ramos-González et al., 2016), citrus sudden death-associated virus (CSDaV) (Maccheroni et al., 2005; Matsumura et al., 2016) and citrus endogenous pararetrovirus (CitPRV) (Roy et al., 2014; Matsumura et al., 2016), which can induce symptoms and cause diseases when the infected cultivar is susceptible to the viral pathogen (Lee, 2015). Thus, the successful use of the RNAi approach in different crops for engineering virus resistance plants brings high expectations for its application in citrus plants. RNA-mediated gene silencing mechanisms might be an efficient strategy to prevent and/or reduce citrus virus infections, which can decrease the viral sources for vector transmission and consequently control the development of plant viral diseases.

6. CRISPR/Cas-mediated citrus genome engineering for resistance against bacteria

CRISPR are essential components of nucleic-acid-based adaptive immune systems that are widespread in bacteria and archaea for protection against invaders such as phage and plasmid nucleic acids (Dupuis et al., 2013). In response to viral and plasmid challenges, bacteria integrate short fragments of foreign nucleic acid (called protospacer) into its own chromosome at one end of a repetitive element known as CRISPR (Mojica et al., 2009). The set of protospacer is transcribed as a pre-crRNA which is matured to crRNA right after with the help of the trans-activating-crRNA (tracrRNA) (Barrangou et al., 2007; Garneau et al., 2010). The paring of tracrRNA/crRNA is necessary to recognize and guide the invading DNA sequence that will be cleaved by a DNA endonuclease Cas protein (s), with or without help of other proteins.

To facilitate the experimental procedure, researchers have developed a single guide RNA (sgRNA), which is a quimeric molecule resulted from the fusion of crRNA and tracRNA. The sgRNA guides the Cas protein to interact with the target sequence only if it has the PAM sequence (Garneau et al., 2010). The Cas protein cleavage generates a double strand break (DSB), which will be repaired by the cell through two mechanisms: non-homologous end joining or homology directed repair. The cell repair culminates in the frameshift modification, resulting in the generation of knockout cells or organisms (Vieira et al., 2016). Thus, targeted genome engineering has been a promising approach in contributing for plant breeding, and improving crop yield, quality, and disease resistance (Andolfo et al., 2016; Arora and Narula, 2017; Tabassum et al., 2017).

Plant genome editing mediated by Cas9/sgRNA has been reported in rice (Feng et al., 2013; Jiang et al., 2013; Mao et al., 2013; Shan et al., 2013), wheat (Shan et al., 2013; Upadhyay et al., 2013), *Arabidopsis* (Feng et al., 2014; Jiang et al., 2014), tobacco (Gao et al., 2014; Nekrasov et al., 2013), sorghum (Jiang et al., 2013), maize (Liang et al., 2014), soybean (Jacobs et al., 2015; Sun et al., 2015), and citrus (Jia et al., 2017, 2016; Peng et al., 2017; Zhang et al., 2017a,b). In the first report of targeted citrus genome modification, the Cas9/sgRNA system was employed to target the *Cs*PDS gene in sweet orange (*Citrus sinensis*, cultivar Valencia) (Jia and Wang, 2014a). *CsPDS* encodes a phytoene desaturase with homologs in rice, *Arabidopsis*, and *Nicotiana benthamiana* (Li et al., 2013). In Valencia sweet orange (*C. sinensis* L. Osb.) and Duncan grapefruit (*Citrus paradisi* Macf.), the Cas9/sgRNA system was successfully used to modify the citrus *CsPDS* via *Xcc*-facilitated agroinfiltration, an optimized transient expression method in citrus (Jia and Wang, 2014a, b). As expected, the wild type *Cs*PDS sequence was confirmed in control citrus leaves, whereas the modified *Cs*PDS sequence was not detected in sweet orange leaves expressing Cas9/sgRNA (Jia et al., 2016). The results indicated that Cas9/sgRNA successfully induced mutations in the targeted gene in citrus.

CsLOB1 is a susceptibility gene for *Xcc*, bacteria that causes citrus canker (Hu et al., 2014). The transcription activator-like effector (TALE) PthA4 was translocated from *Xcc* to host cells, binds to the effector binding elements (EBEPthA4) in the *CsLOB1* promoter region (EBEPthA4-*CsLOBP*), and activates the expression of citrus susceptibility genes that lead to the development of canker symptoms (Hu et al., 2014). Though Cas9/sgRNA-mediated modification of one single allele of *CsLOB1* promoter in Duncan grapefruit could not alleviate canker symptoms (Jia et al., 2016), disruption of two alleles of *CsLOB1* promoters conferred a high degree of resistance to citrus canker in four mutations lines of Wanjincheng orange (Fig. 1C), demonstrating that CRISPR/Cas9-mediated promoter editing of *CsLOB1* is an efficient strategy for generation of canker resistant citrus cultivars (Peng et al., 2017). In addition, Cas9/sgRNA-mediated editing of *CsLOB1* coding region in transgenic Duncan grapefruit conferred resistance to citrus canker (Jia et al., 2017).

Although studies with CRISPR in citrus are still incipient, it is possible predict a great importance in studies of gene function, and development of novel citrus varieties not considered genetically modified organisms (GMOs), decreasing the regulatory time for new varieties and also increasing the disease resistance. CRISPR/Cas promises to be a technique faster than the usual transgenic transformation, which allows early gene-function analysis, specially favoring studies on the perennial plant that takes years to grown. Besides that, it also allows multiplex gene editing by simultaneous expression of two or more sgRNAs, asit has been reported for other crops (Brooks et al., 2014; Liang et al., 2014). This might be important to analyze quantitative characteristics controlled by several genes such as resistance to citrus diseases.

For HLB control, a strategy that could be applied is editing citrus genes associated to response to *Candidatus* Liberibacter asiaticus, such as structural genes involved in the blockage of the phloem vessels (Mafra et al., 2013). Modifying those related genes could restore the transport of nutrients through the phloem and perhaps reduce the severity of HLB symptoms.

7. Challenges faced by using the CRISPR/Cas technology in citrus

Some technical challenges remain in CRISPR/Cas9 technology plants application. Among them, the most debated is the high frequency of off-target mutations. Although mismatches in the PAM-distal region can be tolerated (Fu et al., 2013), DNA sequences that contain an extra base or missing base at several locations along the corresponding sgRNA sequence have been shown to induce off-target cleavage (Lin et al., 2014). Some strategies have been developed to reduce off-target genome editing. But, the most important to consider here is the sgRNA design. Usually, a sgRNA 20-nt length is target site specific (Cong et al., 2013), moreover, sgRNA with two additional guanidine residues at the 5`end can avoid off-target sites more efficiently than normal sgRNAs (Cho et al., 2014). Off-targets can be tested rapidly and costless using some online tools to facilitate the selection of unique target sites (Bortesi and Fischer, 2015). Specificity can also be controlled optimizing nuclease used, once high concentrations of sgRNA and Cas protein can increase off-target mutations (Pattanayak et al., 2013).

Table 1
RNAi applications reports in *Citrus* spp. pathogens, indicating the diversity and importance of RNAi studies in plant-pathogen interactions.

Pathogen	Host Plant	RNAi Application	Gene	Phenotype Effect	Reference
Virus					
Geminiviruses and Insects Viruses	tabacco	HIGS	CYPAE14	Reduced growth	Kumar & Sarin
cucumber mosaic virus (CMV)	*Arabidopsis thaliana*	HIGS	TGBp1	Developmental abnormalities	Duan, Wang and Guo
Barley yellow dwarf virus–PAV (BYDV-PAV)	Barley	HIGS	BYDV-polymerase	Virus resistance	Wang, Abbott and Waterhouse
Bean golden mosaic virus	Bean	HIGS	AC1	Virus resistance	Bonfim et al.
Green mottle mosaic virus	*Nicotiana benthamiana*	HIGS	CGMMV – CP	Virus resistance	Kamachi et al.
Tomato yellow leaf curl virus	Tomato	HIGS	TYLCSV Rep	Virus resistance	Yang et al.
Rice tungro bacilliform virus	Rice	HIGS	ORFIV	Virus resistance	Tyagi et al.
Fungi					
C. graminicola	Maize	Gene function	b-1,3-Glucan Synthase	Cell-wall deformation	Oliveira-Garcia and Deising
C. graminicola	Maize	Gene function	GPI12, GAA1, and GPI8	Cell-Wall integrity and pathogenicity reduces	Oliveira-Garcia and Deising
Rosellinia necatrix	Fruit trees	Gene function	1,8-dihydroxynaphthalene (DHN)	Failed to develop pseudosclerotia	Shimizu, Ito and Kanematsu
A. alternata	Citrus	Gene function	ACT-toxin	Lost pathogenicity	Miyamoto et al.
Blumeria graminis	Barley and Wheat	HIGS	Avra10	Reduction in fungal development	Nowara et al.
Fusarium spp.	Wheat	HIGS	Chs3b	Cell-wall deformation	Cheng et al.
Sclerotinia sclerotiorum	Tabacco	HIGS	Chs	Cell-wall deformation	Andrade et al.
Botrytis cinerea	Arabidopsis and tomato	FIGS	ago1 dcl1, dcl2	Host immunity suppression	Weiberg et al.
Fusarium graminearum	Barley	SIGS	P450 lanosterol C-14α-demethylase	Plant resistance for fungal infection	Koch et al.
Fusarium graminearum	Cereal	HIGS			Machado et al.
Fusarium oxysporum	Tomato	Gene function	Fmk1, Hog1 and Pbs2	Reduced invasive growth and pathogenesis	Pareek and Rajam
Rhizoctonia solani	Rice	HIGS	RPMK1-1 and RPMK1-2	Plant resistance for fungal infection	Tiwari et al.
Fusarium oxysporum	Tomato	Gene function	PEX6	Reduced pigmentation and reduction in sporulation	Tetorya and Rajam
Fusarium verticillioides	Maize	Gene function	FUM1 and FUM8	Reduction in mycotoxin production	Johnson et al.
Oomycetes					
P. infestans	|	Gene Function			Judelson and Whittaker
P. infestans	|	Gene Function			van West et al
P. infestans	tabacco	Gene Function	Cellulose Binding Proteins (CEBL)	Lost defence response	Gaulin et al.
P. infestans	Potato	Gene Function			Blanco and Judelson
P. infestans	|	Gene Function	LIM interactor-interacting factors (NIF)		Judelson and Tani
P. infestans	Potato and Tomato	Gene Function	*inf1* elicitin		Ah-Fong et al.
P. parasitica	*Arabidopsis thaliana*	HIGS	Plasma Membrane ATPase 1 (PnPMA1)		Zhang et al.
P. sojae	Soybean	Host Suppresion	PSR1-Interacting Protein 1 (PINP1)	Defects in plant development and immunity	Qiao et al.

Other challenges to deal with are the DNA delivery and plant transformation issues. In most of the published plant genome-editing approaches the CRISPR/Cas expression cassette is delivered into cells via *Agrobacterium* (Yin et al., 2017). During the stable transformation, the expression cassette integrates into the plant genome and induced the cleavage in the desired chromosomal site. However, only a small proportion of the delivered DNA integrates into the plant genome, and plants stably transformed with CRISPR/Cas technology may contain unwanted insertions of plasmid DNA at both on-target and off-target sites (Basak and Nithin, 2015). In the *Citrus* genome editing, off-targets mutagenesis was not detected for *Cs*PDS and *Cs*LOB1 genes sequences (Jia and Wang, 2014a,b; Jia et al., 2017).

Citrus plants have juvenile phases that result in flowering five to ten years after germination. A vector outbreeding approach in such systems would be lengthy and impractical. However, use of a transient expression system or delivery a pre-assembled Cas/sgRNA may offer viable alternatives (Liu et al., 2017). Addressing DNA delivery and genetic transformation using protoplasts can be an alternative because results in higher gene targeting frequency compared to *Agrobacterium*-mediated transformation (Basak and Nithin, 2015). Two approaches involving DNA-free genome editing in plants were described; these involve delivery of a mixture of Cas-encoding mRNA and sgRNA or pre-assembled ribonucleoproteins (RNPs) (Woo et al., 2015). However, an unintegrated transgenes have expression and function for a short time (Yin et al., 2017), so transient gene expression of CRISPR/Cas may provide an alternative method of citrus genome editing aiming gene function studies, as showed for the *Cs*PDS gene in Duncan grapefruit (Jia and Wang, 2014a). Methods using RNPs enhances the specificity of CRISPR/Cas in plants, reducing significantly off-target mutations. Although transfected RNPs into protoplasts can induce targeted genome modifications, the regeneration rate of transfected protoplasts can be very low (Basak and Nithin, 2015; Zhang et al., 2010). These methods can produce desired genome modifications in the absence of a vector sequence, eliminating the outbreeding and reducing the time and resources required to generate a vector-free plant in cultivars with long juvenility periods and for improving the germplasm of clonally propagated crops (Liu et al., 2017). In *Citrus sinensis* the regeneration efficiency varies according the cultivar, density of protoplasts culture and of the culture medium used (Castro et al., 2011).

Plants stably transformed with CRISPR–Cas9 are often considered to be GMOs and may be tightly regulated in some countries, limiting the use of genome editing in plant biotechnology and sustainable agriculture (Bortesi and Fischer, 2015). If researchers avoid transgenes or use strategies to eliminate the gene-editing machinery from plants, CRISPR-edited plants would similar to plants that acquired genetic mutations naturally. The regulatory process may be important over the long-dated. Agencies around the world have not figured yet the best way to regulate plants edited with CRISPR–Cas9 technology. European regulatory agencies tend to focus on process (how the plants were produced) (Callaway, 2018), while USA regulators tend to focus on the end product (Wolt et al., 2016).

8. Conclusions and future directions

The feasibility of using RNAi and CRISPR technologies in crops protection against pathogens has been a hot topic in biological research in the last few years. These approach holds great future exploited, once it allows a wide range of potential targets in the pathogens. Studies regarding to gene silencing and gene deletion or disruption have become essential to explore the gene function and have also been used to protect crops against diseases. Some of these studies related to citrus pathogens and plants are summarized in the Table 1.

Here, we have shown that the use of RNAi and CRISPR has already been applied to several crops as well as to some of their pathogens; and we discuss the great potential in extend these technologies to citrus plants. Several approaches have shown the great potential of RNAi to contribute toward development of novel management strategies of vectors-borne viral/bacterial diseases in citrus. As example, CTV, CVC and HLB diseases has been successfully controlled by applying RNAi-mediated protection against *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy) (Shang et al., 2016), *Homalodisca vitripennis* (Rosa et al., 2012, 2010) and *Diaphorina citri*, respectively (Andrade and Hunter, 2017; El-shesheny et al., 2013; Galdeano et al., 2017; Hajeri et al., 2014; Killiny et al., 2014; Kishk et al., 2017; Yu et al., 2017). Thus, RNAi and CRISPR could definitely be exploited to development novel management strategies to protect citrus against pathogens and its vectors, bringing benefits to both growers and consumers.

Funding

This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo–Fapesp (Project: 2012/23381-7, 201450880-0) and CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico (465440/2014-2).

References

Alejandra, M., Andrea, R., Gabriel, G., Lorena, R., Noemí, L., Lutz, A., Sillon, M., Mario, L., Rosa, M., Francisco, N., 2017. Relationships between copper content in orange leaves, bacterial biofilm formation and citrus canker disease control after different copper treatments. Crop Prot. 92, 182–189. https://doi.org/10.1016/j.cropro.2016.11.011.

Andolfo, G., Iovieno, P., Frusciante, L., Ercolano, M.R., 2016. Genome-editing technologies for enhancing plant disease resistance. Front. Plant Sci. 7, 1–8. https://doi.org/10.3389/fpls.2016.01813.

Andrade, E.C., Hunter, W.B., 2017. RNAi feeding bioassay : development of a non-transgenic approach to control Asian citrus psyllid and other hemipterans. Entomol. Exp. Apl. 1–8. https://doi.org/10.1111/eea.12544.

Andrade, C.M., Tinoco, M.L.P., Rieth, A.F., Maia, F.C.O., Aragão, F.J.L., 2016. Host-induced gene silencing in the necrotrophic fungal pathogen Sclerotinia sclerotiorum. Plant Pathol. 65, 626–632. https://doi.org/10.1111/ppa.12447.

Andrew, F., Craig, M., 2006. The discovery of RNA interference. Karolinska Inst. 1–10.

Arora, L., Narula, A., 2017. Gene editing and crop improvement using CRISPR-Cas9 system. Front. Plant Sci. 8, 1–28. https://doi.org/10.3389/fpls.2017.01932.

Åsman, A.K.M., Fogelqvist, J., Vetukuri, R.R., Dixelius, C., 2016. *Phytophthora infestans* Argonaute 1 binds microRNA and small RNAs from effector genes and transposable elements. New Phytol. 211, 993–1007. https://doi.org/10.1111/nph.13946.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A., Horvath, P., 2007. CRISPR provides acquired resistance against viruses in prokaryotes. Science 315, 1709–1712. https://doi.org/10.1126/science.1138140.

Basak, J., Nithin, C., 2015. Targeting non-coding RNAs in plants with the CRISPR-Cas technology is a challenge yet worth accepting. Front. Plant Sci. 6, 1001. https://doi.org/10.3389/fpls.2015.01001.

Baulcombe, D., 2004. RNA silencing in plants. Nature 431, 356–363. https://doi.org/10.1038/nature02874.

Beyene, G., Chauhan, R.D., Ilyas, M., Wagaba, H., Fauquet, C.M., Miano, D., Alicai, T., Taylor, N.J., Taylor, N.J., 2017. A virus-derived stacked RNAi construct confers robust resistance to cassava brown streak disease. Front. Plant Sci. 7, 1–12. https://doi.org/10.3389/fpls.2016.02052.

Billmyre, R.B., Calo, S., Feretzaki, M., Wang, X., Heitman, J., 2013. RNAi function, diversity, and loss in the fungal kingdom. Chromosome Res. 21, 561–572. https://doi.org/10.1007/s10577-013-9388-2.

Blanco, F.A., Judelson, H.S., 2005. A bZIP transcription factor from *Phytophthora* interacts with a protein kinase and is required for zoospore motility and plant infection. Mol. Microbiol. 56, 638–648. https://doi.org/10.1111/j.1365-2958.2005.04575.x.

Boina, D.R., Bloomquist, J.R., 2015. Chemical control of the asian Citrus psyllid and of huanglongbing disease in Citrus. Pest Manage. Sci. 71, 808–823. https://doi.org/10.1002/ps.03957.

Bonfim, K., Faria, J.C., Nogueira, E.O., Mendes, E.A., Aragao, F.J., 2007. RNAi-mediated resistance to Bean golden mosaic virus in genetically engineered common bean (*Phaseolus vulgaris*). Mol. Plant Microbe Interact. 20, 717–726. https://doi.org/10.1094/MPMI-20-6-0717.

Bortesi, L., Fischer, R., 2015. The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol. Adv. 33, 41–52. https://doi.org/10.1016/j.biotechadv.2014.12.006.

Bove, J.M., Genomique, D.R., Pathogene, P., Recherche, C.De, Bordeaux, I. De, Bourlaux, E., Cedex, O., Df, A., Pereira, A., Araraquara, P., 2006. Huanglongbing : a destructive, newly-emerging, century · old disease of Citrus. J. Plant Pathol. 88, 7–37.

Brooks, C., Nekrasov, V., Lippman, Z.B., Eck, J.Van, 2014. Efficient Gene Editing in Tomato in the First Generation Using the Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR-Associated9 System. Plant Physiol. 1 (166), 1292–1297. https://doi.org/10.1104/pp.114.247577.

Brunings, A.M., Gabriel, D.W., 2003. *Xanthomonas citri*: breaking the surface. Mol. Plant Pathol. 4, 141–157. https://doi.org/10.1046/j.1364-3703.2003.00163.x.

Callaway, E., 2018. CRISPR plants now subject to tough GM laws in European Union.

Nature 560, 16. https://doi.org/10.1038/d41586-018-05814-6.
Camargos, R.B., Perina, F.J., Diego, D., Carvalho, C., Alves, E., Mascarello, A., Chiaradia-delatorre, L.D., Yunes, R.A., Nunes, R.J., Oliveira, D.F., 2016. CHALCONES TO CONTROL *Alternaria alternata* IN MURCOTT TANGOR FRUITS. Biosci. J. 32, 1512–1521.
Castro, L.M., Mourão Filho, F.A.A., Mendes, B.M.J., 2011. Eficiência de isolamento de plaqueamento de protoplastos de laranja-doce. Rev. Bras. Frutic. 33, 509–516. https://doi.org/10.1590/S0100-29452011005000074.
Chen, L., Tsai, H., Yu, P., Chung, K., 2017. A major facilitator superfamily transporter-mediated resistance to oxidative stress and fungicides requires Yap1, Skn7, and MAP kinases in the Citrus Fungal pathogen Alternaria alternata. PLoS One 12, 1–16. https://doi.org/10.1371/journal.pone.0169103.
Cheng, W., Song, X.S., Li, H.P., Cao, L.H., Sun, K., Qiu, X.L., Xu, Y. Bin, Yang, P., Huang, T., Zhang, J.B., Qu, B., Liao, Y.C., 2015. Host-induced gene silencing of an essential chitin synthase gene confers durable resistance to Fusarium head blight and seedling blight in wheat. Plant Biotechnol. J. 13, 1335–1345. https://doi.org/10.1111/pbi.12352.
Cho, S.W., Kim, S., Kim, Y., Kweon, J., Kim, H.S., Bae, S., Kim, J.-S., 2014. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res. 24, 132–141. https://doi.org/10.1101/gr.162339.113.
Coletta-filho, H.D., Targon, M.L.P.N., Takita, M.A., De Negri, J.D., Pompeu, J., Machado, M.A., 2004. First report of the causal agent of huanglongbing ("Candidatus liberibacter asiaticus") in Brazil. Plant Dis. 88, 1382.
Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A., Zhang, F., 2013. Multiplex genome engineering using CRISPR/Cas systems. Science (80-.) 339, 819–823. https://doi.org/10.1126/science.1231143.
Dang, Y., Yang, Q., Xue, Z., Liu, Y., 2011. RNA interference in fungi: pathways, functions, and applications. Eukaryot. Cell 10, 1148–1155. https://doi.org/10.1128/EC.05109-11.
Duan, C.-G., Wang, C.-H., Guo, H.-S., 2012. Application of RNA silencing to plant disease resistance. Silence 3, 5. https://doi.org/10.1186/1758-907X-3-5.
Dupuis, M.-È., Villion, M., Magada, A.H., Moineau, S., 2013. CRISPR-Cas and restriction–modification systems are compatible and increase phage resistance. Nat. Commun. 4, 1–7. https://doi.org/10.1038/ncomms3087.
El-sheshseny, I., Hajeri, S., El-hawary, I., Gowda, S., Killiny, N., 2013. Silencing abnormal wing disc gene of the asian Citrus psyllid, Diaphorina citri disrupts adult wing development and increases nymph mortality. PLoS One 8, 2–9. https://doi.org/10.1371/journal.pone.0065392.
Escobar, M.A., Civerolo, E.L., Summerfelt, K.R., Dandekar, A.M., 2001. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. Proc. Natl. Acad. Sci. U. S. A. 98, 13437–13442.
Fahlgren, N., Bollmann, S.R., Kasschau, K.D., Cuperus, J.T., Press, C.M., Sullivan, C.M., Chapman, E.J., Hoyer, J.S., Gilbert, K.B., Grünwald, N.J., Carrington, J.C., 2013. Phytophthora have distinct endogenous small RNA populations that include short interfering and microRNAs. PLoS One 8. https://doi.org/10.1371/journal.pone.0077181.
FAO, 2016. Food and Agriculture Organization of The United Nations [WWW Document]. (accessed 11.10.16). http://faostat3.fao.org/browse/Q/QC/E.
Feng, Z., Zhang, B., Ding, W., Liu, X., YAng, D.-L., Wei, P., Cao, F., Shihua, Z., Zhang, F., Mao, Y., Zhu, J.-K., 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat. Biotechnol. 31, 227–229. https://doi.org/10.1038/nbt.2501.
Feng, Z., Mao, Y., Xu, N., Zhang, B., Wei, P., Yang, D.L., Wang, Z., Zhang, Z., Zheng, R., Yang, L., Zeng, L., Liu, X., Zhu, J.K., 2014. Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas-induced gene modifications in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 111, 4632–4637. https://doi.org/10.1073/pnas.1400822111.
Figueiredo, J.G., Goulin, E.H., Tanaka, F., Stringari, D., Kava-cordeiro, V., Galli-terasawa, L.V., Staats, C.C., Schrank, A., Glienke, C., 2010. *Agrobacterium tumefaciens* -mediated transformation of *Guignardia citricarpa*. J. Microbiol. Methods 80, 143–147. https://doi.org/10.1016/j.mimet.2009.11.014.
Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806–811.
Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K., Sander, J.D., 2013. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat. Biotechnol. 31, 822–826. https://doi.org/10.1038/nbt.2623.
Gaj, T., Gersbach, C.A., Barbas, C.F., 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 31, 397–405. https://doi.org/10.1016/j.tibtech.2013.04.004.
Galdeano, D.M., Lopes, S., Falk, W., Machado, M.A., 2017. Oral delivery of double-stranded RNAs induces mortality in nymphs and adults of the Asian citrus psyllid, *Diaphorina citri*. PLoS One 1–15. https://doi.org/10.1371/journal.pone.0171847.
Gao, J., Wang, G., Ma, S., Xie, X., Wu, X., Zhang, X., Wu, Y., Zhao, P., Xia, Q., 2014. CRISPR/Cas9-mediated targeted mutagenesis in *Nicotiana tabacum*. Plant Mol. Biol. 87, 99–110. https://doi.org/10.1007/s11103-014-0263-0.
Garneau, J.E., Dupuis, M.-È., Villion, M., Romero, D.A., Barrangou, R., Boyaval, P., Fremaux, C., Horvath, P., Magadán, A.H., Moineau, S., 2010. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468, 67–71. https://doi.org/10.1038/nature09523.
Gaulin, E., Jauneau, A., Villalba, F., Rickauer, M., Esquerre-Tugaye, M.T., Bottin, A., 2002. The CBEL glycoprotein of *Phytophthora parasitica* var. nicotianae is involved in cell wall deposition and adhesion to cellulosic substrates. J. Cell. Sci. 115, 4565–4575. https://doi.org/10.1242/Jcs.00138.
Gmitter, F.G., Chen, C., Machado, M.A., de Souza, A.A., Ollitrault, P., Froehlicher, Y., Shimizu, T., 2012. Citrus genomics. Tree Genet. Genomes 8, 611–626. https://doi.org/10.1007/s11295-012-0499-2.
Graham, J., Feichtenberger, E., 2015. Citrus *Phytophthora* diseases: management challenges and successes. J. Citrus Pathol. 2, 1–11.
Gray, M.A., Hao, W., Förster, H., Adaskaveg, J.E., 2018. Baseline sensitivities of new fungicides and their toxicity to selected life stages of *Phytophthora* species from Citrus in California. Plant Dis. 102.
Hajeri, S., Killiny, N., El-Mohtar, C., Dawson, W.O., Gowda, S., 2014. Citrus tristeza virus-based RNAi in citrus plants induces gene silencing in *Diaphorina citri*, a phloem-sap sucking insect vector of citrus greening disease (Huanglongbing). J. Biotechnol. 176, 42–49. https://doi.org/10.1016/j.jbiotec.2014.02.010.
Hobbelen, P.H.F., Paveley, N.D., Van Den Bosch, F., 2014. The emergence of resistance to fungicides. PLoS One 9, 1–14. https://doi.org/10.1371/journal.pone.0091910.
Hu, Y., Zhang, J., Jia, H., Sosso, D., Li, T., Frommer, W.B., Yang, B., et al., 2014. Lateral organ boundaries 1 is a disease susceptibility gene for citrus bacterial canker disease. Proc. Natl Acad. Sci. 111, 521–529.
Jacobs, T.B., LaFayette, P.R., Schmitz, R.J., Parrott, W.A., 2015. Targeted genome modifications in soybean with CRISPR/Cas9. BMC Biotechnol. 15, 16. https://doi.org/10.1186/s12896-015-0131-2.
Jia, H., Wang, N., 2014a. Targeted genome editing of sweet orange using Cas9/sgRNA. PLoS One 9. https://doi.org/10.1371/journal.pone.0093806.
Jia, H., Wang, N., 2014b. Xcc-facilitated agroinfiltration of citrus leaves: a tool for rapid functional analysis of transgenes in citrus leaves. Plant Cell Rep. 33, 1993–2001. https://doi.org/10.1007/s00299-014-1673-9.
Jia, H., Orbovic, V., Jones, J.B., Wang, N., 2016. Modification of the PthA4 effector binding elements in Type I CsLOB1 promoter using Cas9/sgRNA to produce transgenic Duncan grapefruit alleviating XccΔpthA4: DCsLOB1.3 infection. Plant Biotechnol. J. 14, 1291–1301. https://doi.org/10.1111/pbi.12495.
Jia, H., Zhang, Y., Orbovic, V., Xu, J., White, F., Jones, J., Wang, N., 2017. Genome editing of these disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. Plant Biotechnol. J. 15, 817–823. https://doi.org/10.1111/ijlh.12426.
Jiang, W., Zhou, H., Bi, H., Fromm, M., Yang, B., Weeks, D.P., 2013. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucleic Acids Res. 41, 1–12. https://doi.org/10.1093/nar/gkt780.
Jiang, W., Yang, B., Weeks, D.P., 2014. Efficient CRISPR/Cas9-mediated gene editing in *Arabidopsis thaliana* and inheritance of modified genes in the T2 and T3 generations. PLoS One 9, 21–26. https://doi.org/10.1371/journal.pone.0099225.
Joga, M.R., Zotti, M.J., Smagghe, G., Christiaens, O., 2016. RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: what we know so far. Front. Physiol. 7, 1–14. https://doi.org/10.3389/fphys.2016.00553.
Judelson, H.S., Tani, S., 2007. Transgene-induced silencing of the zoosporogenesis-specific NIFC gene cluster of *Phytophthora infestans* involves chromatin alterations. Eukaryot. Cell 6, 1200–1209. https://doi.org/10.1128/Ec.00311-06.
Kamachi, S., Mochizuki, A., Nishiguchi, M., Tabei, Y., 2007. Transgenic *Nicotiana benthamiana* plants resistant to cucumber green mottle mosaic virus based on RNA silencing. Plant Cell Rep. 26, 1283–1288. https://doi.org/10.1007/s00299-007-0358-z.
Kamoun, S., Furzer, O., Jones, J.D.G., Judelson, H.S., Ali, G.S., Dalio, R.J.D., Roy, S.G., Schena, L., Zambounis, A., Panabières, F., Cahill, D., Ruocco, M., Figueiredo, A., Chen, X.-R., Hulvey, J., Stam, R., Lamour, K., Gijzen, M., Tyler, B.M., Grünwald, N.J., Mukhtar, M.S., Tomé, D.F.A., Tör, M., Van Den Ackerveken, G., McDowell, J., Daayf, F., Fry, W.E., Lindqvist-Kreuze, H., Meijer, H.J.G., Petre, B., Ristaino, J., Yoshida, K., Birch, P.R.J., Govers, F., 2015. The Top 10 oomycete pathogens in molecular plant pathology. Mol. Plant Pathol. 16, 413–434. https://doi.org/10.1111/mpp.12190.
Khalid, A., Zhang, Q., Yasir, M., Li, F., 2017. Small RNA based genetic engineering for plant viral resistance: application in crop protection. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00043.
Khatoon, S., Kumar, A., Sarin, N.B., Khan, J.A., 2016. RNAi-mediated resistance against Cotton leaf curl disease in elite Indian cotton (*Gossypium hirsutum*) cultivar Narasimha. Virus Genes 52, 530–537. https://doi.org/10.1007/s11262-016-1328-8.
Killiny, N., Hajeri, S., Tiwari, S., Gowda, S., Stelinski, L.L., 2014. Double-stranded RNA uptake through topical application, mediates silencing of five CYP4 genes and suppresses insecticide resistance in *Diaphorina citri*. PLoS One 9, 1–8. https://doi.org/10.1371/journal.pone.0110536.
Kishk, A., Killiny, N., Abdel-raof, T.K., Gowda, S., Hamed, S., 2017. RNA interference of carboxyesterases causes nymph mortality in the Asian citrus psyllid, *Diaphorina citri*. Arch. Insect Biochem. Physiol. 1–13. https://doi.org/10.1002/arch.21377.
Koch, A., Kogel, K.-H., 2014. New wind in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing. Plant Biotechnol. J. 12, 821–831. https://doi.org/10.1111/pbi.12226.
Koch, A., Biedenkopf, D., Furch, A., Weber, L., Rossbach, O., Abdellatef, E., Linicus, L., Johannsmeier, J., Jelonek, L., Goesmann, A., Cardoza, V., McMillan, J., Mentzel, T., Kogel, K.-H., 2016. An RNAi-Based control of *fusarium graminearum* infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. PLoS Pathog. 12, e1005901. https://doi.org/10.1371/journal.ppat.1005901.
Kumar, A., Sarin, N.B., 2013. RNAi : A Promising Approach to Develop Transgenic Plants Against Geminiviruses and Insects. J. Plant Physiol. Pathol. 1, 1–6. https://doi.org/10.4172/jppp.1000103.
Laranjeira, F.F., Amorin, L., Bergamin Filho, A., Aguilar-Vildoso, C.I., Coletta Filho, Hdella, 2005. Fungos, procariotos e doenças abióticas. Citros 511–566.
Laranjeira, F.F., Silva, L.G., Fonseca, E.L., Silva, S.X.B., Rocha, J.B., Santos-Filho, H.P., Ledo, C.A.S., Hau, B., 2008. Prevalence, incidence and distribution of citrus variegated chlorosis in Bahia, Brazil. Trop. Plant Pathol. 33, 339–347. https://doi.org/10.1590/S1982-56762008000500001.
Lee, R.F., 2015. Control of virus diseases of citrus. Advances in Virus Research, 1st ed. Elsevier Inchttps://doi.org/10.1016/bs.aivir.2014.10.002.
Leeuwen, T.Van, Tirry, L., Yamamoto, A., Nauen, R., Dermauw, W., 2015. The economic

importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pestic. Biochem. Physiol. 121, 12–21.
Li, J., Aach, J., Norville, J.E., Mccormack, M., Bush, J., Church, G.M., Sheen, J., 2013. Multiplex and homologous recombination-mediated plant genome editing via guide RNA/Cas9. Nat. Biotechnol. 31, 688–691. https://doi.org/10.1038/nbt.2654.Multiplex.
Liang, Z., Zhang, K., Chen, K., Gao, C., 2014. Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. J. Genet. Genomics 41, 63–68. https://doi.org/10.1016/j.jgg.2013.12.001.
Lin, Y., Cradick, T.J., Brown, M.T., Deshmukh, H., Ranjan, P., Sarode, N., Wile, B.M., Vertino, P.M., Stewart, F.J., Bao, G., 2014. CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences. Nucleic Acids Res. 42, 7473–7485. https://doi.org/10.1093/nar/gku402.
Lindbo, J.A., Dougherty, W.G., 2005. Plant pathology and RNAi: a brief history. Annu. Rev. Phytopathol. 43, 191–204. https://doi.org/10.1146/annurev.phyto.43.040204.140228.
Liu, X., Xie, C., Si, H., Yang, J., 2017. CRISPR/Cas9-mediated genome editing in plants. Methods 121–122, 94–102. https://doi.org/10.1016/J.YMETH.2017.03.009.
Maccheroni, W., Alegria, M.C., Greggio, C.C., Piazza, J.P., Kamla, R.F., Zacharias, P.R., Bar-Joseph, M., Kitajima, E.W., Assumpção, L.C., Camarotte, G., Cardozo, J., Casagrande, E.C., Ferrari, F., Franco, S.F., Giachetto, P.F., Girasol, A., Jordão Jr, H., Silva, V.H., Souza, L.C., Aguilar-Vildoso, C.I., Zanca, A.S., Arruda, P., Kitajima, J.P., Reinach, F.C., Ferro, J.A., da Silva, A.C., 2005. Identification and genomic characterization of a new virus (Tymoviridae Family) Associated with citrus sudden death disease. J. Virol. 79, 3028–3037. https://doi.org/10.1128/JVI.79.5.3028.
Mafra, V., Martins, P.K., Francisco, C.S., Ribeiro-Alves, M., Freitas-Astúa, J., Machado, M.A., 2013. *Candidatus Liberibacter americanus* induces significant reprogramming of the transcriptome of the susceptible citrus genotype. BMC Genomics 14, 247. https://doi.org/10.1186/1471-2164-14-247.
Mallik, I., Arabiat, S., Pasche, J.S., Bolton, M.D., Patel, J.S., Gudmestad, N.C., 2014. Molecular characterization and detection of mutations associated with resistance to succinate dehydrogenase-inhibiting fungicides in *Alternaria solani*. Phytopathology 104, 40–49. https://doi.org/10.1094/PHYTO-02-13-0041-R.
Mann, S.K., Kashyap, P.L., Sanghera, G.S., Singh, G., Singh, S., 2008. RNA interference : an eco-friendly tool for plant disease management. Transgenic Plant J. 111–126 ©2008.
Mao, Y., Zhang, H., Xu, N., Zhang, B., Gou, F., Zhu, J.K., 2013. Application of the CRISPR-Cas system for efficient genome engineering in plants. Mol. Plant 6, 2008–2011. https://doi.org/10.1093/mp/sst121.
Matsumura, E.E., Della, H., Filho, C., Dorta, S.D.O., Nouri, S., Machado, M.A., 2016. Genetic structure and molecular variability analysis of Citrus sudden death-associated virus isolates from infected plants grown in Brazil. Viruses 8, 330. https://doi.org/10.3390/v8120330.
Mendonça, L., Zambolim, L., Badel, J., 2017. Bacterial Citrus diseases : major threats and recent progress bacterial Citrus diseases. J. Bacteriol. Mycol. Monroe Township (Monroe Township) 5, 1–12. https://doi.org/10.15406/jbmoa.2017.05.00143.
Miyamoto, Y., Masunaka, A., Tsuge, T., Yamamoto, M., Ohtani, K., Fukumoto, T., Gomi, K., Peever, T.L., Akimitsu, K., 2008. Functional analysis of a multicopy host-selective ACT-toxin biosynthesis gene in the tangerine pathotype of *Alternaria alternata* using RNA silencing. Mol. Plant Microbe Interact. 21, 1591–1599. https://doi.org/10.1094/MPMI-21-12-1591.
Mlotshwa, S., Voinnet, O., Mette, M.F., Matzke, M., Vaucheret, H., Ding, S.W., Pruss, G., Vance, V.B., 2002. RNA silencing and the mobile silencing signal. Plant Cell (14 Suppl), S289–S301. https://doi.org/10.1105/tpc.001677.
Mojica, F.J.M., Díez-Villaseñor, C., García-Martínez, J., Almendros, C., 2009. Short motif sequences determine the targets of the prokaryotic CRISPR defence system. Microbiology 155, 733–740. https://doi.org/10.1099/mic.0.023960-0.
Moreno, P., Ambrós, S., Albiach-Martí, M.R., Guerri, J., Peña, L., 2008. *Citrus tristeza virus*: a pathogen that changed the course of the citrus industry. Mol. Plant Pathol. 9, 251–268. https://doi.org/10.1111/j.1364-3703.2007.00455.x.
Münch, S., Ludwig, N., Floss, D.S., Sugui, J.A., Koszucka, A.M., Voll, L.M., Sonnewald, U.W.E., Deising, H.B., 2011. Identification of virulence genes in the corn pathogen *Colletotrichum graminicola* by *Agrobacterium tumefaciens*-mediated transformation. Mol. Plant Pathol. 12, 43–55. https://doi.org/10.1111/J.1364-3703.2010.00651.X.
Muniz, M. de F.S., Queiroz, F.M., Menezes, M., 2004. Caracterização de isolados de *Phytophthora* patogênicos a *Citrus sinensis* no Estado de Alagoas. Fitopatol. Bras. 29, 201–204. https://doi.org/10.1590/S0100-41582004000200014.
Nakayashiki, H., Nguyen, Q.B., 2008. RNA interference: roles in fungal biology. Curr. Opin. Microbiol. 11, 494–502. https://doi.org/10.1016/j.mib.2008.10.001.
Narayan, R.D., Blackman, L.M., Shan, W.X., Hardham, A.R., 2010. *Phytophthora nicotianae* transformants lacking dynein light chain 1 produce non-flagellate zoospores. Fungal Genet. Biol. 47, 663–671. https://doi.org/10.1016/j.fgb.2010.04.008.
Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J.D.G., Kamoun, S., 2013. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. Nat. Biotechnol. 31, 691–693. https://doi.org/10.1038/nbt.2655.
Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., Hensel, G., Kumlehn, J., Schweizer, P., 2010. HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen blumeria graminis. Plant Cell 22, 3130–3141. https://doi.org/10.1105/tpc.110.077040.
Oliveira-Garcia, E., Deising, H.B., 2013. Infection structure-specific expression of β-1,3-glucan synthase is essential for pathogenicity of *Colletotrichum graminicola* and evasion of β-glucan-triggered immunity in maize. Plant Cell 25, 2356–2378. https://doi.org/10.1105/tpc.112.103499.
Pattanayak, V., Lin, S., Guilinger, J.P., Ma, E., Doudna, J.A., Liu, D.R., 2013. High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. Nat. Biotechnol. 31, 839–843. https://doi.org/10.1038/nbt.2673.
Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L., Yao, L., Zou, X., 2017. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus. Plant Biotechnol. J. 15, 1509–1519. https://doi.org/10.1111/ijlh.12426.
Prins, M., Laimer, M., Noris, E., Schubert, J., Wassenegger, M., Tepfer, M., 2008. Strategies for antiviral resistance in transgenic plants. Mol. Plant Pathol. 9, 73–83. https://doi.org/10.1111/j.1364-3703.2007.00447.x.
Roy, A., Shao, J., Schneider, W.L., Hartung, J.S., Brlansky, R.H., 2014. Population of endogenous pararetrovirus genomes in carrizo citrange. Genome Announcements 2 (1), 13–e01063.
Qu, F., 2010. Plant viruses versus RNAi: simple pathogens reveal complex insights on plant antimicrobial defense. Wiley Interdiscip. Rev. RNA 1, 22–33. https://doi.org/10.1002/wrna.7.
Ramos-González, P.L., Chabi-Jesus, C., Guerra-Peraza, O., Breton, M.C., Arena, G.D., Nunes, M.A., Kitajima, E.W., Machado, M.A., Freitas-Astúa, J., 2016. Phylogenetic and molecular variability studies reveal a new genetic clade of *Citrus leprosis virus* C. Viruses 8, 1–25. https://doi.org/10.3390/v8060153.
Rodoni, B.C., Ahlawat, Y.S., Varma, a., Dale, J.L., Harding, R.M., 1997. Identification and characterization of *Banana Bract Mosaic Virus* in India. Plant Dis. 81, 669–672. https://doi.org/10.1094/PDIS.1997.81.6.669.
Rosa, C., Kamita, S.G., Dequine, H., Wuriyanghan, H., Lindbo, J.A., Bryce, W., 2010. RNAi effects on actin mRNAs in Homalodisca vitripennis cells. J. RNAi Gene Silencing 6, 361–366.
Rosa, C., Kamita, S.G., Falk, B.W., 2012. RNA interference is induced in the glassy winged sharpshooter *Homalodisca vitripennis* by actin dsRNA. Pest Manage. Sci. 68, 995–1002. https://doi.org/10.1002/ps.3253.
Sánchez-torres, P., Tuset, J.J., 2011. Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus. Postharvest Biol. Technol. 59, 159–165. https://doi.org/10.1016/j.postharvbio.2010.08.017.
Sasaya, T., Nakazono-Nagaoka, E., Saika, H., Aoki, H., Hiraguri, A., Netsu, O., Uehara-Ichiki, T., Onuki, M., Toki, S., Saito, K., Yatou, O., 2013. Transgenic strategies to confer resistance against viruses in rice plants. Front. Microbiol. 4, 1–11. https://doi.org/10.3389/fmicb.2013.00409.
Scott, J.G., Michel, K., Bartholomay, L.C., Siegfried, B.D., Hunter, W.B., Smagghe, G., Yan, K., Douglas, A.E., 2013. Towards the elements of successful insect RNAi. J. Insect Physiol. 59, 1212–1221. https://doi.org/10.1016/j.jinsphys.2013.08.014.
Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., Zhang, K., Liu, J., Xi, J.J., Qiu, J.-L., Gao, C., 2013. Targeted genome modification of crop plants using a CRISPR-Cas system. Nat. Biotechnol. 31, 686–688. https://doi.org/10.1038/nbt.2650.
Shang, F., Xiong, Y., Xia, W., Wei, D., 2016. Identification, characterization and functional analysis of a chitin synthase gene in the brown citrus aphid, *Toxoptera citricida* (Hemiptera, Aphididae). Insect Mol. Biol. 00. https://doi.org/10.1111/imb.12228.
Shimizu, T., Ito, T., Kanematsu, S., 2014. Functional analysis of a melanin biosynthetic gene using RNAi-mediated gene silencing in Rosellinia necatrix. Fungal Biol. 118, 413–421. https://doi.org/10.1016/j.funbio.2014.02.006.
Singh, S., Rajam, M.V., 2009. Citrus biotechnology: achievements, limitations and future directions. Physiol. Mol. Biol. Plants 15, 3–22. https://doi.org/10.1007/s12298-009-0001-2.
Sun, X., Hu, Z., Chen, R., Jiang, Q., Song, G., Zhang, H., Xi, Y., 2015. Targeted mutagenesis in soybean using the CRISPR-Cas9 system. Sci. Rep. 5, 10342. https://doi.org/10.1038/srep10342.
Sunkar, R., Zhu, J.-K., 2004. Novel and stress-regulated miRNAs and other small RNAs from Arabidopsis. Plant Cell 16 \r, 2019–2186. https://doi.org/10.1105/tpc.104.022830.The.
Tabassum, B., Sher, Z., Tariq, M., Khan, A., Shahid, N., Bilal, M., Ramzan, M., 2013. Experimental Agriculture & Horticulture Overview of Acquired Virus Resistance in Transgenic Plants 2. Natural Defense of Plants Against Viruses. pp. 12–28.
Tabassum, A., Shweta Ellur, V., 2017. Applications of CRISPR-Cas system in plant pathology: a review. Int. J. Pure Appl. Biosci. 5, 916–919.
Thakare, D., Zhang, J., Wing, R.A., Cotty, P.J., Schmidt, M.A., 2017. Aflatoxin-free transgenic maize using host-induced gene silencing. Sci. Adv. 3, 1–9. https://doi.org/10.1126/sciadv.1602382.
Trieu, T.A., Calo, S., Nicolás, F.E., Vila, A., Moxon, S., Dalmay, T., Torres-Martínez, S., Garre, V., Ruiz-Vázquez, R.M., 2015. A non-canonical RNA silencing pathway promotes mRNA degradation in basal Fungi. PLoS Genet. 11, 1–32. https://doi.org/10.1371/journal.pgen.1005168.
Tyagi, H., Rajasubramaniam, S., Rajam, M.V., Dasgupta, I., 2008. RNA-interference in rice against Rice tungro bacilliform virus results in its decreased accumulation in inoculated rice plants. Transgenic Res. 17, 897–904. https://doi.org/10.1007/s11248-008-9174-7.
Upadhyay, S.K., Kumar, J., Alok, a., Tuli, R., 2013. RNA guided genome editing for target gene mutations in wheat. G3 Genes|Genomes|Genetics 3, 2233–2238. https://doi.org/10.1534/g3.113.008847.
van West, P., Kamoun, S., van' t Klooster, J.W., Govers, F., 1999v. Internuclear gene silencing in Phytophthora infestans. Mol. Cell 3, 339–348. https://doi.org/10.1016/S1097-2765(00)80461-X.
Vieira, G., Cecílio Nerry, T., Arruda, L., Sales, K., 2016. Visão geral do mecanismo básico de ação. In: Pereira, T.C. (Ed.), Introdução à Técnica de CRISPR. Sociedade Brasileira de Genética, Ribeirão Preto p. 250.
Wang, M., Jin, H., 2017. Spray-induced gene silencing: a powerful innovative strategy for crop protection. Trends Microbiol. xx 2–3. https://doi.org/10.1016/j.tim.2016.11.011.
Wang, M.B., Abbott, D.C., Waterhouse, P.M., 2000. A single copy of a virus-derived transgene encoding hairpin RNA gives immunity to barley yellow dwarf virus. Mol. Plant Pathol. 1, 347–356. https://doi.org/10.1046/j.1364-3703.2000.00038.x.
Wang, M., Weiberg, A., Lin, F.-M., Thomma, B.P.H.J., Huang, H.-D., Jin, H., 2016.

Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nat. Plants 2, 16151. https://doi.org/10.1038/nplants.2016.151.
Wang, N., Pierson, E.A., Setubal, C., Xu, J., Levy, J.G., Zhang, Y., Li, J., Rangel, L.T., Martins Jr, J., 2017. The candidatus liberibacter–host interface: insights into pathogenesis mechanisms and disease control. Annu. Rev. Phytopathol. 55, 1–32.
Weiberg, A., Wang, M., Lin, F.-M., Zhao, H., Zhang, Z., Khaloshian, I., Huang, H.-D., Jin, H., 2014. Fungal small RNAs suppress plant immunity by hijacking host. Science (80-.) 342, 118–123. https://doi.org/10.1126/science.1239705.Fungal.
Wolt, J.D., Wang, K., Yang, B., 2016. The regulatory status of genome-edited crops. Plant Biotechnol. J. 14, 510–518. https://doi.org/10.1111/pbi.12444.
Woo, J.W., Kim, J., Kwon Il, S., Corvalán, C., Cho, S.W., Kim, H., Kim, S.-G., Kim, S.-T., Choe, S., Kim, J.-S., 2015. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat. Biotechnol. 33, 1162–1164. https://doi.org/10.1038/nbt.3389.
Wynant, N., Santos, D., Vanden Broeck, J., 2014. Biological mechanisms determining the success of RNA interference in insects. Int. Rev. Cell Mol. Biol. https://doi.org/10.1016/B978-0-12-800178-3.00005-1. 1st ed, Elsevier Inc.
Yang, Y., Sherwood, T.A., Patte, C.P., Hiebert, E., Polston, J.E., 2004. Use of tomato yellow leaf curl virus (TYLCV) Rep Gene Sequences to engineer TYLCV resistance in tomato. Phytopathology 94, 490–496. https://doi.org/10.1094/PHYTO.2004.94.5.490.
Yin, K., Gao, C., Qiu, J.-L., 2017. Progress and prospects in plant genome editing. Nat. Plants 3, 17107. https://doi.org/10.1038/nplants.2017.107.
Yu, N., Christiaens, O., Liu, J., Niu, J., Cappelle, K., Caccia, S., Huvenne, H., Smagghe, G., 2013. Delivery of dsRNA for RNAi in insects: an overview and future directions. Insect Sci. 20, 4–14. https://doi.org/10.1111/j.1744-7917.2012.01534.x.
Yu, X., Gowda, S., Killiny, N., 2017. Double stranded RNA delivery through soaking, mediates silencing of the muscle protein 20 and increases mortality to the Asian citrus psyllid. Pest Manage. Sci. 73, 1846–1853.
Zhang, F., Maeder, M.L., Unger-Wallace, E., Hoshaw, J.P., Reyon, D., Christian, M., Li, X., Pierick, C.J., Dobbs, D., Peterson, T., Joung, J.K., Voytas, D.F., 2010. High frequency targeted mutagenesis in *Arabidopsis thaliana* using zinc finger nucleases. Proc. Natl. Acad. Sci. 107, 12028–12033. https://doi.org/10.1073/pnas.0914991107.
Zhang, M.X., Wang, Q.H., Xu, K., Meng, Y.L., Quan, J.L., Shan, W.X., 2011. Production of dsRNA sequences in the host plant is not sufficient to initiate gene silencing in the colonizing oomycete pathogen *Phytophthora parasitica*. PLoS One 6, e28114. https://doi.org/10.1371/journal.pone.0028114.
Zhang, F., Leblanc, C., Irish, V.F., Jacob, Y., 2017a. Rapid and efficient CRISPR/Cas9 gene editing in citrus using the YAO promoter. Plant Cell Rep. 36, 1883–1887. https://doi.org/10.1007/s00299-017-2202-4.
Zhang, Y., Wang, N., Schiemann, J.H., Kühn-institut, J., 2017b. Editing Citrus genome via SaCas9/sgRNA system. Front. Plant Sci. 8, 1–9. https://doi.org/10.3389/fpls.2017.02135.
Zhu, L., Zhu, J., Liu, Z., Wang, Z., Zhou, C., Wang, H., 2017. Host-induced gene silencing of rice blast fungus *Magnaporthe oryzae* pathogenicity genes mediated by the brome mosaic virus. Genes (Basel) 8. https://doi.org/10.3390/genes8100241.
Zotti, M., Cagliari, D., Christiaens, O., Tizi, N., Smagghe, G., 2017. RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest Manage. Sci. 1–13. https://doi.org/10.1002/ps.4813.