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REVIEW **Open Access**

Trans-kingdom sRNA silencing in the prevention and control of crop *Fusarium* wilt disease

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Abstract

The *Fusarium oxysporum* species complex (FOSC), comprising a multitude of soil borne fungal pathogens, represent a major group of plant invaders that cause vascular wilt diseases of crops worldwide. Although sexual reproduction is nebulous in the FOSC, horizontal gene transfer (also well known as trans-kingdom) may contribute to the observed diversity in pathogenic strains. Concerning the safety and costly process of generating genetically modified crops, a plant-disease management strategy not reliant on transgenic approaches remains the most economic and environmental-friendly option. Here, we highlight the current scenario via beginning with *F. oxysporum* formae speciales and races as well as caused *Fusarium* wilt disease in field. Subsequently, based on the mechanisms by which small RNA (sRNA) acts in gene silencing, especially in bidirectionally trans-kingdom sRNA silencing between *F. oxysporum* and their hosts in the development of disease, we summarize technological breakthroughs like Spray-Induced Gene Silencing (SIGS) technology in the developing and applying to protect crops from *Fusarium* wilt disease. Given that SIGS has been developed and recently applied in controlling crop *Fusarium* wilt diseases, we propose a potential prevention and control system to exploit crop *Fusarium*-vasculature interaction leading to further study trans-kingdom sRNA silencing.

Keywords *Fusarium oxysporum* species complex, Trans-kingdom sRNA, SIGS, *Fusarium* wilt disease resistance

Background

Global crop production is severely challenged by a variety of fungal diseases, of which caused by soil borne fungal pathogenic *Fusarium oxysporum* species complex (FOSC) is one of the most destructive (Wade 1929; Haware et al. 1978; Smith et al. 1999; Fall et al. 2001). Rather than separate taxa, host-specific strains of *F. oxysporum* are treated such biologic forms as variants of a single species following assigned to *formae speciales* (f. sp.). The *forma speciales* designation had no taxonomic standing but served to facilitate communication among plant pathologists (Snyder and Hansen 1940). Presently, *F. oxysporum* occurs as pathogenic (plant, animal, and human) and non-pathogenic strains. Following entry of plant root, *Fusarium* wilt pathogen colonize in the vascular system to destroy plant root-water continuum resulting in wilting symptoms and death of plant eventually (Schäfer 1994).

Since small RNA (sRNA)-mediated RNA interference (RNAi) discovered as a conserved regulatory mechanism in almost all eukaryotes, trans-kingdom RNAi is convinced playing a critical role in host pathogen interactions (Knip et al. 2014; Huang et al. 2019; Liu et al. 2016). Recently, it is discovered that fungus-derived natural trans-kingdom sRNAs are transmitted into plant hosts during infection, functioning as RNA effectors

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to suppress host immunity. On the other hand, host-derived natural trans-kingdom sRNAs are also confirmed to transmit from host plants into the fungal pathogens (Weiberg et al. 2013; Zhang et al. 2016; Ji et al. 2021). Intriguingly, these discoveries prompt the development of Spray-Induced Gene Silencing (SIGS) for plant disease management. As a non-GMO (genetically modified organisms) alternative to Host-Induced Gene Silencing (HIGS), SIGS attenuates pathogen infection by topical application of dsRNA or sRNA molecules to silence pathogen virulence-related genes (Song et al. 2018; Qiao et al. 2021; Ouyang et al. 2023).

This review synthesizes information from studies of FOSC that pertain to its activities as the cause of field crop *Fusarium* wilt diseases to highlight important gaps in our knowledge. Important considerations for generating successful SIGS strategies against FOSC will also be emphasized as a powerful and eco-friendly method for crop protection from *Fusarium* wilt diseases. These include characteristics of effective gene targets and their pathways, and new insights into sRNAs properties impacting on cellular uptake and silencing efficiency. The extensive literatures on the genetic and biochemical interplay between host and FOSC is beyond the scope of this review. The interested reader can find many useful points to this rapidly expanding body of knowledge.

F. oxysporum formae speciales and races

The latest review recorded *F. oxysporum formae speciales* and races that many scientists still refer to dates back to thirty years (Buxton 1957; Armstrong and Armstrong 1981; Correll et al. 1986). To date, *F. oxysporum* comprises more than 200 host-specific strains (*formae speciales*), including plant pathogens, animal pathogens, and nonpathogens, many of which have worldwide distributions (O'Donnell et al. 2009; Geldner 2013). From a practical point of view, pathogenic FOSC can invade perennial and annual plants, including mostly land-based as well as many aquatic plants. *F. oxysporum* strains lead to either wilts or root/crown rots on economically main field crops (e.g. cotton, banana, and soybean), important market garden crops (e.g. tomato, onion, and melon), grain legumes (e.g. cowpea, chickpea, and faba bean), ornamental crops (e.g. cyclamen, orchids, and gerbera), as well as and even on broom or rapewitchweed (e.g. parasitic plants, and weeds) (Abawi and Lorbeer 1972; Brayford 1996; Olivain and Alabouvette 1999; Gao et al. 2021).

Intriguing individual strains display developing selective pathogenicity to a relative narrow range of host plants, which is defined as a *forma speciales*. For example, strains responsible for *Fusarium* wilt of tomato belong to the *forma speciales lycopersici*. Actually, the concept '*forma speciales*' is first formulated early to distinguish strains of *Puccinia graminis* Pers. similar morphological characteristics but different host ranges (Stakman 1913; Edel-Hermann and Lecomte 2019; Favre et al. 2023). Among known *forma speciales*, more than 100 *formae speciales* are well documented. The host range of these *formae speciales* consists of plants belonging to 45 families (Asteraceae, Cucurbitaceae, Fabaceae, and Solanaceae are the most represented) (Poli et al. 2012; Pastrana et al. 2017). More than 30 *formae speciales* are considered as insufficiently documented. These pathogens isolated from diseased plants and assigned to a *forma speciales* without confirming their pathogenicity, or, host specificity of these *formae speciales* are not analyzed yet (Armstrong and Armstrong 1981; Gordon et al. 2015). Furthermore, 58 host plant species in the literature are recorded and described as susceptible to *F. oxysporum* but whose *forma speciales* has not been characterized so far. Such a wide geographical distribution and wide host spectrum confirms the adaptability of FOCS to the diverse biotic and abiotic environmental conditions encountered worldwide (Steinberg et al. 2016; Edel-Hermann and Lecomte 2019).

However, continuously increasing diversity in FOCS and within *formae speciales* may be revealed over time with the new plant genotype derived from breeding. This growth may reflect a worrying trend, as more and more new varieties are described as special varieties related to the resurgence of diseases in market horticultural and ornamental crops, as well as large-scale crops. For example, many *formae speciales* are well known to be polyphyletic obstructing to identify specific molecular markers (Baayen et al. 2000; Fourie et al. 2009; Koyyappurath et al. 2015). Meanwhile, both soil-borne and endophytic non-pathogenic varieties of *F. oxysporum* exhibit high genetic variability and are closely related to pathogenic isolates (Edel et al. 2001; Inami et al. 2014; Imazaki and Kadota 2015). For this, desirable tools, such as molecular markers, are urgently developed to detect the presence and activity of pathogenic FOCS isolates, as well as discriminate between races and *formae speciales*.

Factors influencing wilt diseases caused by *F. oxysporum*

Pathogenic *F. oxysporum* strains are responsible for two typical types of symptoms, vascular wilting and rotting in roots (without reaching the vascular system). According to the Committee on Standardization of Common names for plant diseases (http://www.apsnet.org), when pathogens penetrate the host roots to reach the xylem vessels, *F. oxysporum* causes vascular wilting which colonizes upwards, resulting in progressive yellowing and wilting of the plant, associating with several disease names as *Fusarium* yellows, *Fusarium* blight, and *Fusarium* wilt. Meanwhile, diseases with rotting symptoms are called basal rot, *Fusarium* stem rot, or crown and root rot (Jarvis and Shoemaker 1978; Olivain and Alabouvette 1999). However, some plants, for example tomato, can be attacked by *formae speciales lycopersici* and *radicis-lycopersici* causing vascular wilting and root rot, respectively (Vakalounakis 1996).

Most of the *Fusarium forma speciales* are considered as hemi-biotrophic plant pathogens, killing host by producing reactive oxygen species (ROS), secreting cell wall degrading enzymes (CWDE), and phytotoxic molecules (Desmond et al. 2008; Fones et al. 2020). During the course of invasion, phytopathogenic fungi are perceived through plant cell surface receptors that recognize pathogen-associated molecular patterns (PAMP), secreted by pathogens or released by the action of lytic enzymes during the interaction with the host. PAMPs (including but not limited phospholipomannan, ß-glucans, mannoproteins, and chitin) further activate the first line of defense well-known as PAMP-triggered immunity (PTI) in plants (Jones and Dangl 2006; Kanyuka and Rudd 2019; Pierzgalski et al. 2021). Phytopathogenic fungi evolve to counteract PTI through the production and secretion of molecules called effectors, which initiate the second line of plant defense called effector-triggered immunity (ETI) through the interaction with host intracellular receptors (Jones and Dangl 2006). In most case, investigation of the function of effectors is impeded by the redundancy of homologous genes, therefore, the most promising alternative is to isolate these effectors to uncover new metabolites and proteins associated with pathogenicity or virulence (Müller et al. 2008).

Fungal virulence is termed as a capacity causing damage to the host masking any obvious phenotype (wilt syndrome). Thus, factors influencing wilt diseases caused by *F. oxysporum* are any molecule that enhances the pathogenicity of the pathogen by interfering with host particular functions. These virulence factors (VF) can be categorized according to the chemical nature, the acting site, and the interaction style. Basically, VF can be classified into two large groups: low molecular weight metabolites (e.g. PAMPs) and proteins (e.g. effectors) (Kraft 1994; Ibrahim et al. 2021). Moreover, nitrogen-containing metabolites, such as ammonium or glutamine, play as critical signal maintaining the expression level of virulence genes in *F. oxysporum* (Wong et al. 2008; López-Berges et al. 2010). Specifically, secreted in xylem (SIX), a group of small effector proteins in the xylem secreted by *F. oxysporum*, are well documented contributing to *F. oxysporum* virulence. So far, fourteen SIXes have been verified, with no surprise, *formae speciales* vary in SIX protein profile and respective gene sequence (Rep et al. 2004; Maldonado Bonilla et al. 2018; Adusei-Fosu and Dickinson 2019; Lyons et al. 2019). The main VFs reported to date specially for *F. oxysporum* f. sp. *lycopersici* are summarized in Table 1. All listed VF are from species *F. oxysporum* f. sp. *lycopersici*, with exception SIX from f. sp. *lycopersici*, *cubense*, *conglutinans*, and *melonis* respectively, and FoEG1 from f. sp. *lycopersici* and *vasinfectum* respectively. Among these VF, PGs, Tomatinase,

Table 1 Main proteins reported as VF from *F. oxysporum* f. sp. *lycopersici*

VF	Annotation	Host	Biological function	References
PGs	Poligalacturonases	Tomato	Degradation of pectin in host plant cell walls	He et al. (2023), Powell et al. (2000)
Tomatinase	Glycosidase	Tomato	A saponin with antifungal activity cleaving Alpha-tomatine into aglycon tomatidine and tetrasaccharide lycotetraose	Carere et al. (2017), Pareja-Jaime et al. (2008), Ito et al. (2004)
FoRnt2	Ribonuclease T2	Tomato	RNA degradation in host plant	Qian et al. (2022b)
FoMep1	Metalloprotease	Tomato	Cleaving host plant chitinases by removing the chitin binding site	Jashni et al. (2015)
FoAPY	Aminopeptidase Y	Tomato	Altering the abundance of host plant proteins	Pautot et al. (2001), Qian et al. (2022a)
FoSep1	Serine protease	Tomato	Cleaving host plant chitinases by removing the chitin binding site	Jashni et al. (2015)
SIX	Secreted in Xylem proteins	Tomato, banana, cabbage, cucumber	Effector to Enhance pathogen virulence and suppresses cell death during plant-pathogen interaction	Rep et al. (2004), Gawehns et al. 2014, Ma et al. 2015, Maldonado Bonilla et al. (2018), Widinugraheni et al. 2018, Adusei-Fosu and Dickinson (2019), Lyons et al. (2019), Jangir et al. 2021
FoEG1	Glycoside hydrolase	Tomato, cotton	Cellulase activity to trigger the hypersensitive response	Zhang et al. (2021)

FoMep1, FoSep1, and FoEG1 are located in the apoplast, FoRnt2, FoAPY, and SIX are translocated into the host cell cytoplasm. However, the functional annotation of these VF (or proteins) is not full addressed yet and it is unknown how they contribute to virulence.

Trans-kingdom sRNA silencing in *Fusarium oxysporum*-plant interactions

Small RNAs (sRNAs) are 20–30 nucleotide (nt)-long non-coding RNA molecules, which are divided into two major classes: microRNAs (miRNAs) and small interfering RNAs (siRNAs). Most miRNAs are 18–24 nt long and derived from single-stranded RNA precursors with imperfectly base-paired hairpin structures. sRNAs are universally distributed in all kingdoms of life from bacteria, archaea to various eukaryotic lives. It is well established that sRNAs contributes to almost all eukaryotic cellular processes, which have not ceased to surprise us throughout the last three decades regarding their functional and compositional diversity (Grosshans and Filipowicz 2008; Carthew and Sontheimer 2009; Guo et al. 2019; Chen and Rechavi 2022). Since the first miRNA reported in *Caenorhabditis elegans* in 1993, researchers have begun to explore the generation mechanism and biological functions of microRNAs, as well as the key role in gene expression regulation (Lee et al. 1993; Reinhart et al. 2000). The Nobel Assembly at Karolinska Institutet awarded the 2024 Nobel Prize in Physiology or Medicine jointly to scientists Victor Ambros and Gary Ruvkun 'for their discovery of microRNA and its role in post-transcriptional gene regulation'. The groundbreaking discovery reveals an entirely new dimension to gene regulation, meanwhile, inspires the wide application in plant protection.

RNA interference (RNAi, or RNA silencing) mechanisms have been studied comprehensively in different organisms in many biological processes (Hannon 2002; Baulcombe 2004). Bidirectional transmission of sRNAs between host plants and fungal pathogens has been documented to influence host-fungus interactions, which provided direct evidence of natural trans-kingdom RNAi, although fungal RNAi mechanisms still require further investigation (Wen et al. 2023). In virus-induced gene silencing (VIGS), the siRNA specificity determinant is derived from the viral RNA. Meanwhile, emerging body of evidence show that plants and filamentous microorganisms employ RNAi to influence each other, which lead to host induced gene silencing (HIGS) affecting gene expression in fungal. However, filamentous organism-induced gene silencing (FIGS) acts in the opposite direction (Baulcombe 2015).

Recently, several studies illustrated that spraying double-stranded RNAs (dsRNAs)/sRNAs on plant surfaces confers efficient crop protection through targeting essential pathogen genes (Koch et al. 2013, 2016; Wang et al. 2016; Qiao et al. 2021; Ouyang et al. 2023). Thus, a termed spray-induced gene silencing (SIGS) is known as a powerful, fast, sustainable, and environmenta-friendly strategy to circumvent the problems in creating GMOs (Wang and Jin 2017). Previously, we explored the RNAi-based SIGS strategy using direct application of *FolRDR1*-dsRNA to protect tomato wilt disease caused by *F. oxysporum* f. sp. *lycopersici* (*Fol*). Our data alternatively demonstrated that *FolRDR1* mediated the pathogen development and pathogenicity. Both *Fol* and tomato efficiently took up *FolRDR1*-dsRNA from the environment through spraying on tomato seedling leaves. Further, exogenous application of *FolRDR1*-dsRNAs significantly alleviated the progress of tomato wilt disease symptoms. More than intriguing, the fluorescence signals of *FolRDR1*-dsRNAs were dominantly localized in the host vascular bundles where microconidia spore are produced and disseminated. Employing the vascular bundles as transport corridor, endogenetic hypha spreading to above ground tissues is critical for disease progression for *Fol* (Ouyang et al. 2023). Similarly, spraying application of 791 nt noncoding *CYP3*-dsRNA alleviates the growth of pathogen using the agronomically barley—*F. graminearum* pathosystem. After uptake via the plant vascular system by the pathogen, CYP3-dsRNAs are processed into small interfering (si)RNAs by fungal DICER-LIKE 1 (FgDCL-1), then target three fungal ergosterol biosynthesis genes (*CYP51A*, *CYP51B*, *CYP51C*) in soil-borne pathogen *F. graminearum* (Koch et al. 2016). These successful SIGS studies show that such specific *Fusarium* pathogen gene-targeting RNAs represents a new generation of environmentally-friendly fungicides to control vascular wilt disease (Fig. 1).

Given the ease of design, high specificity, and applicability to diverse pathogens, and environmente-friendly compared with traditional fungicides and creating GMOs, SIGS immediately attracts the attention of researchers as a new plant protection strategy. To serve as an efficient 'RNA fungicides', a reasonable duration of efficacy is concerned and desired for the SIGS strategy. The most challenge of the SIGS technology is the relative instability of RNAs in the natural environment. Strikingly, application strategies can be improved by inorganic/organic nanoparticles as carriers to stabilize the RNAs and thus increase the strength and duration of plant protection (Joga et al. 2016; Christiaens et al. 2018; Schwartz et al. 2020; Martinez et al. 2021). However, there are still several questions need to be addressed, (i) what are the optical properties (e.g., length, secondary structure formation, etc.) of dsRNA fragment for nanoparticles? (ii) how to avoid off-target silencing which

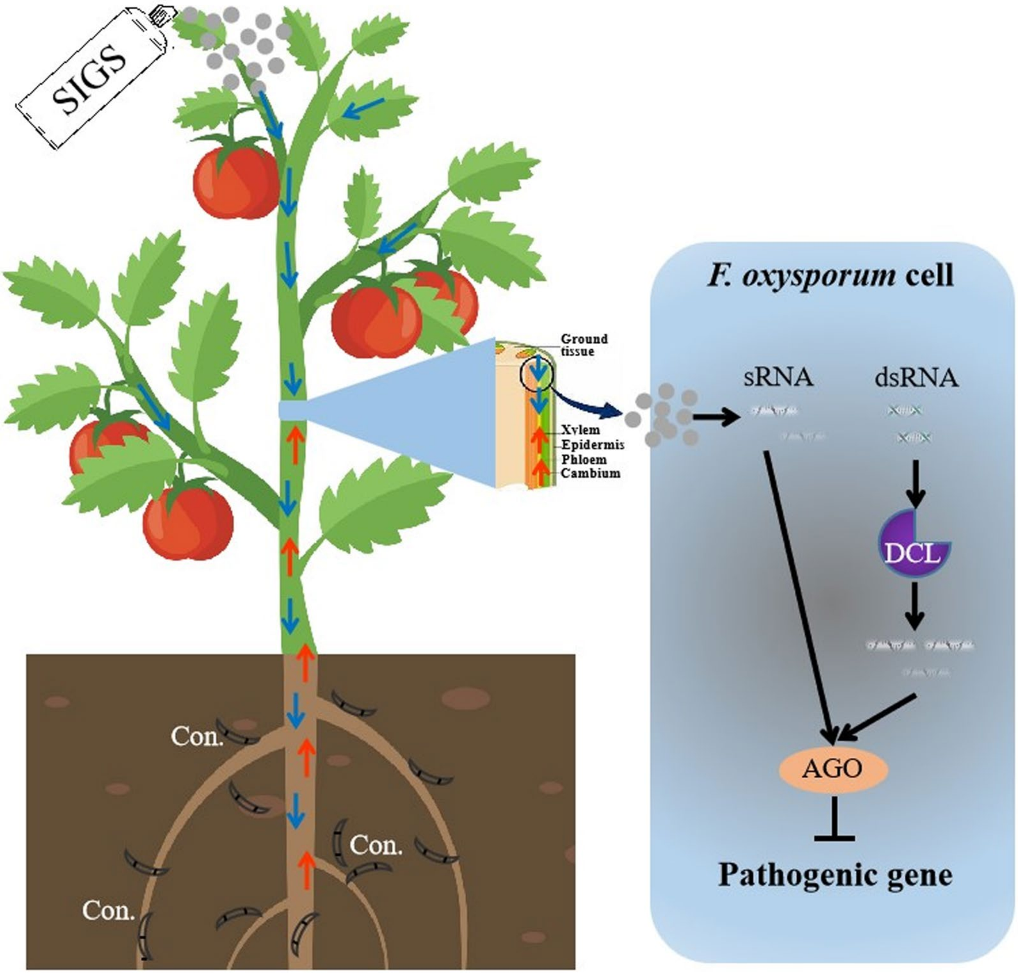

Fig. 1 SIGS strategy to protect crops from the invasion of fungal pathogens *F. oxysporum*. Sprayed exogenous dsRNAs/sRNA can be taken directly into *Fusarium* cells on the plant vascular system or by first passing through the cells of the plant. In this case, the dsRNAs/sRNA are translocated through vascular system before delivery to the fungal hyphae or conidia. Alternatively, the exogenous processed and/or unprocessed dsRNA, as well as sRNA, are taken into the fungal cell either by haustorial feeding, endocytosis, or vesicle trafficking (the certain entry mechanism of dsRNA/sRNA into the fungal cell still to be determined). In *Fusarium* cell, the fungal RNAi machinery is guided by siRNAs derived from the exogenous dsRNA to mediate particular target gene silencing, finally leading to impair the pathogenicity of *Fusarium* pathogens. dsRNAs are processed by DCL family to produce siRNAs and subsequently loaded into the AGO complexes to induce targeted gene silencing (Hannon 2002; Baulcombe 2004; Koch et al. 2016; Ouyang et al. 2023). DCL, Dicer-like protein; AGO, Argonaute protein; Con, conidia; Grey dots, dsRNAs/sRNA. Red arrows, the moving direction of pathogens. Blue arrows, the moving direction of dsRNAs/sRNA

may lead to biosafety concerns? (iii) what's the balance of RNA uptake efficiency between host plant and pathogen?

Conclusion: SIGS in *F. oxysporum* infections—Where do we stand?

Devastating vascular wilt caused by *F. oxysporum*, curtailing the production of economically important crops worldwide, has become a menace to farmers. The emergence of Spray-Induced Gene Silencing (SIGS) is a native strategy for crop protection utilizing exogenously applied non-coding RNA (double-stranded RNA or sRNA) to specifically reduce pathogenic target gene expression. Once a disease-resistant phenotype has been developed with SIGS against an accurate target, further optimization of the applied RNA sequence may further increase efficacy (Sharath Chandra et al. 2019; Kweon et al. 2022). The efficiency of non-coding sRNA-mediated gene silencing depends on uptake, processing to siRNAs, accessibility of the target region of the gene, and forming RNA-induced silencing complex (RISC) together with a member of the Argonaute (AGO) protein family (Reynolds et al. 2004; Lo Presti et al. 2015; Fakhr et al. 2016).

Since *B. cinerea* is found to take up exogenous dsRNAs naturally, SIGS technology has been developed for crop protection against fungal pathogens (Wang et al. 2016; Wang and Jin 2017). In December 22, 2023, the U.S. Environmental Protection Agency (EPA) has registered biopesticide products containing the new active ingredient Ledprona (https://www.epa.gov). Ledprona is a new type of pesticide that relies on RNA interference (RNAi) used to protect against disease in plants and animals. Ledprona is a sprayable dsRNA product that targets the Colorado potato beetle (CPB), a major pest of potato crops, to kill the pest by 'silencing' the CPB gene needed to produce the PSMB5 protein. This RNAi-based pesticide is the first SIGS-dsRNA pesticide in the world allowed to be used commercially and sprayed on plants (Rodrigues et al. 2021; Pallis et al. 2022). With the examples discussed in this review, it is conceivable that SIGS-based technology can be deeply elucidated and exploited to further understand crop *Fusarium* wilt disease. Therefore, the interaction of plants and vascular pathogens is an ideal model for the study of trans-kingdom RNAi, which are relatively more diverse and complex than oomycetes, bacteria, and viruses. Comparative pathogen genomics and deep sRNA sequencing based on microbiome projects would promote exploration of key trans-kingdom sRNAs involved in plant—*F. oxysporum* interactions, providing new efficient targets for crop protection tools such as SIGS.

Abbreviations

AGO	Argonaute protein
Con	Conidia
CPB	Colorado potato beetle
CWDE	Cell wall degrading enzymes
DCL	Dicer-like protein
dsRNA	Double strand RNA
ETI	Effector-triggered immunity
FIGS	Filamentous organism-induced gene silencing
Fol	*Fusarium oxysporum* F. sp. *lycopersici*
FOSC	*Fusarium oxysporum* species complex
GMO	Genetically modified organisms
HIGS	Host induced gene silencing
PAMP	Pathogen-associated molecular patterns
PTI	Pathogen-triggered immunity
RNAi	RNA interference
ROS	Reactive oxygen species
SIGS	Spray-induced gene silencing
SIX	Secreted in xylem
sRNA	Small RNA
VF	Virulence factor
VIGS	Virus-induced gene silencing

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Author contributions

SO contributed to the study conception and design. XH and YW drew the diagrams and tables. YL and YX performed the data collection. SO, XH, and YW analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

Abawi GS, Lorbeer JW. Several aspects of the ecology and pathology of *Fusarium oxysporum* f. sp. cepae. Phytopathology. 1972;62:870–6.
Adusei-Fosu K, Dickinson M. Development of pathogenicity assay and characterization of *Fusarium oxysporum* f. sp. elaeidis (FOE) based on Secreted In Xylem genes and EF-1a. J Plant Pathol. 2019;101:1013–24.
Armstrong GM, Armstrong JK. *Formae speciales* and races of *Fusarium oxysporum* causing wilt diseases. *Fusarium*: Disease, Biology, and Taxonomy. The Pennsylvania State University Press, University Park, London. 1981; 391–99.
Baayen RP, O'Donnell K, Bonants PJ, Cigelnik E, Kroon LP, Roebroeck EJ, et al. Gene genealogies and aflp analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic *formae speciales* causing wilt and rot disease. Phytopathology. 2000;90(8):891–900.
Baulcombe D. RNA silencing in plants. Nature. 2004;431:356–63.
Baulcombe DC. VIGS, HIGS and FIGS: small RNA silencing in the interactions of viruses or filamentous organisms with their plant hosts. Curr Opin Plant Biol. 2015;26:141–6.
Brayford D. *Fusarium oxysporum* f. sp. cepae (Hanzawa) Snyder & Hansen I.M.I. Descr Pathog Fungi Bact. 1996;133:39–40.
Buxton EW. Some effects of pea root exudates on physiologic races of *Fusarium oxysporum* Fr. f. pisi (Linf) Snyder & Hansen. Trans Br Mycol Soc. 1957;40:145–54.
Carere AH, Benfield M, Ollivier CJ, Liu K, Kazan, Gardiner DM. A tomatinase-like enzyme acts as a virulence factor in the wheat pathogen *Fusarium graminearum*. Fungal Genet Biol. 2017;100:33–41.
Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136:642–55.
Chen X, Rechavi O. Plant and animal small RNA communications between cells and organisms. Nat Rev Mol Cell Biol. 2022;23:185–203.
Christiaens O, Tardajos MG, Martinez Reyna ZL, Dash M, Dubruel P, Smagghe G. Increased RNAi efficacy in *Spodoptera exigua* via the formulation of dsRNA with guanylated polymers. Front Physiol. 2018;9:316–25.
Correll JC, Puhalla JE, Schneider RW. Identification of *Fusarium oxysporum* f. sp. apii on the basis of colony size, virulence, and vegetative compatibility. Phytopathology. 1986;76:396–400.
Desmond OJ, Manners JM, Stephens AE, Maclean DJ, Schenk PM, Gardiner DM, et al. The *Fusarium mycotoxin* deoxynivalenol elicits hydrogen peroxide production, programmed cell death and defence responses in wheat. Mol Plant Pathol. 2008;9(4):435–45.

Edel V, Steinberg C, Gautheron N, Recorbet G, Alabouvette C. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. FEMS Microbiol Ecol. 2001;36:61–71.

Edel-Hermann V, Lecomte C. Current status of *Fusarium oxysporum formae speciales* and races. Phytopathology. 2019;109(4):512–30.

Fakhr E, Zare F, Teimoori-Toolabi L. Precise and efficient siRNA design: a key point in competent gene silencing. Cancer Gene Ther. 2016;23:73–82.

Fall AL, Byrne PF, Jung G, Coyne DP. Detection and mapping of a major locus for *Fusarium* wilt resistance in common bean. Crop Sci. 2001;41:1494–8.

Favre F, Jourda C, Grisoni M, Chiroleu F, Dijoux JB, Jade K, et al. First *Vanilla planifolia* high-density genetic linkage map provides quantitative trait loci for resistance to *Fusarium oxysporum*. Plant Dis. 2023;107(10):2997–3006.

Fones HN, Bebber DP, Chaloner TM, Kay WT, Steinberg G, Gurr SJ. Threats to global food security from emerging fungal and oomycete crop pathogens. Nat Food. 2020;1(6):332–42.

Fourie G, Steenkamp ET, Gordon TR, Viljoen A. Evolutionary relationships among the *Fusarium oxysporum* f. sp. cubense vegetative compatibility groups. Appl Environ Microbiol. 2009;75(14):4770–81.

Gao Y, Li SJ, Zhang SW, Feng T, Zhang ZY, Luo SJ, et al. SlymiR482e-3p mediates tomato wilt disease by modulating ethylene response pathway. Plant Biotechnol J. 2021;19(1):17–9.

Gawehns PM, Houterman FA, Ichou CB, Michielse M, Hijdra BJC, Cornelissen M, et al. The *Fusarium oxysporum* effector Six6 contributes to virulence and suppresses I-2-mediated cell death. Mol Plant-Microbe Interact. 2014;27:336–48.

Geldner N. The endodermis. Annu Rev Plant Biol. 2013;64:531–58.

Gordon TR, Swett CL, Wingfield MJ. Management of *Fusarium* diseases affecting conifers. Crop Prot. 2015;73:28–39.

Grosshans H, Filipowicz W. Molecular biology: the expanding world of small RNAs. Nature. 2008;451:414–6.

Guo Z, Li Y, Ding SW. Small RNA-based antimicrobial immunity. Nat Rev Immunol. 2019;19:31–44.

Hannon GJ. RNA interference. Nature. 2002;418:244–51.

Haware MP, Nene YL, Rajeshwari R. Eradication of *Fusarium oxysporum* f. sp. ciceri transmitted in chickpea seed. Phytopathology. 1978;68:1364–7.

He P, Zhang J, Lv Z, Cui P, Xu X, George MS, et al. Genome-wide identification and expression analysis of the polygalacturonase gene family in sweetpotato. BMC Plant Biol. 2023;23(1):300.

Huang CY, Wang H, Hu P, Hamby R, Jin H. Small RNAs - big players in plant-microbe interactions. Cell Host Microbe. 2019;26(2):173–82.

Ibrahim SRM, Sirwi A, Eid BG, Mohamed SGA, Mohamed GA. Bright side of *Fusarium oxysporum*: secondary metabolites bioactivities and industrial relevance in biotechnology and nanotechnology. J Fungi (Basel). 2021;7(11):943.

Imazaki I, Kadota I. Molecular phylogeny and diversity of *Fusarium endophytes* isolated from tomato stems. FEMS Microbiol Ecol. 2015;91:098.

Inami K, Kashiwa T, Kawabe M, Onokubo-Okabe A, Ishikawa N, Pérez ER, et al. The tomato wilt fungus *Fusarium oxysporum* f. sp. lycopersici shares common ancestors with nonpathogenic *F. oxysporum* isolated from wild tomatoes in the Peruvian Andes. Microbes Environ. 2014;29:200–10.

Ito S, Eto T, Tanaka S, Yamauchi N, Takahara H, Ikeda T. Tomatidine and lycotetraose, hydrolysis products of alpha-tomatine by *Fusarium oxysporum* tomatinase, suppress induced defense responses in tomato cells. FEBS Lett. 2004;571(1–3):31–4.

Jangir P, Mehra N, Sharma K, Singh N, Rani M, Kapoor R. Secreted in Xylem Genes: drivers of host adaptation *in Fusarium oxysporum*. Front Plant Sci. 2021;22(12):628611.

Jarvis WR, Shoemaker RA. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology. 1978;68:1679–80.

Jashni MK, Dols IHM, Iida Y, Boeren S, Beenen HG, Mehrabi R, et al. Synergistic action of a metalloprotease and a serine protease from *Fusarium oxysporum* f. sp. lycopersici cleaves chitin-binding tomato chitinases, reduces their antifungal activity, and enhances fungal virulence. Mol Plant Microbe Interact. 2015;28:996–1008.

Ji HM, Mao HY, Li SJ, Feng T, Zhang ZY, et al. *Fol*-milR1, a pathogenicity factor of *Fusarium oxysporum*, confers tomato wilt disease resistance by impairing host immune responses. New Phytol. 2021;232(2):705–18.

Joga MR, Zotti MJ, Smagghe G, Christiaens O. RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: what we know so far. Front Physiol. 2016;7:553–68.

Jones JDG, Dangl JL. The plant immune system. Nature. 2006;444:323–9.

Kanyuka K, Rudd JJ. Cell surface immune receptors: the guardians of the plant's extracellular spaces. Curr Opin Plant Biol. 2019;50:1–8.

Knip M, Constantin ME, Thordal-Christensen H. Trans-kingdom cross-talk: small RNAs on the move. PLoS Genet. 2014;10(9):e1004602.

Koch A, Kumar N, Weber L, Keller H, Imani J, Kogel KH. Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase-encoding genes confers strong resistance to *Fusarium* species. Proc Natl Acad Sci USA. 2013;110(48):19324–9.

Koch A, Biedenkopf D, Furch A, Weber L, Rossbach O, Abdellatef E, et al. 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. 2016;12(10):e1005901.

Koyyappurath S, Conéjéro G, Dijoux JB, Lapeyre-Montès F, Jade K, Chiroleu F, et al. Differential responses of vanilla accessions to root rot and colonization by Fusarium oxysporum f sp radicis-vanillae. Front Plant Sci. 2015;6:1125.

Kraft JM. *Fusarium* wilt of peas a review. Agronomie. 1994;14:561–7.

Kweon Y, Fang M, Shin SY, Lee D, Kim KH, Shin C. Sequence optimization and multiple gene-targeting improve the inhibitory efficacy of exogenous double-stranded RNA against pepper mottle virus in *Nicotiana benthamiana*. Appl Biolog Chem. 2022;65:87–96.

Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843–54.

Liu S, da Cunha AP, Rezende RM, Cialic R, Wei Z, Bry L, et al. The host shapes the gut microbiota via fecal MicroRNA. Cell Host Microbe. 2016;19(1):32–43.

Lo Presti L, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M, Zuccaro A, et al. Fungal effectors and plant susceptibility. Annu Rev Plant Biol. 2015;66:513–45.

López-Berges MS, Rispail N, Prados-Rosales RC, Di Pietro A. A nitrogen response pathway regulates virulence functions in *Fusarium oxysporum* via the protein kinase TOR and the bZIP protein MeaB. Plant Cell. 2010;22(7):2459–75.

Lyons R, Czislowski E, Zeil-Rolfe I, Kaur S, Liu Z, Chen A, et al. Unique secreted in xylem genes in banana-infecting endophytic *Fusarium Oxysporum*. Proceedings. 2019;36(1):180.

Ma L, Houterman PM, Gawehns F, Cao L, Sillo F, Richter H, et al. The AVR2-SIX5 gene pair is required to activate I-2-mediated immunity in tomato. New Phytol. 2015;208(2):507–18.

Maldonado Bonilla LD, Villarruel Ordaz JL, Calderón Oropeza MA, Sánchez-Espinosa AC. Secreted in xylem (Six) genes in *Fusarium oxysporum* f. sp. cubense and their potential acquisition by horizontal transfer. Adv Biotech Micro. 2018;10:555779.

Martinez Z, De Schutter K, Van Damme EJM, Vogel E, Wynant N, Vanden Broeck J, et al. Accelerated delivery of dsRNA in lepidopteran midgut cells by a *Galanthus nivalis* lectin (GNA)-dsRNA-binding domain fusion protein. Pestic Biochem Physiol. 2021;175:104853.

Müller O, Schreier PH, Uhrig JF. Identification and characterization of secreted and pathogenesis-related proteins in Ustilago maydis. Mol Genet Genom. 2008;279(1):27–39.

O'Donnell K, Gueidan C, Sink S, Johnston PR, Crous PW, Glenn A, et al. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. Fungal Genet Biol. 2009;46(12):936–48.

Olivain C, Alabouvette C. Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f. sp. lycopersici in comparison with a non-pathogenic strain. New Phytol. 1999;141:497–510.

Ouyang SQ, Ji HM, Feng T, Luo SJ, Cheng L, Wang N. Artificial trans-kingdom RNAi of FolRDR1 is a potential strategy to control tomato wilt disease. PLoS Pathog. 2023;19(6):e1011463.

Pallis S, Alyokhin A, Manley B, Rodrigues TB, Buzza A, Barnes E, et al. Toxicity of a novel dsRNA-based insecticide to the Colorado potato beetle in laboratory and field trials. Pest Manag Sci. 2022;78(9):3836–48.

Pareja-Jaime Y, Roncero MI, Ruiz-Roldán MC. Tomatinase from *Fusarium oxysporum* f. sp. lycopersici is required for full virulence on tomato plants. Mol Plant Microbe Interact. 2008;21(6):728–36.

Pastrana AM, Kirkpatrick SC, Kong M, Broome JC, Gordon TR. *Fusarium oxysporum* f. sp. mori, a new forma speciales causing Fusarium wilt of blackberry. Plant Dis. 2017;101(12):2066–72.

Pautot V, Holzer FM, Chaufaux J, Walling LL. The induction of tomato leucine aminopeptidase genes (LapA) after *Pseudomonas syringae* pv. tomato infection is primarily a wound response triggered by coronatine. Mol Plant Microbe Interact. 2001;14(2):214–24.

Pierzgalski A, Bryła M, Kanabus J, Modrzewska M, Podolska G. Updated review of the toxicity of selected *Fusarium* toxins and their modified forms. Toxins (Basel). 2021;13(11):768.

Poli A, Gilardi G, Spadaro D, Gullino ML, Garibaldi A. Molecular characterization of *Fusarium oxysporum* f. sp. cichorii pathogenic on chicory (*Cichorium intybus*). Phytoparasitica. 2012;40:383–91.

Powell AL, van Kan J, ten Have A, Visser J, Greve LC, Bennett AB, et al. Transgenic expression of pear PGIP in tomato limits fungal colonization. Mol Plant Microbe Interact. 2000;13(9):942–50.

Qian H, Song L, Wang L, Wang B, Liang W. The secreted FoAPY1 peptidase promotes *Fusarium oxysporum* invasion. Front Microbiol. 2022a;13:1040302.

Qian H, Wang L, Wang B, Liang W. The secreted ribonuclease T2 protein FoRnt2 contributes to *Fusarium oxysporum* virulence. Mol Plant Pathol. 2022b;23(9):1346–60.

Qiao L, Lan C, Capriotti L, Ah-Fong A, Nino Sanchez J, Hamby R, et al. Spray-induced gene silencing for disease control is dependent on the efficiency of pathogen RNA uptake. Plant Biotechnol J. 2021;19(9):1756–68.

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature. 2000;403(6772):901–6.

Rep M, van der Does HC, Meijer M, van Wijk R, Houterman PM, Dekker HL, et al. A small, cysteine-rich protein secreted by *Fusarium oxysporum* during colonization of xylem vessels is required for I-3-mediated resistance in tomato. Mol Microbiol. 2004;53(5):1373–83.

Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. Rational siRNA design for RNA interference. Nat Biotechnol. 2004;22(3):326–30.

Rodrigues TB, Mishra SK, Sridharan K, Barnes ER, Alyokhin A, Tuttle R, et al. First sprayable double-stranded rna-based biopesticide product targets *Proteasome* subunit beta type-5 in colorado potato beetle (*Leptinotarsa decemlineata*). Front Plant Sci. 2021;12:728652.

Schäfer W. Molecular mechanisms of fungal pathogenicity to plants. Annu Rev Phytopathol. 1994;32:461–77.

Schwartz SH, Hendrix B, Hoffer P, Sanders RA, Zheng W. Carbon dots for efficient small interfering rna delivery and gene silencing in plants. Plant Physiol. 2020;184(2):647–57.

Sharath Chandra G, Asokan R, Manamohan M, Krishna KN. Enhancing RNAi by using concatemerized double-stranded RNA. Pest Manag Sci. 2019;75(2):506–14.

Smith SN, Helms DM, Temple SR, Frate C. The distribution of fusarium wilt of blackeyed cowpeas within California caused by *Fusarium oxysporum* f. sp. tracheiphilum Race 4. Plant Dis. 1999;83(7):694.

Snyder WC, Hansen HN. The species concept in *Fusarium*. Am J Bot. 1940;27(2):64–7.

Song XS, Gu KX, Duan XX, Xiao XM, Hou YP, Duan YB, et al. Secondary amplification of siRNA machinery limits the application of spray-induced gene silencing. Mol Plant Pathol. 2018;19(12):2543–60.

Stakman EC. A study in cereal rusts: physiological races. Retrieved from the University Digital Conservancy; 1913. https://hdl.handle.net/11299/178001

Steinberg C, Lecomte C, Alabouvette C, Edel-Hermann, V. Root interactions with non-pathogenic *Fusarium oxysporum*. Hey *Fusarium oxysporum*, what do you do in life when you do not infect a plant? Belowground Defence Strategies in Plants. Springer International Publishing, Switzerland. 2016;281–299.

Vakalounakis DJ. Root and stem rot of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* f. sp. *nov*. Plant Dis. 1996;80:313–6.

Wade BL. Inheritance of *Fusarium* wilt resistance in canning peas. Madison, WI: Agricultural Experiment Station of the University of Wisconsin; 1929. p. 1–32.

Wang M, Jin H. Spray-induced gene silencing: a powerful innovative strategy for crop protection. Trends Microbiol. 2017;25(1):4–6.

Wang M, Weiberg A, Lin FM, Thomma BP, Huang HD, Jin H. Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nat Plants. 2016;2:16151.

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, et al. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science. 2013;342(6154):118–23.

Wen HG, Zhao JH, Zhang BS, Gao F, Wu XM, Yan YS, et al. Microbe-induced gene silencing boosts crop protection against soil-borne fungal pathogens. Nat Plants. 2023;9(9):1409–18.

Widinugraheni S, Niño-Sánchez J, van der Does HC, van Dam P, García-Bastidas FA, Subandiyah S, et al. A SIX1 homolog in *Fusarium oxysporum* f. sp. cubense tropical race 4 contributes to virulence towards Cavendish banana. PLoS ONE. 2018;13(10):e0205896.

Wong KH, Hynes MJ, Davis MA. Recent advances in nitrogen regulation: a comparison between *Saccharomyces cerevisiae* and filamentous fungi. Eukaryot Cell. 2008;7:917–25.

Zhang T, Zhao YL, Zhao JH, Wang S, Jin Y, Chen ZQ, et al. Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. Nat Plants. 2016;2(10):16153.

Zhang L, Yan J, Fu Z, Shi W, Ninkuu V, Li G, et al. FoEG1, a secreted glycoside hydrolase family 12 protein from *Fusarium oxysporum*, triggers cell death and modulates plant immunity. Mol Plant Pathol. 2021;22(5):522–38.