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Cross-Kingdom Interactions Between Plant and Fungal Viruses

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Abstract

The large genetic and structural divergences between plants and fungi may hinder the transmission of viruses between these two kingdoms to some extent. However, recent accumulating evidence from virus phylogenetic analyses and the discovery of naturally occurring virus cross-infection suggest the occurrence of past and current transmissions of viruses between plants and plant-associated fungi. Moreover, artificial virus inoculation experiments showed that diverse plant viruses can multiply in fungi and vice versa. Thus, virus cross-infection between plants and fungi may play an important role in the spread, emergence, and evolution of both plant and fungal viruses and facilitate the interaction between them. In this review, we summarize current knowledge related to cross-kingdom virus infection in plants and fungi and further discuss the relevance of this new virological topic in the context of understanding virus spread and transmission in nature as well as developing control strategies for crop plant diseases.

INTRODUCTION

The discovery of an infectious agent that passed through bacterial filters in tobacco plants showing mosaic disease later identified to be tobacco mosaic virus (TMV)—a positive-sense (+) single-stranded (ss) RNA virus of the family *Virgaviridae*—around the end of the nineteenth century is considered the beginning of the field of virology (1). Since then, many plant diseases have been found to be caused by viruses, and a vast number of diverse viruses have been identified in various taxa of vascular land plants as well as algae (2–6). Considering the high prevalence of viruses in the plant kingdom (Plantae, earth's phytosphere), including in their associated organisms, plant viruses may represent one of the most abundant virus groups in the environmental ecosystem; nevertheless, it seems that we still have not reached a comprehensive understanding of the abundance, biodiversity, and distribution of plant viruses in the global ecosystem (7–9).

A plant virus is generally defined as a virus that is naturally found in plants and able to stably replicate its genome in plant cells. However, there are a number of plant viruses that are also able to infect and replicate in their insect vectors (10). The majority of crop disease–related plant viruses are known to be transmitted by arthropods such as aphids, whiteflies, leafhoppers, planthoppers, thrips, mealybugs, and mites (11, 12), although only a limited number of plant viruses replicate in their insect vectors as described above. In addition, a number of plant viruses are transmitted in a nonreplicating or unknown manner by soil-inhabiting organisms such as plasmodiophorids (Protists), *Olpidium* spp. (a fungal genus in the order Olpidiales), and nematodes (13–15). The infection of plant viruses in nonplant species, particularly those outside the context of virus-vector relationships, remains obscure and has not been deeply investigated. However, intriguingly, animal metagenomic studies revealed the presence of plant viruses or plant virus–related sequences in the virome associated with bats (mammals) and various invertebrates such as bees and mosquitoes (16–20). As plants are closely associated with animals and microbes in the ecosystem, these findings suggest that to some extent, plant viruses may spread beyond their known conventional hosts in nature.

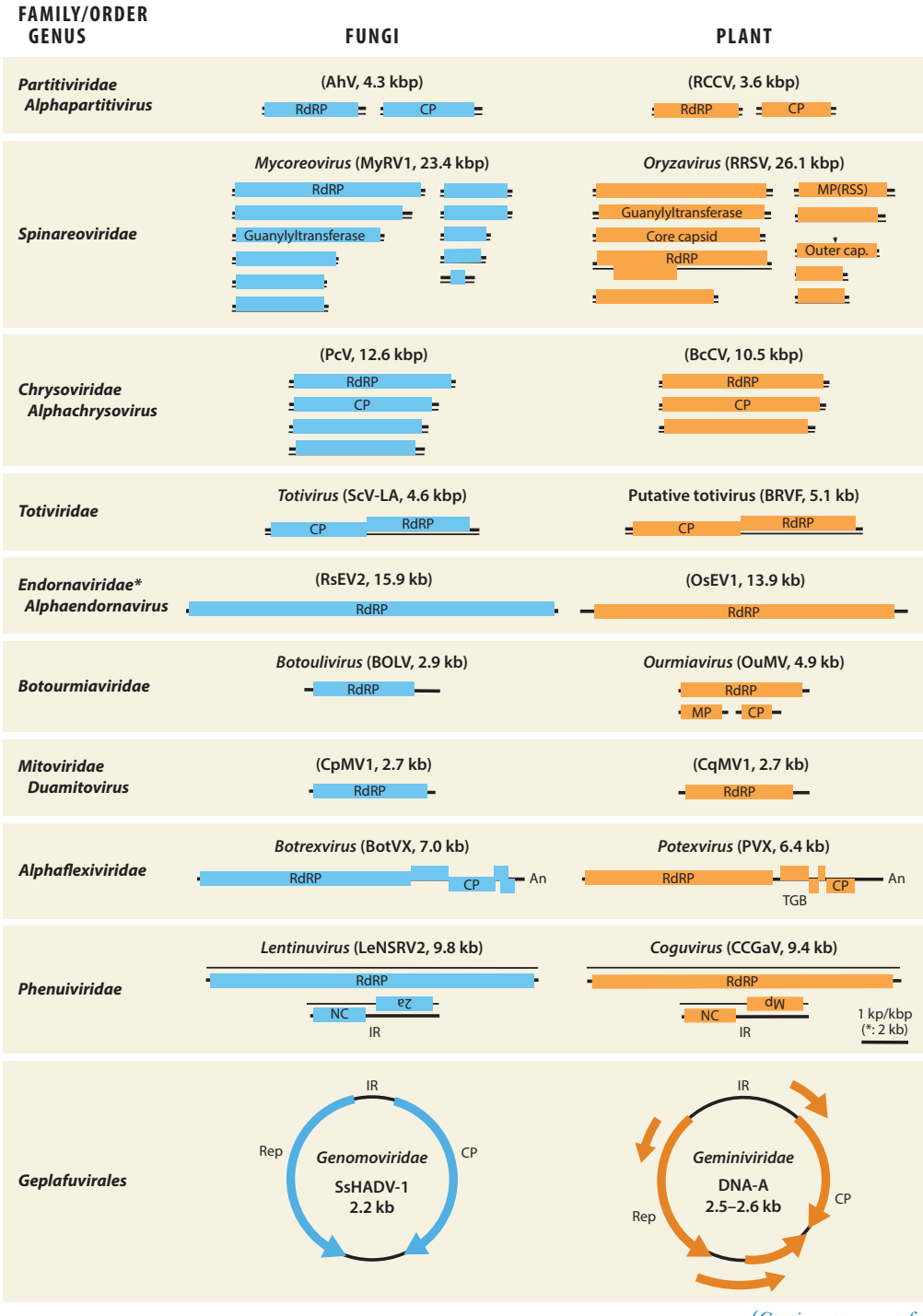
Compared to viruses (or phages) that infect plants, animals, and bacteria, viruses infecting fungi, called fungal viruses or mycoviruses, were explored much later and relatively less extensively during the early period, with some notable discoveries of fungal virus further instigating interests in fungal virology. Fungal viruses were first identified in the diseased fruiting bodies of the cultivated basidiomycete mushroom *Agaricus bisporus* (order Agaricales) in 1962 (21, 22). In *Saccharomyces cerevisiae* (unicellular yeast fungus) strains that secrete killer toxins that are lethal to other sensitive yeast strains, the toxic proteins were found to be encoded by satellite double-stranded (ds) RNAs that require a helper dsRNA virus (totiviruses, family *Totiviridae*) for their replication (23–26). Viral dsRNA, which was identified to be Penicillium chrysogenum virus (family *Chrysoviridae*), was observed to induce interferon activity in several fungal species in the genus *Penicillium* (27, 28). Another notable discovery is the identification of a capsidless (+)ssRNA virus, Cryphonectria hypovirus 1 (CHV1) (family *Hypoviridae*), in hypovirulent strains of an ascosmycetous fungus, *Cryphonectria parasitica* (order Diaporthales), a fungal pathogenic agent of chestnut blight disease (29, 30). Subsequently, the observation that CHV1 infection causes the hypovirulence (reduced pathogenicity) of *C. parasitica* strains and the successful application of CHV1 to control chestnut blight disease further stimulated the identification of viruses in other diverse plant pathogenic fungi in the hope of finding potential biological agents used to control crop fungal diseases (31, 32). However, recently, in fungal virology, particularly since the advent of high-throughput sequencing technologies, the exploration of viruses in the fungal world has also expanded to nonpathogenic fungi including fungal endophytes and mycorrhizae (33). Indeed, studies on fungal viruses greatly contribute to the knowledge of wide-ranging virological topics, including virus diversity and evolution and virus-virus/virus-host interactions (21, 22, 33–36).

Although plants and fungi are intimately associated through parasitic or symbiotic relationships with the active exchange of cellular contents between the two organisms, the biological separation between plants and fungi at the taxonomic kingdom level may impose restrictions on the transmission of viruses between these two organisms to a certain degree. However, the extensive identification of viruses in various fungi and artificial virus inoculation have revealed that many plant and fungal viruses are taxonomically related and display host interchangeability. First, considerable numbers of plant and fungal viruses share similar genome characteristics, and some of them are phylogenetically closely related (35, 37). Second, fungal strains isolated from plants collected from the fields were discovered to carry plant viruses; moreover, artificial virus inoculations in the laboratory demonstrated the compatibility of fungi as hosts of plant viruses and vice versa (37–39). Together, these observations suggest the common occurrence of past and current transmissions of viruses between plants and fungi as well as possible interactions between these two virus groups. The study of cross-kingdom virus infection (via horizontal virus transmission) in plants and fungi is still in its infancy but has the potential to enhance our knowledge of many aspects of virology, including replication, emergence, spread, evolution, pathology, and plant disease control, as well as elucidate the mechanism of macromolecule transfer between plants and fungi. In this review, we summarize the currently available information related to this new virological topic. We also extend the review by discussing the possible mechanisms of virus transmission between plants and fungi and the potential contribution of these studies to the development of control strategies for crop diseases.

DIFFERENCES AND SIMILARITIES BETWEEN PLANT AND FUNGAL VIRUSES

In 2022, the taxonomy list by the International Committee on Taxonomy of Viruses (ICTV Master Species List 2021.v3, **https://ictv.global/msl**) showed that plant virus species span 35 families and 155 genera, including 2 families of subviral agents, viroids, while fungal virus species span 26 families and 62 genera, including 3 unassigned genera (**Supplemental Table S1**). Both virus groups consist of viruses having linear (+)ssRNA, negative-sense (−)ssRNA, dsRNA genomes, circular ssDNA, and reverse-transcribing genomes, whereas viroids, which have noncoding circular ssRNA genomes with self-cleaving ribozymes (in some viroids), have so far been identified only in plants. However, a recent finding expands our knowledge of the spread of viroid-like self-cleaving elements other than plant infective agents: A group of unassigned mycoviruses (ambiviruses) appearing to have hybrid circular ssRNA genomes with self-cleaving elements and novel viroid-like RNAs was found to naturally infect a filamentous fungus (40–42) (**Supplemental Table S1**). Virus families that contain both plant and fungal virus members include *Metaviridae* and *Pseudoviridae* with reverse-transcribing genomes; *Chrysoviridae*, *Totiviridae*, *Reoviridae*, *Amalgaviridae*, and *Partitiviridae* with dsRNA genomes; *Alphaflexiviridae*, *Endornaviridae*, *Botourmiaviridae*, and *Mitoviridae* with (+)ssRNA genomes; and *Phenuiviridae* with (−)ssRNA genomes (**Figure 1**). Notably, several genera, including *Metavirus* (family *Metaviridae*), *Pseudovirus* (family *Pseudoviridae*), *Alphachrysovirus* (family *Chrysoviridae*), *Totivirus* (family *Totiviridae*), *Alphapartitivirus/Betapartitivirus* (family *Partitiviridae*), and *Alphaendornavirus* (family *Endornaviridae*), consist of both plant and fungal viruses, indicating high levels of similarity in their genome sequences and structures (**Figure 1**). Particularly in the case of *Alphapartitivirus* and *Alphaendornavirus*, which have persistent life cycles and likely no effect on host phenotypes (generally asymptomatic and transmitted vertically through reproductive cells), phylogenetic analyses showed that in addition to clades that contain only plant or fungal viruses, some clades contain both plant and fungal viruses; thus, some plant viruses are more similar to fungal viruses than other plant viruses, and vice versa (37, 43). Fungal viruses belonging to the genus *Botrexvirus* (family *Alphaflexiviridae*) and plant viruses belonging to

Supplemental Material >



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Schematic genome structures of representative plant and fungal viruses with close taxonomic relationships. AhV: Atkinsonella hypoxylon partitivirus (accession no. NC_003470/NC_003471), RCCV1: red clover cryptic virus 1 (NC_022616/NC_022617), MyRV1: Mycoreovirus 1 (AY277888-AY277890, AB179636-AB179643), RRSV: rice ragged stunt virus (AF020334–AF020337, U66712–U66714, L46682, L38899), PcV: Penicillium chrysogenum virus (AF296439-AF296442), BcCV1: Brassica campestris chrysovirus 1 (KP782029-KP782030), ScV-LA: Saccharomyces cerevisiae virus LA (NC_003745), BRVF: black raspberry virus F (NC_009890), RsEV2: Rhizoctonia solani endornavirus 2 (KT823701.1), OsEV1: Oryza sativa endornavirus (D32136), BOLV: Botrytis ourmia-like virus (NC_028476), OuMV: ourmia melon virus (NC_011068-NC_011070), CpMV1: Cryphonectria parasitica mitovirus 1 (NC_004046), CqMV1: Chenopodium quinoa mitovirus 1 (MF375475), BotVX: Botrytis virus X (AY055762), PVX: potato virus X (D00344), LeNSRV2: Lentinula edodes negative-strand virus 2 (LC466008/LC466009), CCGaV: citrus concave gum-associated virus (NC_035759/NC_035754), SsHADV-1: Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (GQ365709). A representative DNA-A segment of plant begomoviruses was shown in the order *Geplafuvirales*. Abbreviations: 2a, 2a protein; An, poly(A) tail; CP, capsid protein; IR, intergenic region; MP, movement protein; NC, nucleocapsid protein; RdRP, RNA-dependent RNA polymerase; Rep, replicase; RSS, RNA silencing suppressor; TGB, triple gene block.

the genus *Potexvirus* (family *Alphaflexiviridae*) are also strikingly similar (44–46). Likewise, fungus-infecting phenuiviruses (genus *Lentinuvirus*, family *Phenuiviridae*) share their ambisense genomes (two coding regions with the opposite direction) with plant-infecting phenuiviruses (genus *Coguvirus*), both of which encode putative proteins similar to plant viral movement proteins (MPs) (30K superfamily) (33, 47). Notably, the rolling-circle replication initiation proteins encoded by fungal viruses belonging to genus *Gemycircularvirus* (circular ssDNA genomes, family *Genomoviridae*) and plant-infecting geminivirus (family *Geminiviridae*) share unique sequence motifs and form a sister group in phylogenetic analyses (48, 49). Taken together, these data suggest an evolutionary link between plant and fungal viruses and also suggest that transmission of viruses between plants and fungi may have occurred in the past as well as in relatively recent times. These data might also reflect the close and mutualistic association between plants and fungi throughout the long evolutionary time span, whose interconnections include the promotion of nutrient uptake, plant growth, and stress tolerance (50).

Except for plant mitoviruses and endornaviruses (persistent viruses), most of the genomes of plant RNA/DNA viruses are encapsidated by one or multiple units of capsid proteins (CPs) (structural proteins) or condensed by proteins into a nucleocapsid, although generally, they are not enclosed in an enveloped virion [except for some plant (−)ssRNA virus groups], which is common for animal viruses. Plant viruses usually encode the structural CP in their genomes, but there are also examples, as observed for umbraviruses (family *Tombusviridae*), in which the genome does not encode the CP but the virus is encapsidated by the CP of a helper luteovirus within the same family (*trans*-encapsidation) for its horizontal transmission through aphid insect vectors (51). Notably, a high proportion of fungal RNA viruses do not encode CPs, and thus their genomes are not encapsidated (capsidless nature) or it is unclear whether their genome is encapsidated (33). From an evolutionary perspective, this is likely related to the major transmission of fungal viruses through sporulation and hyphal anastomosis, which are devoid of an extracellular phase (21), whereas the majority of plant viruses are transmitted by biological vectors (3). Interestingly, similar to plant umbraviruses, genome *trans*-encapsidation by unrelated helper viruses (several types of dsRNA viruses within the order *Ghabrivirales*) was also observed for some fungal (+)ssRNA viruses belonging to the family *Yadokariviridae*, whose hetero-encapsidation likely required for replication resembles that of dsRNA viruses (52–54).

Aside from replication-associated and structural proteins that are commonly encoded by both plant and fungal viruses, proteins having similar characteristics to plant viral proteins with unique functions, such as MPs (55) or vector transmission-associated proteins (14), have not been commonly identified in fungal viruses (except for lentinuviruses, which have an MP-like gene as mentioned above). While the majority of plant viruses are transmitted by arthropod vectors (mainly

sucking insects) (11), in fungal viruses, so far only one example of insect-mediated transmission has been found: Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1), an ssDNA virus of the family *Genomoviridae*, is acquired by the mushroom fly *Lycoriella ingenua*, replicates in this insect, and uses it as a vector (56). As the horizontal transmission of fungal viruses through hyphal anastomosis is often obstructed by vegetative incompatibility between fungal strains or species (57), it is interesting to note that Sclerotinia sclerotiorum mycoreovirus 4 (dsRNA virus, family *Spinareoviridae*) could suppress non–self-recognition of the fungal host to facilitate horizontal virus transmission between vegetatively incompatible fungi (58). Like in plants and animals where virus accumulation is commonly suppressed by RNA silencing (RNA interference), a sequence-specific RNA downregulation mechanism mediated by small interfering RNAs (siRNAs) (59, 60), fungal viruses are also targeted by the antiviral RNA silencing mechanisms of fungal hosts (61–69). Thus, similar to plant viruses, fungal viruses also encode the RNA silencing suppressor protein that functions to counter host RNA silencing-mediated defenses (64, 70–74).

CROSS-INFECTION OF PLANT VIRUSES AND VIROIDS IN FUNGI

Around five decades ago, TMV was reported to associate with rust and powdery mildew (caused by fungi in the orders Pucciniales and Erysiphales, respectively), but it was unknown whether the virus replicated in fungal cells (75, 76). In the early 1990s, the replication system of brome mosaic virus [(+)ssRNA virus, family *Bromoviridae*] using *S. cerevisiae* cells was established (77). Since then, a replication system using budding yeast was also developed for other plant RNA and DNA viruses (78–81). Through artificial inoculation in the laboratory, TMV was shown to stably replicate in the phytopathogenic ascomycetous fungus *Colletotrichum acutatum* (order Glomerellales) (82). More recently, infections of TMV and other (+)ssRNA plant viruses in various fungi and an oomycete (*Phytophthora infestans*, a fungus-like organism within the kingdom Chromista) by artificial inoculation were also reported (39, 83–85). Together, studies that involve virus artificial inoculation demonstrated the compatibility of fungi as hosts of diverse plant viruses.

The first evidence of natural cross-infection of a plant virus in a fungus was presented by our discovery of cucumber mosaic virus (CMV) [(+)ssRNA virus, family *Bromoviridae*] in a phytopathogenic basidiomyceteous fungus strain, *Rhizoctonia solani* (order Cantharellales), isolated from a potato plant grown in the field (39). Fungal inoculation experiments on plants [potato and *Nicotiana benthamiana* (a wild tobacco) plants] in the laboratory clearly demonstrated the two-way transmission of CMV between the plant and fungus. First, when a CMV-free *R. solani* strain was inoculated in plants infected with CMV and reisolated from the plants after allowing fungal colonization, many fungal strains carried CMV, indicating that *R. solani* could acquire CMV during infection. Next, when a CMV-infected *R. solani* strain was inoculated in CMV-free plants, CMV was detected in the upper systemic leaves of the plants 4 or 12 days after fungal inoculation (*N. benthamiana* and potato plant, respectively), indicating that *R. solani* could transmit CMV to the plant during infection (39). Interestingly, CMV infection did not negatively affect fungal growth but rather enhanced fungal virulence. A CMV-infected *R. solani* strain induced more severe stem rot disease than a CMV-free *R. solani* strain when inoculated in potato or *N. benthamiana* plants (39) (**Figure 2**). Thus, plant virus infection could render the fungal hosts more aggressive. From the phytopathological perspective, this is an important observation revealing the possible emergence of highly pathogenic fungal strains due to nongenetic/nonchromosomal factors.

To address whether the cross-infection of plant viruses in plant-associated fungi commonly occurs in nature, our research group recently carried out a systematic screening for fungal strains harboring plant viruses (38). A large number of fungal strains were isolated from the leaves of various vegetable plants such as radish, napa cabbage, leaf mustard (order Brassicales), spinach (order Caryophyllales), celery (order Apiales), and other vegetables infected with known

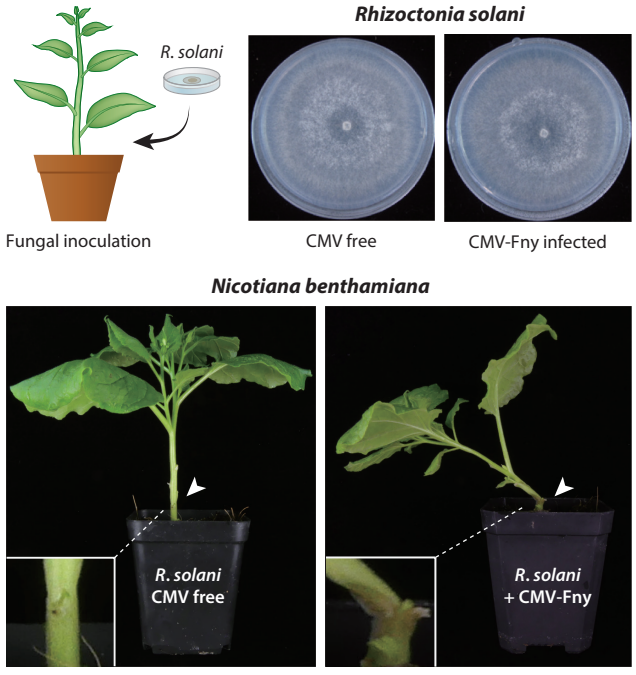


Figure 2

Enhanced severity of stem rot disease in a wild tobacco (*Nicotiana benthamiana*) plant infected with a phytopathogenic basidiomycete fungus *Rhizoctonia solani* carrying a plant positive-sense single-stranded RNA virus [cucumber mosaic virus Fny (CMV-Fny)]. Lower stems were wounded and mycelia-containing gel plugs from the edge of the culture colony were placed on the wounded area. Plants were photographed 4 days after fungal inoculation. Close-up views of the inoculated stems are presented (*insets*).

(+)ssRNA plant viruses belonging to different families or genera such as *Cucumovirus*, *Potyvirus*, *Polerovirus*, *Fabavirus*, and *Waikavirus*, as well as novel (+)ssRNA and (−)ssRNA viruses. Reverse transcription-polymerase chain reaction (RT-PCR) detection of plant viruses in the fungal strains revealed that around half of the 169 fungal strains tested carried plant viruses representing 10 different virus species (8 virus genera) (38). Species identification of the fungal strains carrying plant viruses indicated that they were most likely endophytic fungi, with the majority being *Sarocladium kiliense* species (order Hypocreales), while a small number of fungal strains were identified as being *Lecanicillium coprophilum* (order Hypocreales) and *Alternaria* sp. (order Pleosporales). Overall, these results reveal the common spread of plant viruses to plant-associated fungi in the agroecosystem.

Viroids are parasitic agents having a small, nonencapsidated, noncoding, covalently closed, circular ssRNA genome (86, 87). Currently, viroids are classified as subviral agents consisting of two families, *Avsunviroidae* and *Pospiviroidae* (88, 89). Previously, viroids were known to naturally infect only plant species and cause agriculturally important diseases (90, 91); however, using artificial inoculation, some studies have shown that viroids can replicate in nonplant hosts such as *S. cerevisiae* (92), cyanobacteria (93), filamentous fungi (94), and *P. infestans* (95). Our research group introduced seven viroids representing two families to three phytopathogenic filamentous fungi, namely *C. parasitica*, *Valsa mali* (order Diaporthales), and *Fusarium graminearum* (order Hypocreales), through the transfection of fungal spheroplasts with viroid RNA transcripts (94). Among 21 viroid-fungus combinations, three viroids were found to stably accumulate in at least one

of the fungi for at least eight successive subcultures, namely hop stunt viroid (HSVd) (family *Pospiviroidae*), avocado sunblotch viroid (ASBVd) (family *Avsunviroidae*), and iresine viroid 1 (family *Pospiviroidae*). HSVd infection drastically reduced the growth and virulence of *V. mali*, while the other viroid-fungus combinations were asymptomatic. Moreover, the genomic RNA of HSVd and ASBVd that were maintained in the RNA silencing key gene knockout mutants (Δ*dicer-like 2*, *dcl2*) of *F. graminearum* and *C. parasitica*, respectively, after eight subcultures showed the presence of nucleotide sequence substitutions (96). This observation suggests that genome evolution or adaptation may occur during viroid multiplication in fungi. Using HSVd-*F. graminearum* as the viroid-fungal host system, the bidirectional transfer of HSVd between the plant and fungus was also demonstrated (94). Similarly, other studies demonstrated the bidirectional transmission of potato spindle tuber viroid (family *Pospiviroidae*) between *P. infestans* and potato or tomato plants under laboratory conditions (95). These results suggest the possible occurrence of the cross-kingdom transmission of viroids from plants to fungi under natural conditions as previously observed for plant viruses. To explore this possibility, in a subsequent study, our research group (97) isolated a large number of filamentous fungi from apple trees infected with apple scar skin viroid (ASSVd) (family *Pospiviroidae*). RT-PCR detection indicated that 69.2% of the 117 fungal strains tested carried ASSVd (97). As the majority of the isolated fungal strains were *Alternaria alternata* (order Pleosporales), most of the ASSVd-carrying fungi were *A. alternata*, while the others included *Epicoccum nigrum* (order Pleosporales), *Botryosphaeria dothidea* (order Botryosphaeriales), and *Diaporthe phaseolorum* (order Diaporthales). The phenotypic comparison of ASSVd-free and ASSVd-carrying fungal isogenic lines showed that ASSVd markedly reduced the growth of *A. alternata* and *E. nigrum* but not *B. dothidea* on medium plates, while fungal inoculation on apple leaves showed that ASSVd reduced the virulence of *E. nigrum* but not that of the two other fungal species (97). This study provides evidence of the cross-infection of viroids in fungi under natural settings and further demonstrates that viroids could cause symptoms in certain susceptible filamentous fungi, thus indicating the potential for the use of viroids as biocontrol agents of fungal diseases, although this may raise concerns because viroids are pathogenic agents of plants.

Taking advantage of the availability of mutant fungal strains lacking genes encoding key proteins in the RNA silencing pathway, we demonstrated that the RNA silencing mechanism operates to inhibit plant virus and viroid accumulation in fungi (85, 94). The ribonuclease III–like enzymes Dicer or Dicer-like (DCL) are one of the key factors in the RNA silencing pathway that function to generate siRNAs through the processing of highly base-paired RNA or dsRNAs (60). Ascomycetous fungi usually encode two *dcl* genes, *dcl1* and *dcl2* (98). In *F. graminearum*, DCL1 and DCL*2* redundantly contribute to the suppression of TMV (85). The defense role of fungal DCLs was also observed for viroid infection. DCL2 primarily plays a role in defense against HSVd in *F. graminearum* (94). Likewise, ASBVd accumulation was also enhanced in a *C. parasitica dcl2* mutant (94). The observation that CMV and TMV siRNAs accumulated in infected *R. solani* and *F. graminearum*, respectively (99), provided further evidence of antiviral RNA silencing responses against plant virus infection in fungi. Intriguingly, the characteristics of CMV and TMV siRNAs in fungi differ from the general characteristics of siRNAs of fungal viruses but are similar to those of CMV and TMV siRNAs generated in plant hosts (99). Together, these observations suggest the adaptivity of fungal RNA silencing machinery to recognize and target infecting plant virus and viroid genomes.

INTERACTIONS BETWEEN PLANT AND FUNGAL VIRUSES

The finding that plant viruses can replicate in fungi prompted our research group to examine whether fungal viruses can also replicate in plants, thus substantiating the view that both plant

and fungal viruses can cross kingdom barriers between plants and fungi. Indeed, our investigation using CHV1 as the fungal virus tested led to interesting observations regarding interactions between plant and fungal viruses (85). A previous report showed the replication of fungal dsRNA viruses isolated from *Penicillium aurantiogriseum* var. *viridicatum* (order Eurotiales), a marine endophytic fungus associated with seaweed, in plant cells (protoplasts) (100), but the infection of intact plants by fungal viruses was not demonstrated. A recent report demonstrated the transfer of a partial genome sequence of a mitovirus from the phytopathogenic necrotrophic fungus *Botrytis cinerea* (order Helotiales) to cucumber plants following a fungal inoculation experiment in the laboratory, but in this study, replication of the mitovirus in the plants was unclear (101). Mechanical rub inoculation of CHV1 (+)-strand RNA transcripts on the leaves of an experimental model plant, *Nicotiana tabacum*, showed CHV1 accumulation and replication in the inoculated leaves 1 to 5 days post inoculation (dpi), but CHV1 RNA was not detected in uninoculated upper leaves at 7 and 14 dpi (85). Systemic infection of CHV1 in the upper leaves was observed in plants infected with a (+)ssRNA virus, namely TMV, CMV, potato virus X (family *Alphaflexiviridae*), and potato virus Y (family *Potyviridae*) or in transgenic plants expressing the TMV 30K MP gene (*N. tabacum* 30K) (102). These suggest that the infection of plant virus facilitates CHV1 systemic infection in plants through the function of virus-encoded MP. Co-infection with TMV also increased CHV1 RNA accumulation in the *N. tabacum* 30K plants. In a fungal inoculation experiment in which a CHV1-infected *F. graminearum* strain was inoculated in virus-free plants or plants infected with TMV or other plant viruses, CHV1 RNA was detected in the upper uninoculated leaves of plant virus–infected plants but not in virus-free plants, showing that plant viruses can facilitate the transmission of CHV1 from fungi to plants. The reciprocal interaction can occur as well. When TMV was artificially introduced to *F. graminearum* by spheroplast transfection, co-infection with CHV1 markedly enhanced TMV accumulation in *F. graminearum* and TMV transmission through the fungal spores. Furthermore, in a fungal inoculation experiment to assess the acquisition of TMV by *F. graminearum*, the presence of CHV1 in *F. graminearum* enhanced the efficiency of TMV transmission from the plant to *F. graminearum*.

Overall, the above-described observations showed that plant and fungal viruses can confer facilitative and synergistic effects on each other exclusively in their respective plant and fungal hosts. Many synergistic interactions observed in plant and fungal viruses are related to the suppression of host antiviral RNA silencing responses by virus-encoded proteins (34, 36, 66, 103, 104). The replicase encoded by TMV and other tobamoviruses suppresses RNA silencing activity via direct binding or the modification of siRNAs (105–107), while the p29 protein encoded by CHV1 functions as an RNA silencing suppressor by inhibiting the high induction of key gene transcriptions involved in RNA silencing in fungi (64, 108). Considering some mechanistic differences between RNA silencing in plants and fungi (64, 68, 74, 109, 110), it is possible that the silencing suppressors encoded by TMV and CHV1 are not sufficiently effective in the nonconventional hosts (fungi and plants, respectively), and this may explain why the plant and fungal viruses accumulate at relatively low levels in fungal and plant hosts, respectively, and confer synergistic effects only in their conventional well-adapted hosts. The observation of facilitative interactions between plant and fungal viruses that stimulate virus transmission between plants and fungi presents biological implications for the route and extent of spread of fungal and plant viruses in nature. As plant virus infection in plants could facilitate fungal virus transmission to and systemic infection throughout the plant, the fungal virus can then be acquired by other fungi that co-colonize the plant. Thus, by this route, fungal viruses can be transmitted to vegetatively incompatible strains or different fungal species, as demonstrated in our study (85). In fact, fungal virus–related sequences were often found in the virome of plant samples infected with plant viruses (111–113). However, more detailed analyses are necessary to determine whether the fungal virus sequences are derived from virus replication

products in plant cells or the contamination of fungal materials. On the other hand, fungal viruses can enhance the efficiency of plant virus transmission to fungi; this conceivably promotes the role of fungi as alternative biological reservoirs and transmission vectors of plant viruses in nature.

POSSIBLE MECHANISMS OF VIRUS AND VIROID TRANSMISSIONS BETWEEN PLANTS AND FUNGI

According to their infection strategy, plant-colonializing fungi or fungal-like organisms can be classified into three groups: necrotrophs (kill host cells and acquire nutrients from the dead cells/tissues), biotrophs (alter host physiology and take up nutrients from living cells), and hemibiotrophs (biotrophic phase during initial infection followed by a necrotrophic phase) (114). The import of nutrients into fungal cells is regulated by a variety of nutrient transporters located in the cell plasma membranes (115). Regardless of the type of fungus, during the colonization of the plant, fungi extracellularly secrete various molecules such as toxins, cell metabolites, hormones, peptides, enzymes, effector proteins, and small RNAs to facilitate infection through the destruction of cells/tissue or reprogramming of host cell physiology and defense responses (116–119). Biotrophic and hemibiotrophic filamentous fungi usually develop specialized feeding structures such as haustoria, arbuscules, and invasive hyphae that invaginate the host plasma membrane to enable the effective transfer of molecules between fungi and hosts (116). In coping with fungal invasion, plants also secrete a variety of compounds/molecules, including secondary metabolites, nucleotides, peptides, proteins, RNAs, and DNAs (119). Therefore, both plants and fungi release various molecules into the extracellular space outside the cell plasma membrane, generally called apoplast. This is the major location of interactions between plant and fungal factors (120).

The eukaryotic secretory pathway transports proteins into the extracellular space. In the conventional secretory pathway, N-terminal-specific transit peptide (signal peptide)-containing cargo proteins are delivered using secretory vesicles via the endoplasmic reticulum (ER)-Golgi route to the plasma membrane and then released through exocytosis. In the unconventional pathway, vesicles are directly transported to the plasma membrane from the ER or proteins are directly secreted through transporters or pores in the plasma membrane (121–123). Recently, a mechanism of extracellular secretion involving membrane-associated exosomes or extracellular vesicles (EVs) that formed inside multivesicular bodies (MVBs) (late endosomes) and were released outside the cell following the fusion of the MVBs with the plasma membrane was intensively studied in plants and fungi (124–126). These lipid-bilayer compartments (EVs) play a pivotal role in intercellular communication between plants and microbes through the transport and exchange of a variety of cargo molecules (127–129).

The occurrence of the transfer of macromolecules across the boundary of plants and fungi is particularly exemplified by the transfer of effector proteins and RNAs between these two organisms. To promote infection, fungi secrete various effector proteins to modulate the host cell metabolism and repress defense responses (130, 131). According to their location of action, apoplastic fungal effectors function in the extracellular space, while symplastic (cytoplasmic) fungal effectors enter plant cells and function therein (132). The majority of fungal effector proteins are secreted through the conventional ER-Golgi pathway, while some effectors are extracellularly discharged via unconventional vesicular or nonvesicular pathways (117). The hyphal tips and feeding structures such as haustoria and invasive hyphae are known to be the secretion sites of effector proteins (132). However, the molecular mechanism of uptake of fungal effector proteins (including oomycete effectors) into plant cells is not well understood, although a mechanism involving endocytosis has been proposed (133, 134).

Some small RNAs produced in the fungus *B. cinerea* are translocated from fungi to plant cells and induce RNA silencing of the host genes involved in antifungal immunity (135). Conversely,

the expression of small RNAs or dsRNAs that are specific for the genes encoded by the fungal pathogens in plants could lead to the suppression of fungal pathogenicity and growth (136–138). Together, these observations indicate the bidirectional transfer of small RNAs between plants and fungi (cross-kingdom RNA silencing), but how these regulatory RNAs translocate across plants and fungi is not fully understood. It was found that plants secrete EVs containing RNAs or small RNAs that are taken up by fungal cells (136, 139, 140). Some plant RNA-binding proteins were found to be involved in the loading of small RNAs into vesicles (141). Notably, a recent study showed that small RNAs in apoplastic fluids are associated with proteins but mostly located outside vesicles (142). Similarly, fungi are known to secrete EVs that contain various RNAs, including messenger RNAs and small RNAs (143). Overall, although both plants and fungi are known to extracellularly secrete RNAs, how these RNAs are taken up by the cells of colonizing fungi or plant hosts is still unknown. Interestingly, some fungi were shown to directly take up naked external small RNA or dsRNA molecules, but the mechanism of absorption is unknown (138, 144).

As viruses and viroids consist of proteins and/or nucleic acids (DNA or RNA), they may be delivered to the extracellular space through secretory pathways similar to those involved in the secretion of proteins and RNAs (**Figure 3**). In fact, plant RNA viruses are known to recruit host membranes and utilize secretory pathways for intra- and intercellular trafficking (145). During the infection of turnip mosaic virus [(+)ssRNA virus, family *Potyviridae*], viral RNA is associated with MVBs. These viral RNA-containing vesicles are then released into the extracellular space after fusion with the plasma membrane (146). In PVX-infected plants, virus particles and RNAs were detected in the apoplast fluid, but PVX particles were not associated with vesicles (147). Besides CMV and TMV, other plant RNA viruses were found to be able to cross-transmit to fungi (38), implying that it may be common for a variety of plant viruses and viroids to be delivered to the extracellular space and taken up by plant-associated fungi. Furthermore, similar mechanisms may be involved in the delivery of fungal viruses to the extracellular space of fungi. In fact, our ongoing study showed the association of fungal viruses with EVs (L. Sun et al., unpublished results). Thus, it is important to further study the biological relevance of the extracellular transfer of plant and fungal viruses, in particular, its role in virus transmission across different organisms (**Figure 3**). Notably, some fungi were shown to be able to directly take up virus particles and viroid RNAs (82, 94, 148), suggesting that fungi could efficiently absorb virus particles or RNAs and other macromolecules that are secreted into the apoplast. However, it is still unclear how macromolecules such as virus particles and RNAs secreted by the fungi can pass through plant cell plasma membranes and enter plant cells. Thus, further studies are necessary to address this question.

PROSPECTS FOR THE MANAGEMENT AND CONTROL OF CROP PLANT DISEASES

As curative treatments with chemicals are not available, the control of plant virus and viroid diseases commonly relies on the utilization of natural genetic resources, biotechnology, and intervention in the virus transmission pathway, along with other agronomical and preventive practices (14, 149–152). Therefore, understanding the distribution, host range, biological reservoirs, and transmission routes of plant viruses/viroids in the natural and agricultural environment would provide the basis for the development of control and management strategies for crop viral diseases. The discovery that plant viruses and viroids transmit between plants and fungi implicates fungi as living sources and transmission vectors of plant viruses/viroids in nature. Plant viruses and viroids could be horizontally transmitted through hyphal anastomosis and vertically transmitted through spores (39, 85, 94, 97). These observations suggest that once plant viruses and viroids cross-infect fungi, they could be transmitted among fungal strains and maintained in the fungal population across generations; they also could be retransferred to the plants during the

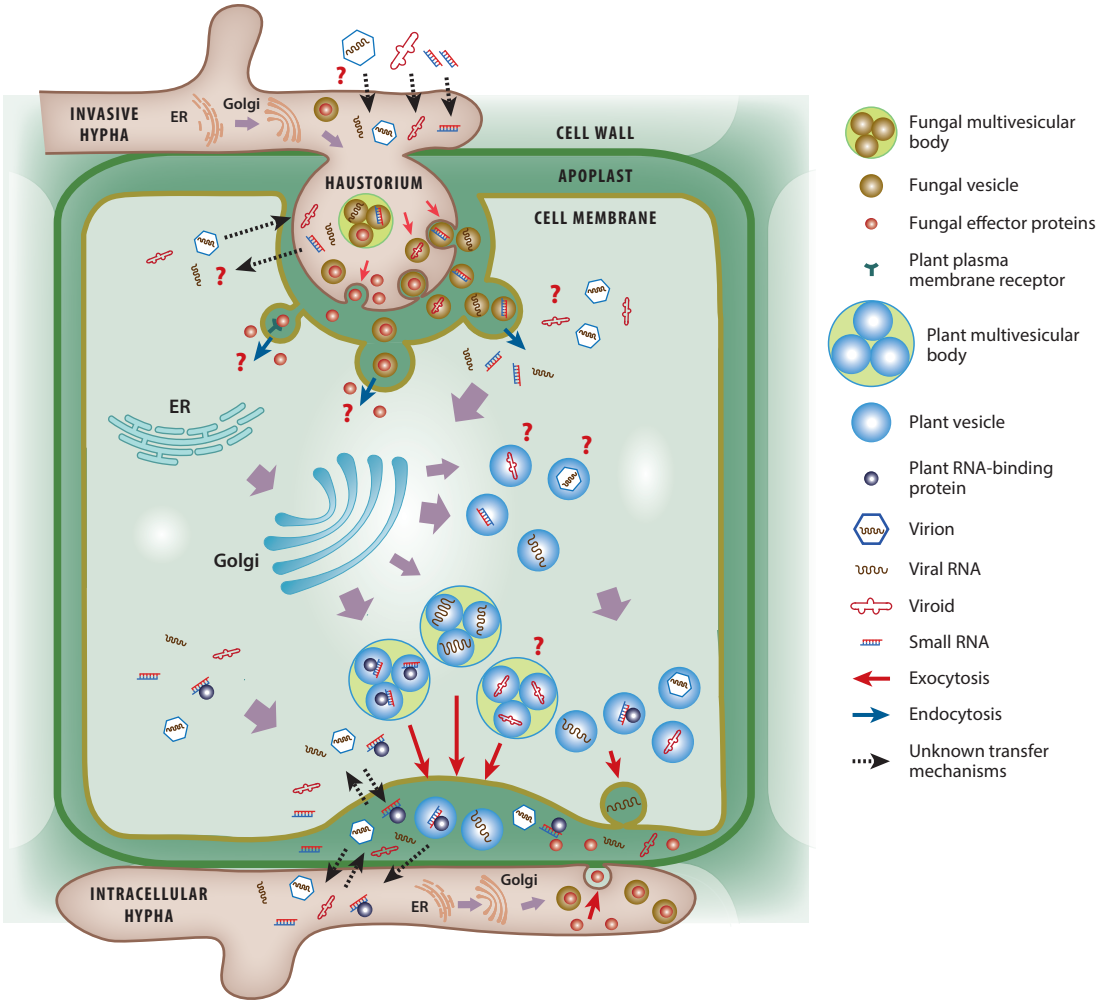


Figure 3

An illustration depicting the transfer of effector proteins, small RNAs, viruses, and viroids across the boundaries of plant and fungal cells. Fungal effector proteins are extracellularly secreted through the conventional endoplasmic reticulum (ER)-Golgi pathway, multivesicular body, or nonvesicular pathways. Feeding structures such as haustoria and invasive hyphae, along with hyphal tips, are the secretion sites of effector proteins. The molecular mechanism of uptake of fungal cytoplasmic effector proteins into plant cells is not well understood, although a mechanism involving endocytosis has been proposed, for example, via plasma membrane receptor–mediated endocytosis. Fungal small RNAs and fungal virus RNAs are secreted through extracellular vesicles, but how they are taken up by the plant cell is unclear. Plant small RNAs bind to RNA-binding proteins and are secreted into the apoplast through the vesicular pathway or other unknown pathways, but it is unclear how these small RNAs enter the fungal cell. Plant viral (also viroid) RNAs or virions are delivered to the extracellular space through vesicular or unknown nonvesicular pathways. Fungal viruses (also viroids) are likely also secreted into the extracellular space from fungal cells. How plant and fungal viruses (also viroids) that exist in the apoplast can cross the plasma membrane and enter fungal and plant cells is unknown. Notably, some fungi were shown to be able to directly take up small RNAs, viroids, and virions applied to the mycelia.

fungal colonization of the plants. In this regard, it seems necessary to involve fungal species (and may also include oomycetes and other protists) in epidemiological and ecological studies of plant viruses and viroids. Notably, many fungi and oomycetes produce resting spores, thick cell-walled spores that can stand harsh environmental conditions for long periods. Thus, transmission to

fungi and oomycetes may facilitate the survival of plant viruses in an unfavorable environment. The transmission vectors of many plant viruses (and also viroids), such as those infecting perennial trees, are still unidentified (152, 153). It is worth exploring whether plant-associated fungi play a role in the dissemination of these viruses in the field, and therefore, mycology-based approaches may contribute to the management of these plant viral diseases.

Due to the genetic diversity of fungal strains in the field, the application of hypovirulence-inducing fungal viruses to control crop fungal diseases is hampered by vegetative incompatibility between fungal strains that restricts the transmission of the virus from a hypovirulent fungal strain to a virulent strain (31). As an alternative for fungal virus transmission through the hyphal contact, direct application of fungal virus particles to the fungal mycelia was successfully demonstrated for SsHADV-1 (148). Likewise, we demonstrated the effective inoculation of viroids through the direct application (spraying) of viroid RNAs to the fungal mycelia (94). Thus, exogenous inoculation through the direct application of the fungal virus/viroid may be a promising method for the biocontrol of crop fungal diseases in the field. However, because the viruses or viroids are applied exogenously in unprotected extracellular conditions, the stability of the inoculants, particularly for a long time period, may limit the efficacy of this method. Based on the current knowledge described in this review, we propose an alternative method to utilize fungal viruses as biocontrol agents by establishing fungal virus infection in the plant (**Figure 4**). In this case, the plant needs

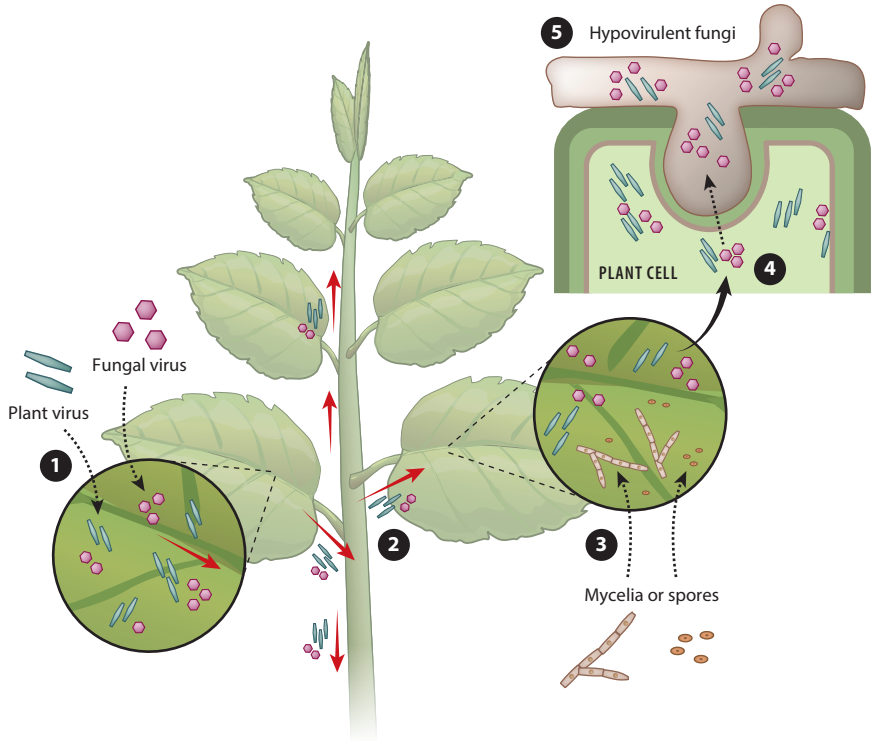


Figure 4

Illustration of the inoculation of fungal viruses in plants to protect the plants against fungal diseases. ① The plant is inoculated with fungal and plant viruses. ② Plant virus infection facilitates the systemic infection of the fungal virus throughout the plant. ③ Phytopathogenic fungi colonize the plant. ④ Fungal virus (possibly also plant virus) transmits from plant to fungus. ⑤ Fungal virus causes hypovirulence in the fungal host.

to be co-infected with a plant virus (asymptomatic to the plant) to facilitate systemic infection of the fungal virus throughout the plant. When the phytopathogenic fungus colonizes the plant, it is expected that the fungal virus cross-infects the fungus and induces hypovirulence. Thus, the plant is protected against severe diseases caused by the infection of phytopathogenic fungi. We surmise that this method may be more suitable when applied to perennial or woody plants because they have long lifespans. The feasibility and effectivity of this approach need to be thoroughly tested under laboratory and field conditions with proper fungal virus agents and phytopathogenic fungus targets. We have found that some fungal hypoviruses other than CHV1 are able to systemically infect transgenic plants expressing viral MP (85; L. Sun et al. unpublished data). More fungal viruses from various taxa should be tested for their infectivity in plant cells. Moreover, with a similar strategy, viroids could be used as hypovirulence-inducing agents to protect plants, but this approach might be compromised by the limited range of compatibility between viroids and fungal species.

CONCLUDING REMARKS

Accumulating evidence suggests that as intracellular parasites, viruses and viroids are commonly transferred between plants and fungi along with the exchange of various molecules during the fungal colonization of the plant, which may also facilitate interactions between plant and fungal viruses. This new understanding of virus transmission between plants and fungi may redefine the concept of plant and fungal viruses. As plant viruses that replicate in their insect vectors can be considered insect viruses, plant viruses that commonly cross-infect fungi may also be considered fungal viruses and the other way around. Indeed, this is a new and interesting research area in which plant and fungal virology converge. Certainly, further related studies would expand our knowledge of many important aspects of virology, mycology, plant pathology, and other related scientific fields.

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