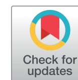

Phytopathogenic fungus hosts a plant virus: A naturally occurring cross-kingdom viral infection

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The transmission of viral infections between plant and fungal hosts has been suspected to occur, based on phylogenetic and other findings, but has not been directly observed in nature. Here, we report the discovery of a natural infection of the phytopathogenic fungus *Rhizoctonia solani* by a plant virus, cucumber mosaic virus (CMV). The CMV-infected *R. solani* strain was obtained from a potato plant growing in Inner Mongolia Province of China, and CMV infection was stable when this fungal strain was cultured in the laboratory. CMV was horizontally transmitted through hyphal anastomosis but not vertically through basidiospores. By inoculation via protoplast transfection with virions, a reference isolate of CMV replicated in *R. solani* and another phytopathogenic fungus, suggesting that some fungi can serve as alternative hosts to CMV. Importantly, in fungal inoculation experiments under laboratory conditions, *R. solani* could acquire CMV from an infected plant, as well as transmit the virus to an uninfected plant. This study presents evidence of the transfer of a virus between plant and fungus, and it further expands our understanding of plant–fungus interactions and the spread of plant viruses.

plant virus | fungus | transmission | cross-kingdom

Viruses, as obligate parasites that infect cellular organisms, strictly depend on compatibility with the cellular components for viral multiplication in the host (1–3). Viruses usually spread from one host to another in the environment but, depending on the host range and transmission pathways, certain viruses may be limited to a few or only specific host species, while other viruses could infect various organisms belonging to the same biological class/phylum (4). In fact, a number of viruses are known to infect organisms from different taxonomic kingdoms, usually involving an insect as one of the host species. This is exemplified by some plant viruses belonging to the families *Bunyaviridae*, *Rhabdoviridae*, *Reoviridae*, and genus *Tenuivirus* that infect and propagate in their insect vectors (5, 6), as well as a recent report on a fungal virus that infects an insect and uses it as a transmission vector (7). Furthermore, cross-kingdom infections can be experimentally established for some virus–host systems in the laboratory (8–13), suggesting that more viruses may cross the kingdom barrier in nature if the appropriate conditions, such as close association/contact and compatibility with the potential novel host, are available to facilitate such an event. Indeed, the occurrence of horizontal virus transfers across kingdoms is one of the theories suggested to account for the presence of many viruses that are taxonomically related but exist in organisms belonging to different taxonomic kingdoms (14–16).

Virtually all flowering plants are exposed to attack by pathogenic fungi (17). Necrotrophic fungal pathogens kill host tissue and absorb nutrients from the dead host cells, while biotrophic and hemibiotrophic fungal plant pathogens colonize the living host, and some commonly develop a specialized organ for uptake of sugars and other nutrients (18–21). To promote infection and disease development, fungal pathogens secrete or inject effector proteins that interfere with the host immunity and other physiological processes (22–24). Interestingly, numerous studies recently reported the occurrences of horizontal transfer of small RNAs, which are usually in the form of micro-RNAs or small interfering RNAs, from fungi and also other microbes to plants, and vice versa (25–27), showing that the infecting fungus and plant could exchange their genetic materials. Fungal virus (mycovirus) infections widely occur in fungi, including in plant pathogenic fungi. Several mycoviruses have been characterized to reduce the virulence of their fungal hosts (28). Notably, some mycoviruses have relatively close sequence identities to plant viruses (29–32). These observations give rise to speculation about the transmission of viruses between plant and fungi in the not so distant past. Nevertheless, no contemporary cross-infection of a plant virus to a phytopathogenic fungal host has been described.

Rhizoctonia solani Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is a soil-borne plant pathogenic fungus that has a wide host range, including field crops, vegetables, ornamental plants, alfalfa, shrubs, and fruit trees, and spreads throughout the world (33). The disease symptoms may vary depending on the host plants, but the most common symptoms are root or stem rot, stem canker, and damping-off of seedlings (34). *R. solani* is a basidiomycete fungus that does not produce asexual spores (conidia) and exists primarily as vegetative mycelium and/or sclerotia (resting bodies) in nature (35). Several studies have shown that *R. solani* hosts diverse mycoviruses, including some of the novel viruses that do not belong to any established virus families or genera (36–41).

In this study, we discovered an infection of a plant virus, cucumber mosaic virus (CMV), in an *R. solani* strain isolated from potato plants (*Solanum tuberosum* L.) in the field. CMV is a positive single-stranded RNA [(+)ssRNA] virus belonging to the genus *Cucumovirus* in the family *Bromoviridae* (42). CMV is known to have the largest host range of any plant virus and is vectored

Significance

Virus cross-infection is an important topic in understanding the course of virus dissemination and evolution. Viruses may spread between the same host species or into taxonomically distinct organisms. The occurrences of cross-kingdom viral infection for certain virus groups are suggested by the current virus taxonomic data. In particular, several plants and fungal viruses show close phylogenetic relationships, but productive transmission of virus between plant and fungal hosts in nature has not been directly demonstrated. Here, we describe the natural infection of *Rhizoctonia solani* fungus by a plant virus, cucumber mosaic virus (CMV). We further demonstrate that *R. solani* can acquire and transmit CMV during plant infection. Our findings are evidence of cross-kingdom virus transmission from the plant to fungus.

MICROBIOLOGY

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mainly by aphid insects in a nonpropagative manner (43, 44). CMV has a tripartite genome composed of RNA1, RNA2, and RNA3, each encapsidated within isometric virus particles (44). Furthermore, we were able to demonstrate the transmission of CMV from plant to *R. solani*, as well as the opposite direction from *R. solani* to plant under laboratory conditions. We discuss the significance of our findings in the context of understanding the host range expansion, spread, and evolution of plant viruses in nature.

Results

Discovery of a CMV Isolate Infecting an *R. solani* Strain. To investigate the occurrence of mycoviruses in *R. solani*, we screened 29 *R. solani* strains isolated from potato plants grown in several fields in Inner Mongolia Province of China for the presence of double-stranded RNA (dsRNA), which is a molecular marker for RNA virus infection. Eleven strains were found to contain dsRNAs with diverse sizes, and seven fungal strains representing each distinct dsRNA pattern were selected for further analysis (*SI Appendix*, Fig. S1*A*). Those dsRNA-containing strains showed similar colony morphology (*SI Appendix*, Fig. S1*B*). Sequence analysis of the intergenic spacer region (ITS) of rRNA indicated that those *R. solani* strains belong to the hyphal anastomosis group (AG)-3 (45). The dsRNAs extracted from those seven strains were pooled and then subjected to next-generation sequencing (NGS). BLASTp searches using the contig sequences assembled from the NGS reads revealed a number of mycovirus-related sequences. Surprisingly, in addition to these, three contigs (DN711_c0_gl–DN711_c0_g3) showed high sequence identity with RNA1, 2, 3 of CMV (*SI Appendix*, Table S1). The CMV sequences identified in *R. solani* (hereafter referred to as CMV-Rs) show a typical CMV genome organization in which RNA1 encodes 1a (replicase component), RNA2 encodes 2a (replicase component) and 2b (RNA-silencing suppressor), and RNA3 encodes 3a (movement protein, MP) and coat protein (CP) (Fig. 1*A*). BLAST analysis indicated that the CMV-Rs sequences are most closely identical (>98% identity) to those of CMV isolates from East Asia countries that belong to the subgroup Ia.

RT-PCR and sequence analysis of the amplification products confirmed that CMV-Rs is present in one *R. solani* strain designated as Ra1 (Fig. 1*B*). Consistently, this strain, but not the cured strain (Ra1/CMV-cured), contains some dsRNA segments (3.3, 3.0, 2.3, and 1.2 kbp) that resemble the pattern of dsRNAs extracted from the leaves of CMV-infected plants (Fig. 1*C*). RT-PCR analysis using primer sets specific for the virus-related sequences showed that the Ra1 strain also contains at least three (+)ssRNA mycoviruses related to endornaviruses and mitoviruses (Fig. 1*C* and *SI Appendix*, Table S1). The complete analysis of mycoviruses identified from our current screening on *R. solani* strains will be published elsewhere.

To validate that CMV-Rs is indeed a plant virus, the total RNA extracted from Ra1 (CMV-Rs–carrying strain) was used as inoculum for mechanical rub-inoculation of the leaves of *Nicotiana benthamiana* plants. Starting from 7 days after inoculation (dai), inoculated plants showed a typical mosaic symptom, which is similar to the symptom induced by a subgroup I CMV isolate (Fny) (46, 47) in *N. benthamiana* plants (Fig. 1*D*), and virus accumulations in upper leaves of inoculated plants were confirmed by RT-PCR (Fig. 1*E*). These results strongly suggest that CMV-Rs is a plant virus that has crossed the host-specificity barrier and infects phytopathogenic fungus *R. solani*.

Introduction of CMV into *R. solani* and Other Fungi. We then questioned whether *R. solani* could also host other CMV isolates. To answer this, the CMV-Fny isolate, which has 95% amino acid sequence identity to CMV-Rs in 1a proteins (*SI Appendix*, Fig. S2), was introduced into dsRNA-free *R. solani* strain (Rs/dsF) by transfection of fungal protoplasts with purified virus particles. After transfection and regeneration of *R. solani* protoplasts, CMV-Fny accumulation was detected by either RNA or Western blotting (Fig. 2 *A* and *B*) and dsRNA analysis (Fig. 2*C*), indicating that *R. solani* supports the replication of another CMV isolate. Moreover,

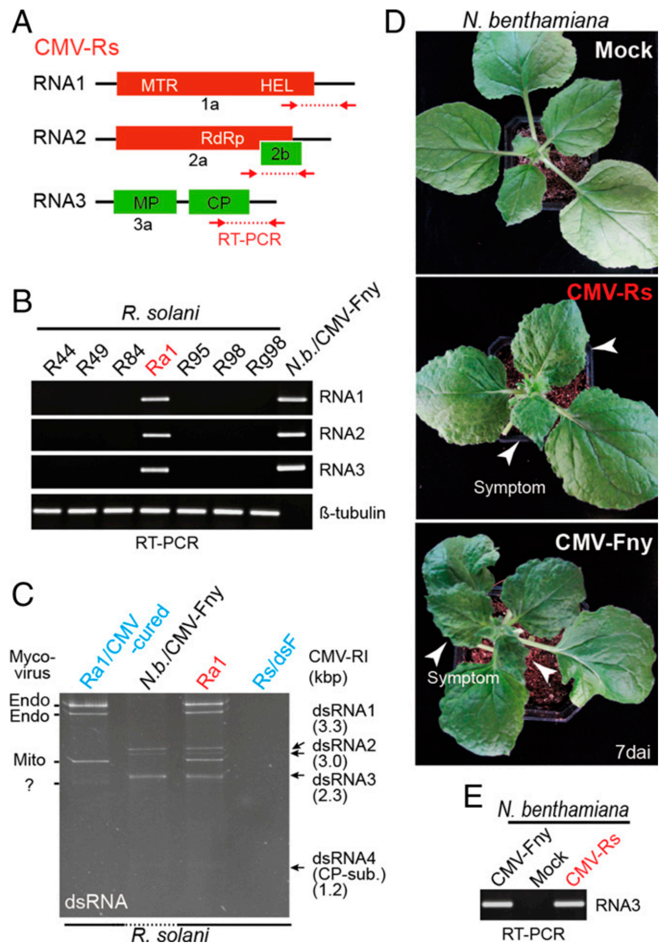

Fig. 1. CMV infection in *R. solani*. (*A*) Schematic representation of genome structure of CMV-infecting *R. solani* (not to scale). Colored boxes and lines indicate the reading frame (ORF) and untranslated regions (UTR), respectively. (*B* and *E*) RT-PCR detection of CMV in *R. solani* strains (*B*) or *N. benthamiana* plants (*E*). ssRNA fraction extracted from *R. solani* strains or *N. benthamiana* leaves were used for RT-PCR using primer sets specific for CMV RNA 1, 2, 3 and *R. solani* beta-tubulin. PCR products were run on agarose gel electrophoresis and stained with ethidium bromide (EtBr). (*C*) dsRNA profiles of *R. solani* strain infected with CMV or cured. dsRNAs were extracted from total RNAs of fungi, run on polyacrylamide gel electrophoresis, and stained with EtBr. dsRNA extracted from leaves of CMV-infected *N. benthamiana* plants was included in the gel electrophoresis (Nb/CMV-Fny). (*D*) Virus symptom expressions in *N. benthamiana* plants mechanically rub-inoculated with CMV-Fny virions or total RNA extracted from *R. solani* carrying CMV-Rs. Plants were photographed at 7 dai. CP-sub, CP subgenomic RNA; N.b., *N. benthamiana*.

CMV-Fny was stably maintained in this *R. solani* transfectant during successive cultures (*SI Appendix*, Fig. S3). Nevertheless, CMV-Fny infection did not affect the morphology and growth of *R. solani* on potato dextrose agar (PDA) medium (Fig. 2*D*).

As mycoviruses are commonly transmitted horizontally via hyphal anastomosis and vertically through sporulation (48), we investigated if CMV also undergoes such transmissions in *R. solani*. First, in a horizontal transfer experiment, where Rs/dsF and CMV-Fny–infected *R. solani* (Rs/CMV-Fny) strains were cocultured side-by-side on a PDA medium, CMV-Fny was efficiently transmitted to Rs/dsF through hyphal fusion (Fig. 2 *E* and *F*). A similar result was obtained when the Ra1 strain was used as a donor in a hyphal fusion experiment (*SI Appendix*, Fig. S4). Because *R. solani* does not produce asexual spores (conidia), we examined CMV transmission through basidiospores (sexual spores). Of 100 basidiospore progenies of Ra1, none was found to contain CMV-Rs, whereas the

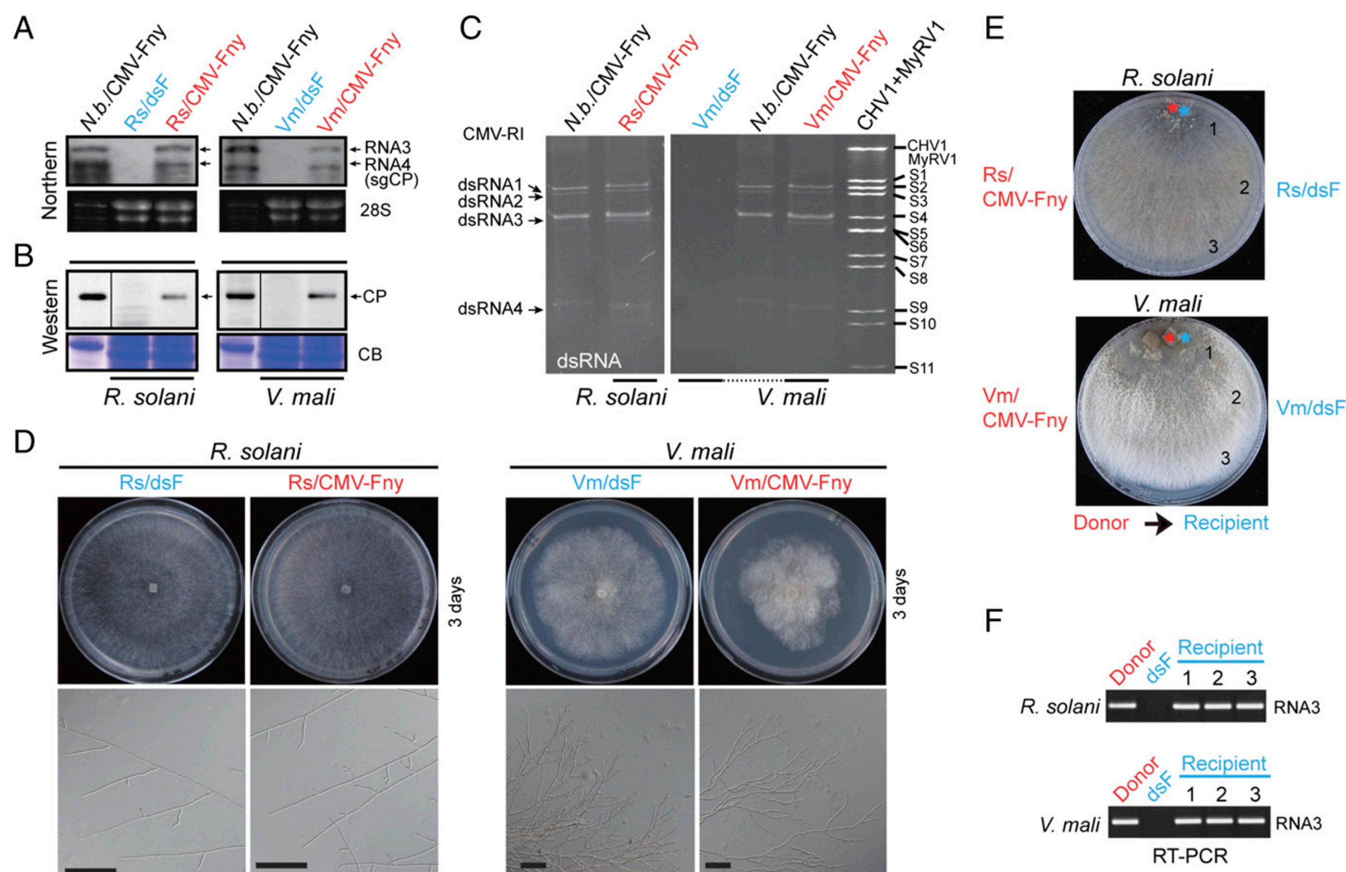

Fig. 2. The introduction of CMV into fungal protoplasts. (*A*) RNA blot analysis of CMV RNA3 accumulation in *R. solani* and *V. mali* strains regenerated from protoplasts transfected with CMV-Fny virions. An RNA sample derived from CMV-Fny–infected leaves of *N. benthamiana* plants was included in the analysis. The RNA gel was stained with ethidium bromide, and 28S rRNA is shown as a loading control (28S). (*B*) Western blot analysis of CMV CP accumulation in fungal strains and leaves described in *A*. Vertical lines indicate that two separate lanes in the same blot were spliced together. To confirm protein loading amount, coomassie blue-stained total proteins (CB) run on separated gels are shown as loading controls (*Bottom*). (*C*) dsRNA profiles of *R. solani* and *V. mali* strains infected with CMV. dsRNA extracted from leaves of CMV-infected *N. benthamiana* plants was included in the gel electrophoresis (Nb/CMV-Fny). dsRNA of Cryphonectria hypovirus 1 (CHV1) and mycoreovirus 1 (MyRV1) were used as the size marker. (*D*) Colony and hyphal morphology of *R. solani* and *V. mali* strains infected with CMV-Fny on PDA medium. Fungi were grown for 3 d and photographed. (Bars, 100 μm.) (*E*) Coculturing of virus-infected and virus-free fungal strains on PDA medium for investigating virus horizontal transfer through hyphal fusion. The transmission of the virus from donor to recipient fungi is indicated by arrows. Number (1–3) indicates the positions where the mycelia of fungal recipients were taken for subsequent culture. Plates were photographed at 6 d after coculture. (*F*) RT-PCR detection of CMV RNA3 accumulation in fungal recipients described in *E*. N.b., *N. benthamiana*.

presence of endornaviruses was consistently detected in the progenies (*SI Appendix*, Fig. S5). Thus, unlike endornaviruses, CMV is not transmitted through sexual spores.

To investigate the ability of CMV to replicate in other fungi, CMV-Fny virions were transfected to protoplasts of three phytopathogenic ascomycete fungi, *Valsa mali*, *Cryphonectria parasitica*, and *Fusarium graminearum*, which are known as the causal agents of apple canker, chestnut blight, and wheat head blight diseases, respectively. CMV-Fny accumulation was detected in *V. mali* (Fig. 2 *A*–*C*), and this virus was stably maintained and transmitted horizontally via hyphal anastomosis in the transfectant (Fig. 2*E* and *SI Appendix*, Fig. S3). CMV-Fny–infected *V. mali* developed more aerial hyphae than virus-free strain (Vm/dsF) on PDA medium (Fig. 2*D*), and in a virulence assay on apple fruits and apple twigs, Vm/CMV-Fny produced bigger lesions than Vm/dsF (*SI Appendix*, Fig. S6). In contrast, a very low level of CMV-Fny accumulation was detected by RT-PCR in the first-generation transfectants of *C. parasitica* and *F. graminearum*, and later, the virus was undetectable after subsequent subcultures (*SI Appendix*, Fig. S7), indicating that these two fungi are not suitable hosts for CMV.

CMV Transferred from Plant to *R. solani*. Our finding of CMV infection in a field strain of *R. solani* raises the important question of whether CMV could be transferred from plant to *R. solani* during infection in the natural environment. To explore this possibility, we examined virus acquisition by *R. solani* under laboratory conditions. In this experiment, potato and *N. benthamiana* plants infected with CMV-Fny were then inoculated with Rs/dsF in the lower stem. After allowing *R. solani* to colonize the stem, the fungus was then reisolated from the plants, cultured, and subjected for virus detection using RT-PCR (Fig. 3*A*). Strikingly, Rs/dsF induced much more severe stem rot disease in CMV-infected than in virus-free potato and *N. benthamiana* plants, showing that CMV infection enhances the plant susceptibility to *R. solani*. At 14 or 4 d after *R. solani* inoculation in potato and *N. benthamiana* plants, respectively, the stems of CMV-infected plants were heavily rotted (damping off), and the plants were collapsed, while virus-free plants retained vigor (Fig. 3*B* and *SI Appendix*, Fig. S8*A*). By RT-PCR, we found that 38% and 28% of fungal strains isolated from virus-infected potato and *N. benthamiana* plants, respectively, were carrying CMV-Fny (Fig. 3 *C* and *D* and *SI Appendix*, Fig. S8*B*), demonstrating that *R. solani* could acquire CMV during infection.

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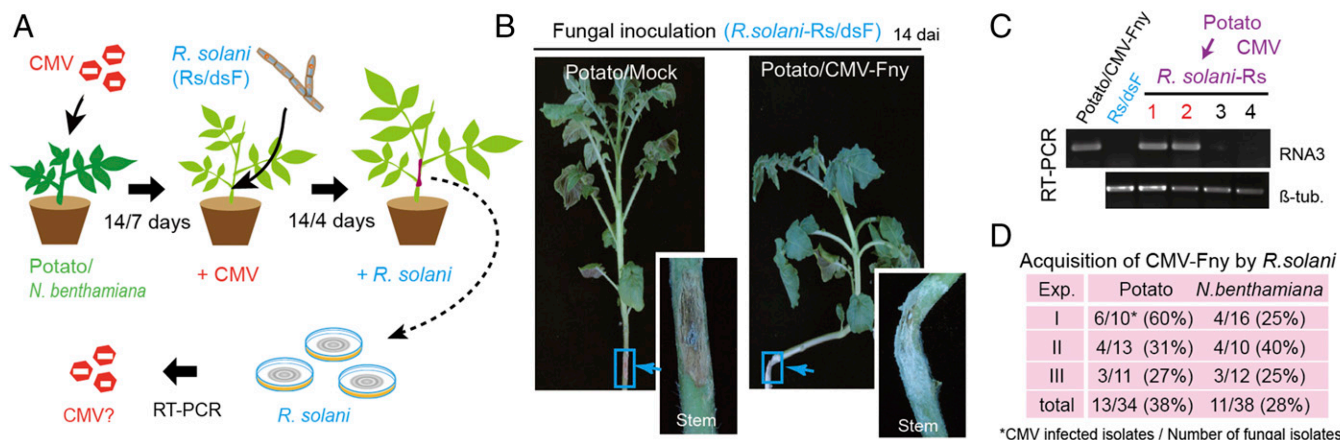

Fig. 3. Acquisition of CMV by *R. solani*. (*A*) A cartoon illustration describing the experimental procedure for investigating CMV acquisition by *R. solani*. (*B*) Stem rot disease in virus-free and CMV-infected potato plants inoculated with virus-free *R. solani* (Rs/dsF). (*Insets*) Close-up views of the inoculated stems. (Magnification: 4.5×.) Plants were photographed at 14 d after fungal inoculation. (*C*) RT-PCR detection of CMV RNA3 accumulation in *R. solani* strains isolated from inoculated stem of potato plants. (*D*) Efficiency of CMV-Fny acquisition by *R. solani* from potato and *N. benthamiana* plants. Exp, experiment; tub, tubulin.

R. solani **Transmits CMV to the Plant.** Next, we examined whether CMV could be transmitted to the plant by inoculating CMV-infected *R. solani* to the stem of potato or *N. benthamiana* plants, and then the virus accumulation in upper leaves was analyzed. Interestingly, severe stem rot disease was induced by Rs/CMV-Fny and Ra1 infections, leading to plant collapse on day 12 (potato) or 4 (*N. benthamiana*) after inoculation, while virus-free Rs/dsF caused only mild stem rot, and the plants remained erect (Fig. 4*A* and *SI Appendix*, Fig. S9*A*), similar to previous observations (Fig. 3*B* and *SI Appendix*, Fig. S8*A*). At this point, the plants infected with Ra1 and Rs/CMV-Fny did not show any obvious virus mosaic symptoms, but CMV accumulations were detected by RT-PCR in the uppermost leaves of around half of the potato plants and all *N. benthamiana* plants (Fig. 4 *B* and *C* and *SI Appendix*, Fig. S9*B*). When the same uppermost leaves were placed on medium, no growth of *R. solani* colony was observed (*SI Appendix*, Fig. S10), suggesting that the fungus did not systemically spread to the upper leaves. This result shows that *R. solani* could transmit CMV to the plant during infection.

Discussion

In light of the potential application for the biological control of crop fungal diseases, extensive efforts have been made to identify and characterize viruses infecting phytopathogenic fungi (28, 48). To our knowledge, natural infection of a plant virus in a fungus has not been reported previously. A plant virus, tobacco mosaic virus [TMV, genus *Tobamovirus*, a monopartite (+)ssRNA genome] or its relatives has long been known to associate with some fungi, such as rust and powdery mildew, but the direct evidence of virus replication in fungal cells was not reported (49, 50). Many mycoviruses and plant viruses share similar characteristics, especially in terms of replication and coding strategies of their genome, but notably, a number of mycoviruses are capsidless, and an extracellular phase is generally absent or unknown in the mycoviruses life cycle, while the majority of plant viruses are spread by biological vectors, which are mainly arthropods (51–53). The compatibility between plant virus and fungus as a host was first demonstrated experimentally by replication of brome mosaic virus [BMV, genus *Bromovirus*, a tripartite (+)ssRNA genome] in yeast *Saccharomyces cerevisiae* (12), and later, a similar yeast system has been successfully applied to replication of other plant viruses (9). More recently,

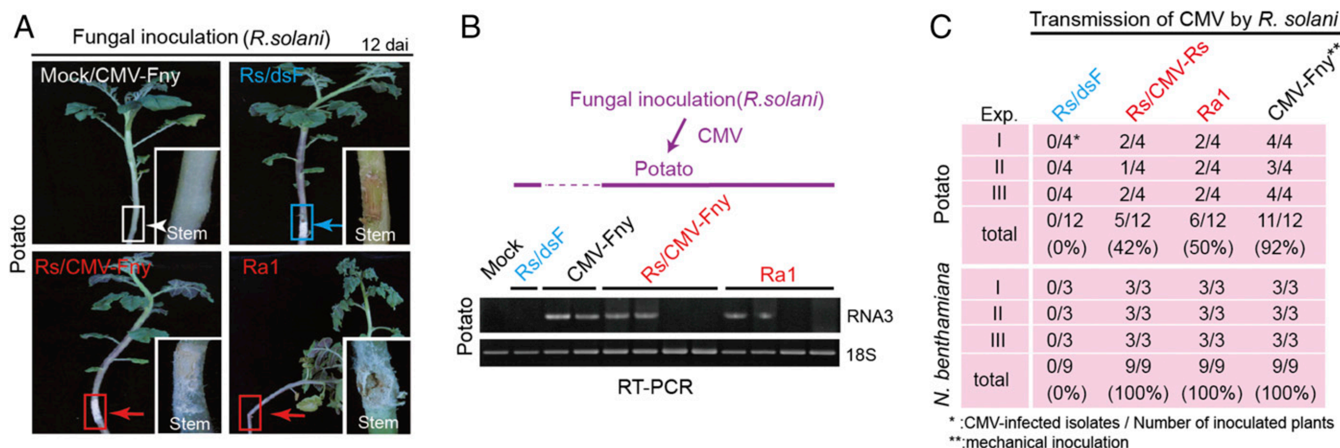

Fig. 4. Transmission of CMV from *R. solani* to the plant. (*A*) Development of stem rot disease in potato plants infected with virus-free (dsF) and virus-infected *R. solani* strains. (*Insets*) Close-up views of the inoculated stems. (Magnification: 3×.) Plants were photographed at 12 dai. (*B*) Detection of CMV RNA3 accumulation in uppermost leaves of potato plants by RT-PCR. (*C*) Efficiency of CMV transmission by *R. solani* to the potato and *N. benthamiana* plants. Exp, experiment.

TMV was shown to replicate in ascomycete fungus *Colletotrichum acutatum* (8). Likewise, we have demonstrated that two quite divergent filamentous fungi, *R. solani* (a basidiomycete) and *V. mali* (a ascomycete), are compatible hosts for CMV multiplication (Fig. 2). Thus, many phytopathogenic fungi may be able to support the multiplication of plant viruses, in particular, those belonging to the alpha-like (+)ssRNA virus superfamily such as CMV, TMV, and BMV (14). Considering the high probability of coinfection of an individual plant with phytopathogenic fungi and plant viruses in nature, we anticipate that the cross-infections of a plant virus to fungi are not merely isolated cases and could occur more frequently.

The observation that *R. solani* could acquire and transmit CMV during infection presents a finding that parasitic materials could be translocated bidirectionally between plant and pathogenic fungus during infection. Unlike plant biotrophic fungi, such as rust and powdery mildew fungi that commonly absorb nutrients from the host cells using feeding organs called haustoria without penetrating the plant plasma membrane, *R. solani* is a necrotrophic pathogen that actively decomposes the host cells for uptake of nutrients. This possibly allows the flow of bigger molecules, such as virus particles that are present in the plant cell contents into fungal cells. The previous report showed that necrotrophic ascomycete fungus *Sclerotinia sclerotiorum* could be infected by an ssDNA mycovirus, *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1, genus *Gemycircularvirus*), when virions were applied extracellularly to hyphae, but the mechanism of SsHADV-1 entry into fungal cells remains unclear (54). Similarly, TMV enters and replicates in *C. acutatum* (hemibiotrophic) through the addition of virus particles to liquid medium (8). It has been suggested that TMV enters the cell through cell membrane damage that occurs during hyphae grow or through pinocytosis (55). Nevertheless, it is also possible that CMV is translocated between the plant and fungus in the form of naked RNA or ribonucleoprotein. It is hypothesized that small-RNA molecules are transferred from plant to fungus through an exosomal pathway (56). Further studies are needed to elucidate the mechanism by which CMV translocates between plant and fungus.

A question arises whether *R. solani* is a potential vector transmission of CMV. In fact, a number of plant viruses are transmitted by zoosporic soil-borne fungi (chytrids) or plasmodiophorids (protists) to the roots (51, 57). Nonetheless, zoosporic vectors are not known to be the host of virus multiplication, and the association of the virus with a vector is mediated by direct attachment of virus particles to the surface of vector or facilitated by viral-encoded protein through unknown mechanisms (51, 58). Moreover, those soil-borne vectors are not considered serious pathogenic threats to the crop plant. *R. solani* is also a soil-borne fungus that infects roots, stems, and underground plant organs (34). The efficiency of CMV acquisition and transmission by *R. solani* should be tested under field conditions, and extensive screening of *R. solani* strains for the CMV infection would reveal the actual prevalence of viruliferous fungus in the field. Compared with virus transmission by common soil-borne vectors, transmission of a plant virus by a phytopathogenic fungus may pose a greater threat in the sense that simultaneous infection of two different pathogens could elicit more serious damage to the plant (59). Interestingly, our experiment showed synergistic effects of CMV infection on *R. solani* virulence in potato and *N. benthamiana* plants (Figs. 3*B* and 4*A* and *SI Appendix*, Figs. S8 and S9). CMV 2b protein, which interferes with basal plant defense-signaling pathways (60, 61), might contribute to this synergism. CMV infection does not affect the growth and morphology of *R. solani* (Fig. 2*D*); thus, in terms of pathogenicity, acquiring CMV seems to benefit *R. solani*.

Plant and fungal partitiviruses (bipartite dsRNA virus, picornavirus-like superfamily)/endornaviruses [alpha-like (+)ssRNA virus superfamily] show close phylogenetic relationships (31, 32). Likewise, some mycoviruses classified as members of the family *Flexiviridae* [alpha-like (+)ssRNA virus superfamily], which previously contains only plant virus members, were also found (62–65). Considering the compatibility of certain plant alpha-like (+)ssRNA viruses with fungal hosts as demonstrated by this and previous studies, future investigations of viruses infecting fungi may further uncover the occurrence of past and ongoing transmissions of this virus group into the fungal hosts. Collectively, our data therefore supports the view that the transmission of viruses between plant and fungal hosts contributes to the evolution and genetic diversities of plant and fungal viruses. Mycovirus with a close phylogenetic relationship to CMV has so far not been found in fungi. A long-term study is necessary to monitor CMV adaptation and evolution within the fungal host. Lastly, from an ecological perspective, cross-infection of plant viruses to fungus could extend the reservoir of plant viruses in the environment. Intriguingly, some plant viruses including CMV were recently found in some invertebrates unrelated to plant-virus insect vectors by metatranscriptomic analyses (66). Taken together, these observations imply that certain plant viruses may actually spread beyond their known conventional hosts.

Experimental Procedures

Fungal Strains, Virus Isolates, and Plant Materials. A total of 319 *R. solani* strains were isolated from potato tubers with black scurf disease randomly collected from different potato-planting areas located in the middle and western parts of Inner Mongolia Province of China from year 2008–2015. All fungal strains were maintained at laboratory. For identification of *R. solani* strains, fungal DNA was isolated using standard phenol–chloroform extraction and ethanol precipitation and used for PCR amplification of the ITS1 and ITS2 regions of ribosomal RNA (67). The amplified ITS sequences were subjected to Nucleotide BLAST search. Twenty-nine fungal strains were selected for dsRNA screening. CMV-Rs sequences have been submitted to NCBI with the accession number, MG025947 (RNA1), MG025948 (RNA2), and MG025949 (RNA3). The CMV-cured Ra1 strain is obtained by a single basidiospore isolation. *C. parasitica* strain EP155 infected with Cryphonectria hypovirus 1 or mycoreovirus 1 and virus-free were generous gifts from Nobuhiro Suzuki (Okayama University, Japan). *V. mali* YL strain was isolated from an apple orchard in Shaanxi Province, China. *F. graminearum* PH1 strain was provided by Zhonghua Ma (Zhejiang University, China). CMV-Fny virions were provided by X. B. Wang (China Agricultural University, China). CMV isolates were maintained in *N. benthamiana* plants and used for inoculation.

All fungal strains were grown on PDA medium for 3–6 d at 24–26 °C for morphological observation or on cellophane-covered PDA medium for RNA, DNA, and protein extractions. Potato cultivar Shepody was used in this study and provided by Bolin Liu (Northwest A&F University, China). Potato and *N. benthaniana* plants were grown in a growth room at 22 ± 2 °C with a photoperiod of 16 h/8 h (day/night).

Virus and Fungal Inoculation. For mechanical virus inoculation, virions, total RNAs, or CMV-infected leaves homogenated in 0.1 M phosphate buffer (pH 7.0) were used as the inoculums and rubbed onto carborundum-dusted leaves of potato and *N. benthamiana* plants. For inoculation of *R. solani* to potato and *N. benthamiana* plants, lower stems were wounded using sterilized toothpicks, and mycelia-containing gel plugs (around 0.5 × 1 cm), which were picked up from the edge of a 3-d old culture colony, were placed on the wounded area. The inoculated part of the stem was wrapped with parafilm for 24 h. Inoculated plants were grown at 22 ± 2 °C, 70–80% humidity, and a photoperiod of 16 h/8 h (day/night).

Virus Acquisition by *R. solani*. To investigate CMV acquisition by *R. solani*, potato and *N. benthamiana* plants were first mechanically rub-inoculated with CMV (12–16 plants were inoculated in each experiment), and, after virus infection was confirmed by RT-PCR (at 14 and 7 dai in potato and *N. benthamiana* plants, respectively), virus-free *R. solani* was then inoculated to the lower stems. Fourteen (potato) or four (*N. benthamiana*) dai, *R. solani* was retrieved from the inoculated plants with the procedures as follow; small pieces (roughly 0.2 × 0.5 cm) of stem tissue colonized by the fungus were cut and washed using 75–80% ethanol solutions and followed by several rinses with sterilized water. The stem tissue pieces (about 15 pieces from one stem) were then put on PDA medium containing streptomycin, ampicillin, and tetracycline (50 μg/mL). After the fungi had grown, colonies were subcultured on fresh PDA medium. *R solani* strains were reconfirmed by PCR amplification and sequence analysis of the ITS regions.

Next-Generation Sequencing and Bioinformatics Analysis. Preparation of the cDNA library for next-generation sequencing was performed using NEBNext UltraTM RNA Library Prep Kit for Illumina (New England Biolabs) and

MICROBIOLOGY

sequenced on the Illumina HiSEq 4000 platform (Illumina). Raw reads were cleaned by removing adapter sequences, low-quality bases (PHRED quality scores ≤5) were trimmed with a Trimmomatic package with default parameter, and truncated reads smaller than 35 bp were discarded. All clean reads were then assembled using the de novo assembly program Trinity (trinityrnaseq.github.io) with K-mer value = 25. All assembled transcripts were subjected to BLASTx searches with a cutoff of E ≤ 1e-5.

Fungal protoplast isolation, virus transfection, RNA extraction, RT-PCR detection, RNA blot analysis, and Western blot analysis methods were described in *SI Appendix, SI Experimental Procedures*.

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