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BACTERIAL, FUNGAL AND VIRUS MOLECULAR BIOLOGY - REVIEW

Mycovirus-induced hypovirulence in notorious fungi Sclerotinia: a comprehensive review

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Abstract

Members of the genus *Sclerotinia* are notorious plant pathogens with a diverse host range that includes many important crops. A huge number of mycoviruses have been identified in this genus; some of these viruses are reported to have a hypovirulent effect on the fitness of their fungal hosts. These mycoviruses are important to researchers from a biocontrol perspective which was first implemented against fungal diseases in 1990. In this review, we have presented the data of all hypovirulent mycoviruses infecting *Sclerotinia sclerotiorum* isolates. The data of hypovirulent mycoviruses ranges from 1992 to 2023. Currently, mycoviruses belonging to 17 different families, including (+) ssRNA, (−ssRNA), dsRNA, and ssDNA viruses, have been reported from this genus. Advances in studies had shown a changed expression of certain host genes (responsible for cell cycle regulation, DNA replication, repair pathways, ubiquitin proteolysis, gene silencing, methylation, pathogenesis-related, sclerotial development, carbohydrate metabolism, and oxalic acid biosynthesis) during the course of mycoviral infection, which were termed differentially expressed genes (DEGs). Together, research on fungal viruses and hypovirulence in *Sclerotinia* species can deepen our understanding of the cellular processes that affect how virulence manifests in these phytopathogenic fungi and increase the potential of mycoviruses as a distinct mode of biological control. Furthermore, the gathered data can also be used for in-silico analysis, which includes finding the signature sites [e.g., hypovirus papain-like protease (HPP) domain, "CCHH" motif, specific stem-loop structures, p29 motif as in CHV1, A-rich sequence, CA-rich sequences as in MoV1, GCU motif as in RnMBV1, Core motifs in hypovirus-associated RNA elements (HAREs) as in CHV1] that are possibly responsible for hypovirulence in mycoviruses.

Keywords *Sclerotinia sclerotiorum* · Mycovirus complex · Differential expressed genes · Neo-lifestyles of mycoviruses · Fungal pathogenicity · Biocontrol

Introduction

Sclerotinia pathogens are a well-known and devastating plant pathogen in the Ascomycota group and are responsible for stem rot disease. It has a wide host range and can infect different varieties of oilseed rape, sunflower, chickpea, soybean, lettuce, and almost all dicotyledonous plants and vegetables, resulting in massive yield losses worldwide [1]. This fungus produces sclerotia that can survive in soil for several years, allowing it to persist and infect crops in subsequent growing seasons. The rotted stems, leaves, and pods of the affected plants can cause significant yield losses. The fungus can also infect flowers, causing them to wilt and turn brown [2]. Plants infected with this fungus have softer tissues, which eventually lead to necrosis due to oxalic acid and hydrolytic enzyme production [3]. Crop rotation, sanitation, fungicides, and organic soil amendments cultural practices (such as planting resistant varieties, avoiding overplanting, and promoting good air circulation) are some standard procedures that farmers typically use. Fungicides were initially considered necessary because no known cultivar was resistant to them. Resistance to carbendazim and dimethachlon has been reported in a strain of *S. sclerotiorum* [4].

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Fungicide residues have purposefully harmed the pristine nature of the environment, harming the biology of natural enemies, pollution at its peak, and the socioeconomic welfare of consumers and producers. Some pesticides can remain in the soil for up to 10 years, posing an insidious threat to the environment. However, its widespread use has resulted in the evolution of resistant fungal strains [5]. Secondly, these chemicals can possibly cause cancer, skin allergies, and other potentially fatal complications in humans and animals. Traditional control methods, on the other hand, are time-consuming and labor-intensive [6]. However, none of these strategies have been proven to be effective (Fig. 1). However, using microorganisms to limit these infections is a more advanced, safe, and environmentally friendly method [11]. As a result, implementing biological control can change the scenario to the level desired for sustainable agriculture. Despite the presence of vegetative incompatibility in the population, which creates a barrier to using mycovirus as a biocontrol tool, mycovirus has been extensively studied at the molecular level [12, 13]. Their ability to destroy virulent strains has piqued the interest of researchers, and drawbacks can be mitigated with advanced, sophisticated protocols.

The phenomenon of mycovirus-associated hypovirulence was first described by Antonio Biraghi with the aim of developing a hypovirus called BCA to control fungal infections. However, mycoviruses have not been commercialized as biocontrol agents due to the limitations of vegetative incompatibility (VIC) and the lack of extracellular routes of entry [14]. However, the idea of using mycoviruses as external fungicides sounds promising, similar to commercial products based on other microorganisms such as phages, fungi, and bacteria [15]. The potential of mycoviruses to be used as biocontrol agents has been demonstrated in various studies. Controlling the chestnut blight fungus (*Cryphonectria parasitica*) is the best demonstration of mycoviral-induced biological control [16]. Other hypoviruses from different families have been reported, including Mycoreovirus 1 (MyRV-1) and Cryphonectria mitovirus 1 (CpMV1) [17, 18]. A Rosellinia necatrix megabirnavirus 1 (RnMBV1) has also been reported to have excellent biocontrol capabilities [19]. Several mycovirus families with either an RNA or DNA genome have been found to have hypovirulence-associated mycoviruses that infect *S. sclerotiorum* (details of hypovirulence-associated mycoviruses are summarized in Table 1). *Sclerotinia* genera have also yielded a number of hypovirulent mycoviruses. The available literature lacks comprehensive details about these viruses. Given the importance of these viruses in the field, we present detailed information on all mycoviruses reported from *Sclerotinia* genera thus far. The collected data will allow researchers to compare genomic properties, biological attributes, and the effects associated with them to previously studied fungal or viral strains. This review covers all the necessary details of mycoviruses inducing hypovirulence in *S. sclerotiorum*. Gathered information will provide an in-depth insight into those viruses with the potential to be an effective biocontrol strategy for the management of *S. sclerotiorum*, as well as the extent to which various protection strategies are used. Furthermore, the collective information will be helpful for the researchers who are working with this pathogen and its virome.

Incidence of mycoviruses in hypovirulent isolates of *Sclerotinia* genus

Early research for mycoviral screening of 30 isolates of *S. sclerotiorum* using conventional methods of agarose gel electrophoresis (AGE) identified 12 dsRNA-positive isolates, but only 5 isolates were associated with hypovirulence.

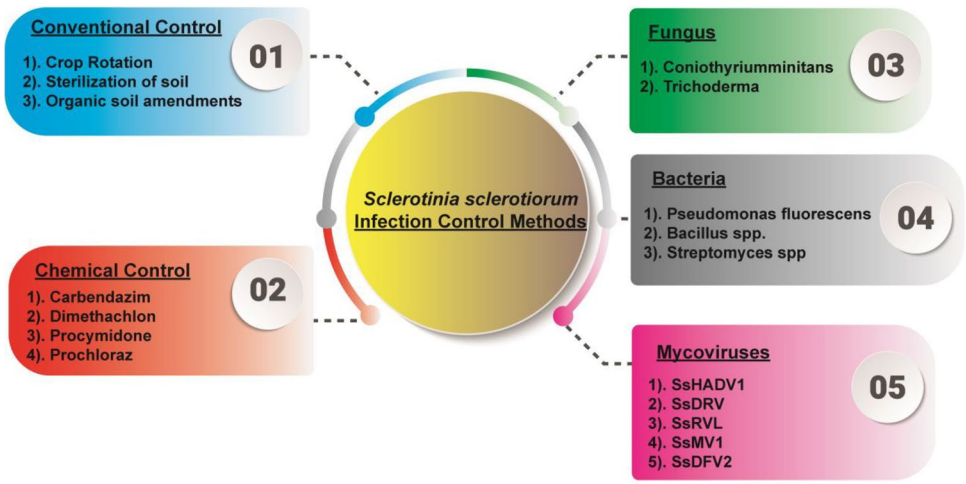

Fig. 1. A depiction of various control strategies used against *S. sclerotiorum* infection. In summary, three types of control strategies are used against *S. sclerotiorum* infection, i.e., conventional [7], chemical [2], and biological control including other fungal strains, bacterial, and mycoviruses [8–10]

Table 1 List of mycoviruses associated with hypovirulence in *Sclerotinia* species

Sr.	Mycovirus Name	Abbreviation	Genome	Isolate name	Family	Segments (size)	Genomic accessions	References
1.	Sclerotinia sclerotiorum dsRNA virus	SsDV	dsRNA	91	Unclassified	–	Not provided	[20]
2.	Sclerotinia sclerotiorum debilitation-associated RNA virus	SsDRV	(+) ssRNA	Ep-1PN	*Alphaflexiviridae*	5470 bp	AY147260.1	[21]
3.	Sclerotinia sclerotiorum RNA virus L	SsRV-L	dsRNA	Ep-1PN	*Closteroviridae*	6043 bp	EU779934.1	[22]
4.	Sclerotinia sclerotiorum deltaflexivirus 2	SsDFV2	(+) ssRNA	228	*Deltaflexiviridae*	6735 bp	MH299810.1	[23]
5.	Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1	SsHADV-1	ssDNA	DT-8	*Genomoviridae*	2166 bp	NC_013116.1	[10]
6.	Sclerotinia sclerotiorum hypovirus 1	SsHV1	dsRNA	SZ-150	*Hypoviridae*	10,438 bp	JF781304.1	[24]
7.	Sclerotinia sclerotiorum hypovirus 2	SsHV2	dsRNA	5472	*Hypoviridae*	14,581 bp	KF525367.1	[25]
8.	Sclerotinia sclerotiorum hypovirus 2 Lactuca	SsHV2L	dsRNA	328	*Hypoviridae*	14,581 bp	NC_022896.1	[25, 26]
9.	Sclerotinia sclerotiorum hypovirus 2	SsHV2	dsRNA	SX247	*Hypoviridae*	15,239 bp	KJ561218.1	[24, 27]
10.	Sclerotium rolfsii hypovirus 1	SrfHV2	dsRNA	BLH-1	*Hypoviridae*	3558 bp 2781 bp	KU885931.1 KU885932.1	[28]
11.	Sclerotinia sclerotiorum mitovirus 1	SsMV1	dsRNA	KL-1	*Mitoviridae*	2513 bp	JQ013377.1	[29]
12.	Sclerotinia sclerotiorum mitovirus 2	SsMV2	dsRNA	KL-1	*Mitoviridae*	2421 bp	JQ013378.1	[29]
13.	Sclerotinia sclerotiorum mitovirus 2	SsMV2	(+) ssRNA	16235	*Mitoviridae*	2445 bp	NC_040434.1	[29, 30]
14.	Sclerotinia sclerotiorum mitovirus 1	SsMV1	dsRNA	HC025	*Mitoviridae*	2530 bp	KJ463570.1	[31, 32]
15.	Sclerotinia sclerotiorum mitovirus 2	SsMV2	(+) ssRNA	16235	*Mitoviridae*	2438 bp	JX401536.1	[30]
16.	Sclerotinia sclerotiorum mitovirus 3	SsMV3	(+) ssRNA	16235	*Mitoviridae*	2617 bp	JX401537.1	[30]
17.	Sclerotinia sclerotiorum mitovirus 4	SsMV4	(+) ssRNA	16235	*Mitoviridae*	2752 bp	JX401538.1	[30, 33]
18.	Sclerotinia Homoeocarpa mitovirus	ShMV	(+) ssRNA	Sh12B	*Mitoviridae*	2632 bp	AY172454.1	[34]
19.	Sclerotinia sclerotiorum narnavirus 4	SsNV4	(+) ssRNA	HC025	*Narnaviridae*	3031 bp	OK165495.1	[31]
20.	Sclerotinia sclerotiorum narnavirus 5	SsNV5	(+) ssRNA	SCH733	*Narnaviridae*	2463 bp 2472 bp	OK573450.1 OK573451.1	[35]
21.	Sclerotinia sclerotiorum negative-stranded RNA virus 1	SsNSRV-1	(−) ssRNA	AH98	*Mymonaviridae*	10,002 bp	KJ186782.1	[36]
22.	Sclerotinia sclerotiorum partitivirus	SsPV1	dsRNA	WF-1	*Partitiviridae*	2292 bp 2334 bp	JX297510.1 JX297511.1	[37]
23.	Sclerotinia sclerotiorum megabirnavirus 1	SsMBV1	dsRNA	SX466	*Megabirnavirus*	16,715 bp 7909 bp	NC_027221.1 NC_027222.1	[38]
24.	Sclerotinia sclerotiorum botybirnavirus 2	SsBRV2	dsRNA	AH16	*Botourmiaviridae*	6159 bp 5872 bp	KT962972.1 KT962973.1	[33]
25.	S. sclerotiorum botybirnavirus 1	SsBRV1	dsRNA	SCH941	*Botourmiaviridae*	12,422 bp	NC_027138.1	[39]
26.	Sclerotinia sclerotiorum ourmia-like virus 22	SsOLV22	(+) ssRNA	HC025	*Botourmiaviridae*	3987 bp	OK165499.1	[31]

Table 1 (continued)

Sr.	Mycovirus Name	Abbreviation	Genome	Isolate name	Family	Segments (size)	Genomic accessions	References
27.	Sclerotinia sclerotiorum ourmiavirus 17	SsOV17	(+) ssRNA	GF3	*Botourmiaviridae*	2802 bp	MW452526.1	[40]
28.	Sclerotinia minor endornavirus 1	SmEV1	dsRNA	LC22	*Endornaviridae*	12,626 bp	MG255170.1	[41]
29.	Hubei sclerotinia RNA virus 1	HuSRV1	(+) ssRNA	277	*Solemoviridae*	4492 bp	MK889164.1	[42]
30.	Sclerotinia sclerotiorum mycoreovirus 4	SsMV4	dsRNA	SX10	*Reoviridae*	24,899 bp	KU128375.1 KU128386.1	[43]
31.	Uncharacterized virus	–	–	XG36-1	Unclassified	–	Not provided	[44]
32.	Uncharacterized virus	–	–	S10	Unclassified	–	Not provided	[45]
33.	Sclerotinia sclerotiorum rhabdovirus 1	SsRhV1	(−)ssRNA	SX276	*Rhabdoviridae*	11,356 bp	MT706019.1	[46]

Interestingly, the associated hypovirulence was lost upon repeated subculturing, and the phenotype was recovered [47]. Another study investigated the pathogenicity and checked for the presence of dsRNA in 132 *S. homoeocarpa* isolates. Surprisingly, 13 of 132 isolates (9.8%) were deemed hypovirulent because they did not produce dollar-spot lesions. In contrast to the other wild-type isolates, six *S. homoeocarpa* isolates grew slowly, had thin colonies, and were unable to develop a characteristic black stroma. Only 6 (46.2%) of the 13 isolates had dsRNA found on agarose gel, indicating that there was no intermediate replication of dsRNA related to other isolates [48, 49]. Recent studies have demonstrated that the titer of some mycoviruses is beyond the resolution of agarose gel electrophoresis and cannot be detected using conventional AGE, but can only be detected by next-generation sequencing (NGS). Diverse and novel mycoviruses have been identified using high-throughput sequencing technologies [50–52].

Mycovirus induced hypovirulence in *Sclerotinia* genus

A wide number of fungal isolates belonging to the genus *Sclerotinia* are found to be associated with hypovirulence (Table 1). The Canadian strain S10 of *S. sclerotiorum* (isolated from sunflower in 1979) was reported to show the phenotype of hypovirulence. This isolate developed tan sclerotia and albino apothecia, whereas a typical isolate would produce black sclerotia and light brown apothecia. When incubated on damp sand, its sclerotia lacked dormancy, and more than 85% of them grew mycelially [53]. The virulent strain killed sunflower seedlings, although isolate S10 had a poor prognosis and was very vulnerable to infection by the hyperparasite *Coniothyrium minitans*. Compared to the *S. sclerotiorum* isolate (Ep-1PN; another hypovirulent isolate), the Ep-1PN isolate showed stronger hypovirulence. DsRNAs were identified in the Ep-1PN but not in the S10 isolate. Isogenic lines derived from single spore isolation were used to evaluate pathogenicity on canola plant leaves, which showed that the virus-free isolates exhibit more pathogenicity. Virus-infected isolates were distinguished from virulent isolates by reduced hyphal development, increased aberrant mycelial branches, decreased sclerotial formation, improved brown pigment production, and decreased oxalic acid production [54, 55].

Hypovirulence associated with these isolates was capable of transmitting to other virulent isolates (S10–2A-11) and suppressing their growth, which confirmed that this hypovirulence phenomenon was directly associated with mycoviral infection and can be transmitted to a virus-free strain through hyphal anastomosis. The phenotype remained stable after further subculture. The hypovirulence in S10-2A-11 was transmissible at a lower rate than isolated Ep-1PN. Subsequently, hypovirulence was also reported from isolate 91 (*S. sclerotiorum*) that infected plants in Canada during the year 1992. It showed retarded growth, altered morphology, and small lesion size [20, 56, 57]. Furthermore, its sclerotia had fewer protein bodies and a more granular appearance compared to virulent isolates. Virion particles were not purified from mycelial homogenates, suggesting their capsidless nature. Virus-infected isolates produced less dry mycelium than virulent isolates [57]. The virus was not cured after treatment with a combined approach of thermotherapy and cycloheximide, which depicts the persistent nature of this virus in its parental strain. The Sclerotinia sclerotiorum dsRNA virus was responsible for this hypovirulent phenotype and was effectively transferred to a compatible recipient strain and induced the same phenotypic changes [20], but later studies have also shown interspecific transmission of mycovirus-induced hypovirulence from *S. sclerotiorum* (isolate Ss275) to *S. minor* (Sm10) [58]. The XG36-1 was identified from a rapeseed stem in China. On PDA medium, it displayed debilitating characteristics such as poor growth, extensive hyphal tip branching, a lack of apothecia, and the

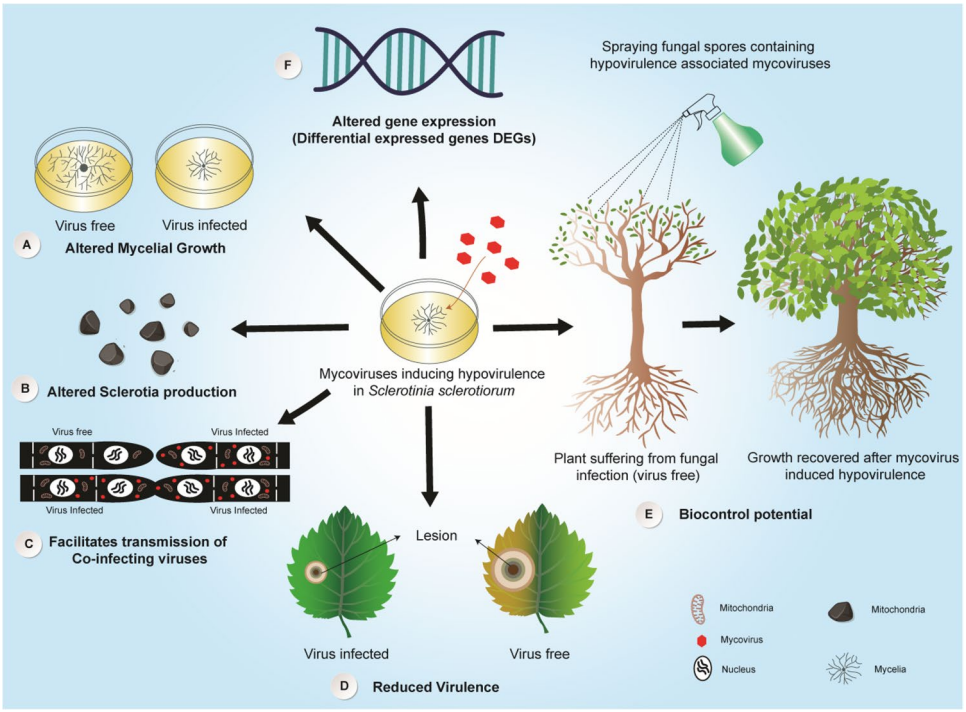

Fig. 2 Mycoviruses inducing hypovirulence in *Sclerotinia sclerotiorum* produce variety of effects on their hosts. (A) Altered mycelial growth: Mycoviruses infected fungal isolates may exhibit slower growth rates and reduced hyphal branching compared to non-infected strains. These alterations in mycelial growth can affect the pathogen's ability to spread and colonize host tissues, leading to reduced disease progression. (B) Changes in sclerotia formation: Sclerotia are compact, melanized structures produced by *Sclerotinia sclerotiorum* that serve as survival and dissemination structures. Infected strains may produce fewer smaller sclerotia, abnormal or reduced formation of apothecia, which are cup-shaped fruiting bodies responsible for the release of spores, thus affecting the long-term survival and potential for disease recurrence. (C) Facilitators of co-infecting viruses: mycoviruses facilitates the transmission od co-infecting viruses to other fungal strain despite vegetative incompatibility. These viruses are capable of suppressing the VIC genes. (D) Reduced virulence: hypovirulent mycoviruses significantly reduce the virulence of *Sclerotinia sclerotiorum*. The presence of hypoviruses within the fungal cells leads to a decrease in pathogenicity, resulting in reduced disease severity in infected plants. This reduction in virulence is due to various mechanisms, including the attenuation of fungal growth, reduced production of pathogenic enzymes, and impaired ability to penetrate and colonize host tissues. (E) Potential for biological control: Hypovirulent mycoviruses have shown potential as biocontrol agents against *Sclerotinia sclerotiorum*. By infecting the pathogen and reducing its virulence, hypovirulent strains can be utilized as biological control agents to manage white mold disease. This approach offers an environmentally friendly alternative to chemical fungicides, contributing to sustainable disease management strategies. (F) Genetic alterations: mycovirus-induced genetic changes can disrupt the expression of virulence-related genes, impair the production of pathogenic factors, and interfere with various cellular processes essential for infection. The specific mechanisms underlying these genetic alterations are still being investigated [59].

development of fewer sclerotia (Fig. 2). Disintegration of mitochondrial and nuclear membranes and a decrease in the number of mitochondria occurred along with destruction and granulation of the cytoplasm in strain XG36-1. The abnormal phenotype of strain XG36-1 was cured using protoplast regeneration [44].

The Sclerotinia sclerotiorum mycovirus complex

Viral co-infection is a natural phenomenon and has been commonly observed in a variety of fungal strains. Viral co-infections may promote recombinations or horizontal gene transfer, which ultimately results in viral evolution together with unique adaptive features for survival. The interactions between mycoviruses may affect the symptoms of viral diseases [59]. The vegetative incompatibility between fungal isolates is a limiting barrier to the spread of mycovirus among distantly related fungal strains. It should be noted that similar co-infections of mycoviruses (either related or distant) have been reported more frequently. Consider *Beauveria bassiana*, which has 11 mycoviruses [60]; *Agaricus bisporus*, which has 16 mycoviruses [61]; *Fusarium poae*, which has 16 mycoviruses [62]; and *Fusarium mangiferae*, which has 11 mycoviruses [51]. *S. sclerotiorum* has been found to harbor a similar mycovirus complex. In the EP-1PN isolate, EP-1PN, three different dsRNAs of varying sizes were discovered and named L, M, and S. This isolate was discovered in China

and exhibited altered morphology, retarded growth, and a small lesion size. The hypovirulence of this isolate was also confirmed by anastomosis with the recipient strain (virulent strain). Further analysis also demonstrated that aside from retarding fungal growth, M dsRNA (namely, Sclerotinia sclerotiorum debilitation-associated RNA virus SsDRV) infection also elevates production of oxalic acid and regulates pathogenicity-associated genes [63, 64]. Reduced growth was most likely caused by downregulation of genes involved in the cell wall biosynthetic pathway, which was later confirmed by a study in which 150 genes involved in protein synthesis, metabolism, stress response, nutrient transport, and signal transduction were downregulated [65]. The S dsRNA, a satellite RNA, was also associated with SsDRV infection and had a genome size of 1.0 kb. Curing these mycoviruses with cycloheximide and isolating single spores revealed that the hypovirulence and debilitation phenotypes were caused by M dsRNA (Sclerotinia sclerotiorum debilitation-associated RNA virus (SsDRV)). SsDRV has a 5419 nt genome and a single open reading frame (ORF) encoding RNA-dependent RNA polymerase (RdRP) with conserved methyl transferase, helicase, and RdRp domains. There were no virion particles found to be associated with viruses, indicating that it is capsidless. Phylogenetic analysis confirmed its close relationship to Botrytis virus F (BVF) and other *Flexiviridae* members, but SsDRV was distinct in that it did not encode for any coat or movement proteins [21].

L dsRNA (Sclerotinia sclerotiorum RNA virus L; SsRV-L) had a genome size of 6043 nucleotides with a single long ORF that encodes methyltransferase, helicase, and RNA-dependent RNA polymerase. It is reported to be the first (+) stranded RNA virus to which resembles human hepatitis E virus. The L-infected strains of the Sclerotinia sclerotiorum RNA virus showed reduced growth but had significant pathogenicity compared to the Ep-1PN strain (containing all three dsRNAs). Furthermore, sclerotia growth was also normal, suggesting that a low level of hypovirulence was associated with L-dsRNA. Phylogenetically, it was placed in the family *Closteroviridae*, and it is still unclear if there is any interaction (synergistic or antagonistic) existing between SsRV-L and SsDRV during their co-infection. More research is needed to reach any conclusion [22]. In another study, curing attempts generated three strains, namely, PB (strain that lacked virulence), PK (strain with reduced virulence), and A5 (virulent strain). Oxalate production was negligible for strain PB, while both A5 and PB produced oxalate, but the quantity in strain PB was lower than that in strain A5. The growth of mycelial biomass production also showed a similar pattern. Compared to strain A5, strains PB and PK were more vulnerable to *C. minitans* infection [66]. The analysis of virus-free and infected Ep-1PNA strains showed differentially expressed genes with a wide range of biological functions, including signal transduction, protein synthesis and transport, carbon and energy metabolism, and stress response [65].

Another hypovirulent isolate of *S. sclerotiorum* (isolate HC025) was identified from a soybean plant in China. Isolate HC025 harbored a complex of five mycoviruses belonging to different mycoviral families, namely, Sclerotinia sclerotiorum narnavirus 4 (SsNV4), Sclerotinia sclerotiorum mitovirus 1 (SsMV1), Sclerotinia sclerotiorum negative-stranded RNA virus 1 (SsNSRV1), Sclerotinia sclerotiorum ourmia-like virus 14 (SsOLV14), and SsOLV22 [67]. The protoplast regeneration technique did not cure the fungus from mycovirus, which was similar to most other mitoviruses [29]. SsMV1 was successfully transferred to Ep-1PNA367RV and 1980RV using a horizontal transmission assay, resulting in slower growth and decreased pathogenicity in detached soybean, tomato, and rapeseed leaves. The SsMV1 infected isolates had more branched hyphal tips with swollen mitochondria [32]. However, further research revealed that all mycoviruses, except for SsNSRV1, could transfer horizontally to Ep-1PNA367 (virulent strain), thus transferring the hypovirulence [31].

The DsRNA profile of Isolate SX276 revealed six distinct dsRNA segments, with sizes ranging from 1.6 to 23 kbp. Nine different mycoviruses were found in the isolate. Nine mycoviruses were examined, and three showed unusual genome organization and evolutionary position. Based on their genome characterizations, those nine mycoviruses temporarily were designated as SsHV1, SsBV3, SsOV4, SsOV5, SsDFV3, SsEV3, SsMTV1, SsMAV1, SsRhV1, SsHV1, SsBV3, SsMTV1, and SsOV4, and SsHV1 showed almost 100% identities with previously known viruses. SsOV5 showed a 44% identity with SsOLV2. Its genome consisted of a single larger ORF that encoded a putative RdRP. The hypovirulence of *S. sclerotiorum* strain SX276 was linked to an endornavirus. On potato dextrose agar (PDA), strain SX276 showed a decreased growth rate and abnormal colony morphology compared to the virulent isolate, strain Ep-1PNA367, which is the normal strain. On detached rapeseed leaves, strain Ep-1PNA367 produced typical lesions, but strain SX276 was unable to infect the leaves. Additionally, the hyphal tips of strain SX276 were found to be curvier than those of Ep-1PNA367 and to extend primarily through branching. According to these findings, strain SX276 is an example of a typical hypovirulent strain [68].

Another interesting study showed that the virome in *S. sclerotiorum* isolates growing in a rapeseed field changed after every year. The prevalence of mycoviruses in these isolates was unique in all 3 years of study survey. A total of 68 mycoviruses were identified, among which 24 were detected during all three consecutive years which were declared as core virome. These viruses have a high transmission rate among these isolates in field conditions. The

number of novel mycoviruses was identified as 28. These viruses belong to different evolutionary lineages thus demonstrating the diverse variety of mycoviruses in *Sclerotinia* population. This study also provides some clues regarding the evolution of mycoviruses; however, more research is needed to be done in the future to get a better insight [69].

Rhabdoviruses in *Sclerotinia sclerotiorum*

Rhabdoviruses are a family of single-stranded RNA viruses that infect animals and humans [70]. They are shaped like bullet or rod-shaped particles, hence the name "rhabdo" which means rod-like. Rhabdoviruses are known to cause a variety of diseases in animals, including rabies, vesicular stomatitis, and viral hemorrhagic septicemia [71]. They are also known to infect vertebrates, invertebrates, and plants. To date, not many rhabdoviruses have been identified in kingdom Fungi. Currently, only one novel rhabdovirus has been identified in *S. sclerotiorum* (isolate SX276 named Sclerotinia sclerotiorum rhabdovirus 1 (SsRhV1). SsRhV1 having a genome size 11,356 nts in length. The terminal sequences of both strands (3′ and 5′ end) showed complementarity. SsRhV1 genome encodes five major ORFs (ORFs I-V). The virus showed bullet-shaped virions under transmission electron microscope. ORF I encodes the nucleocapsid protein (N) 467 aa-protein (52.98 kDa). ORF IV encodes glycoprotein (G) a 493 aa protein (56.68 kDa). The ORF V encodes L protein a larger 2093-aa protein, which is vital for the formation of the mRNA cap structures. ORF II and ORF III encode two hypothetical small proteins of 32.7 kDa and 24.3 kDa, respectively [46]. On potato dextrose agar (PDA), strain SX276 grew at a slower rate and had an aberrant colony shape, with much fewer and smaller sclerotia than normal strain Ep-1PNA367 (the virulent isolate). The strain Ep-1PNA367 induced typical lesions on rapeseed leaves, whereas strain SX276 did not. Furthermore, when compared to Ep-1PNA367, the hyphal ends of strain SX276 were curved and extended mostly through branching. These findings indicated that SX276 is a typical hypovirulent strain. Unfortunately, after using different techniques of elimination and transfection, the team was unable to generate strains infected by SsRhV1 alone. Thus, the biological effects of SsRhV1 on *S. sclerotiorum* must be investigated further in the future [68].

Hypoviruses in *Sclerotinia sclerotiorum*

Hypoviruses are a group of small RNA viruses that infect fungi, particularly species in the genus *Cryphonectria*. They are commonly known for their ability to reduce the virulence of their fungal hosts, making them potential candidates for use in biological control of fungal diseases [72]. So far, a total of three hypoviruses have been reported causing debilitating effects in *S. sclerotiorum.* Sclerotinia sclerotium hypovirus 1 (SsHV1) was reported from *S. sclerotiorum* strain SZ-150, which was identified from rapeseed plant in China. Sclerotinia sclerotiorum hypovirus 2 (SsHV2) was identified from isolate SX247 [24, 27], isolate 328 [26], and isolate 574 [25], which were identified from lettuce plants in North America. Apart from hypoviruses, these isolates also harbored co-infection of other viruses, which is commonly observed in almost all fungal taxonomic groups [51, 68]. Isolate SZ-150 harbored a co-infection of Sclerotinia sclerotiorum botybirnavirus 3 (SsBV3) and Sclerotinia sclerotiorum mycotymovirus 1 (SsMTV1) [73]. Similarly, isolate 328 had a co-infection of Sclerotinia sclerotiorum endornavirus 1 (SsEV1) [26]. Isolate SX247 was also found to co-infected with SsHV2 and Sclerotinia sclerotiorum debilitation-associated RNA virus (SsDRV) [27], SsHV1 (SZ-150) and SsHV2 (SX247, 328 and 574) had a genome size of 10,438 nts, 15,219 bp, 13,786-nt-long, and 15,219 bp long, respectively. They all encode a single long ORF encoding a polyprotein with conserved domains, including papain-like protease, UDP glucose/sterol glycosyltransferase, RNA-dependent RNA polymerase, and viral RNA Helicase. SsHV1 also had a satellite dsRNA (S-dsRNA) having a genome size of 3643 nts having a single large ORF encoding a protein of 639 aa (71 kDa) with no replicase domain. It showed similarities to the 5′ UTR of SsHV1. Pathogenicity tests showed that only satellite-like segment was responsible for hypovirulent phenotype [24]. For SsHV2 (a gammahypovirus), no differences related to growth in the medium were observed, but a significant decrease in the diameter of the lesion was observed during pathogenicity tests. The results of transmission electron microscopy (TEM) demonstrated the integrity of the interior structures [25]. The assembled nucleotide sequence of SsHV2L (328) was up to 92% similar to two recently described SsHV2 strains, although it had an insertion of 524 nucleotides (nt) that was distantly linked to Valsa ceratosperma hypovirus 1 (VcHV1) and a deletion of more than 1.2 kb at its 5′ terminus. This shows that the newly discovered isolate is a heterologous recombinant of SsHV2 and a hypovirus that has not yet been identified. The novel strain was given the name Lactuca by Sclerotinia sclerotiorum hypovirus (SsHV2L). Isolate DK3 (virus free) was infected with an infectious clone of SsHV2L and showed a hypovirulent phenotype. It also showed delayed sclerotia production. The cured 328 strain was compared with (SsEV1 and SsHV2L) virus infected. A dark pigmentation and large lesion size was observed in cured strain [26].

The *Sclerotium rolfsii* isolate BLH-1 was isolated from *Macleaya cordata* in China. It was discovered that strain BLH-1 had 8–10 segments of dsRNA with sizes ranging from 1 to 15 kbp. Moreover, BLH-1's hyphal cells contained

virus-like particles that were < 100 nm in size. Sclerotium rolfsii hypovirus 1 (SrHV1) was identified from this strain and had altered morphology, less pathogenicity, and no sclerotia formation. Internal organ deterioration and fragmented hyphae were seen in virus-infected strains. In BLH-1, the mitochondria were considerably less and enlarged, indicating a degradation syndrome. It was shown to have three viruses in total, two of which belonged to unclassified families and one of which was known as Sclerotinia sclerotiorum hypovirus 2 (SsHV2). Hyphal tipping and protoplast mixed approach was used for curing. The virus-free strain was named BLH-1-P1. Pathogenicity tests confirmed the hypovirulent nature of BLH-1 with small lesion size compared to virus-free strain. Attempts to transfer virus through horizontal transfer from BLH-1-P1 to LJ-01 (virus-free *S. sclerotiorum*) failed due to vegetative incompatibility, but the three viruses were transferred to BLH-1-P1 (which showed hypovirulent properties). In comparison to the virus-infected strain, the virus-free strain was shown to have greater laccase activity. The yield of oxalic acid produced by strain BLH-1 was substantially greater than that of strain BLH-1-P1 in cultures maintained in PD for 5 days [28]. The Sclerotinia sclerotiorum strain GB375 (isolated in the USA) was found to harbor a new + ssRNA virus, namely Sclerotinia sclerotiorum hypovirus 9 (SsHV9). The complete genome comprises 14,067 nucleotides in length with a single large ORF encoding a polyprotein of 4196 amino acids. The polyprotein contains papain-like protease, a protein of unknown function, an RNA-dependent RNA polymerase, and an RNA helicase. The virus-infected strain showed slow normal growth with irregular margins without any sclerotia. Strain GB375 did not cause any obvious lesions on rapeseed leaves [74].

Mitoviruses in *Sclerotinia sclerotiorum*

Mitoviruses are small, single-stranded RNA viruses that infect fungi. They are named "mitoviruses" because they are found in the mitochondria [75]. They have a genome that ranges from 2 to 3.5 kb in length. They also have potential applications in biotechnology and agriculture, as they may be used to control fungal diseases or improve the growth and productivity of crops [76]. Four mitoviruses with hypovirulent phenotypes were identified in *S. sclerotiorum*. Sclerotinia sclerotiorum mitovirus 1 and 2 have been identified from isolate KL-1 (USA) while sclerotiorum mitovirus 2, 3, and 4 were identified from isolate NZ1 (New Zealand). SsMV4 was also identified from isolate AH16 of *S. sclerotiorum* [33]. They had a genome size of 2513 nts 2421 nts 2438 nts, 2588 nts, and 2744 nts respectively. The 5′ and 3′ untranslated region 5 and 3 (UTR) of these mitoviruses was 418, 311 313, 297, and 465 and 16, 82, 88, 152, and 84 nt. All mitoviruses had single long ORF encoding for RdRP with 691 aa (79.43 kDa), 676 aa (75.75 kDa), 678 aa (76.662 KDa), 712 aa (81.052 kDa). and 731 aa (85.608 kDa). In contrast to SsMV2/KL1, SsMV2/NZ1 has no 3′ poly(A) but ends in four Cs. A change in pigmentation with reduced growth was observed in strain KL-1 while only reduced virulence was observed for strain NZ1. Using a dual culture technique, strain 1980 (VF) acquired the hypovirulent features of strain KL-1 by hyphal anastomosis. Thermotherapy, hyphal tipping, single sclerotia isolation, cycloheximide, and chloramphenicol treatments failed to eliminate viral infection, which shows the stability of SsMV1 and 2 in their parental isolate (KL-1). The pathogenicity test showed a large size of the lesion for virus-free strain (strain 1980hyg), but a small lesion was observed for KL-1 [29, 77]. Curing was successful for isolate NZ1. The effect of each individual mycovirus was observed by using ascospore progeny, which concluded that all three mycoviruses, when present simultaneously, had the most hypovirulent effect, rather than individual ones. It was assumed that they could have synergistic effect. This portion of the hyphae seen by electron microscopy included enlarged and deformed mitochondria without or with twisted cristae. Furthermore, another study investigated the impact of SsMV1/HC025 mycoviral infection on the virulence of *Sclerotinia sclerotiorum*. It was noted that virus-infected isolate exhibited reduced virulence and slower growth compared to non-infected isolates. This observation indicates a potential correlation between SsMV1 infection and hypovirulence in *Sclerotinia sclerotiorum* [32].

Partitiviruses in *Sclerotinia sclerotiorum*

Partitiviruses are small, double-stranded RNA viruses that infect fungi, plants, and some protozoa. They are named "partitiviruses" because their genome is divided into two segments, which are packaged separately into virus particles [78]. They typically have a genome size ranging from 2.8 to 4.8 kb and encode two or three proteins [79]. Partitiviruses can cause a range of symptoms in their hosts, including stunted growth, reduced yield, and necrosis. However, some partitiviruses are known to have beneficial effects on their hosts, such as enhancing their tolerance to environmental stress [80]. In addition, some partitiviruses are asymptomatic and do not cause any noticeable effects in their hosts. Partitiviruses are interesting from a scientific perspective because they can be used as a model system to study virus evolution and host-virus interactions [81]. They are also being investigated for their potential as biocontrol agents for plant pathogens, as they can infect and suppress the growth of pathogenic fungi. The *S. sclerotiorum* isolate WF-1 (identified from *Cirsium japonicum* in Japan) was found to harbor a partitivirus infection named Sclerotinia

sclerotiorum partitivirus 1 (SsPV1). Isolate WF-1 failed to produce apothecium and showed retarded growth, reduced virulence, and an expected increased conidial production. SsPV1 was previously reported in the Sunf-M, where it had a cryptic profile [82]. It has a bi-segmented genome, segment 1 comprises 2287 nts, and a single large open reading frame (ORF) encoding RdRP protein with 704 aa (82 kDa) and segment 2 comprises 2274 nts with a single ORF encoding for a putative coat protein 678-aa (74 kDa). The virion particles were spherical and nearly 40 nm in diameter. Internal organs were damaged several times. The hypovirulent nature of SsPV1 was confirmed by hyphal anastomosis with compatible strains (VF) and incompatible strains (VF). The hypovirulence associated with SsPV1 successfully transferred to *S. sclerotiorum*, *Sclerotinia nivalis*, and *Sclerotinia minor*. Furthermore SsPV1 successfully transfected *Botrytis cinerea* strain (KY-1) which demonstrates its wide host range. Pathogenicity testing on detached leaves of *Arabidopsis thaliana* with different isolates of *B. cinerea* showed reduced virulence. Most *B. cinerea* conidia that were germinating had hyphal growth suppression as a result of SsPV1 infection, and infected hyphae ultimately died as very few new colonies could form after germ tube development. The outer surface topography of the SsPV1/WF-1 resembles that seen for other partitiviruses including PsV-F and FpV1. Capsid was found to be convoluted with depressions at each icosahedral symmetry axes. Normally, the nature of mycoviruses belonging to the family *Partitiviridae* is cryptic (associated with latent infections) [37, 83]. But now, both hypo and hypervirulent partitiviruses are known to infect different fungal strains [84, 85].

Megabirnaviruses in *Sclerotinia sclerotiorum*

Megabirnaviruses are large, double-stranded RNA viruses that infect fungi. They are named "megabirnaviruses" because they have one of the largest known viral genomes, ranging from 8.2 to 13.5 kb in size [19]. They typically have two segments in their genome, encoding for four or five proteins and are known to infect a wide range of fungi, including plant pathogenic fungi, edible mushrooms, and yeast. They are also known to have a unique morphology, with a characteristic rod-shaped structure that can be seen under electron microscopy [86]. They have been shown to cause a range of symptoms in their fungal hosts, including stunted growth, reduced yield, and necrosis. However, some megabirnaviruses have been shown to have beneficial effects on their hosts, such as improving their tolerance to environmental stress. They are interesting from a scientific perspective because they challenge the traditional notion that RNA viruses have small genomes. They are also being investigated for their potential as biocontrol agents for plant pathogens, as they can infect and suppress the growth of pathogenic fungi. Sclerotinia sclerotiorum megabirnavirus 1 (SsMBV1) was isolated from *S. sclerotiorum* isolate SX466 (isolated from rapeseed plants in China). The SsMBV1 genome consisted of L1 and L2 segments (dsRNA) that have lengths of 8806 (nt) and 7909 bp, respectively. ORF1 in segment L1 encoded a protein with 1250 aa (137 kDa), while ORF2 encoded a protein with 1116 aa (125 kDa). The L2 dsRNA was implicated in pathogenicity, replication, and genome packing. In the L2 region, a conserved papain-like protease domain was found that resembled the p29 of CHV1. Reduced growth on PDA and altered morphology was observed when virus-infected strain SX466 and Ep-1PNA367 (virus-free) were compared. While the Ep-1PNA367 strain was highly pathogenic and caused extensive lesions on rapeseed leaves, the virus-infected SX466 strain was less virulent and generated a smaller lesion. This research also showed that, even in the absence of L2-dsRNA, SsMBV1 has a minute impact on the host fungus characteristics [38].

Botybirnavirus in *Sclerotinia sclerotiorum*

Botybirnaviruses are a group of small, double-stranded RNA viruses that infect fungi. They belong to the family *Botybirnaviridae*, which is a relatively new taxonomic group that was first proposed in 2018. These viruses are characterized by their unique genome structure, which consists of two segments of RNA encoding a few genes [87]. The name "Botybirna" is derived from the acronym of "Botrytis virus nomenclature" and the suffix "-birna," which indicates a bisegmented RNA genome. These viruses have been identified in a variety of fungal hosts, including plant pathogenic fungi, saprophytic fungi, and mycorrhizal fungi [88]. However, much remains unknown about the biology and pathogenicity of these viruses. Some studies suggest that botybirnaviruses may play a role in regulating fungal growth and virulence, while others suggest that they may be harmless or even beneficial to their hosts. Further research is needed to better understand the diversity and function of botybirnaviruses. The *S. sclerotiorum* strain SCH941 (isolated from rapeseed in China) was found to have Sclerotinia sclerotiorum botybirnavirus 1 (SsBRV1). Virus particles have a rough surface and a spherical shape (38 nm in diameter). Phylogenetically, it was assigned to the family *Botybirnaviridae*. Its genome consists of three dsRNA segments ranging from 1.2 to 6.5 kbp in size. Sequence analysis revealed that the fungus harbored two distinct mycoviruses, one of which is a member of the *Reoviridae* family. Both dsRNAs have a genomic size of 6457 (dsRNA1) and 5965 bp (dsRNA2), respectively. ORF1 encoded for RdRP. ORF2 (nts 577–5848) on the dsRNA2 encoded for a large polyprotein (195 kDa). This polyprotein, designated p2, displays

sequence similarities with the putative protein encoded by the ORF2 of Botrytis porri botybirnavirus 1 (BpRV1). The length of dsRNA3 was 1647 bp, and its GC content is 46.9%. The dsRNA3 did not include any significant ORFs, but a short ORF that encoded a peptide with a mass of 13.7 kDa (121 aa), which did not share any significant similarity to the viral proteins already reported. Furthermore, there were no sequence relationships between the three dsRNAs. The dsRNA3 was lost during protoplast transfection utilizing a PEG-mediated mechanism. As a result, dsRNA3 is not essential for the SsBRV1 virus, thus considered a satellite-like RNA (SatlRNA). However, dsRNA3 lowers the virulence and growth rates of transfectants when present. As a result, SsBRV1 with SatlRNA can make the fungus *S. sclerotiorum* less virulent [89]. Both *S. sclerotiorum* and *B. porri* are members of the same taxonomic family Sclerotiniaceae and share a number of developmental and physiological characteristics as well as a taxonomic connection [90]. It is worth noting that despite being members of the same virus family, SsBRV1 and BpRV1 have very different biological effects on their fungal hosts [39].

In AH16 of *S. sclerotiorum*, a co-infection of two viruses was observed, Sclerotinia sclerotiorum mitovirus 4 (SsMV4) (+ ssRNA) (mono-segmented) and Sclerotinia sclerotiorum botybirnavirus 2 (SsBRV2) (dsRNA) (bisegmented). SsBRV2 comprised two segments (L1-dsRNA and L2-dsRNA). Segment 1 has a length of 6159 nts and was encoding for RdRp (1868 aa). Segment 2 has a length of 5872 nts and encodes the 1778 aa protein. SsBRV2 produces 40-nm-diameter spherical virions, as shown by electron microscopy. Mycovirus was unable to infect vegetatively incompatible Ep-1PNA367R by horizontal transmission. SsBRV2 was successfully introduced into Ep-1PNA367R (virus free strain) of *S. sclerotiorum* but SsMV4 failed to transfect. The transfected was named Ep-1PNA367RVT. Regeneration of the protoplast was used to cure mycovirus fungus. Ep-1PNA367RVT and AH16 showed reduced growth and produced small lesions on the detached soybean leaves. SsBRV2/ Ep-1PNA367RVT showed more hypovirulence than SsBRV2 + SsMV4/AH16. It was probably caused by the host and virus's different genetic backgrounds and unidentified interactions, or SsBRV2 and SsMV4 might have antagonistic effects in a single host. Further studies are needed to study the effects of SsMV4. This study was the first report on the coexistence of dsRNA and ssRNA in the same isolate [33].

Botourmiavirus in *Sclerotinia sclerotiorum*

Botourmiavirus is a type of virus that belongs to the family *Tombusviridae*. This family includes several genera of positive-sense single-stranded RNA viruses that infect plants, fungi, and bacteria [91]. Botourmiaviruses have been isolated from the plant species *Arabidopsis thaliana* and are known to cause mild symptoms such as mosaic patterns on leaves [92]. Botourmiaviruses have a small, non-enveloped spherical structure and a genome of approximately 4–5 kb. They replicate in the cytoplasm of infected cells and are transmitted between plants by mechanical inoculation or through infected seeds [46]. Although they are not considered to be major plant pathogens, botourmiaviruses can potentially affect crop yields and the quality of plant products. Isolate HC025 was found to harbor SsOLV22. The complete genome comprises 3987 nucleotides having a single ORF encoding RdRp having 951 amino acids (110 kDa). Another hypovirulent strain GF3 harbored Sclerotinia sclerotiorum ourmiavirus 17 (SsOV17) had a complete genomic sequence of 2802 nucleotides encoding an ORF with 663 amino acids (75kDa) [40]. The Sclerotinia sclerotiorum ourmia-like virus 4 (SsOLV4) was identified from PX14A1 and PX14A4 where it existed both in cytoplasm and mitochondria. It has a genome size of 2982 nt with a single large ORF encoding RdRp. The comparison of virus-free and virus-infected isolates demonstrated that SsOLV4 was causing hypovirulence in this isolate. Strain PX14A4 has a slower hyphal growth rate and cannot produce sclerotia on potato dextrose agar (PDA) medium, and it could only induce lesions that were much smaller than those of the virulent strain Ep-1PNA367 (virus free) on the detached rapeseed leaves. The strain was cured was protoplast preparation. This study also suggested that SsOLV4 has little effect on host growth and virulence. Furthermore, virus-infected strain showed smaller lesion compared to virulent strain [93].

Genomovirus in *Sclerotinia sclerotiorum*

Genomoviruses are a group of small, circular single-stranded DNA viruses that belong to the family *Genomoviridae*. They are characterized by their small genome size, ranging from 2.5 to 3.2 kb, and their ability to infect a wide range of hosts, including insects, fungi mammals, and humans [94]. The first genomovirus was discovered in 2012 in fecal samples of a human patient, and since then, several genomoviruses have been identified in various hosts and environments [95]. Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1), a DNA mycovirus, was identified from *S. sclerotiorum* isolate DT-8 (from rapeseed plant in China). The virus genome size is 266 nucleotides and has two ORFs encoding coat protein (CP) and replication-associated protein (Rep). The size of the virion particles was 20–22 nm in diameter. This strain showed a more prominent hypovirulent phenotype compared to the Ep-1PN strain. Curing of virus was successful *via* hyphal tipping and protoplast

regeneration. Mostly, DNA mycoviruses have movement protein (MP), which is a key feature of plant genomoviruses which they used for cell-to-cell transfer, but in the case of SsHADV-1, no movement protein was observed; the reason might be that mycoviruses are resident in the cytoplasm of fungus, and they move with the movement of cytoplasm in hyphae, as they are interconnected [10]. A variant of SsHADV-1 reported by [96] from New Zealand showed 98% similarity to the already reported virus, but it was isolated from river sediments. This virus also showed a hypovirulent phenotype in its host isolate [96]. Soybean leaf-associated gemygorvirus-1 (SlaGemV−1) was also found to regulate some important pathways in *S. sclerotiorum* including replication, cell cycle, melanin biosynthesis, pathogenesis, and oxalic acid catabolism. The understudied genes were Dss1, DNA Pol ε subunit 2, Ago-4, β-fructofuranosidase, cellobiohydrolase, Mcm4, PCNA, Qde1, RNase H2 subunit C, Rrp3, Sad1, Top3, and ubiquitin-conjugating enzyme E2 H [94, 97].

Endornaviruses in Genus *Sclerotinia*

Endornaviruses are a group of plant viruses that contain a single-stranded RNA genome. The genome size of endornaviruses is various, with a range of 9.7–17.6 kb [98]. They are known to infect a variety of plant species, including nematodes, mites, insects, legumes, cereals, and fruit trees, and are generally considered to be non-pathogenic. However, recent studies have suggested that endornaviruses may have beneficial effects on host plants, such as enhancing their tolerance to abiotic stress and promoting plant growth [99, 100]. They typically have a large open reading flame (ORF) that encodes a polyprotein with methyltransferase (MTR), helicase (Hel), glycosyltransferase (GTR), and RNA-dependent RNA polymerase (RdRp) [101]. Sclerotinia minor strain LC22 was found to harbor Sclerotinia minor endornavirus 1 (SmEV1). The genomic comprises 12,626 nts which was encoding for a putative protein of 4020 aa. Cysteine-rich region (CRR), viral methyltransferase (MTR), putative DEXDc, viral helicase (Hel), and RNA-dependent RNA polymerase (RdRp) domains were all identified in SmEV1. SmEV1 lacks the site-specific nick seen in most previously characterized endornaviruses. The hypovirulence associated with strain LC22 was easily transferred to other vegetative compatible isolates. Additionally, it was observed that SmEV1 in strain LC22 may spread vertically through sclerotia. Interestingly, virus-infected mycelia also showed anti *S. minor* activity. When PCR using MAT primers, several strains were tested for compatibility with vegetative growth. Horizontal transmission was achieved using strain LC22. All stains acquired SmEV1 from another. The virus-infected strain had aberrant colony shape and a small number of sclerotia. The pathogenicity of oil seed rape was tested, and the results revealed that although the virus-infected strain displayed a tiny lesion (less virulent), the virus-free strain, LC41, had a high size of lesion (more virulent). Protoplast regeneration, hyphal tipping, and plant inoculation and re-isolation were unsuccessful in curing the fungus. Due to *S. minor*'s myceliogenic germination process, a single spore also failed to germinate. It is the first account of an endornavirus related with pathogenicity debilitation in *S. minor*. Field experiments are required to fully exploit the potential for biocontrol [41]. The isolate SX276 was found to harbor SsEV3 that has a complete genome of 12,661 nts that encodes a single ORF with the conserved domains of Mtr, Hel, phytoreo_S7 (S7) and RdRp. A mix of chemical treatment and protoplast preparation was used to get singly infected SX276 with SsEV3 infection. A comparison of virus-free and virus infected isogenic lines showed that virus-infected strain has slow attenuated growth and small lesion size [68]. Isolate XY79 showed an infection of Sclerotinia sclerotiorum endornavirus 11 (SsEV11) with a coinfection of other three viruses. Sclerotinia sclerotiorum hypovirus 7, Sclerotinia sclerotiorum deltaflexivirus 2-WX, and Sclerotinia sclerotiorum ourmia-like virus 15. The complete genome sequence of SsEV11 comprised 11,906 nts with a single large ORF containing a conserved viral MTR domain, a cysteine-rich region (CRR), RdRp_2, and DEADc domain. Phylogenetic analysis placed it with betaendornaviruses. Symptoms included slow growth, delayed sclerotia formation, and maturation with a small lesion size [101].

Sobemovirus in *Sclerotinia sclerotiorum*

Sobemoviruses are a group of plant viruses that belong to the family *Secoviridae*. They are small, icosahedral viruses that contain a single-stranded positive-sense RNA genome of approximately 4.5–5.5 kb [102, 103]. They are known to infect a wide range of plants, including cereal crops such as wheat and barley, and legumes such as soybeans and beans. They are transmitted by aphids and other insect vectors, as well as through mechanical means such as seed transmission [102]. They can cause various symptoms in infected plants, including yellowing and necrosis of leaves, stunting, and reduced yields. However, they can also have beneficial effects on plants, such as enhancing their resistance to abiotic stress and improving seed germination [104]. *S. sclerotiorum* strain 277 was collected from *Brassica napus* plant in China. It was identified with a Hubei sclerotinia RNA virus 1 (HuSRV1). Its genome consists of four ORFs with the highest of 4492 nts without a poly A tail at the 3′ end. Protease and a transmembrane domain and RdRP were encoded by ORF1 while ORF 2 encodes the coat protein. The ORF3 and ORF4 encoded an unidentified protein. Phylogenetic analysis

placed it in the *Sobemoviridae* family. The particles were around 30 nm in diameter and were capable of transfecting a virulent strain of Ep-1PNA367 and producing hypovirulence. HuSRV1-purified particles successfully transfected the protoplast of virulent strain Ep-1PNA367. Resultant strain displayed debilitation phenotypes, including slower growth on PDA and less virulence on detached rapeseed leaves. However, HuSRV1 was not capable of transmitting vegetatively incompatible strains [42].

Deltaflexivirus in *Sclerotinia sclerotiorum*

Deltaflexiviruses are a group of plant viruses that belong to the family *Flexiviridae*. They are flexuous, filamentous viruses that contain a single-stranded positive-sense RNA genome of approximately 8–10 kb [105]. They are known to infect a wide range of plants, including fruit trees, citrus crops, and ornamental plants. They are transmitted by insect vectors, such as aphids and whiteflies, as well as through mechanical means such as pruning and grafting [70]. Deltaflexiviruses can cause various symptoms in infected plants, including mosaic patterns on leaves, stunting, and reduced yields. They can also cause more severe symptoms such as necrosis and death in some plant species [106]. Sclerotinia sclerotiorum deltaflexivirus 2 (SsDFV2) was isolated from a hypovirulent strain of *S. sclerotiorum*, namely isolate 228 which was collected from diseased rapeseed in China. SsDFV2 has a genome size of 6735 nucleotides and was capable of transmitting to vegetatively incompatible strain by horizontal transmission. This study was the first report of transmission of +ssRNA mycovirus to a vegetative incompatible strain. A single large open reading frame (ORF) was predicted to encode for a putative viral RNA replicase. The strains, 1980hph and Ep-PNA367^{hph} (both vegetatively incompatible strains), were used for horizontal transmission of mycovirus. Only SsDFV2 was transferred to the recipient strain. Ep-1PNA367VI (virus infected) was also used to transfer virus to strain 1980hph (VF) to check whether the transmission depended on the host or not. But it was successfully transferred, which showed that it was independent of the host. SsDFV2 showed reduced colony morphology, small lesion size, and very little sclerotia production. SsDFV3 isolated from strain SX276 has a tripartite genome of five ORFs (ORF I to ORF V). Segments 1–3 were found to always co-exist during transfection in recipient fungal isolate and share conservations at 5′-terminal sequences. S1 comprised 7435 nts encoding two putative ORFs, a polyprotein (Mtr, Hel, RdRP) and an unknown protein respectively. S2 consisted of 2566 nts encoding two ORFs (ORF III and ORF IV) with hypothetical proteins. S3 has single small ORF (ORF V) encoding second helicase domain (Hel-2). Phylogenetic analysis placed it with members of *Deltaflexiviridae*. SsDFV2 was capable of transmitting to incompatible (virus free Ep-1PNA367 and 1980hph) strains during dual-culture transmission assays. SsDFV2-infected strains grew at a slower rate than virus-free strains, and sclerotial development was delayed. The lesser lesions generated by strain Ep-1PNA367VI on new rapeseed leaves indicated that it had a slightly lower pathogenicity than its virus-free counterpart. As a result, SsDFV2 appears to have a slight effect on the virulence and development of its hosts [23].

Unclassified viruses inducing hypovirulence in *Sclerotinia sclerotiorum*

The isolate AH98 harbored the negative-stranded RNA virus known as SsNSRV-1. The 10,002 nts long genome has six linearly spaced, non-overlapping ORFs. SsNSRV-1 possesses a filamentous 1000-nm-long enveloped virion with 25–50 nm diameter. A virulent strain Ep-1PNA367 was successfully transfected with purified SsNSRV-1 particles, resulting in a hypovirulent phenotype (including retarded growth on PDA, no sclerotia production and pathogenicity loss). A possible loss of growth polarity was also indicated by the uniform zigzag development of the hyphal ends in these recently transfected strains. The biomass production of newly infected strains decreased dramatically, and the strains accumulated more acidic chemicals. Phylogenetic analysis placed it between *Bornaviridae* and *Nyamiviridae* families. In terms of biological characteristics, isolate AH98 differed somewhat from Ep-1PNA367 in that it did not considerably lose growth polarity. This also suggests an antagonistic relationship between SsNSRV-1 and SsHV-1 [36]. The isolate SX276 was found to harbor SsMAV1 that has a genome size of 7531 nts and has a single large ORF encoding a polyprotein with three conserved domains (Mtr, Hel and RdRp). Phylogenetic analysis placed it with the members of proposed family *Mycoalphaviridae* and genus Scleroalphavirus [68].

Mycoviruses alter gene regulation

Mycoviruses interact favorably or unfavorably with their hosts and other mycoviruses. Consider the example of a co-infection system in which one mycovirus inhibits the non-self-recognition of its fungus host, enabling heterologous mycovirus transmission. It is common for a fungus to exhibit non-self-recognition or allorecognition, which allows them to identify one another. Heterokaryon incompatibility, which occurs when two isolates of fungi have distinct mycelial compatibilities, leads to compartmentalization and programmed cell death (PCD)

which ultimately prevent hyphal fusion [107]. Sclerotinia sclerotiorum mycoreovirus 4 (SsMYRV4) was found to suppress vegetative incompatibility and promote the transmission of viruses in different fungal strains [43]. SsMYRV4 is known to suppress heterotrimeric G proteins, guanine nucleotide binding proteins (G proteins), and *het* or *vic* genes related to vegetative incompatibility and cellular reactive oxygen species (ROS) [43, 108]. SsHADV-1 infection showed differential expression of 187 genes with 114 genes showing upregulation and just 73 showing downregulation. These genes were involved in metabolic pathways, antibiotic biosynthesis, secondary metabolite production, Ras-small G protein signal transduction, DNA replication, DNA damage response, carbohydrate and lipid metabolism, ribosomal assembly, and translation [109, 110]. ORF1 infection transcriptomic analysis of Sclerotinia sclerotiorum negative stranded virus 1 (SsNSRV-1) showed 686 DEG in total, of which 267 showed upregulated and 419 were downregulated. Altered protein production, secretory proteins, transmembrane transport, metabolism, and pathogenicity pathways had a modified expression. SsHV2L infection showed alteration in 958 mRNAs and 835 sRNA-producing loci, which were involved in transporting lipids and carbohydrate metabolism [111]. Soybean leaf–associated gemycircularvirus-1 (SlaGemV-1) reduced the expression of genes involved in the production of cell walls, the creation of microtubules, and the metabolism of steroids and natural antibiotics. In the presence of SlaGemV-1, methyltransferase genes, kinesin domain genes, and genes associated with cytochrome P450 were shown to be downregulated in *S. sclerotiorum* [94].

Functions of argonautes and dicer in the antiviral RNA silencing of *Sclerotinia sclerotiorum*

The suppression of gene expression by RNA silencing occurs both before and after transcription. It has long been known as adaptive defense mechanism against foreign nucleic acids i.e., when viruses infect mamals, fungi, plants and others. RNA silencing serves a variety of functions [112, 113]. To date, the evolved RNA silencing in fungi has shown that, in contrast to animals and plants, it is essentially unnecessary for the endogenous regulation of gene expression because gene disruption mutants frequently grow normally. Instead, the antiviral role of those genes is only revealed when the RNA silencing gene mutants are infected with a virus [114, 115]. A study demonstrated that an argonaute mutant isolate of *S. sclerotiorum* was transfected with (SsHV2-L) and compared to wild-type–infected strain analysis that showed that AGL-2 is crucial for vegetative growth and antiviral defense, whereas the biological purpose is still unknown. Application of dsRNA externally was successful in targeting *agl-2* as an RNA pesticide to dose-dependently slow the infection. Before virus infection, the *agl-2* mutant had noticeably slower growth and virulence, but not the *agl-4* mutant. Similar to this, the *agl-2* mutant, but not the *agl-4* mutant, displayed more debilitation when infected with the virus than uninfected strains. In another study, an infectious clone of SsHADV-1 was transfected (*dcl-1*, *dcl-2* and both *dcl-1/dcl-2* mutant strains). Compared to *S. sclerotiorum* in its wild-type state, disruption of *dcl-1* or *dcl-2* had no effect on the phenotype; however, the double dicer mutant strain grew noticeably more slowly. Additionally, the double mutant showed much more severe debilitation after virus infections, including phenotypic changes such as slower growth, reduced pigmentation, and delayed sclerotial formation. This mutant was slow growing without virus infection. The single mutant *dcl-1* and *dcl-2* lacked these phenotypic alterations. The viral susceptibility to the wild-type state was reversed by the complementation of a single dicer gene. From virus-infected wild-type strains, small RNAs with a bias toward the negative sense strand were accumulated. The results of these studies suggest that *S. sclerotiorum* has strong RNA silencing mechanisms that process both DNA and RNA mycoviruses and that invasive nucleic acids can significantly reduce the virulence of this fungus when both dicers are silenced [116].

Redirecting harmful fungal infection to beneficial; mycoviruses as a switch

There are numerous examples of parasitic or endosymbiotic relations that affect their host behavior either directly or indirectly. Considering the example of Ophiocordyceps fungus (entomopathogenic fungi), which modify the behavior of their insect hosts for increasing the dispersal of their spores [117]. Similarly, the growth habits of fungi can also be altered by endohyphal bacteria [118], e.g., by promoting the endophytic way of life [119]. Similarly, viruses can also alter the functioning of their hosts. It has been documented that a virus may control insects without harming the host, either directly [120] or indirectly [121]. It has previously been demonstrated that a mycovirus that infects an endophytic fungus can provide temperature tolerance to plants [122]. Infection of mycovirus-mediated hypervirulent strain of *Leptosphaeria biglobosa* produced systemic resistance against *L. biglobosa* in *Brassica napus* [123].

Biological control is a viable option for crop protection since it uses live organisms to fight pests, as has already

been described [124]. Seed biopriming has previously been made accessible as one of the delivery methods for biological control, particularly for the management of soil-borne diseases. Faba bean seeds (*Vicia fabae*) that have been bioprimed with a variety of antagonistic bacterial and fungal agents can effectively and long-term suppress root rot [125]. Another method for lowering airborne diseases is seed bio-priming. After being bioprimed with *Pseudomonas fluorescens* (Fig. 1), pearl millet showed resistance to downy mildew, which increased the yield [126]. Through bio-priming using *S. sclerotiorum* (strain DT-8), Sclerotinia stem rot (SSR) severity of symptoms was significantly decreased, and the field productivity was increased. It is just another example of how seed bio-priming prevents airborne diseases. The ability of mycoviruses to switch from virulent fungal strains to hypovirulent ones is the main advantage of using mycoviruses to combat plant pathogens [127] (Fig. 3). SsHADV-1 is reported to reduce pathogenicity and inhibit sclerotia production in a lab environment [10], which reduces the main cause of infections in SSR [90]. To long-term manage SSR, rapeseed can be bioprimed with the DT-8 *S. sclerotiorum* isolate DT-8 [110]. SsHADV-1 decreases the capacity of *S. sclerotiorum* to express several genes linked to pathogenicity during infection. Whilst the plant is growing, the SsHADV-1-infected strain DT-8 tightly regulates the expression of rapeseed genes involved in defense, hormone signaling, and circadian rhythm pathways. In response, this promotes plant growth and increases resistance to plant disease. Spraying DT-8 isolate in the early stages of flowering can reduce the severity of rapeseed stem rot disease by 67.6% and increase the yield by 14.9%. The researchers also discovered that SsHADV-1 could infect additional strains of *S. sclerotiorum* on DT-8-inoculated plants and that DT-8 could be recovered from dead plants. These findings suggest that mycoviruses may affect the genesis of endophytisms. According to research results, mycoviruses may have an impact on the way endophytis arises and may even offer a cutting-edge disease

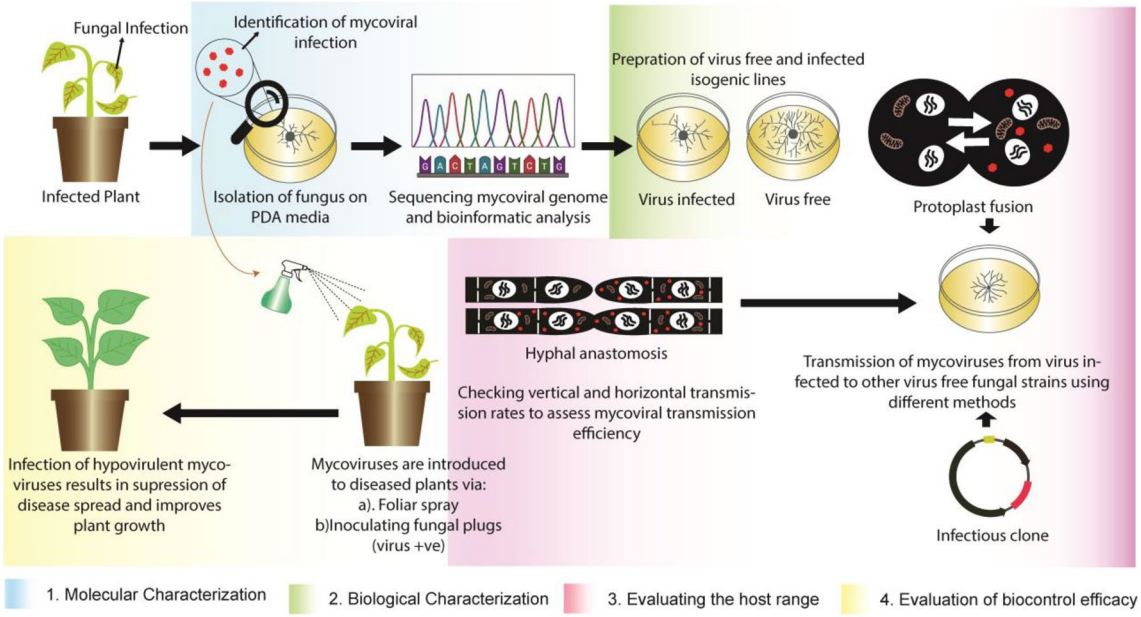

Fig. 3 Using mycoviruses as biocontrol agents involves several procedures, depending on the specific objectives and target pathogens. (A) Mycovirus isolation and characterization: The first step is to the molecular characterization which involves the isolation and screening of hypovirulence-associated mycoviruses from their fungal hosts. This typically involves culturing the fungus in a laboratory setting, extracting the viral particles and sequencing their genome for identification. (B) Once isolated, the mycoviruses are biologically characterized to understand their properties, such as its mode of transmission, and potential effects on the host fungus. (C) After hypovirulent-associated mycovirus is identified, its host range is evaluated. A good biocontrol agent should have wide host range. Different techniques including co-cultivation, protoplast fusion, hyphal anastomosis, and construction of infectious clones are used for host range extension with persistent infections. (D) Evaluation of biocontrol efficacy: assess the impact of the mycovirus infection on the target pathogens. This involves evaluating the virulence, growth, and reproductive abilities of the pathogenic fungi in the presence of the mycovirus. Laboratory experiments and controlled field trials can help determine the effectiveness of the mycovirus as a biocontrol agent. Once the biocontrol potential of the mycovirus is confirmed, methods for mass production need to be established. Large-scale production of mycoviruses can be achieved by growing the host fungi under optimized conditions. The mycovirus particles can then be harvested and formulated into suitable formulations for application. The last step is the determination of the most appropriate method and timing for applying the mycovirus-based biocontrol agents. This may include foliar sprays, soil drenches, seed treatments, or other delivery methods, depending on the target pathogens and their infection routes [128]

control strategy that leverages mycovirus-infected variants to improve crop quality and distribute mycoviruses in the landscape. SsHADV-1 decreases the expression of critical pathogenicity factor genes in *S. sclerotiorum* during infestation. While proliferating in rapeseed, the SsHADV-1-infected strain DT-8 tightly regulates the expression of rapeseed genes involved in defense, hormone signaling, and circadian rhythm pathways. As a result, plant growth is promoted, and disease resistance is increased [129].

Neo-life style of *Sclerotinia sclerotiorum* mycoviruses

The "Neo-life style" of mycoviruses refers to the way in which these viruses can impact the ecology and evolution of their fungal hosts, leading to novel interactions between fungi, other viruses, and their environment. These benefits are thought to arise through various mechanisms, including the regulation of gene expression within the fungal host, changes to cell signaling pathways, the suppression of fungal immune responses, and synergistic and antagonistic interactions among mycoviruses. Such interactions highlight the potential for viruses to play important roles in shaping the biology of fungi and the ecosystems in which they reside. In the case of *S. sclerotiorum*, some mycoviruses have been shown to reduce the virulence of the fungus, while others have been shown to enhance it. For example, a mycovirus called SsHADV-1 has been shown to reduce the virulence of *S. sclerotiorum*, making it less damaging to plants. On the other hand, another mycovirus called SsDRV-1 has been shown to increase the virulence of the fungus, making it more damaging to plants [130].

S. sclerotiorum isolate SCH941 harbored a co-infection. Sclerotinia sclerotiorum yadokarivirus 1 (SsYkV1; *yadokariviridae*) genome replicates in a heterocapsid of sclerotiorum botybirnavirus 3 virions (SsBV3). This study provides the evidence that mycoviruses can depend on each other for their survival. There are some other examples where members of yadokariviruses are dependent on yadonoushiviruses for their replication and protection [131]. However, more studies are needed to further explain the survival mechanisms. The exact mechanisms by which mycoviruses affect their fungal hosts are not fully understood, but it is thought that they may interfere with gene expression, alter the morphology of the fungal cells, or affect the interaction between the fungus and its environment. The discovery of these mycoviruses and their impact on *S. sclerotiorum* has important implications for crop protection and management, as it may be possible to use mycoviruses as a tool to control fungal plant pathogens [132] (Fig. 3).

Conclusion and future directions

Mycoviruses are a diverse group of viruses that infect fungi. Over the years, research on mycoviruses has revealed many interesting aspects of their biology, including their potential as biocontrol agents, their role in modulating fungal virulence, and their impact on fungal ecology. One of the most fascinating aspects of mycoviruses is their ability to modulate the phenotypic traits of their fungal hosts. Mycoviruses have been shown to influence fungal growth rates, pigmentation, and pathogenicity. Due to their hypovirulence inducing properties, mycoviruses have attained much attention in the fields of fungal ecology, biotechnology, and agriculture. This paper provides a comprehensive overview of the current understanding of *Sclerotinia* mycoviruses. It covers their classification, genome organization, transmission, and effects on the host fungi. The paper also discusses the potential applications of these mycoviruses in biological control of plant diseases. Some of the reported *S. sclerotiorum* isolates associated with hypovirulence are summarized in Fig. 4. The details of their genomic properties are summarized in Table 1.

The non-infectivity and non-symptomatic characteristics of some mycoviruses have made mycovirus research exceedingly difficult and challenging. In the upcoming years, molecular biology progress will make new methods based on in silico approaches and availability of whole genome sequences of different fungal strains including *A. bisporus*, *B. cinerea*, and *S. sclerotiorum* which will be highly helpful for studying mycovirus-host interaction at molecular level. The insight of hypovirulence associated with mycoviruses would be helpful in finding a potential biocontrol agent to control devastating fungal infections. Mycoviruses like SsHADV-1 (which can be transmitted extracellularly) can be engineered to be used as potential biocontrol agents. On the other hand, genes encoding movement proteins from plant or animal viruses can also be integrated into mycoviruses to provide them with an extracellular means of transmission that would be a good option to use them as biocontrol agents. Furthermore, understanding both host and viral behavior in the real world is crucial for this goal. To increase mycovirus potential for biocontrol, understanding mycovirus population biology is just as important as understanding fungal population biology. There is a need for such bioinformatics tools which could be used for the identification of hypovirulent mycoviruses, as assessing the nature of mycovirus is a tedious and time-consuming process. Furthermore, an interdisciplinary approach should be used to target mycoviruses using mycovirus-specific drugs or ligands, which will target specific sites and block mycoviral replication. This will ultimately help in curing mycoviral infection for the preparation of virus-free and infected isogenic lines.

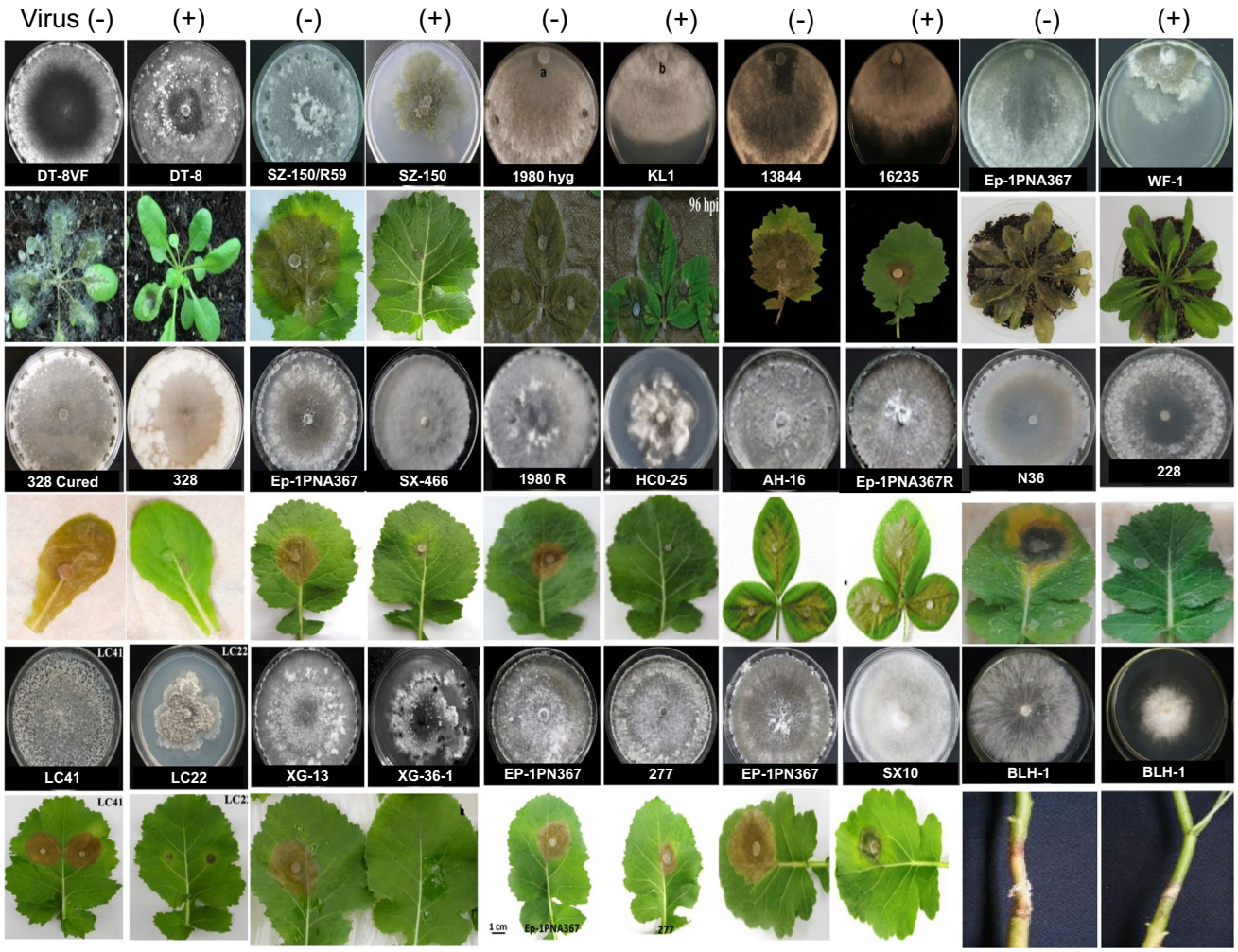

Fig. 4 A comparative representation of *Sclerotinia sclerotiorum* isolates associated with hypovirulence (virus free and virus infected) together with the pathogenicity tests. The isolates on the left side in a group are virus free and on the right side are virus infected. Isolate DT-8 [10], Isolate 328 [26], Isolate LC22 [41], SZ-150 [24], SX-466 [38], Isolate XG-13 [44], Isolate KL1 [29], Isolate HCO25 [32], Isolate 277 [42], Isolate 16235 [30], Isolate AH-16 [33], Isolate SX10 [43], Isolate WF-1[37], Isolate 228 [23], Isolate BLH-1 [28]

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Author contribution HAK: conceptualization; HAK, M.M, MFB: data collection and writing — original draft, HAK: review and edit of the original draft.

Declarations

Conflict of interest The authors declare no competing interests.

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