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Harnessing hypovirulence: Mycovirus-induced modulation of *Sclerotinia sclerotiorum* pathogenicity in mustard

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Abstract
Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of stem rot in Indian mustard (*Brassica juncea*) and other oilseed Brassicas, is a cosmopolitan necrotrophic pathogen capable of inflicting yield losses of up to 80% in severely affected fields (Rai *et al*., 2024). Conventional management relying on partial host resistance, cultural practices, and systemic fungicides has proven inconsistent and environmentally costly, prompting interest in mycovirus-mediated hypovirulence as an alternative biological control strategy. This review synthesizes two decades of research on hypovirulence-inducing mycoviruses infecting *S. sclerotiorum*, with particular emphasis on the single-stranded DNA virus *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1) and co-infecting RNA mycoviruses such as endornaviruses and hypoviruses. Evidence from laboratory, greenhouse and field studies demonstrates that these agents attenuate fungal growth, sclerotial development, and virulence-factor expression through RNA-silencing suppression, disruption of G-protein-coupled signal transduction, and transcriptional reprogramming of pathogenicity genes, in some cases converting the pathogen into a beneficial endophyte that promotes plant growth and induces systemic resistance. Unlike most mycoviruses, SsHADV-1 is unusual in being infectious extracellularly, allowing it to bypass the vegetative-incompatibility barriers that have historically constrained biocontrol efforts built around hypoviruses such as CHV-1 in *Cryphonectria parasitica*. Despite these advances, translation to mustard fields remains constrained by formulation instability, incomplete understanding of natural transmission routes, and the near-total absence of dedicated studies in *Brassica juncea* rather than *B. napus.* This review critically evaluates the mechanistic basis and comparative advantages and limitations of virocontrol relative to conventional approaches and proposes priority research directions, including vector-mediated transmission, seed bio-priming, and mycovirus prospecting in Indian isolates to accelerate deployment of hypovirulence-based management in mustard-growing regions.

Keywords: Mycovirus, hypovirulence, SsHADV-1, Brassica juncea, biological control, RNA silencing

Introduction
Mustard (*Brassica juncea*) ranks among the principal oilseed crops of the Indian subcontinent, and rapeseed-mustard as a group is the third most important oilseed commodity globally after soybean and oil palm, with India contributing a substantial share of world acreage under this crop group (Rakesh *et al*., 2016) [14]. Among the many biotic constraints affecting rapeseed–mustard, Sclerotinia stem rot, caused by the necrotrophic ascomycete , has progressively shifted from a disease of minor consequence to one of the most economically damaging constraints on productivity, particularly in the cool, moist winter-growing tracts of northern India (Rakesh *et al*., 2016; Rai *et al*., 2024) [13, 14]. Field surveys report disease incidence and associated yield losses as high as 80% in parts of Punjab, with substantial losses also recorded in Rajasthan, Uttar Pradesh and Haryana, and an increasing incidence now being noted in the eastern states, including Bihar (Rai *et al*., 2024) [13]. Beyond India, *S. sclerotiorum* is recognized as one of the most polyphagous plant pathogens known, attacking several hundred plant species, and inflicting comparable economic damage on rapeseed, canola, soybean, sunflower and numerous vegetable crops in Canada, China, Europe, Australia and the United States (Rakesh *et al*., 2016; Gupta *et al*., 2019) [3, 14].

Management of Sclerotinia stem rot has historically relied on an integrated combination of cultural practices (deep ploughing, timely sowing, optimized spacing), partial host resistance, botanical extracts, and chemical fungicides such as carbendazim, tebuconazole, and Boscalid-pyraclostrobin combinations. However, complete resistance to *S. sclerotiorum* has not been identified in any cultivated Brassica genotype to date, and only partial or moderate resistance has been reported in selected *B. napus* and *B. juncea* lines (Rakesh *et al*., 2016) [14]. Fungicidal control, meanwhile, is compromised by the prolonged soil survival of sclerotia, the late-season timing of infection relative to the practicality of spraying, the risk of fungicide-resistant isolates, and the environmental and health costs associated with repeated chemical application (Rakesh *et al*., 2016; Gupta *et al*., 2019) [3, 14]. These limitations have driven sustained interest in biological control strategies that exploit natural antagonism, including bacterial and fungal biocontrol agents, and, more recently, mycoviruses capable of inducing hypovirulence in the pathogen itself.

Mycoviruses are viruses that infect and replicate within fungi, and are now recognized as ubiquitous, genetically diverse inhabitants of the fungal kingdom, spanning double-stranded RNA (dsRNA), positive- and negative-sense single-stranded RNA, and single-stranded DNA genomes

(Mu *et al*., 2021). Although most mycovirus infections are cryptic and asymptomatic, a subset markedly reduces the fitness and disease-causing capacity of their fungal hosts, a phenomenon termed hypovirulence (Nuss, 2005) [10]. The paradigm for virus-induced hypovirulence was established through work on Cryphonectria hypovirus-1 (CHV-1) and its role in the natural recovery of European chestnut populations from chestnut blight, caused by *Cryphonectria parasitica* (Nuss, 2005; Turina & Rostagno, 2007) [10, 16]. This precedent inspired the search for analogous hypovirulence-inducing agents in other economically important pathogens, culminating in the identification of a diverse mycovirome within *S. sclerotiorum*, including the unusual single-stranded DNA virus SsHADV-1, which uniquely among characterized mycoviruses is infectious when applied extracellularly to fungal hyphae and can therefore be deployed as a spray-applied "natural fungicide" (Yu *et al*., 2010, 2013).

Despite two decades of mechanistic progress, translation of mycovirus-mediated hypovirulence into a field-deployable management tool for Sclerotinia stem rot of mustard remains incomplete. The great majority of foundational studies have been conducted in Chinese populations of S. *sclerotiorum* infecting *Brassica napus* (oilseed rape), and comparatively little dedicated work has addressed Indian isolates or the specific agro-ecological context of *Brassica juncea* cultivation. Given the scale of yield losses attributable to this disease in Indian mustard, and the urgent need for environmentally sustainable alternatives to chemical fungicides, a critical synthesis of the current evidence base for mycovirus-induced hypovirulence, its mechanisms, demonstrated efficacy, constraints, and applicability to mustard cropping systems is timely. This review accordingly aims to: (i) trace the historical development of the hypovirulence concept from chestnut blight to Sclerotinia biocontrol; (ii) critically synthesize current understanding of the diversity, biology and mechanisms of hypovirulence-inducing mycoviruses in *S. sclerotiorum*; (iii) evaluate recent advances (2015-2026) in extracellular transmission, transcriptomic characterization and field application, (iv) compare mycovirus-based virocontrol against conventional management approaches and (v) identify priority knowledge gaps and research questions specific to deployment of this technology in mustard.

Historical Background and Development

The concept of hypovirulence originated from a field observation rather than a deliberate search for biocontrol agents. In the 1950s, the Italian phytopathologist Antonio Biraghi noted that chestnut trees (*Castanea sativa*) in parts of Italy were spontaneously recovering from cankers caused by the introduced fungus *Cryphonectria* (then *Endothia*) *parasitica*, the causal agent of chestnut blight that had already devastated American chestnut (Castanea dentata) populations (Biraghi, 1953) [1]. French researchers subsequently demonstrated that this recovery was not attributable to host resistance but to a debilitated, hypovirulent state of the fungus itself, transmissible between compatible fungal strains through hyphal anastomosis (as reviewed in Turina & Rostagno, 2007) [16]. A landmark study using nuclear auxotrophic markers confirmed hypovirulence as a maternally inherited, horizontally transmissible fungal trait, establishing its potential as a biological control strategy (as reviewed in Turina & Rostagno, 2007) [16]. The molecular basis was resolved only decades later, when the causal agent was identified as a mycovirus, subsequently classified within the family Hypoviridae and shown to be sufficient, on its own, to confer hypovirulence when introduced into virulent fungal strains as an infectious cDNA clone (Nuss, 2005) [10].

In parallel, the broader field of mycovirology traces its origins to 1962, with the first description of a virus associated with disease in the cultivated mushroom *Agaricus bisporus*, subsequently attributed to a double-stranded RNA virus (Zhang *et al*., 2009) [21]. Over the following decades, mycoviruses were progressively identified across all major fungal phyla, and a minority were found to modulate the fitness or virulence of their hosts (Mu *et al*., 2021).

Within *S. sclerotiorum* specifically, the first indications of virus-associated hypovirulence emerged from naturally occurring debilitated isolates carrying dsRNA elements (Boland, 1992) [2]. Molecular characterization of Sclerotinia mycoviruses accelerated substantially thereafter, culminating in the isolation, from the naturally hypovirulent strain DT-8, of a small circular single-stranded DNA mycovirus SsHADV-1, phylogenetically related to the geminiviruses but morphologically and organizationally distinct from them (Yu *et al*., 2010; Gupta *et al*., 2019) [3]. Independently, Zhang *et al*. (2009) [21] characterized another hypovirulent isolate, XG36-1, isolated from a rapeseed stem lesion, and demonstrated by transmission electron microscopy that infection was associated with disintegration of nuclear and mitochondrial membranes and granulation of the fungal cytoplasm. The debilitated phenotype was transmissible through hyphal anastomosis but was lost during sexual reproduction. 104 single-ascospore progenies of XG36-1 all reverted to wild-type growth and virulence, indicating a cytoplasmically inherited, extrachromosomal causal agent consistent with a mycovirus (Zhang *et al*., 2009) [21]. The subsequent demonstration that purified SsHADV-1 particles were infectious when applied directly and extracellularly to fungal hyphae or to plant leaves (Yu *et al*., 2013) [19] represented a conceptual turning point, challenging the long-standing generalization that mycoviruses lack an extracellular phase and are restricted to intracellular transmission via spores or hyphal fusion. Since 2015, this foundation has been substantially extended through metatranscriptomic surveys revealing complex multi-virus communities within single *S. sclerotiorum* strains (Mu *et al*., 2021), functional characterization of the mechanisms by which SsHADV-1 reprograms its host into a beneficial endophyte (Zhang *et al*., 2020) [12], and field validation of bio-priming approaches in rapeseed (Qu *et al*., 2020) [12]. In India, the trajectory of mycovirology has been comparatively recent and narrower in scope, culminating from around 2016-2019 onward in the characterization of Himachal Pradesh isolates of *S. sclerotiorum* and the first documented identification of a hypovirulence-associated mycovirus in an Indian isolate, recovered from a survey spanning cauliflower, pea and mustard (Kapatia *et al*., 2016; Gupta *et al*., 2019) [3, 5].

Current Understanding:

1. Biology and Pathogenicity of

S. sclerotiorum is a homothallic ascomycete of the family Sclerotiniaceae, that persists in soil primarily as sclerotia a

melanized, multilayered resting structures capable of surviving for extended periods under adverse conditions (Rakesh *et al*., 2016) [14]. Sclerotia germinate either myceliogenically, producing infectious hyphae that attack plant bases directly, or carpogenically, producing apothecia that release airborne ascospores capable of infecting senescent floral tissue (Rakesh *et al*., 2016) [14]. Pathogenesis proceeds through a well-characterized sequence involving surface adhesion, formation of compound appressoria, secretion of cell-wall-degrading enzymes (polygalacturonases, cutinases, xylanases) and oxalic acid, and suppression of host immune responses via secreted effector proteins, ultimately producing rapidly expanding, water-soaked necrotic lesions (Zhu *et al*., 2024) [22]. Numerous individual pathogenicity determinants have been functionally validated by targeted gene disruption, including transcription factors regulating hyphal growth and appressorium formation (SsSte12, SsNsd1), MAPK-pathway components (SsSm1, Smk3), oxalic-acid metabolism genes (Ssoah1, SsODC1/2), and secreted effectors such as SsCP1 and SsSSVP1 (Zhu *et al*., 2024) [22]. Collectively, these studies establish that *S. sclerotiorum* virulence is polygenic and tightly coupled to conserved fungal signal-transduction cascades, a feature directly relevant to understanding how mycoviruses subsequently disrupt pathogenicity, since several of the same signalling nodes are also targets of virus-induced dysregulation.

2. Diversity and classification of mycoviruses infecting

S. sclerotiorum harbours an unusually rich and continually expanding mycovirome. Early molecular surveys identified a handful of dsRNA elements, but the application of next-generation sequencing to individual hypovirulent strains has revealed far greater complexity than previously appreciated. In a single strain (SX276), Mu *et al*. (2021) characterized nine distinct mycoviruses spanning eight taxonomic lineages seven (+)ssRNA viruses, one dsRNA virus, and one (-)ssRNA virus including a deltaflexivirus with an unusual tripartite genome interpreted as an evolutionary intermediate between segmented and non-segmented RNA viruses, and the first confirmed naturally occurring fungal rhabdovirus, for which the authors proposed a new genus, Sclerorhabdovirus. Of the nine co-infecting viruses in that study, only the endornavirus SsEV3 was confirmed to be independently associated with hypovirulence, underscoring that co-infection complexity does not necessarily equate to a proportional number of biologically active hypovirulence determinants (Mu *et al*., 2021). Khan *et al*. (2023) [6], synthesizing the wider literature, catalogued hypovirulence-associated mycoviruses infecting Sclerotinia isolates across 17 different viral families identified between 1992 and 2023[2, 6], spanning (+)ssRNA, (-)ssRNA, dsRNA and ssDNA genome types a diversity considerably broader than that documented for any other single hypovirulence-biocontrol system, including *C. parasitica*.

Several individually characterized isolates illustrate this diversity at the strain level. The Canadian isolate S10, recovered from sunflower in 1979, was among the earliest hypovirulence-associated isolates reported, exhibiting abnormal tan-coloured sclerotia, albino apothecia, reduced pathogenicity, and heightened susceptibility to the mycoparasite Coniothyrium minitans (Huang, 1981) [4]. The Chinese isolate Ep-1PN was subsequently found to harbour three dsRNA elements of differing size associated with altered colony morphology, reduced growth, and shortened lesion length; one of these elements, debilitation-associated RNA virus (SsDRV), was shown to alter expression of pathogenicity-associated genes, with the reduced-growth phenotype linked to downregulation of genes involved in cell-wall biosynthesis, protein synthesis, metabolism, stress response, nutrient transport and signal transduction (Li *et al*., 2008). A further Chinese isolate, HC025, recovered from soybean, was found to carry five mycoviruses from distinct viral families: mitovirus 1 (SsMV1), negative-stranded RNA virus 1 (SsNSRV1), narnavirus 4 (SsNV4), *Sclerotinia sclerotiorum* ourmia-like virus 14 (SsOLV14), and *Sclerotinia sclerotiorum* ourmia-like virus 22 (SsOLV22); all but SsNSRV1 were shown to co-transfer horizontally from HC025 into the virulent strain Ep-1PNA367, converting the recipient to a hypovirulent phenotype (Wang *et al*., 2022) [17]. The subsequently sequenced strain SX276 extended this picture further, with its nine co-infecting viruses individually identified as SsEV3, SsHV1, SsMTV1, SsOV4, SsDFV3S1-3, SsOV5, SsMAV1, SsBV3S1-2 and SsRhV1 (Mu *et al*., 2021). Collectively, these isolate-level findings demonstrate that hypovirulence in *S. sclerotiorum* arises repeatedly and independently across taxonomically diverse mycoviruses rather than being attributable to any single virus family.

Within this diversity, the ssDNA virus SsHADV-1 remains the most extensively characterized and functionally consequential agent. Its genome is a compact, circular ssDNA molecule of approximately 2.2 kb encoding a replication-initiation protein (Rep) and a coat protein (CP); despite phylogenetic affinity to the geminiviruses on the basis of Rep sequence, SsHADV-1 differs fundamentally from geminiviruses in genome organization, particle morphology, and critically the absence of any known insect vector or plant-infecting capability at the time of its discovery (Yu *et al*., 2010; Gupta *et al*., 2019) [3]. It was subsequently designated the type species of a new virus family, Genomoviridae (Zhang *et al*., 2020) [12]. By contrast, dsRNA hypoviruses and endornaviruses more closely resemble the CHV-1 paradigm in their reliance on intracellular transmission.

3. Mechanism of Hypovirulence: RNA Silencing and Signal Transduction

Two broad, non-mutually-exclusive mechanistic frameworks have been proposed to explain how mycovirus infection translates into reduced fungal virulence. The first invokes the fungal RNA-interference (RNAi) pathway as an antiviral defence system that mycoviruses must counteract, and in doing so, perturb host physiology. Fungi possess Dicer-like (DCL) and Argonaute-like (AGL) proteins that process viral dsRNA into small interfering RNAs, which guide an RNA-induced silencing complex to cleave viral RNA (Nuss, 2005) [10]. To persist, several mycoviruses most thoroughly studied in CHV-1 of *C. parasitica* encode RNA-silencing suppressor proteins, such as the papain-like protease p29, that bind and sequester dsRNA/siRNA intermediates, preventing the host's Dicer/RISC machinery from clearing the virus. Functional studies in *C. parasitica* show that disruption of a single Dicer gene (dcl2) or a single Argonaute gene (agl2) dramatically increases both viral RNA accumulation and the severity of the debilitated hypovirulent phenotype, providing strong evidence that RNAi functions as an antiviral defence whose subversion is

mechanistically coupled to hypovirulence (Nuss, 2005) [10]. In *S. sclerotiorum*, functional redundancy appears more pronounced. Double mutants lacking both fungal Dicer genes show a similarly debilitated phenotype when infected with either a hypovirus or SsHADV-1, and this debilitation could be reverted by complementation with a single Dicer gene, indicating partial redundancy between the two DCL paralogues in this species (Khan *et al*., 2023) [6]. Collectively, these findings position the RNAi pathway as a genuine antiviral immune system in fungi, whose molecular "arms race" with the virus is mechanistically linked to the debilitated, hypovirulent phenotype.

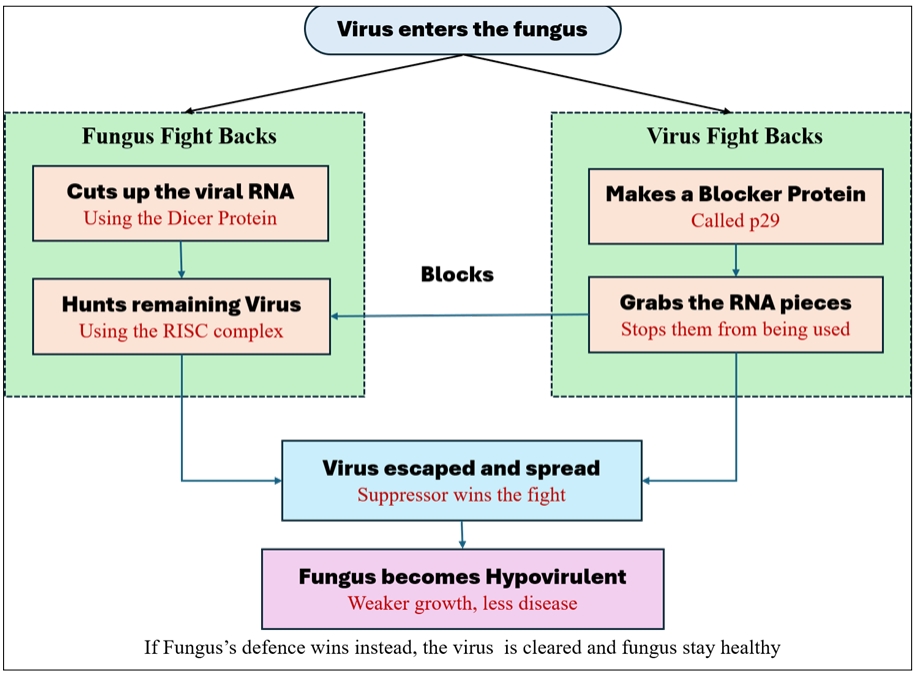

Fig 1: Counter RNA-silencing mechanism of mycovirus infection in. Schematic showing the fungal RNA-interference (RNAi) pathway, Dicer-mediated processing of viral dsRNA into siRNAs and RISC-guided degradation of viral RNA opposed by a virus-encoded silencing-suppressor protein (e.g., p29), illustrating how successful suppression allows the virus to persist and ultimately induce hypovirulence.

The second framework implicates disruption of heterotrimeric G-protein and downstream MAPK signal-transduction cascades that, independently of virus infection, are already established regulators of fungal pathogenicity. In *C. parasitica*, CHV-1 infection reduces accumulation of G-α-subunit proteins, thereby attenuating downstream cAMP–PKA and PI-PLC–PKC signalling required for laccase production, pigmentation, sporulation and, ultimately, virulence (Nuss, 2005; Turina & Rostagno, 2007). Turina and Rostagno (2007) [10, 16] caution, however, that despite two decades of molecular dissection, no single downstream target or pathway has been unambiguously shown to be both necessary and sufficient to explain virus-induced hypovirulence in its entirety, describing the phenomenon as "still an unresolved conundrum" and emphasizing that multiple, possibly redundant, cellular processes are perturbed simultaneously. This caution is important context for interpreting mechanistic claims made for Sclerotinia mycoviruses, where equivalent gene-knockout dissection of individual signalling components remains comparatively less complete than in the *C. parasitica* system.

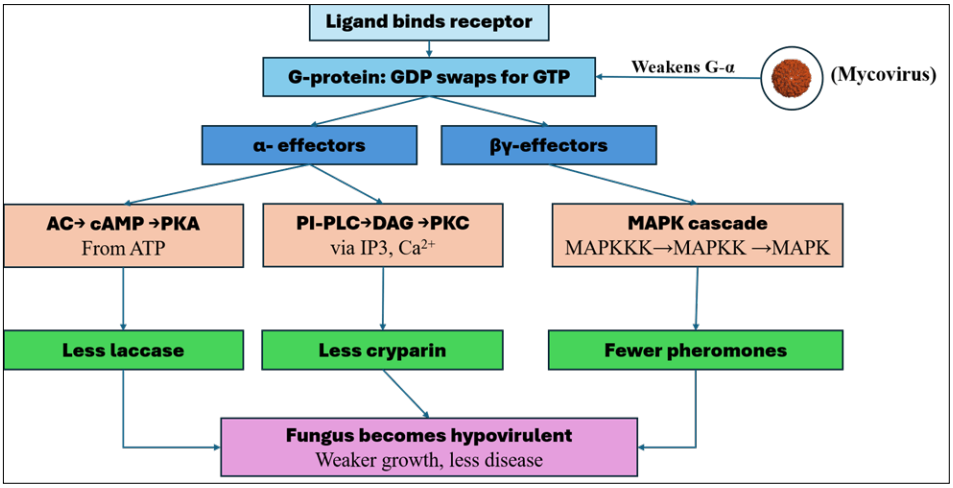

Fig 2: Disruption of heterotrimeric G-protein and downstream signal-transduction cascades by mycovirus infection. Schematic showing depletion of the Gα subunit attenuating the downstream cAMP–PKA and PI-PLC–PKC branches, reducing laccase, cryparin and pheromone-associated outputs, and resulting in a hypovirulent phenotype.

4. Extracellular Transmission and the SsHADV-1 Exception

Mycoviruses are conventionally considered non-infectious as free extracellular particles, restricted instead to vertical transmission through spores or horizontal transmission through hyphal anastomosis between vegetatively compatible isolates (Yu *et al*., 2013). This constraint imposed by the rigid fungal cell wall, thought to block uptake of free virions, has historically been considered the single greatest obstacle to field-scale mycovirus-based biocontrol, since vegetative-incompatibility (VIC) systems restrict anastomosis, and therefore viral spread, to genetically compatible subsets of a fungal population (Turina & Rostagno, 2007; Zhang *et al*., 2020) [12, 16]. Yu *et al*. (2013) demonstrated that this generalization does not hold for SsHADV-1, when purified virus particles were applied directly to hyphae on agar, or sprayed onto detached leaves and whole plants of *Arabidopsis thaliana* and *Brassica napus*, successfully established infection, converted virus-free recipient strains to the hypovirulent phenotype, and did so irrespective of the vegetative compatibility group of the recipient isolate. This extracellular infectivity required whole virus particles (naked viral DNA alone was not infectious), was narrow in host range (infecting *S. minor* and *S. nivalis* but not *Botrytis cinerea*, *Coniothyrium minitans* or *Magnaporthe oryzae*), and conferred both protective and curative activity in planta: pre-application to leaves reduced lesion development following subsequent fungal challenge, while post-infection application halted lesion expansion with efficacy comparable to the chemical fungicide carbendazim (Yu *et al*., 2013). Field trials on naturally infected rapeseed further confirmed that spraying a suspension of virus-infected hyphal fragments reduced both disease incidence and severity, and increased yield to a degree statistically comparable with the fungicide prochloraz manganese (Yu *et al*., 2013). Virus particles were also shown to be environmentally stable on leaf surfaces, with detectable viral DNA persisting up to 15 days post-application on unwounded leaves (Yu *et al*., 2013).

Recent Advances

1. Reprogramming the Pathogen: SsHADV-1 as a "Plant Vaccine"

The most consequential recent advance is the demonstration that SsHADV-1 does not merely attenuate *S. sclerotiorum* but can convert it, under appropriate conditions, into a beneficial plant endophyte. Zhang *et al*. (2020) [20] showed that the SsHADV-1-infected strain DT-8 forms compound appressoria and colonizes rapeseed leaf and root tissue without inducing necrosis, in marked contrast to the virus-cured isogenic strain, which retains full necrotrophic virulence. Comparative transcriptomic analysis demonstrated that virus infection downregulates a coordinated suite of pathogenicity associated genes including those encoding cell-wall-degrading enzymes, secreted effector-like proteins, and enzymes of the oxalic-acid biosynthetic pathway, while genes involved in compound-appressorium development show more complex, stage-dependent regulation (Zhang *et al*., 2020) [12]. Functionally, endophytic colonization by DT-8 promoted rapeseed growth, evidenced by increased fresh weight and enhanced rootlet proliferation, and primed plant defence pathways, including MAPK signalling and the ethylene/jasmonic-acid hormone axes, conferring enhanced resistance not only to subsequent challenge by virulent, virus-free *S. sclerotiorum* but also to the unrelated necrotroph *Botrytis cinerea* (Zhang *et al*., 2020) [12]. This "plant vaccine" effect a virus-mediated conversion of a lethal necrotroph into a growth-promoting, cross-protective endophyte represents a qualitatively distinct mode of biocontrol from simple virulence attenuation and has since been extended to other delivery formats, with seed-coating approaches reported to broaden protection to additional pathogens beyond *Sclerotinia sclerotiorum* itself (Shang *et al*., 2024) [15].

2. Field-Level Validation: Bio-Priming and Yield Outcomes

Building on the endophyte-conversion findings, Qu *et al*. (2020) conducted field trials applying strain DT-8 as a bio-priming agent on rapeseed and reported that spraying at early flowering reduced Sclerotinia stem rot severity by approximately 68% and increased yield by nearly 15%, while also demonstrating that the DT-8-treated plant-associated microbiome showed enhanced connectivity and network strength relative to untreated controls. This implies that the endophytic hypovirulent strain reshapes the broader plant-associated microbial community in ways that may contribute to disease suppression beyond direct pathogen antagonism and constitutes among the first demonstrations that a mycovirus-modified fungal strain can function as a self-propagating, field-applicable biocontrol and growth-promotion agent rather than merely a laboratory curiosity.

3. Expanding Mycovirus Diversity and the Endornavirus Contribution

Metatranscriptomic sequencing has substantially expanded the known Sclerotinia mycovirome since 2020. Mu *et al*. (2021) [11] identified nine co-infecting mycoviruses across eight lineages within a single hypovirulent strain, including novel evolutionary intermediates and the first natural fungal rhabdovirus, and confirmed that among this complex community, the endornavirus SsEV3 was independently sufficient to confer hypovirulence. Follow-up mechanistic work by Mu *et al*. (2025) demonstrated that SsEV3 infection reduces sclerotial production, abolishes infection-cushion formation, increases hyphal vacuolation, and heightens sensitivity to abiotic stress. Transcriptomic profiling linked these phenotypes to downregulation of the gene Sssnf1, whose deletion independently impairs infection-cushion formation and virulence and to upregulation of RNAi pathway components, with the fungal Dicer gene Ssdcl2 shown to restrict SsEV3 accumulation. The same study identified a previously uncharacterized host protein, Sshp1, that binds the viral RNA-dependent RNA polymerase domain and appears to function as a novel antiviral factor independent of the canonical RNAi machinery, indicating that *S. sclerotiorum* 's antiviral defence repertoire extends beyond RNAi alone (Mu *et al*., 2025). Collectively, this work establishes endornaviruses historically considered largely cryptic in fungi as a second, mechanistically distinct class of hypovirulence determinant in *S. sclerotiorum*, complementing the ssDNA (SsHADV-1) and dsRNA hypovirus systems.

4. First Reports from Indian Isolates

Directly relevant to mustard cultivation in South Asia, a small but growing body of Indian research has begun to

characterize the genus locally. Kapatia *et al*. (2016) [5] established molecular and RAPD-based genetic diversity profiles of *S. sclerotiorum* isolates from cauliflower and pea in Himachal Pradesh, providing baseline isolate-level resolution. Building on this, Gupta *et al*. (2019) [3] reported, to their knowledge, the first identification of a hypovirulence-associated ssDNA mycovirus within an Indian *S. sclerotiorum* isolate showing growth and sclerotial defects, screened from a survey of 308 isolates collected from cauliflower, pea and mustard in Himachal Pradesh, and subsequently reviewed the broader biocontrol potential of Sclerotinia mycoviruses. While these studies remain preliminary relative to the depth of mechanistic characterization achieved in Chinese *B. napus* systems, they establish that hypovirulence-associated mycoviruses occur naturally in Indian *S. sclerotiorum* populations, including isolates recovered directly from mustard, providing a foundation for future virocontrol development specific to Indian cropping conditions.

Integration and Future Directions

Several converging lines of evidence suggest that the most promising path to field deployment lies not in deploying purified mycovirus particles as a stand-alone spray, though this route retains value where curative, fungicide-comparable action is needed against active infections (Yu *et al*., 2013), but in combining viro-control with existing integrated disease management (IDM) frameworks. The demonstrated capacity of SsHADV-1-infected DT-8 to function simultaneously as a biocontrol agent, a growth promoter, and an inducer of systemic and cross-pathogen resistance (Zhang *et al*., 2020; Qu *et al*., 2020) [12] positions seed or seedling bio-priming as a particularly attractive delivery route for mustard, since it would circumvent the canopy-penetration problem that limits foliar fungicide efficacy in this crop, and would align with existing agronomic practice for seed treatment. Integration with partially resistant *Brassica* genotypes could plausibly provide additive protection, since host resistance and viro-control act through independent mechanisms and would not be expected to select for cross-resistance in the way that repeated single-mode fungicide use does. Combining hypovirulent-strain application with reduced-rate fungicide use, rather than outright fungicide replacement, may offer a pragmatic transitional strategy that lowers overall chemical load while hedging against current uncertainty in the field persistence of virocontrol agents.

A second important direction concerns transmission biology. Reports that SsHADV-1 can utilize a mycophagous fungus-gnat vector for transmission open the possibility of naturally self-propagating spread through insect populations associated with sclerotia-infested fields, a mechanism that, if confirmed and quantified under Indian field conditions, could substantially reduce the need for repeated re-application. Prospecting for additional insect or arthropod vectors, and for naturally occurring extracellularly transmissible mycoviruses beyond SsHADV-1, represents a high-value avenue given how disproportionately the vector-independent, VIC-bypassing biology of SsHADV-1 has driven practical progress relative to intracellularly transmitted dsRNA hypoviruses.

Finally, given the near-complete concentration of existing mechanistic and field data in Chinese *B. napus* systems, a clear integration priority is the systematic screening of Indian *S. sclerotiorum* populations across mustard-growing states including Punjab, Rajasthan, Haryana, Uttar Pradesh and increasingly Bihar for naturally occurring hypovirulent isolates and their associated mycoviromes, building on the preliminary work of Gupta *et al*. (2019) and Kapatia *et al*. (2016) [3, 5], to establish whether locally adapted hypovirulent strains might offer superior field fitness and persistence relative to introduced strains such as DT-8.

Major Knowledge Gaps and Research Priorities

Despite substantial mechanistic progress, several fundamental gaps constrain translation of mycovirus-induced hypovirulence into a deployable management tool for Sclerotinia stem rot of mustard, summarized in **Table 1** below and elaborated in the surrounding text.

First, transmission and field persistence remain incompletely understood. Vegetative incompatibility continues to restrict horizontal spread of most Sclerotinia mycoviruses (all except SsHADV-1) between genetically diverse field populations, and even for SsHADV-1, the relative contributions of hyphal contact, environmental particle stability, and any natural vector-mediated transmission to sustained field spread as opposed to a single controlled application have not been fully quantified under diverse agro-climatic conditions (Zhang *et al*., 2020; Turina & Rostagno, 2007) [12, 16].

Second, formulation and application technology lags behind mechanistic understanding. Existing demonstrations rely on hyphal-fragment suspensions or laboratory-purified virus particles applied under controlled greenhouse or limited field conditions; no validated commercial-scale production, protective formulation, or delivery protocol suited to smallholder mustard cultivation currently exists (Zhang *et al*., 2020) [12].

Third, there is a marked geographic and host-specificity gap: essentially all field-validated efficacy and mechanistic transcriptomic data derive from *B. napus* systems in China, whereas *B. juncea* the dominant oilseed Brassica of the Indian subcontinent has been the subject of only preliminary isolate-level mycovirus surveys (Gupta *et al*., 2019; Kapatia *et al*., 2016) [3, 5], with no published dedicated field-efficacy trial of SsHADV-1 or DT-8-type bio-priming specifically in Indian mustard agro-ecosystems.

Fourth, the ecological consequences of converting a necrotroph into an endophyte are not fully characterized: while Zhang *et al*. (2020) and Qu *et al*. (2020) [12] demonstrate beneficial growth-promotion and cross-protection effects, the long-term stability of the endophytic relationship, its behaviour under the variable conditions of Indian dryland and irrigated mustard cultivation, and its interactions with the native soil and phyllosphere microbiome remain to be established.

Fifth, the precise mechanistic contribution of RNA silencing versus signal-transduction disruption versus direct transcriptional reprogramming remains only partially resolved even in the best-studied *C. parasitica* system, and comparably detailed gene-knockout dissection has not been completed for the majority of Sclerotinia-infecting mycoviruses beyond SsHADV-1 and SsEV3 (Turina & Rostagno, 2007[16]; Mu *et al*., 2025).

Table 1: Key knowledge gaps and research priorities

Domain	Current status	Priority research need	Priority
Transmission/persistence	VIC restricts most mycoviruses; SsHADV-1 route only partly quantified in field	Field-scale transmission and persistence studies across agro-climatic zones	High
Formulation/delivery	Lab-scale hyphal-fragment or purified-particle application only	Commercial-scale, UV-stable formulation and delivery protocols	High
Host/geographic specificity	Almost all data from B. napus, China	Dedicated B. juncea isolate surveys and field trials in India	High
Endophyte ecology	Growth promotion and cross-protection shown in controlled trials	Long-term field stability and microbiome interaction studies	Medium
Mechanistic dissection	RNAi and signalling roles partly resolved for SsHADV-1/SsEV3 only	Gene-knockout dissection across additional Sclerotinia mycoviruses	Medium

Conclusion

Two decades of mycovirology research have transformed from a pathogen studied primarily for its own virulence mechanisms into a demonstrated proof-of-concept system for virus-mediated biological control, anchored by the unusual biology of SsHADV-1 and complemented by an increasingly well-characterized diversity of co-infecting RNA mycoviruses. The capacity of these agents to attenuate virulence through convergent disruption of RNA-silencing, signal-transduction and virulence-gene transcriptional networks and, in the case of SsHADV-1, to reprogram the pathogen into a growth-promoting, cross-protective endophyte represents a genuinely novel mode of crop protection distinct from conventional fungicidal, cultural or classical biological control approaches. For Indian mustard specifically, where Sclerotinia stem rot continues to expand in both geographic range and severity and where existing management options remain only partially effective, the evidence reviewed here indicates that mycovirus-based virocontrol, and particularly SsHADV-1/DT-8-type bio-priming, merits urgent, dedicated field investigation rather than continued reliance on the extrapolation of results obtained in Chinese oilseed-rape systems. Realizing this potential will require coordinated progress across mycovirus prospecting in Indian isolates, transmission and formulation research, and rigorously designed multi-location field trials but the underlying biology, as synthesized in this review, provides a substantive and scientifically credible foundation on which to build.

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