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HISTORY, TAXONOMY, SCOPE, AND CHALLENGES OF RESEARCH ON MYCOVIRUSES IN PAKISTAN

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ABSTRACT

Fungi are often infected by mycoviruses. Mycoviruses, like their plant and animal counterparts, can only replicate within healthy host cells. They are missing with a mobility protein that is required for animals and plant viruses. They can only spread from cell to cell through biological processes including cell division, sporulation, and fusion. The vast majority of mycoviruses are unable to infect cells through the extracellular matrix. Double-stranded RNA (dsRNA) makes up most of the mycoviruses genomes, whereas positive, single-stranded RNA (+ssRNA) makes up just a tiny percentage. Recently, DNA mycoviruses have been discovered. New mycoviruses are detected and identified using recently discovered metagenomic approaches. Although evidence of the presence of these viruses exists in nearly every phylum of fungi, the vast majority are still a mystery. Despite their significance, mycoviruses are typically asymptomatic, which makes them very useful. Uneven development, unusual colouration, and even alterations to sexual reproduction in some hosts have all been attributed to Mycoviruses. Their primary effect a reduction in the virulence of their host has given them increased significance for controlling plant diseases. The term "hypovirulence" is used to describe diminished pathogenicity. Mycoviruses have gained prominence due to the hypovirulence phenomenon, which can potentially lessen the damage to crops and trees caused by their host fungus. In Pakistan, few mycoviruses have been discovered. This review article discusses the history, taxonomy, and usage of mycoviruses as biological agents, their interaction with the fungal host, reports of mycoviruses, and challenges of research on mycoviruses in Pakistan.

Keywords: Hypovirulence, dsRNA, ssRNA, biological control, plant pathogen

INTRODUCTION

Mycoviruses infect fungi but seldom cause disease because they are dormant in the host organism (Bozarth, 1972). Only a small number of mycoviruses are known to cause significant alterations in their fungal hosts (Xie

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and Jiang, 2014; Ghabrial *et al.*, 2015; Jiang *et al.*, 2015) when they do, the results are readily apparent in the form of aberrant growth, altered pigmentation, and distorted sexual reproduction. One of the most useful characteristics of mycoviruses for controlling fungal diseases is their ability to reduce the virulence of plant pathogenic fungi, or hypovirulence. As a result of its usefulness in controlling fungus-based forest and agricultural diseases, this characteristic has recently gained a lot of attention (Tonka *et al.*, 2022). Knowledge of mycoviruses has progressed over the last 50 years

thanks to intensive research. Most mycoviruses are to be associated with latent infection in their host fungi. They are transmitted naturally by hyphal anastomosis and heterokaryosis, called lateral transmission. Some of them are transmitted through spores called serial transmission. Fungi typically exchange cytoplasm during their long-life cycles, and the majority of the fungi produce sexual and asexual spores (Bocos-Asenjo *et al.*, 2022). Mycoviruses, efficiently use these ways and transmit to another host. Though they don't have a cellular phase, they seem to be successfully present in almost all the fungal taxa, especially in the filamentous group of fungi. Though these viruses have been discovered recently, phylogenetic analysis revealed that the mycoviruses are of ancient origin (Ghabrial *et al.*, 2015). There has also been research on their relationship with the plant-pathogenic fungi that serve as their hosts. Hypovirus CHV1 and *Cryphonectria parasitica*, the causal agent of chestnut blight, were the first known organisms to interact with one another. Infected with CHV1 virus, *C. parasitica* fungus showed aberrant coloration and stunted development. Hypovirulence in chest nut blight was caused by the mycovirus CHV1. Later, researchers examined a number of Mycovirus-infected fungus species. Besides *P.*

graminearum, *Fusarium graminearum* has been investigated extensively since it is an important plant pathogenic fungi (Jiang *et al.*, 2013). *F. graminearum* is rendered non-virulent after infection with the mycovirus FgV1. Reduced vegetative growth, abnormal pigmentation, and the production of mycotoxin were observed in *F. graminearum* after infection with FgV1 (Cho *et al.*, 2013). Mycovirus FgV1 is remarkable for a number of reasons, the most important being that it can be transferred to other fungal species like *C. parasitica*, where it promotes more severe hypovirulence than its Mycovirus CHV1 counterpart (Xie and Jiang, 2014). There has been a lot of interest in using mycoviruses as a biocontrol agents because of their ability to lower the virulence of their host fungus. Mycoviruses that produce fungal hypovirulence are increasingly being documented, and they come from a broad range of taxonomic families. How well these viruses disseminate in wild populations of the disease-causing organism is crucial to their use in disease control. Furthermore, these mycoviruses make us think that they can change the trophic mode of several fungal plant pathogens and make them beneficial for crops. In conclusion, with the judicious use of these mycoviruses, crop health can be improved.

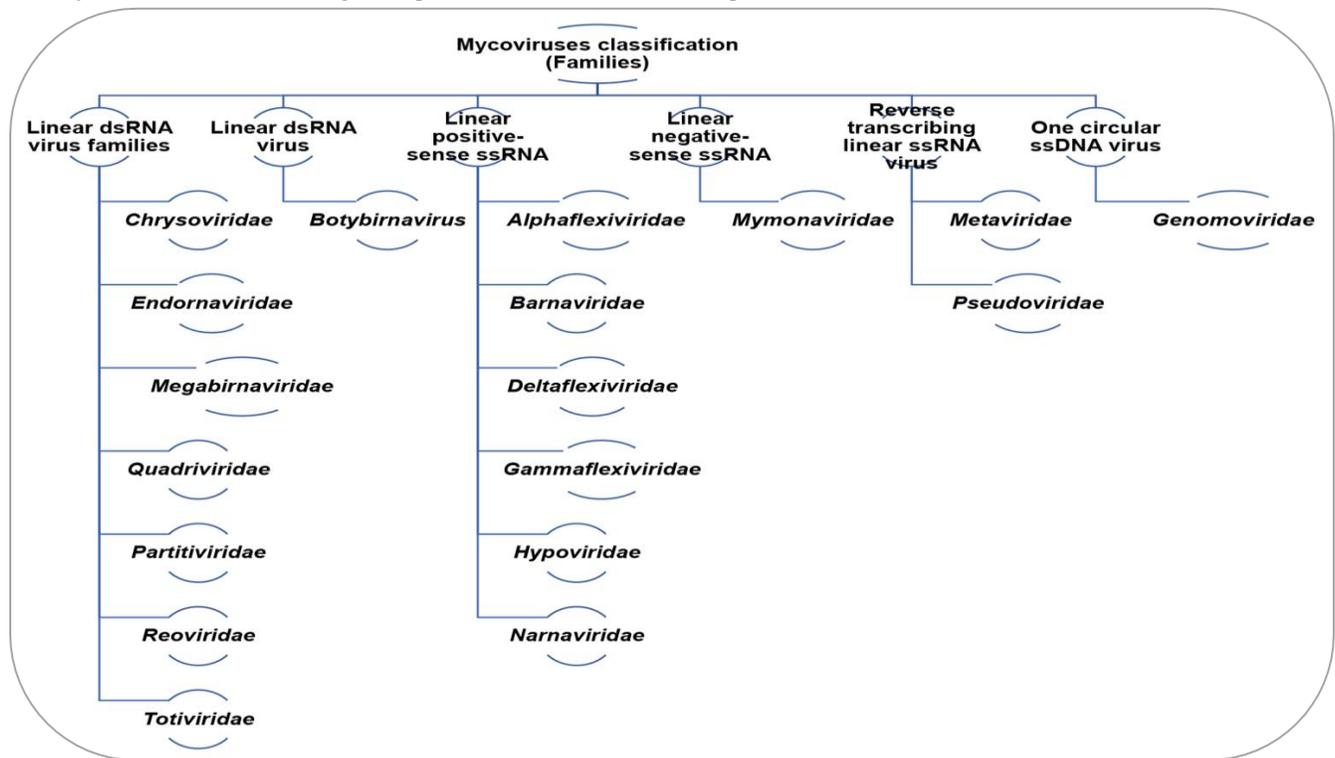


Figure 1. Classification of mycovirus families. Source; The Online (10th) Report of the ICTV; https://talk.ictvonline.org/ictv-reports/ictv_online_report/

History of Mycoviruses: Mycoviruses, as was previously said, are the viruses associated with fungi. Mycoviruses are a special class of viruses that share certain characteristics with viruses found in animals and plants but also possess their own distinct traits. Mycoviruses, for instance, are neither airborne nor waterborne like their animal and plant counterparts but rather propagate by cell division, sporulation, and fusion. Researchers have shown that the majority of Mycoviruses have a double-stranded RNA (dsRNA) genome, while around 30% have a positive, single-stranded RNA (+ssRNA) genome (Sahin *et al.*, 2021). Group Gemini virus-related DNA mycoviruses have recently been identified for the first time (Kotta-Loizou, 2021). Mycoviruses have been found in the cells of all five fungal phyla e.g., Zygomycota, Ascomycota, Deuteromycota, Basidiomycota, and Chytridiomycota. The majority of Mycoviruses are yet to be identified. At now, numerous Mycoviruses may be identified and detected using transcriptome techniques.

Taxonomy of Mycoviruses: According to the tenth report of the International Committee for Taxonomy of Viruses (ICTV), fungal viruses or mycoviruses have been classified into different families as shown (Figure 1). However, a DNA virus was recently identified from the fungal plant pathogen *Sclerotinia sclerotiorum*, which suggests that fungi may host both RNA viruses and DNA viruses (Lefkowitz *et al.*, 2018).

Because many mycoviruses produce dsRNA or dsRNA replicative intermediates in their fungal hosts, Mycoviruses have often been discovered after the isolation of dsRNA molecules. Many mycoviruses were discovered after profiling the purified dsRNA. Fungal isolates known to possess dsRNA were found to exhibit several dsRNA patterns (Li *et al.*, 2019), which may reflect the partition of viral genomes. Our understanding of phylogeny has led us to conclude that members of the same taxonomic family may infect a wide variety of hosts, including fungi, plants, mammals, and protozoa (Pearson *et al.*, 2009). This indicates that the dsRNA and ssRNA estimates about the number of mycoviruses may be too high. Recent taxonomic work has shown that the Partitiviridae family includes dsRNA viruses with the ability to infect plants, fungi, and protozoa. Mycoviruses have a common evolutionary ancestry with other plant viruses; which includes CHV1-4, FgV1, and Botrytis virus X, among others. Their genomic layout and expression technique are similar to those of plant poty viruses or

potex-related viruses, according to the results of a genome study (Pearson *et al.*, 2009; Jiang *et al.*, 2013; Ghabrial *et al.*, 2015; Jiang *et al.*, 2015). In addition, *Sclerotinia sclerotiorum* hosts the RNA virus L, which is very similar to human infections like hepatitis E virus and Rubi virus (Xie *et al.*, 2006). There are two main hypotheses presented for the beginning of mycoviruses. The "ancient coevolution theory" proposes that the unknown mycovirus ancestry is reflective of a lengthy period of coevolution. Mycoviruses, according to the alternative plant virus theory, evolved recently from other plant viruses; the first mycovirus likely began in plants and then traveled to fungi inside the same host plant (Xie *et al.*, 2006). Since none of the two proposed mechanisms for mycovirus development is really convincing, its genesis remains a mystery. Mycoviruses with minimal virulence include those linked with *Sclerotinia sclerotiorum*, *Helminthosporium victoriae*, and *Rosellinia necatrix*. Several mycoviruses were identified and studied by using reverse genetic techniques. Several transfection mechanisms have been investigated despite the absence of an extracellular phase in these mycoviruses. These viruses have been studied using pure virus particles, full-length viral cDNA clones, and *in vitro* RNA transcripts (Nuss, 2005). Experiments with infectious mycoviruses let researchers identify the viral and/or host factors responsible for symptom induction and virus multiplication in many different host-virus interactions. These techniques may also be used to learn more about the host ranges of many other mycoviruses. As was previously mentioned, mycoviruses may either spread by hyphal anastomosis or spores, depending on the kind of cell they infect. Fungal vegetative incompatibility is a barrier to interstrain viral transmission (VIC). A significant drawback of using hypovirulent mycoviruses as biocontrol agents is that they are not plant-compatible. Novel evidence from recent investigations indicates that seven vic genes (vegetative incompatible genes), distributed over five or six loci in the fungus species *C. parasitica*, showed incompatibility in viral transmission (Pearson *et al.*, 2009). The yeast *Saccharomyces cerevisiae* is also the source of many dsRNA and ssRNA viruses (van Regenmortel *et al.*, 2000). Although most studies of mycoviruses have concentrated on its lack of virulence against plant-pathogenic fungi. Of these viruses, the *Saccharomyces cerevisiae* virus L-A (ScV-L-A) is the most consequential. The *Agaricus bisporus* mushroom

business owned by the La France brothers of Pennsylvania, United States, was wiped out by a disease in 1948. Malformed fruiting bodies and a severe drop in output were attributed to a disease dubbed "L France disease," and similar outbreaks were soon detected in Europe, Japan, and Australia. This revelation first sparked interest in the search for mycoviruses among researchers. There were three different viruses identified by Hollings in the spores of the mushroom back in 1962. Myco-virology was launched on the back of Hollings's report (Hollings, 1962). Later many dsRNA of mycoviral origin was recovered from the cultural filtrates of *Penicillium* spp. Hence, there was a motivation for further search for fungal viruses worldwide. The myco-virology, as compared to other virology disciplines was novel. Many mycologists would simply remove fungal colonies that have evident abnormalities since mycoviruses create little or no outward symptoms in their fungal hosts. As a result, mycovirus research lagged behind that of other viruses (Pearson *et al.*, 2009). The double-stranded RNA (dsRNA) viruses have the majority in the mycoviruses category. Recently single-stranded RNA (ssRNA) and spherical-shaped ssDNA genomes viruses have also been discovered (Ghabrial *et al.*, 2015). Some of the mycoviruses are covered in rigid particles, and some do not have typical virus morphology.

Mycoviruses as biocontrol agents: As animal and plant viruses are deleterious to the health of animals and plants; however, mycoviruses do not show symptoms in fungi, and most of them have beneficial effects. However, some of them are deleterious and change the phenotype of fungi and morphology; as a result, those altered shape fungi cannot infect plants because their virulence has decreased. Technically the decrease in virulence is called hypovirulence. Some of the mycoviruses become very severe on their host and increase the virulence called hypervirulence. The first successful biological control was the usage of ssRNA mycovirus *Cryphonectria hypovirus 1* to manage chestnut blight disease caused by *Cryphonectria parasitica* (Nuss, 2005). Later biological control of *Sclerotinia sclerotiorum* was achieved by using ssDNA mycovirus called hypovirulence-associated DNA virus, which has effectively controlled rape rot disease (Wang *et al.*, 2016). Recently few dsRNA mycoviruses have been identified in the anastomosis groups (AG-2 to -13) of *Rhizoctonia solani* (Das *et al.*, 2016). For instance, three viruses infecting *R. solani* have been discovered,

and these are RsRV1 (*Rhizoctonia solani* dsRNA virus 1) (Zheng *et al.*, 2013), RsPV2 (*Rhizoctonia solani* partitivirus 2) (Zheng *et al.*, 2013) and RsRV-HN008 (Zhong *et al.*, 2015). Recently Li *et al.*, (2018) screened 43 strains of *R. solani* from China and found 16 strains contain dsRNAs (Li *et al.*, 2018). These studies on mycoviruses of fungal pathogens were preliminary, and most of them were limited to *in vitro* studies, and few were used in the greenhouse and fields. However, recently, the research on mycoviruses has revealed that these mycoviruses, upon infecting plant pathogens, make that pathogen beneficial to the plants. Zhang *et al.* (2020) used a mycovirus (SsHADV-1) containing a small amount of DNA against *S. sclerotiorum*, which is a very severe plant pathogen of rapeseed in China. The mycovirus upon infecting *S. sclerotiorum* directly targeted its pathogenicity genes, and the strain was called DT-8 (Lyu *et al.*, 2018; Zhang *et al.*, 2020). Consequently, the downregulation of these pathogenicity genes transformed the *S. sclerotiorum* from a severe necrotrophic plant pathogen to a beneficial endophytic fungus. Further, upon spraying the DT8 at the flowering stage, the expression of growth hormones, pathways including the circadian rhythm and defense system, were upregulated (Qu *et al.*, 2020). Similarly, under field conditions, spraying of DT-8 enhances the yield and reduces the severity of stem rot disease of rapeseed (Qu *et al.*, 2020). The other researcher from China used the same strain DT-8 against another severe wheat disease, namely *Fusarium* head blight disease in the field conditions. The strain enhanced wheat yield of about 4-18% in the field and reduced 40-60% *Fusarium* head blight disease (Tian *et al.*, 2020).

Interaction of Mycoviruses with genes of fungal hosts: RNA Genome expression analysis showed that four distinct mycoviruses infected *F. graminearum* transcriptomes in various ways (Jiang *et al.*, 2013). Neither FgV3 nor FgV4 of these mycoviruses seemed to have any discernible effect on host characteristics. Comparatively, other viruses, such as FgV1 and FgV2, resulted in more blatant alterations to host phenotypes. The comprehensive research will also help us better understand how these mycoviruses interact with their fungal hosts. Mycoviruses cause metabolic reprogramming in their hosts and are hence obligatory intracellular parasites. Antiviral reactions rely on them as well. By identifying the critical variables that govern

their interaction, we may learn about their function in the fungal host's life cycle (Zhu *et al.*, 2018; Zoll *et al.*, 2018; Zhang *et al.*, 2020). Currently, scientists apply whole genome techniques, especially in research on mycoviruses of *Cryphonectria parasitica*, *Fusarium graminearum*, and *Sclerotinia sclerotiorum* (Zhang *et al.*, 2020). Infected and uninfected fungal isolates were found to have different gene expression levels. It has been noted that they differ across viruses from different families. The data also demonstrates that these mycoviruses are dependent on a broad variety of host factors, as well as cellular functions and pathways, such as those involved in cellular transport, metabolism, RNA processing, and other RNA signaling. Acylation of the major coat protein of yeast viruses like ScV-L-A is essential for viral assembly, and this acetylation can only occur in the presence of the N-acetyl transferase host protein Mak3p. The process of RNA silencing, or "quelling," in fungus has been studied in *Neurospora crassa*, and there are reports of similar processes in *Candida parasitica* and *Aspergillus nidulans* (Zoll *et al.*, 2018). Due to their RNA composition, the genomes of Mycoviruses hold great promise for protecting their hosts against infection by other Mycoviruses; yet, this potential may be inhibited by a process called quelling. Important for RNA-silencing during CHV1 infection in *C. parasitica* are the dc12 and ag12 genes (Nuss, 2010). *F. graminearum* infected with *Fusarium graminearum* viruses also showed upregulation of genes involved in silence (rdr1, dcl1, dcl2, and agl2) (Nuss, 2011). The p29 protein of mycovirus CHV1 and the S10 gene product of *Rosellinia necatrix* Mycoreovirus are two examples of viruses that have developed novel strategies to inhibit RNA silencing (Pearson *et al.*, 2009).

Reports of Mycoviruses from Pakistan: Until far, there have only been a few of reports of mycoviruses in Pakistan. Cankers, fruit rot, and leaf spots are all caused by the cosmopolitan phytopathogenic fungus *Diplodia seriata*, which belongs to the family *Botryosphaeriaceae*. This fungus is a major problem in Pakistan since it affects many commercially important plant species. A single strain of *Diplodia seriata* in Pakistan was found to be infected with eight distinct mycoviruses, and this strain was characterized in this study. Based on the species delineation criteria supplied by the ICTV or the significant degree of sequence variability among these mycoviruses, DsCV1, DsPV1, DsSpV1, DsBOV1, and DsAV1 seem to constitute unique mycovirus species

(Khan *et al.*, 2022). *Fusarium mangiferae* from Pakistan were subjected to a comprehensive screening survey for the detection of viruses. As a result of testing 396 fungal samples using a traditional dsRNA isolation technique, we observed that 9 percent, or 36 isolates, contained double-stranded (ds) RNA. The plant pathogenic fungus *Fusarium mangiferae* was studied by analyzing the virome of strain SP1, which was one of 36 dsRNA-positive strains. The SP1 strain is co-infected by 11 different viruses, and at least seven of them should be described as new taxa at the species level according to the ICTV (International Committee on the Taxonomy of Viruses) species demarcation criteria, as shown by both next-generation sequencing and the classical Sanger sequencing method used to complete the genome. *F. mangiferae* hosts a total of eight different mitovirids, including three unuamitoviruses (FmMV2, FmMV4, FmMV6), one duamitovirus (FmMV5), and two unidentified mitovirids (FmMV1, FmMV3). Two partitivirids, a betapartitivirus (FmPV1) and a gammapartitivirus (FmPV2), have also been found (FmBOV2) (Khan *et al.*, 2021). *Alternaria alternata* is a phytopathogenic ascomycetous fungus, and a recent research identified a new double-stranded RNA (dsRNA) virus, designated *Alternaria alternata* botybirnavirus 1 (AaBbV1), infecting a Pakistani strain, 4a, of the fungus (Shamsi, 2019). The virus was shown to be a unique member of the genus Botybirnavirus after its genome was sequenced using both next-generation and traditional terminal-end sequencing methods. The virus has two parts (dsRNA1 and dsRNA2), with a combined length of 6127 base pairs. The RNA-dependent RNA polymerase domain of the dsRNA1-encoded protein showed 61% identity to its counterpart in botybirnavirus 1, with lower levels of amino acid similarity with other putative botybirnaviruses and the fungal dsRNA viruses, including members of the families *Totiviridae*, *Chrysoviridae*, and *Megabirnaviridae*. The dsRNA2 encoded protein was shown to have similarities to proteins encoded by botybirnaviruses. The Pakistani researchers recently discovered a victorivirus from *Alternaria alternata*, which they have abbreviated as AalVV1. Molecular and phylogenetic studies distinguished AalVV1 from other victoriviruses that have been described. In place of a stop/restart key mediator, AalVV1 seems to contain a sequence signature necessary for the 1 frame-shifting at the ORF1/2 junction region. Purified virion preparations were analyzed by SDS-

polyacrylamide gel electrophoresis and peptide mass fingerprinting, both of which supported the production of two protein products rather than a CP-RdRp fusion product (Jamal, 2019).

Mycovirus Research: Future Obstacles: Mycoviruses were formerly deemed undesirable due to the damage they caused to the commercial mushroom industry. Later, it was discovered that they were helpful because they could be used as biocontrol agents against fungal diseases in the most economically significant plants (Pearson *et al.*, 2009; Nuss, 2010; 2011; Jiang *et al.*, 2013; Jiang *et al.*, 2015). The artificial introduction of spores of the fungus *C. parasitica* harboring Hypovirus into fungal populations was the first method used to manage illnesses; this was done in the 1980s to combat chestnut blight disease. Orchards in eastern North America and Europe found this strategy utterly fruitless (Pearson *et al.*, 2009; Nuss, 2010; 2011; Jiang *et al.*, 2013; Jiang *et al.*, 2015). The hypovirus characteristics or the vegetative compatibility of the different fungal isolates are responsible for the observed effectiveness differences (Lee *et al.*, 2014). Based on these findings, it seems that a combination of host and viral features, among other aspects, may be necessary to use mycoviruses as biological control agents successfully. Rapeseed stem rot disease has been recently managed using *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1. Dispersed hyphal fragments or viral particles were used in the treatment (Lee *et al.*, 2011; Lefkowitz *et al.*, 2018; Li *et al.*, 2019). The use of hypovirulent strains to manage plant-pathogenic fungi faces several obstacles. Following are some of the difficulties:

Several factors, including impede mycovirus transmission from a hypovirulent strain to a susceptible strain are:

- Vegetative incompatibility
- The possible lack of fitness of the hypovirulent strain
- Most mycoviruses have just a mild effect on the fungi they infect

All of this, points to the possibility that these viruses have evolved to coexist with their host for extended periods. It's possible that the mycovirus and its host may benefit from working together in this way.

A recently observed phenomenon in yeast is the so-called "killer phenomenon" or "killer hypothesis." This is due to the inheritance of a dsRNA virus in the cytoplasm, together with satellites or DNA VLEs (Khan *et al.*, 2021).

These viruses do not cause symptoms in their hosts yet have significant biological effects on the host.

Conclusion, future prospects and current challenges of research on Mycoviruses in Pakistan: It is difficult for researchers to establish the source and impact of mycoviruses and the fungi they infect since they do not yet know how to commence an infection. For several mycoviruses, scientists have created trans-infection techniques and reverse genetic systems. Future reverse genetic methods may aid in deciphering the molecular biology of Mycoviruses, paving the way for their use as stable biological control agents or virus-based expression vectors. Mycoviruses are essential in fungal hosts, and future research will likely disclose crucial answers about this. Additional study of mycoviruses and the fungi that host them will shed light on a subject which is now mainly in the dark. In developed countries, nucleic acid profiles are the generic method to detect mycovirus in fungi. In Pakistan, the identification and detection of mycoviruses have been hampered by the absence of sequence data. Here, viruses are purified and antiserum is generated, but these traditional methods have proved far less successful with mycoviruses. Furthermore, in developed nations, molecular techniques, including protocols and automatic sequencing machines, are available. The researchers of those developed nations can discover greater diversity of mycoviruses in fungi, but unfortunately, in Pakistan, no such facilities are present. PCR-based assays can also provide greater virus specificity through specific primers and the potential to sequence PCR products. Sensitive and specific PCR-based techniques also allow detailed investigation of virus transmission. However, in Pakistan, such techniques are not available. Furthermore, virus-encoded RNAs and proteins can be used to understand virulence, sporulation, pigmentation, and mycotoxin production. Technologies are very important to know the interaction of these phenotypes with the virus at the cellular and molecular levels. Furthermore, RNA silencing and differentially expressed genes of mycovirus and host fungi are important to understand the biological function of mycoviruses against biotic and abiotic stresses.

The development of transformation techniques such as DNA-mediated transformation is also essential and this type of advanced research doesn't seem to be possible in Pakistan in the future. Moreover, labeling of mycoviruses to understand their movement in plants

needs an advanced microscope. A few researchers from the Crop Diseases Research Institute, National Agricultural Research Centre (NARC), Islamabad, Pakistan, and the Atta-ur-Rahman School of Applied Biosciences (ASAB) National University Of Sciences & Technology (NUST), Islamabad, Pakistan are nowadays working on mycoviruses, and the funds for the research are mainly provided by foreign countries.

REFERENCES

- Bocos-Asenjo, I. T., J. Niño-Sánchez, M. Ginésy and J. J. Diez. 2022. New Insights on the Integrated Management of Plant Diseases by RNA Strategies: Mycoviruses and RNA Interference. *International Journal of Molecular Sciences*, 23: 9236.
- Bozarth, R.F. 1972. Mycoviruses: a new dimension in microbiology. *Environmental Health Perspectives*, 2: 23-39.
- Cho, W. K., K. M. Lee, J. Yu, M. Son and K. H. Kim. 2013. Insight into mycoviruses infecting *Fusarium* species. *Advances in Virus Research*, 86: 273-288.
- Das, S., R.E. Falloon, A. Stewart and A.R. Pitman. 2016. Novel mitoviruses in *Rhizoctonia solani* AG-3PT infecting potato. *Fungal Biology*, 120: 338-350.
- Ghabrial, S. A., J. R. Castón, D. Jiang, M. L. Nibert and N. Suzuki. 2015. 50-plus years of fungal viruses. *Virology*, 479: 356-368.
- Hollings, M. 1962. Viruses associated with a die-back disease of cultivated mushroom. *Nature*, 196: 962-965.
- Jamal, A., Y. Sato, S. Shahi, W. Shamsi, H. Kondo and N. Suzuki. 2019. Novel victorivirus from a Pakistani isolate of *Alternaria alternata* lacking a typical translational stop/restart sequence signature. *Viruses*, 11: 577-581.
- Jiang, D., Y. Fu, L. Guoqing and S. A. Ghabrial. 2013. Viruses of the plant pathogenic fungus *Sclerotinia sclerotiorum*. *Advances in Virus Research*, 86: 215-248.
- Jiang, Y., T. Zhang, C. Luo, D. Jiang, G. Li, Q. Li, T. Hsiang and J. Huang. 2015. Prevalence and diversity of mycoviruses infecting the plant pathogen *Ustilaginoidea virens*. *Virus Research*, 195: 47-56.
- Khan, H. A., P. Telengech, H. Kondo, M. F. Bhatti and N. Suzuki. 2022. Mycovirus Hunting Revealed the Presence of Diverse Viruses in a Single Isolate of the Phytopathogenic Fungus *Diplodia seriata* From Pakistan. *Frontier in cellular and infection Microbiology*, 12: 702-710.
- Khan, H. A., W. Shamsi, A. Jamal, M. Javaied, M. Sadiq, T. Fatma, A. Ahmed, 2021. Assessment of mycoviral diversity in Pakistani fungal isolates revealed infection by 11 novel viruses of a single strain of *Fusarium mangiferae* isolate SP1. *The Journal of general virology*, 102: 001690.
- Kotta-Loizou, I. 2021. Mycoviruses and their role in fungal pathogenesis. *Current Opinion in Microbiology*, 63: 10-18.
- Lee, K. M., W. K. Cho, J. Yu, M. Son, H. Choi and K. Min. 2014. A comparison of transcriptional patterns and mycological phenotypes following infection of *Fusarium graminearum* by four mycoviruses. *PLoS One*, 9: 100989.
- Lee, K. M., J. Yu, M. Son, Y. W. Lee and K. H. Kim. 2011. Transmission of *Fusarium boothii* mycovirus via protoplast fusion causes hypovirulence in other phytopathogenic fungi. *PLoS One*, 6: 21629.
- Lefkowitz, E. J., D. M. Dempsey, R. C. Hendrickson, R. J. Orton, S. G. Siddell and D. B. Smith. 2018. Virus taxonomy: the database of the International Committee on Taxonomy of Viruses (ICTV). *Nucleic Acids Research*, 46: 708-717.
- Li, P., P. Bhattacharjee, S. Wang, L. Zhang, I. Ahmed and L. Guo. 2019. Mycoviruses in *Fusarium* species: An update. *Frontiers in Cellular and Infection Microbiology*, 9: 257-264.
- Li, Z., L. I. U. Chen, Z. Meiling, Y. Mei and Z. Erxun. 2018. Diversity of dsRNA viruses infecting rice sheath blight fungus *Rhizoctonia solani* AG-1 IA. *Rice Science*, 25: 57-60.
- Lyu, R., Y. Zhang, Q. Tang, Y. Li, J. Cheng, Y. Fu, T. Chen, D. Jiang and J. Xie. 2018. Two alphapartitiviruses co-infecting a single isolate of the plant pathogenic fungus *Rhizoctonia solani*. *Archives of virology*, 163: 515-520.
- Nuss, D. L. 2005. Hypovirulence: mycoviruses at the fungal-plant interface. *Nature Reviews Microbiology*, 3: 632-642.
- Nuss, D. L. 2010. Mycoviruses. *Cellular and Molecular Biology of Filamentous Fungi*, 145-152.
- Nuss, D.L. 2011. Mycoviruses, RNA silencing, and viral RNA recombination. In *Advances in Virus Research*, 80: 25-48.
- Pearson, M. N., R. E. Beever, B. Boine and K. Arthur. 2009. Mycoviruses of filamentous fungi and their relevance to plant pathology. *Molecular Plant Pathology*, 10: 115-128.

- Qu, Z., H. Zhao, H. Zhang, Q. Wang, Y. Yao, J. Cheng, Y. Lin, J. Xie, Y. Fu and D. Jiang. 2020. Bio-priming with a hypovirulent phytopathogenic fungus enhances the connection and strength of microbial interaction network in rapeseed. *Biofilms and Microbiomes*, 6: 1-13.
- Sahin, E., E. Keskin and I. Akata. 2021. Novel and diverse mycoviruses co-inhabiting the hypogeous ectomycorrhizal fungus *Picoa juniperi*. *Virology*, 552: 10-19.
- Shamsi, W., Y. Sato, A. Jamal, S. Shahi, H. Kondo, N. Suzuki and M.F. Bhatti. 2019. Molecular and biological characterization of a novel botybirnavirus identified from a Pakistani isolate of *Alternaria alternata*. *Virus Research*, 263: 119-128.
- Tian, B., J. Xie, Y. Fu, J. Cheng, B.O. Li, T. Chen, Y. Zhao, Z. Gao, P. Yang, M. J. Barbetti, and B. M. Tyler. 2020. A cosmopolitan fungal pathogen of dicots adopts an endophytic lifestyle on cereal crops and protects them from major fungal diseases. *The ISME Journal*, 14: 3120-3135.
- Tonka, T., L. Walterová and V. Čurn. 2022. Biological control of pathogenic fungi: Can mycoviruses play an important role? *Journal of Central European Agriculture*, 23: 540-551.
- Van Regenmortel, M. H. V., C. M. Fauquet, D. H. L. Bishop, E. B.Carstens, M. K. Estes, S. M. Lemon. 2000. Virus taxonomy: classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses. Academic Press.
- Wang, L., J. Zhang, H. Zhang, D. Qiu and L. Guo. 2016. Two novel relative double-stranded RNA mycoviruses infecting *Fusarium poae* strain SX63. *International Journal of Molecular Sciences*, 17: 641-649.
- Xie, J. and D. Jiang. 2014. New insights into mycoviruses and exploration for the biological control of crop fungal diseases. *Annual Review of Phytopathology*, 52: 45-68.
- Xie, J., D. Wei, D. Jiang, Y. Fu, G. Li and S. Ghabrial, 2006. Characterization of debilitation-associated mycovirus infecting the plant-pathogenic fungus *Sclerotinia sclerotiorum*. *Journal of General Virology*, 87: 241-249.
- Zhang, H., J. Xie, Y.Fu, J. Cheng, Z. Qu and Z. Zhao. 2020. A 2-kb mycovirus converts a pathogenic fungus into a beneficial endophyte for *Brassica* protection and yield enhancement. *Molecular Plant*, 13: 1420-1433.
- Zheng, L., H. Liu, M. Zhang, X. Cao and E. Zhou. 2013. The complete genomic sequence of a novel mycovirus from *Rhizoctonia solani* AG-1 IA strain B275. *Archives of virology*, 158: 1609-1612.
- Zhong, J., C.Y. Chen and B.D. Gao. 2015. Genome sequence of a novel mycovirus of *Rhizoctonia solani*, a plant pathogenic fungus. *Virus Genes*, 51: 167-170.
- Zhu, J. Z., H. J. Zhu, B. D. Gao, Q. Zhou and J. Zhong. 2018. Diverse, novel mycoviruses from the virome of a hypovirulent *Sclerotium rolfsii* strain. *Frontiers in Plant Science*, 9: 1738.
- Zoll, J., P. E. Verweij and W. J. G. Melchers. 2018. Discovery and characterization of novel *Aspergillus fumigatus* mycoviruses. *PloS One*, 13: 0200511.

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