Hungarian University of Agriculture and Life Sciences (MATE)

VIRUS DIAGNOSTICS OF WEEDS ON CROP FIELDS AND INVESTIGATION OF THEIR ROLE AS VIRUS RESERVOIR

Evans Duah Agyemang

Keszthely

2024

DOCTORAL (PhD) DISSERTATION

Evans Duah Agyemang

Keszthely

2024

Name of Doctoral School: Festetics Doctoral School

Discipline: Plant Sciences (Virology)

Head of Doctoral School:

Prof. Dr. Angéla Anda

Hungarian University of Agriculture and Life Sciences

Georgikon Campus

Department of Meteorology and Water Management

Supervisors:

Dr. habil. András Péter Takács

Hungarian University of Agriculture and Life Sciences

Georgikon Campus

Institute of Plant Protection, Keszthely

Dr. Éva Várallyay

Hungarian University of Agriculture and Life Sciences

Institute of Plant Protection, Department of Plant Pathology, Genomics Research Group

Gödöllő.

……	…….	……
Prof. Dr. Angéla Anda	Dr. habil. András Peter Takács	Dr. Éva Várallyay

The dissertation is presented for obtaining a doctoral (Ph.D.) degree in Georgikon campus, Festetics Doctoral School of Environmental Sciences, in the field of Plant science.

TABLE OF CONTENTS

List of Abbreviations

Ldef	Leaf deformations
Chl	Chlorosis
P	Purple colorations
N	Necrosis
TN	Tip necrosis
Stu	Stunting
MM	Mild mosaic
Mo	Mottle
Vc	Vein clearing
US	"Újmajor susnyás"
U	"Újmajor"
BA	"Büdös árok"
M	Millet
ECG	*Echinocloa crus-galli*
SVCD	*Setaria viridis* and *Cynodon dactylon*
SH	*Sorghum halepense*
ECGSV	*Echinocloa crus-galli* and *Setaria viridis*
P. milliaceum	*Panicum milliaceum*
Ma	Maize plants (*Zea mays*)
WSMV	Wheat streak mosaic virus
BYSMV	Barley yellow striate mosaic virus
BVG	Barley virus G
ApGlV1	Aphis glycine virus 1
LDV1	Ljubljana dicistrovirus 1
dsRNAs	double-stranded RNAs
siRNAs	small interfering RNAs

RISC	RNA Induced Silencing Complex
RNAi	RNA Interference
VSRs	Viral-silencing suppressors
ELISA	Enzyme-linked Immunosorbent Assay
DAS-ELISA	Double antibody sandwich Enzyme-linked Immunosorbent Assay.
PCR	Polymerase chain reaction
(RT)-PCR	Reverse transcriptase polymerase chain reaction.
cDNA	complementary DNA
DNA	Deoxyribose nucleic acid
RNA	Ribonucleic acid
ssRNA	Single-stranded ribonucleic acid
dsRNA	Double-stranded ribonucleic acid
sRNAs	Small ribonucleic acids
TNA	Total nucleic acid
dNTP	Deoxynucleotide triphosphate
rpm	Revolutions per minute
BCMNV	Bean common mosaic necrosis virus
BCMV	Bean common mosaic virus
SBMV	Southern bean mosaic virus
CPMMV	Cowpea mild mottle virus
TYLCV	Tomato yellow leaf curl virus
IYSV	Iris yellow spot virus
YDV	Yellow dwarf virus
CYDV	Cereal yellow dwarf virus
BYDV	Barley yellow dwarf virus

ml	Milliliter
µl	Microliter
TNA	Total nucleic acid
bp	Base pair
mins	Minutes
s/sec	Seconds
nt	Nucleotide
TBE	Tris base, boric acid and Ethylenediaminetetraacetic acid.
LIG	Ligation mixture
LB+AMP	Lysogeny Broth + ampicillin
RPM	Reads per million
MIMV	Maize Iranian mosaic virus
RTV	Rice tungro virus
RTBV	Rice tungro bacilliform virus
RTSV	Rice tungro spherical virus
ERSV	Echinochloa ragged stunt virus
RRSV	Rice Ragged stunt virus
STV	Sanya tombus-like virus
GNIV2	Guiyang narna-like virus 2
RSV	Rice stripe virus
WMV	Watermelon mosaic virus
MaYMV	Maize yellow mosaic virus
SCMV	Sugarcane mosaic virus
TPAV	Thin paspalum asymptomatic virus
MDMV	Maize dwarf mosaic virus
BGLV	Bermudagrass latent virus
WDV	Wheat dwarf virus

MSV	Maize streak virus
SpMoV	Spartina mottle virus
CCSV	Cynodon chlorotic streak virus
GFLV	Grapevine fanleaf virus
IJMV	Iranian johnsongrass mosaic virus
JVG	Johnsongrass virus
MCMV	Maize chlorotic mottle virus
ulaRNAs	Umbravirus-like associated RNAs
JgULV	Johnsongrass umbra-like virus
HTS	High-throughput sequencing
sRNA HTS	Small RNA high-throughput sequencing

1. INTRODUCTION

Weeds contribute significantly to yield loss and reduction in yield quality in our agricultural dispensation, competing with the crop for essential resources like light, water, and nutrients. Also, crop plants often grow side by side with weeds and wild plants. These plants constitute potential reservoirs for viruses that may spread into cultivated crops, thereby leading to epidemics or the emergence of novel viruses (Elena et al., 2014). Spread from reservoirs into a new environment, establishing productive infections and effective between-host transmission are necessary steps for virus emergence to occur. Most plant viruses are transmitted by insect vectors, which may increase the possibilities for virus transmission across landscapes and the distances over which viruses can be transmitted (Ng and Falk, 2006).

Climatic variations could contribute to the successful spread of newly introduced viruses or their vectors, and the presence of these organisms in new environs that were previously unfavourable for them. Global climate change is one of the main factors for the increased economic impact of aphids in temperate regions, where these aphids can acclimate to new environmental conditions rather quickly (Hullé et al., 2010, Forsius et al., 2013). Non-native plants and insects may also spread out their geographic ranges and hereby securing communities where they were previously not present. These species might constitute reservoir hosts or vectors of plant viruses capable of causing epidemics in nearby crops (Canto et al., 2009).

Also, plant viruses could spill over in both directions between weed plants and crops with potential adverse effects in both managed and natural ecosystems. Spillover of viruses onto cultivated plants usually occurs at the border of plant ecosystems (Elena, 2011; Stobbe and Roossinck, 2016). In most cases, the virus cannot adapt to further transmission in the organism of the new host, or its titers in plants become insignificant (low intensity of virus reproduction). However, sometimes "introduced" viruses can adapt to further transmission in the new host, leading to new emerging viral infections (Alexander et al., 2014).

Plant virus detection over the years centered on economically important crops, but to effectively manage plant viral infections associated with weeds, research has revealed the need to equally investigate surrounding weeds of crop fields so that strategic management and control practices are devised to manage infestations in crop fields. Plant virus diagnosis is crucial for developing effective and sustainable crop management systems (Biswas et al., 2016), especially considering

that several biotic and abiotic factors also produce virus-like symptoms in plants (Yadav and Khurana, 2016). The use of appropriate integrated control strategies upon virus identification could prove effective in mitigating the spread of the virus, thereby reducing further crop damage and yield loss (López et al., 2009). The methods of detection and identification of viruses developed fall into serological techniques, molecular methods, microscopic and physical observations (Biswas et al., 2016).

Detection techniques such as the enzyme-linked immunosorbent assay and polymerase chain reaction have been commonly used for routine testing (López et al., 2009; Lacroix et al., 2016). High sensitivity, specificity, reliability and cost effectiveness are some of the determinant factors of the success of any method of detection (Lacroix et al., 2016). In terms of plants propagated in a vegetative manner, sensitive diagnosis is necessary for revealing the presence of all of the presenting pathogens in the investigated sample.

High-throughput sequencing (HTS) is a rapidly evolving technique, delivering novel opportunities for diagnosis and epidemiology. This technique enables the sequencing of millions of DNA molecules in a short time, which facilitates the detection of the most viral pathogens in the sample (Elbeaino et al., 2018). Due to the possibility of sequencing millions of nucleotide sequences, it could uncover a global spectrum of occurring strains or species of pathogens. The multiplication of pathogens in plant cells could affect the health condition whether we diagnose or fail to diagnose pathogens (Elbeaino et al., 2018).

1.1 Aim and objectives of the research

Globalization and climate change occurrences keep plant host-virus ecosystems in a continual change. This can induce sensitivity of not only the new, but also the traditionally grown crops for viruses. Virus-crop ecosystem can also be severely affected by endemic and invading weeds. These plants, beside their competitive effect, could also serve as virus reservoirs and can help spread and initiate the persistence of different plant viruses.

The aim of the research is to investigate virus infection in monocotyledonous weeds present at crop fields and to uncover their role in epidemics. Virus diagnostics was carried out not only by using RT-PCR based methods, but also small RNA High throughput sequencing (sRNA HTS), which is able to disclose presenting plant viruses in the investigated sample. The research was achieved undertaking the following objectives:

a) To conduct a survey of monocotyledonous weeds showing virus like symptoms on agricultural experimental fields in Keszthely
b) Investigation of potential plant virus reservoir role played by weeds of the agricultural study location. In a previous project of Pasztor et al. (2020) surveying two millet (*Panicum milliaceum*) populations, wheat streak mosaic virus (WSMV), barley yellow striate mosaic virus (BYSMV) and barley virus G (BVG) were detected in millet samples of the experimental fields. This research further investigates the possible virus reservoir role and virus persistence in millet samples of these fields using sRNA HTS.
c) To validate the outcome or results of the sRNA HTS obtained using independent RT-PCR based method.
d) To conduct phylogenetic analysis of the presenting viral strains, and to assess diversity of virus strains.

2. LITERATURE REVIEW

2.1. Viruses (plant viruses)

Viruses could be defined as an infectious agent often highly host-specific, consisting of genetic material surrounded by a protein coat. For their survival they have to encode at least a replicase for genome proliferation, a coat protein responsible for virion formation and a movement protein to be able to move in the host plant. Viruses have been identified in most plants including vegetables, legumes, cereals, fruit crops, ornamentals and wild plants, constituting approximately one-third of plant disease-causing agents (Makkouk et al., 2014; Suzuki et al., 2015; Malmstrom and Alexander, 2016; Tolin and Fayad, 2016; Mitrofanova et al., 2018; Umer et al., 2019). The viral genome is made of the genetic material either deoxyribose nucleic acid (DNA) or ribonucleic acid (RNA) (Table 2.1). Viruses are not free-living, as they cannot reproduce on their own. Instead, they use host cell machinery to make both the viral genome and capsid of the newly formed viruses. Plant-infecting viruses are of significant concern in agriculture and represent a substantial threat to food security worldwide. Plant viruses are believed to be rated second only to fungal diseases in terms of the economic damage this pathogen could cause (Otim-Napea et al., 2003). Plant virus particles vary in shapes and sizes. They are obligatory intracellular parasites made of a single or multiple DNA or RNA genomic segments enclosed within a protein shell called the capsid. Most plant-infecting viruses are made up of non-enveloped virions apart from members of the families *Rabdoviridae, Firmoviridae* and *Tospoviridae* (Walker et al., 2018). *Potyviridae* has the largest family of RNA plant-infecting viruses and the second-largest plant virus family after *Geminiviridae*, which comprises of some of the most damaging and widespread viruses of agronomic crops. Such losses have had devastating socio-economic consequences for farmers, producers, distributors and consumers (Ivanov et al., 2014; Wylie et al., 2017).

The host plant cuticle and the cell wall provide a solid natural physical protection that has to be broken to create virus entry point to the plant cell of a susceptible host and cause disease. This is generally achieved through mechanical wounds or the action of vectors such as insects and nematodes while feeding on the plants. Following the entry into a host cell, the infectious cycle includes translation and replication of the viral genome, assembly of virus particles, generalized invasion of the host through cell-to-cell and long-distance movements of viral particles or ribonucleoprotein complexes and finally, transmission to new hosts by vectors (Nicaise, 2014).

Virus transmission also occurs through the use of infected plant propagation materials, grafting and contaminated tools.

Table 1. Genomic properties among viruses (Lázár and Bisztray, 2011).

Property	Characteristics
Nucleic acid	Either a DNA or RNA
Shape or Form	Circular, linear or segmented forms
Strandedness	Single- stranded
	Double-stranded
Sense	Positive sense (+)
	Negative sense (-)
	Ambisense (+/-)

2.2. Plant viruses of wild plants

The rapid agricultural advancement, in addition to the benefits of cultural and technological progress, produced imbalances in natural ecosystems. Plant cultivation promoted the corresponding selection in viral populations. Selective pressure, acting simultaneously on both hosts and pathogens, leads to significant evolutionary changes, which happen much faster in agroecosystems compared with natural systems. Breeding of new plant varieties and intensification changes in agricultural practice resulted in the emergence of new pathogens and significantly altered their populations that already existed in the wild ancestors of cultivated plants (Stukenbrock and McDonald, 2008).

Knowledge about the occurrence and diversity of viruses in wild plants can offer insight into factors that promote long-term coexistence between hosts and viruses in nature, as well as the evaluation of the disease emergence risk (Susi et al., 2019). It is also relevant for revealing virus diversity, prevalence and dynamics in wild plant populations so as to better understand virus epidemiology and emergence in crops. Many crop viruses are generalists that can also infect wild or unmanaged plant populations, which we define as non-cultivated plant populations in which changes in population size or genetic composition are not under direct human control (Alexander

et al., 2014). Wild host reservoirs of plant viruses could be passed unto domestic host species aiding transmission and adaptation of plant viruses into the domestic hosts (Figure 1).

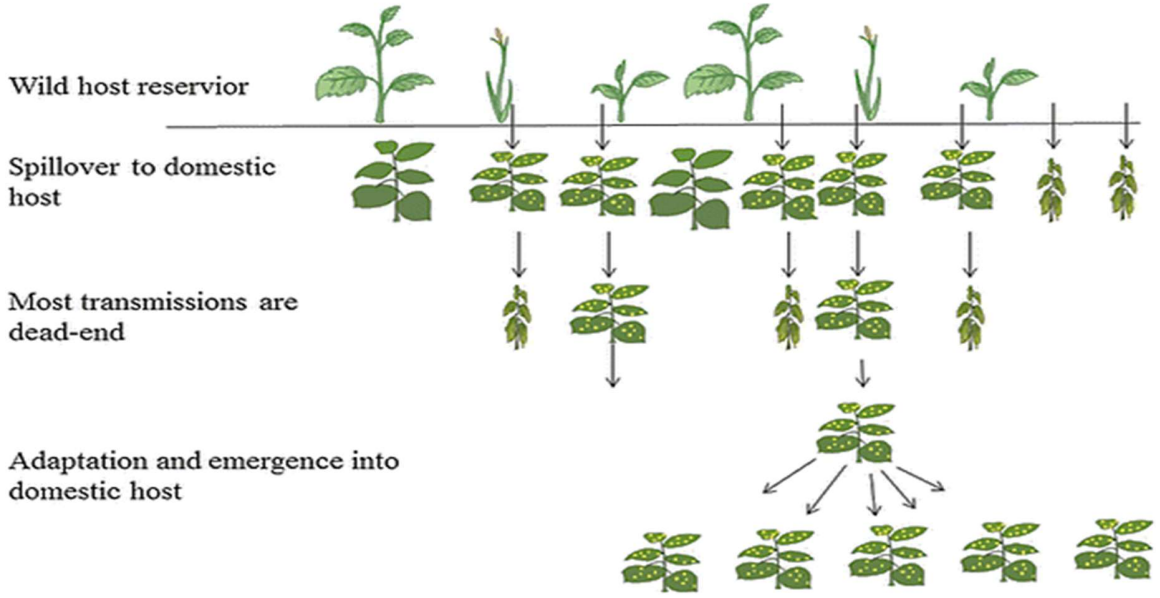

Figure 1. Spillover of plant viruses from wild species to crops (Stobbe and Roossinck, 2016).

2.3. Plant defense mechanism against plant viruses

During plant virus infections, the RNAi-based defense mechanism of the plant is activated. During this mechanism small interfering RNAs (siRNA) having indistinguishable sequences to the infecting viruses are produced, consequently, these siRNAs could be isolated to investigate virus presence (Nicaise, 2014; Pooggin, 2018). When an active plant virus is present in a plant cell, double-stranded RNAs (dsRNAs) are formed. These dsRNAs are cleaved by DICER enzymes into siRNAs. One strand of these siRNAs is incorporated into an RNA Induced Silencing Complex (RISC) which gives specificity and locates all RNAs which have sequence complementarity to the bound siRNAs, and will target the virus, as it was produced from the present plant virus (Pooggin, 2016; Fang and Qi, 2016).

The genome of the specific target RNA (in this case, the virus itself) could be cleaved and its activity blocked in the RISC. If this mechanism works efficiently, low amount of intact virus would be present in the plant, but high amount of virus specific siRNAs would be present. These virus

specific siRNAs can be isolated and used in plant virus diagnosis to investigate if the virus present and active in the plant (Figure 2).

Moreover, it is worth noting that, the plant has the chance of surviving viral infections if the process works efficiently, but that is mostly not the case because different viruses encode different viral-silencing suppressors (VSRs) proteins, which can block the RNAi pathway at different points. For instance, viral particles can bind the siRNAs which then cannot be loaded into the RISC, so the activity is blocked. Also, viruses could inhibit the activity of DICERS or the ARGONAUTE proteins which makes them very active and efficient (Fang and Qi, 2016). This is why co-infections with several viruses in plants result in more severe symptoms. Moreover, another associated reason could be attributed to different viruses encoding different viral-silencing suppressors which can block this mechanism at different steps. If the process is blocked either at one or several points, the effect of the virus infections could be damaging (Cao et al.,2014).

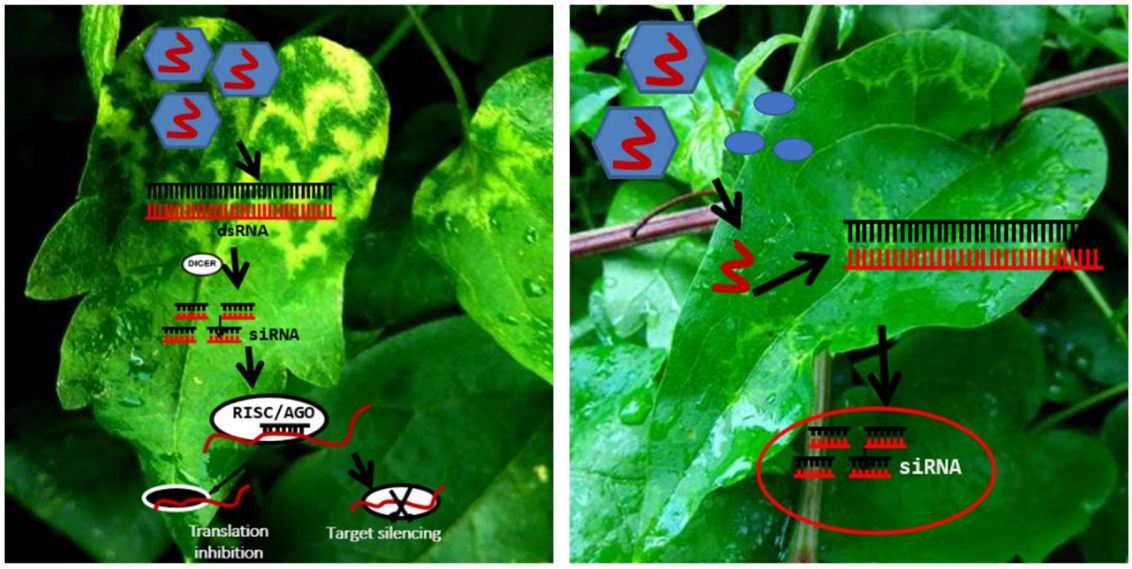

Figure 2. RNA Interference. The siRNAs isolated from the infected plant can be used to diagnose plant virus present (Photo by Pal Salamon).

2.4. Plant infecting viruses of crops

2.4.1. Wheat streak mosaic virus (WSMV)

Wheat streak mosaic virus (WSMV) causes wheat streak mosaic, a disease of cereals and grasses that threatens wheat production worldwide. WSMV infections on leaves begin as light green streaks which progress to form yellow to pale green stripes, forming a mosaic pattern running parallel to the leaf veins as symptoms advance (Sánchez-Sánchez et al., 2001; Price et al., 2010; Workneh et al., 2010). Since its first discovery in 1922 in Nebraska, WSMV has periodically caused severe epidemics across most of the Great Plains of the United States (Stenger and French, 2009). It is a monopartite, positive-sense, single-stranded RNA virus and the type member of the genus *Tritimovirus* in the family *Potyviridae*. WSMV is transmitted by the wheat curl mite (WCM, *Aceria tosichella*) as the only known vector (French and Stenger, 2002). As a result of its windborne dispersal, the mite is widely distributed in cereal fields and grasslands, which boosts the ability of WSMV to spread within cereal-producing regions worldwide. The capability of WCMs to successfully colonize new plants is remarkable. After landing on new plants, WCMs are able to multiply very rapidly (Kiedrowicz et al., 2017a).

The virus is widely distributed in most wheat-growing regions of the world, including the USA, Canada, Mexico, Brazil, Argentina, Europe, Turkey, Iran, Australia and New Zealand (Hadi et al., 2011; Navia et al., 2013). WSMV is hosted by many plant species of the family *Poaceae*, including wheat (*Triticum aestivum*), oat (*Avena sativa*), barley (*Hordeum vulgare*), maize (*Z. mays*), millet (Panicum), Setaria and Echinochloa spp., and several other grasses (Chalupnıkova et al., 2017; Drab et al., 2014). WSMV has a diverse host range, and grasses serve as one of the important natural reservoirs of the virus (Singh and Kundu, 2017; Singh et al., 2018). Also infected volunteer cereals and grasses surviving outside the wheat growing season constitute reservoirs from which WSMV and its eriophyid mite vector spread to wheat crops (Thomas and Hein, 2003). Given the potentially devastating impact of WSMV on affected cereal crops, the occurrence of this disease in wheat has been a cause for concern because losses can range from minimal to complete crop failure (French and Stenger, 2003).

WSMV infection has historically been detected by means of symptoms on leaves. However, symptoms on leaves are not enough for the confirmation of WSMV because other viruses can cause similar symptoms. In terms of serological methods employed for the detection of WSMV,

ELISA types such as the double antibody sandwich-ELISA (DAS-ELISA) and triple antibody sandwich-ELISA (TAS-ELISA) have been the most utilized method for the monitoring of WSMV (Coutts et al., 2011; Schubert et al., 2015). WSMV has also been detected by nucleic acid-based methods, such as RT-PCR or qRT-PCR (Gadiou et al., 2009; Drab et al., 2014; Schubert et al., 2015) and by the use of small RNA HTS in the study of Pasztor et al. (2020).

The management of WSMV involves cultural practices such as removal of volunteer wheat in a field, and grassy weeds in and close to fields should be controlled with tillage or herbicides. An integrated disease management approach that combines as many as possible these disease control strategies and tactics will most effectively reduce losses caused by WSMV, as illustrated in McMechan and Hein (2016), who showed that cultivar resistance and delayed planting improved the yields of three winter wheat cultivars under high WSMV intensity. As WSMV continues to pose global threat to cereal production, research has revealed the need to continue embracing improve techniques which aides deeper diagnosis to assist in making informed decisions on the control and management of WSMV.

2.4.2. Barley yellow striate mosaic virus (BYSMV)

Plant pathogenic rhabdoviruses infect monocot hosts, including weeds and major crops such as rice, maize and wheat. They induce a variable symptomatology ranging from latent infections with no visible symptoms, to stunting, yellowing, mosaic on leaves and chlorosis of systemically infected tissues, that can give rise to necrosis followed by the plant death (Jackson et al., 2005; Almasi et al., 2010). To date, plant rhabdoviruses are classified in four genera: *Nucleorhabdovirus, Cytorhabdovirus, Dichorhavirus and Varicosavirus* (Walker et al., 2018).

Barley yellow striate mosaic virus (BYSMV) is a member of the genus *Cytorhabdovirus*. The plant virus is transmitted by the small brown planthopper, *Laodelphax striatellus*, in a persistent-propagative manner when this vector feed on their hosts. BYSMV was first isolated from planthoppers in Italy (Conti,1969), and was subsequently reported in other European countries, Africa, Australia, Iran and Syria (Izadpanah et al., 1991; Makkouk et al., 2004; Almasi et al., 2010; Di et al., 2014; Yan et al., 2015). BYSMV was identified by electron microscopy and by serological assays with BYSMV antisera from Italy and Morocco (Izadpanah et al., 1991). It causes

considerable losses in cereal crops such as wheat, maize, rice, oats and millet (Izadpanah et al., 1991; Cao et al., 2018).

BYSMV management mostly relies on early field detection, vector control and selection of resistant germplasm. Suitable agronomic practices may be adopted, such as eliminating weed reservoirs for the plant hopper vector and avoiding the coincidence of cereals emergence with the vector spring migration. For an effective management program, it is important to characterize the factors underpinning BYSMV specificity and virulence. Moreover, knowledge of the functional activity that BYSMV genes induce in the defense systems of infected plants may be useful when selecting germplasm with enhanced tolerance or resistance levels (Rabieifaradonbeh et al., 2021).

2.4.3. Barley virus G (BVG)

BVG is a single stranded, positive sense RNA virus, belonging to the genus *Polerovirus*. BVG was categorized in the *Luteoviridae* family but has since been reclassified as belonging to the *Solemoviridae* family according to the 2021 release of the Virus Taxonomy report by the International Committee on Taxonomy of Viruses (ICTV). The name Barley virus G was given by Zhao et al. (2016), who were the first to identify this virus.

The first evidence of BVG was found in Gimje, Korea Republic. Presently where this virus came from, how it was introduced and disseminated throughout time remains unknown (Zhao et al., 2016). Moreover, BVG was also detected in a 34-year-old oat sample from Australia (Nancarrow et al., 2019a), suggesting its possible presence in the oat sample for some time but had gone unnoticed. Thus far, BVG has been found in the following countries: the Netherlands (Kumar et al., 2018); Victoria, Australia (Nancarrow et al., 2019a, b); western Hungary (Pasztor et al., 2020); Kenya (Wamaitha et al., 2018); California in the United States (Erickson and Falk, 2021); Gimje and Uiseong provinces of the Korea Republic (Zhao et al., 2016; Oh et al., 2017; Park et al., 2017; Jo et al., 2018); and Thermi, Greece (Gavrili et al., 2021). BVG, like all *poleroviruses*, is transmitted by aphid vectors in a circulative, non-propagative manner (Latourrette et al., 2021). So far, the only aphid vectors identified for BVG are *Rhopalosiphum maidis* and *Rhopalosiphum padi*, both of which are widely dispersed throughout the world and thus could potentially easily spread this virus to new locations (Erickson and Falk, 2022).

Symptom based identification along with surveys using RT-PCR, high throughput and Sanger sequencing techniques have so far been utilized for investigating and identifying the presence of BVG (Malmstrom and Shu, 2004; Erickson and Falk, 2022). By using sequencing-based surveys, BVG has mainly been found in plant hosts that are members of the *Poaceae* family such as maize (*Z. mays*), barley (*H. vulgare*), foxtail millet (*S. italica*), proso millet (*P. miliaceum*), oats (*A. sativa*), wheat (*T. aestivum*) and switch grass (*P. virgatum*) (Zhao et al., 2016; Oh et al., 2017; Park et al., 2017; Kumar et al., 2018; Jo et al., 2018; Nancarrow et al., 2019 a, b; Pasztor et al., 2020; Erickson and Falk, 2021; Gavrili et al., 2021).

Typical management strategies to curb infestations include application of chemical insecticides to reduce aphid vector populations, planning seeding times that will prevent overlap of the crop growth period with periods of high aphid activity, cultural practices such as removal of volunteer and weedy plant species that may serve as virus reservoirs, and breeding resistant varieties (Walls et al., 2019).

2.4.4. Aphis glycines virus 1 (ApGlV1)

The soybean aphid (*Aphis glycines*), which is widespread in the soybean-growing regions, is the only aphid able to develop large colonies on soybean. Its potential as a vector of plant viruses is recognized. The widespread dispersion of soyabean aphid leads experts to believe that it was in the Midwest for a number of years before to first being identified (Ragsdale et al., 2004). Apart from the harm inflicted by aphid feeding, the soybean aphid has the ability to spread plant viruses that are known to naturally infect soybean (Clark and Perry, 2002). Intense feeding by *A. glycines* causes symptoms of chlorosis, rolled leaves, stunted plants, early maturity and defoliation (Wang et al., 1996; Wu et al.,2004).

Aphis glycines virus 1 is a bicistronic virus of the *Picornaviridae* family, order *Picornavirales*. The virus ApGlV1 has been described with HTS from soyabean and *A. glycines* insect vectors. The in-depth study of tomato and weed viromes which reveals undiscovered plant virus diversity in an agroecosystem led to the identification of ApGlV1 in symptomatic tomatoes sample using HTS in Slovenia (Rivarez et al., 2023).

Current management strategies for *A. glycines* populations depend primarily on the use of chemical insecticides. Breeding of cultivars that encode *A. glycines* resistance genes (the "resistance to *A. glycines*") show promise, but aphid biotypes resistant to those cultivars have been identified. Identification of viruses that infect *A. glycines* would enable investigation of their potential use in management of *A. glycines* and their associated viruses (Michel et al., 2011).

2.4.5. Ljubljana dicistrovirus 1 (LDV1)

The description of Ljubljana dicistrovirus 1 (LDV1) was reported in the study of Rivarez et al. (2023). The virus belongs to the *Dicistroviridae* family, order *Picornavirales* with mainly aphids as the natural hosts.

2.5. Detection of plant viruses

2.5.1. Contribution of serological methods

Serological method refers to the traditional method of detection of plant viruses which is based on the use of antibodies raised in animals that are capable of binding to specific virus antigens. Before the inception of these methods, electron microscopy had contributed a great deal in viral diagnosis. According to Madeley in 1997, electron microscopy was devised in Germany in order to visualize objects too small to be determined clearly by light microscope in the 1930s. Viruses were among the first pathogens to be seen and morphologically characterized within a short time. Application of electron microscope in basic virology and routine viral diagnosis enabled rapid diagnosis of infections which was manageable in terms of cost and safety. Nevertheless, this exceptionally simple method helps to visualize most of the viruses, provided the pathogen is present in at least a required concentration (Biel et al., 2004).

Serological-based methods are of great significance in identifying and classifying viruses except in case of woody plants where there is lack of sensitivity for routine diagnosis. It is a simple method based on the recognition of antigens with antibodies raised against them. With the introduction of immunoenzymatic technique (Enzyme-linked Immunosorbent Assay), which uses antibodies conjugated with an enzyme in order to greatly amplify the signal of the presence of specific amounts of viral antigens, a remarkable improvement in sensitivity was attained (Clark and Adams, 1977). ELISA, a diagnostic technique used for detecting plant viruses, presents a number

of improvements such as sensitivity for identifying very small amount of viruses, specificity for distinguishing serotypes, scale of function, prospects of obtaining quantitative measurements and speed of reaction (Crowther, 2001). This technique can be used for testing multiple plants for a single virus using one well per plant sample, or otherwise a single plant can be concurrently tested for many viruses on a single plate with different antibodies coated to each well in duplicate or triplicate for reproducibility (Webster et al., 2004).

Pursuing the steps towards advancement in sensitivity, some ELISA types, such as double antibody sandwich (DAS)-ELISA, were reported. In this test, virus-specific polyclonal antibodies coated on the wells of a microtiter plate are covered by enzyme attached to a secondary antibody (such as antibody-alkaline phosphatase conjugate). Addition of the substrate induces a colorimetric reaction i.e. from colourless to p-nitrophenol (yellow in alkaline solution) that reveals the presence of bound enzyme, and could be very sensitive (Torrance, 1998). Another type of ELISA is also known as immunoblots or dot blots. Dot blot ELISA tends to be rapid, easy to perform and conservative of reagents and often more sensitive than ELISA carried out in a microtitre plate (Banttari and Goodwin, 1985). Despite its sensitivity, serological methods that rely on the production of virus-specific antibodies cannot be employed for virus and viroids detection of unknown origin.

2.5.2. Nucleic acid-based detection methods

The emerging variants of plant viruses that have broad host range but are often symptomless or produce symptoms showing resemblance with other viruses require techniques for diagnosis that are more sensitive and reproducible and allow the typification of more isolates (Garcia-Arenal et al., 2001). Nucleic acid-based methods founded on amplification or hybridization, are sensitive and specific that allows genetic relationships to be determined. These methods have transformed the way of plant virus detection and identification. Specificity is directly related both to design of primers and analytical procedures.

2.5.2.1. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) has become popular and a fairly expensive technique used for the detection and discovery of pathogenic plant viruses. Detection of viruses in a given sample by PCR does not only rely on the performance of PCR assay but also on the efficiency of the procedure used to extract the nucleic acid from the plant materials, as inhibitors that are present in

the extract of nucleic acids, reduce the sensitivity of detection. It is highly recommended to employ commercially available nucleic acid extraction kits that overcome time consuming and complicated protocols for nucleic acid extraction. Efficiency of PCR is controlled by parameters such as polymerase type, buffer composition, stability, concentration of dNTPs, cycling procedures as well as the characteristics of starting template.

In contrast to traditional methods, PCR offers several advantages, because pathogens do not need to be cultured before their detection, it offers high sensitivity, enabling a single target molecule to be detected in a complex mixture. Technological advances in PCR-based methods enable fast, accurate detection, quantification and characterization of plant pathogens. Different variants of PCR have increased the accuracy of detection and diagnosis, thereby opening more understanding into knowledge of ecology and population dynamics of many pathogens, providing a valuable tool for basic and applied studies in plant pathology.

2.5.2.2. Reverse transcriptase polymerase chain reaction (RT)-PCR

Reverse transcriptase polymerase chain reaction (RT)-PCR is a standard method for the detection of RNA viruses, which involves an initial step of reverse transcription that converts single stranded RNA to cDNA. This procedure is extremely sensitive and requires skill to perform. In the case of RNA viruses, oligonucleotide primers, adjoining part of the genome of the virus, are extended by a thermostable DNA polymerase in a cycle of denaturation, annealing and extension steps that exponentially increase the target DNA. The sensitivity of this technique is its major advantage. An RT-PCR assay of cucumber mosaic virus in banana was able to reliably identify infections even in the absence of visible symptoms (Khan et al., 2011). As such, (RT)-PCR-based detection procedures of cucumber mosaic virus infections appreciably improved monitoring and forecasting of banana mosaic epidemics. The possible setbacks of (RT)-PCR include need for a thermocycler and sequence information for designing primers. As initial knowledge of the nucleotide sequence is an important requirement for designing oligonucleotide primers, it cannot be employed in discovering an unknown virus.

2.5.3. Metagenomics

Metagenomic studies detects the genome not of a single organism but a set of organisms or viruses. The analysis consists of sequencing the total metagenomic DNA or individual genes and

bioinformatics to process huge arrays of data acquired. The metagenomic approach was first applied to examine viral populations in the aquatic environment and in mammals, and it later spread to viral ecology and pathology (Delwart, 2007; Zablocki et al., 2016). The main approaches in metagenomics of plant viruses are based on the analysis of main classes of nucleic acids such as total RNA or DNA, dsRNAs and siRNAs (Roossinck et al., 2015).

Metagenomic studies of viruses permit studying the diversity of viruses found globally, detecting new previously unknown viruses and to delve into the mutualistic relationships between plants, vectors and viruses (Stobbe and Roossinck, 2014). The findings of metagenomic studies are of strategic significance as studies outcomes could assist in preventing the infection of crops with potential pathogens from natural reservoirs and thus, support higher yields and subsequently, food security (MacDiarmid et al., 2013).

Metagenomics has expanded plant viral diagnosis by enabling concurrent diagnosis of multiple viruses regardless of their genomic nature (Jones et al., 2017). Metagenomic studies are now being utilized to analyze the distribution and genetic variability of plant viruses in distinct landscapes, the effects of ecosystem popularization on viral pathogenicity or emergence, and the dynamics of spillover from reservoirs to other hosts. Even though the number of metagenomic studies in weeds and wild plants is increasing, the proportion of novel viruses discovered in these plants remains high, suggesting that there are many viruses yet to be identified and described in wild plant ecosystems (Bernardo et al., 2018).

2.5.3.1. Small RNA HTS

Small RNA high-throughput sequencing (sRNA HTS) together with its bioinformatics analysis of investigated plant samples brings an exceptional avenues to uncover the occurrence of any virus present in the sample, including new ones never described or identified (Kreuze et al.,2009). Total RNAs are isolated from the collected sample, after which small RNA fractions are purified. To prepare the small RNAs and make them sequenceable, adapters are ligated to both RNA ends, thus permitting reverse-transcription and PCR-based library preparation. After sequencing and quality control, sequenced reads are aligned to viral reference genomes by bioinformatics pipelines, which helps to discover the occurrence of viral pathogens in the sample (Figure 3). Plants can be infected with a large number of plant viruses, most of which are ssRNA viruses. Whilst traditional

serological tests and RT-PCR can only uncover the presence of the investigated plant virus, sRNA HTS technique can disclose the presence of all pathogens in a sample.

The small RNA approach works well for detecting viruses in a range of hosts, as one vital immune response against viruses is RNA silencing, where virus-specific dsRNA that is produced in most virus infections activates a plethora of host responses that result in the sequence-specific degradation of RNA (Dunoyer and Voinnet, 2005). The sequencing of sRNAs from the host is an essential activity that drives the accomplishment of virus detection using this technique. Plant virus research encouraged unearthing RNA-based regulation at the beginning of the twenty first century. Besides first discovery of 21-24 nt long sRNAs, the key element of RNA regulation pathways was first identified in virus-infected plants. Furthermore, experiments studying RNAi processes has shown that plant viruses can limit the successful function of antiviral silencing by their viral suppressor proteins encoded in their genome (Várallyay et al., 2012).

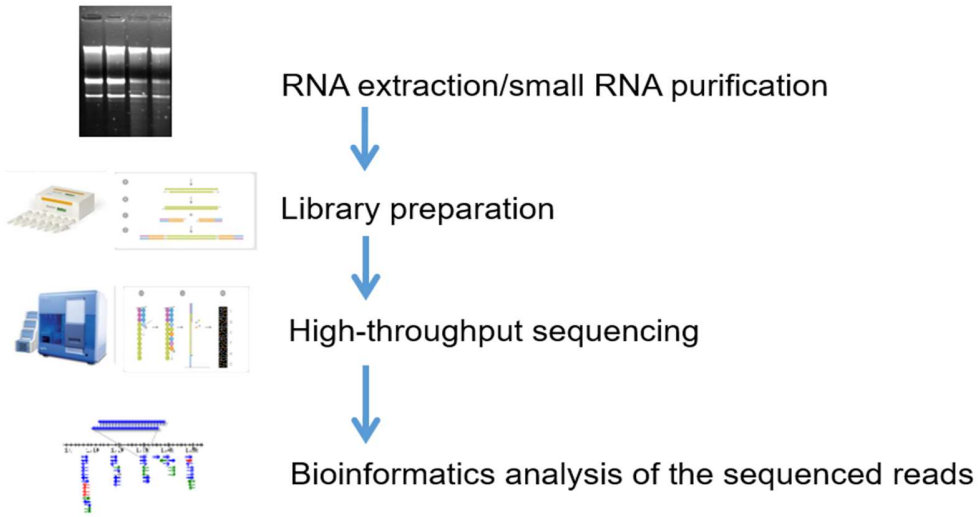

Figure 3. Workflow of small RNA High throughput sequencing (Varallayay Eva)

2.6. The use of HTS in Africa for the detection of plant-infecting viruses

In most of Africa, plant virology is still a growing science. When it comes to adopting HTS technologies, there is limited expenditure in research and equipment, skilled labour, infrastructural problems and other pertinent challenges (Helmy et al., 2016). Successful collaborations among African countries have contributed to the increase in the number of HTS-related studies over the years. Collaborative projects require equal contributions among all who are collecting and

analyzing the data. For plant virus data sets, this usually involves virologists, botanists, sequencing facilitators and bioinformaticians, computer scientists and field scientists who collect data about the study locations. African countries such as South Africa, Kenya, Senegal, Ghana and Nigeria have contributed their quota in studies using HTS and have obtained global recognition for setting up HTS platforms and bioinformatic systems (Mlotshwa et al., 2017; Karikari, 2015).

Studies using HTS to identify plant viruses have been conducted in African countries. At least 29 host plants, including various economically important crops, ornamentals and medicinal plants representing 18 different families have been used in HTS for virus detection and diagnostics (Barba et al., 2014). The studies resulted in the detection of previously known and novel viruses from almost any host, confirming the wide distribution of plant viruses in different ecosystems and suggesting the importance of knowledge on the diversity, prevalence and spatial distribution of viruses. For example, four novel protein-encoding single-stranded DNA viruses were detected in wild and medicinal plant samples from *Poaceae* and *Apiaceae* species collected in the Western Cape region of South Africa (Richet et al., 2019).

In the aspect of crop plants, the prevalence of *Poleroviruses* infecting maize in East Africa was investigated through studies conducted on maize growing areas in Uganda, Kenya, Rwanda and Tanzania (Massawe et al., 2018). HTS of small RNA from common beans in Tanzania and Zambia revealed the presence of bean common mosaic necrosis virus (BCMNV), bean common mosaic virus (BCMV), southern bean mosaic virus (SBMV) and cowpea mild mottle virus (CPMMV). SBMV had never been diagnosed to infest common bean in Zambia. RT-PCR results generated using virus specific primers were consequently performed to validate the HTS findings, confirming the prevalence of SBMV in Zambia (Nordenstedt et al., 2017; Mulenga et al., 2020). Plant virus diseases have been recognized as the second-most significant biotic limitation to sweet potato production. Metagenomic approach was embraced in South Africa to understand the incessant deterioration of yield and quality of sweet potato crops suffered by farmers across the country. Leaf samples collected from different surveys in the major growing regions were subjected to total RNA and small RNAs extraction. The findings indicated the presence of two *badnaviruses*, sweet potato badnavirus A (SPBVA) and sweet potato badnavirus B (SPBVB), which had never been reported alongside other commonly occurring plant viruses (Nhlapo et al., 2018a; Nhlapo et al., 2018b). The continuation of plant viral presence unknown in African could in the long term, have

disturbing consequences for agricultural production, hence the need to support research projects involving these emerging and modern methods in Africa.

2.7. Monocotyledonous weeds and their impact on agricultural crop fields

Monocotyledonous weeds present a severe threat to global food production in terms of its competition with crops and the potential of these weeds as essential inoculums sources of plant viruses (Mehmood et al., 2014; Marwal et al., 2014). Monocot weeds found in crop fields could serve as reservoirs of plant pathogenic viruses. They perform this action by harboring plant viruses and acting as hosts not only for the viruses but also for their potential insect vectors, opening a possibility of viral transfer between them. The plant viral reservoir role of weeds in crop fields could result in virus persistence and infection outbreaks causing significant yield and quality losses in cultivated crops (Elena et al., 2014; Prajapat et al., 2014).

It is evident that, agricultural growth and evolution have potential to instigate significant occurrences and variations in the plant viral spread. This happened for example during the rapid increase or multiple spread of potyviruses such as the PPV in stone fruits that simultaneously happened with the earliest improvement of agricultural crop production mechanisms (Gibbs et al., 2008). Viruses could spread from their natural sources to crops as it was reported for rice yellow mottle virus (RYMV) infecting various rice species both as weeds and in agricultural systems (Fargette et al., 2006) and for iris yellow spot virus (IYSV) found in annual or perennial weeds responsible for initiating significant yield losses in onion (*Allium cepa*) (Hsu et al., 2011). In temperate regions, perennial grasses are reservoirs of viruses that cause serious diseases. The quest to investigate this occurrence revealed that, the wild orchard grass (*Dactylis glomerata*) has shown to act as reservoir of cereal yellow dwarf virus (CYDV) (Pallett et al., 2010).

2.7.1. Plant viral infections of monocotyledonous weeds

2.7.1.1. Viruses that have been found to infect *Echinocloa crus-galli*

Barnyardgrass (*Echinochloa crus-galli*) is a serious weed in major cropping systems, especially on cereal fields such as rice. The wild grass believed to have originated from Tropical Asia possesses great competition prowess with crops on the field (Rodenburg et al., 2011). *E. crus-galli* could harbor and serve as host of plant viruses (Table 2). Maize Iranian mosaic virus (MIMV)

is a *Nucleorhabdovirus* that infects maize, wheat, barley and some perennial weeds in Iran. MIMV has been reported as one of the most common plant viruses affecting maize crops in the maize-growing regions of Iran, responsible for necrotic streaks, chlorosis and leaf stripes on the plant (Izadpanah et al., 1983a; Izadpanah et al., 1983b). The whole genome sequence of MIMV has been reported (Massah et al., 2008; Ghorbani et al., 2017). Moreover, genetic diversity studies of MIMV in Iran have identified three significant isolates of MIMV that were also detected in *E. crus-galli* showing mosaic and chlorotic stripes (Hortamani et al., 2018). Plant rhabdoviruses are not seed transmissible but can spread through vegetative propagation and by planthopper vectors (Hortamani et al., 2018). Perennial plants, like *E. crus-galli,* offer the chance to sustain a persistent infection and effectively transmit viruses (Walia et al., 2014).

Yellow dwarf viruses (YDVs) can affect common wheat, barley, oats, rye, maize, millet, rice and sorghum. The barley yellow dwarf (BYD) disease is initiated by any one of the closely related BYDV (*Luteovirus)* and CYDV (*Poleovirus*) (Du et al., 2007). The most characteristic symptom associated with the disease includes dwarfism and bright yellowish coloration. The disease causes significant reduction in cereal grain production, as a result of its tenacity in yield losses which could be eighty percent or more over time (Pike,1990; Perry et al., 2000; McKirdy et al., 2002). Additionally, the disease is capable of infecting grasses and perennial weed plants, the majority of which belong to the *Poaceae* family (Perry et al., 2000; McKirdy et al., 2002). The identification of YDVs and the rate of infections investigated detected BYDV-PAV as the most virulent strain and identified CYDV-RPV also an important cause of the disease (Ilbagi et al., 2018). The investigation of YDVs and their associated over-summering and wintering *Poaceae* weed host species in the Trakya Region of Turkey revealed that *E. crus-galli* could be an important source of BYDV infections, playing a key role in constituting reservoirs of YDV (Ilbagi et al., 2018).

Tomato yellow leaf curl virus (TYLCV) belongs to the family *Geminiviridae* and genus *Begomovirus*. TYLCV is one of the most important plant viruses that cause devastating losses particularly in tomatoes. Monocotyledonous weed species including *E. crus-galli* was reported to have serve as hosts of TYLCV from leaf and root samples using a TYLCV IRspecific primer set by PCR and amplicon sequencing (Kil et al., 2021).WSMV a member of the genus *Tritimovirus*, and family *Potyviridae* is a serious threat to plants in the *Poaceae* family (Rabenstein et al., 2004). *E. crus-galli* which also belongs to the *Poaceae* family has been confirmed to act as reservoir for WSMV (Chalupniková et al., 2017).

Tungro virus infections cause delayed flowering time and panicle exertion in rice plants. Plants affected by tungro viruses show symptoms of reduced tillering, yellowing and stunting. Rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) are semi persistently spread by the green leafhopper, *Nephotettix virescens*. *E. crus-galli* has been found to host RTBV and RTSV (Rosida et al., 2023). Moreover, researchers have also reported the incidence of a plant virus which was first described in *E. crus-galli*, the Echinochloa ragged stunt virus (ERSV). ERSV was first found in *E. crus-galli* in Taiwan in 1980. Infected plants exhibit severe dwarfing with serrated dark leaves similar to symptoms observed in plants infected with rice ragged stunt virus (RRSV). RRSV is an important rice virus causing a lot of damage in Asia. RRSV was first discovered in Indonesia and the Philippines in the 1970s (Yan et al., 1994; Lacombe et al., 2008).
During the earlier days of plant virus diagnosis of gramineous species in the Korea Republic through the seedling inoculation method, rice black-streaked dwarf fijivirus was reported. The virus causes disease in wheat, maize, barley, some millet and weed species in the field fueled by its viruliferous insect vectors. *E. crus-galli* was identified as one of the new host additions of rice black-streaked dwarf fijivirus at the time (Choi et al., 1989).
In a comprehensive high-throughput survey of the viromes of weeds in rice fields, 224 RNA viruses, and 39 newly identified viruses among them were detected. *E. crus-galli* hosted two of them: sanya tombus-like virus (STV) and Guiyang narna-like virus 2 (GNlV2) putative members of *Tombusviridae* and *Narnaviridae*, respectively. Viruses belonging to *Tombusviridae* and *Narnaviridae* have positive-sense single-stranded RNA genomes (Rochon et al., 2012). The genomes of *Narnaviridae* viruses encode just one polypeptide with an RNA-dependent RNA polymerase and are the simplest representatives of all RNA viruses. Whilst *Tombusviridae* have been reported in plant hosts, *Narnaviridae* are mycoviruses. Members of the *Narnavirus* genus have been identified in the oomycete *Phytophthora infestans* and the yeast *Saccharomyces cerevisiae,* and not in any plant host so far (Hillman and Cai, 2013). It is very likely that GNlV2, identified in this study on *E. crus-galli* originated from a fungus, inhabiting the weed, however, Chao and coworkers could not exclude the possibility that the detected mycoviruses directly infected the weeds (Chao et al., 2022).
Members of the plant *Tenuivirus* genus are associated with significant disease occurrences in their hosts. During the investigation of infections caused by Rice stripe virus (RSV) on weeds and to discover more alternative hosts around rice cultivated fields in South Korea, *E. crus-galli* was one

of the weed species that were found to be infected by RSV (Yoon et al., 2009). In the roots of *E. crus-galli*, tobacco rattle virus (TRV) was identified which became the first report of this weed species as natural host and prospective virus reservoir in the field (Kegler et al., 1989). Also viruses such as watermelon mosaic virus (WMV) (865bp fragment) from Belgium, maize yellow mosaic virus (MaYMV) and sugarcane mosaic virus (SCMV) (complete genome) from China have been identified in *E. crus-galli* host with the Sanger sequenced GenBanks records in these cases (Table 2).

Table 2. List of plant viruses detected in *Echinocloa crus-galli*

virus names	abbrev		method (Sanger/HTS)	Genbank accession number	Geographical origin	Citation
Maize Iranian mosaic nucleorhabdovirus	MIMV		Sanger dideoxy sequencing	MG367447,MG242377, MG242375	Iran	Hortamani et al., 2018
Barley yellow dwarf virus	BYDV		DAS-ELISA, RT-PCR	KJ816653	Turkey	Ilbagi et al., 2018
Cereal yellow dwarf virus	CYDV		DAS-ELISA, RT-PCR	KT923457	Turkey	Ilbagi et al., 2018
Tomato yellow leaf curl virus	TYLCV		PCR	no sequence in GenBank	South-Korea	Kil et al., 2021
Wheat streak mosaic virus	WSMV		TAS-ELISA and RT-PCR	no sequence in GenBank	Czech Republic	Chalupnikova et al., 2017
Rice tungro bacilliform virus	RTBV		RT-PCR	no sequence in GenBank	Indonesia	Rosida et al., 2023
Rice tungro spherical virus	RTSV		vector transmission	no sequence in GenBank		Khan et al., 1991
Echinochloa ragged stunt virus	ERSV		EM, serology	no sequence in GenBank	Taiwan	Yan et al., 1994
Rice black-streaked dwarf fijivirus	SRBSDV		seedling inoculation	no sequence in GenBank	South-Korea	Choi et al., 1989
Sanya tombus-like virus	STlV		Illumina	OM514394,OM514434, OM514426, OM514421	China	Chao et al., 2022
Guiyang narna-like virus 2	GNlV		Illumina	OM514595	China	Chao et al., 2022
Rice stripe virus	RSV		ELISA	no sequence in GenBank	South-Korea	Yoon et al., 2009
Tobacco rattle virus	TRV		ELISA and bioassay	no sequence in GenBank	Germany	Kegler et al., 1989
Watermelon mosaic virus isolate GBVC_WMV_33	WMV		Sanger dideoxy sequencing	KP980661	Belgium	only GenBank
Maize yellow mosaic virus	MaYMV		Sanger dideoxy sequencing/RT-PCR	OP846588, OP846589, OP846590, OP846591	China	only GenBank
Sugarcane mosaic virus	SCMV		Sanger dideoxy sequencing	MN586599	China	only GenBank

2.7.1.2. Plant viral disease incidence in *Setaria viridis*

Setaria viridis is commonly known as “green foxtail”. The weed is a kind of annual grass with its origination attributed to Eurasia. It belongs to the *Poaceae* family and is regarded as an invasive plant that could be found in most regions of the globe. This is due to the fact that *S. viridis* produces a large number of seeds and can grow quickly from the vegetative stage to flowering (Defelice, 2002; Holm et al., 1991b). The weeds are mostly found growing in pastures, fallow fields, crop fields and gardens (Holm et al., 1991b; Yatskievych, 1999). Due to its capacity to sprout in late

spring or early summer, elude early cultivation, and finish its life cycle quickly, green foxtail is highly suited for survival in conventional cropping systems (Holm et al., 1991b).

S. viridis has been reported as an alternate host for a number of insects and plant viruses that attack crops (Douglas et al., 1985; Holm et al., 1991b). Foxtail mosaic virus was detected in the United States in *S. viridis* in 1967 using electron microscopy and serology (Paulsen and Niblett, 1977). Although the virus has been found to affect a broad host range consisting of grasses, the virus had not been associated with any detrimental yield loss (Paulsen and Niblett, 1977). BYDVs have been found to be present in *S. viridis* using double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Remold, 2002). Thin paspalum asymptomatic virus (TPAV) was described from a metagenomic survey of plant viruses in Osage County, Oklahoma, USA, where *S. viridis* was identified as host of this virus producing symptomless infections. TPAV is a panicovirus known to affect plants in the *Poaceae* family (Scheets, 2013).

IYSV is a serious virus pathogen in onion bulb and seed crops (Gent et al., 2006). Besides onion and other susceptible crops, weeds could be performing as potential reservoir sources of IYSV inoculum. A study of IYSV infection of grass species *S. viridis* was conducted using DAS-ELISA. Total nucleic acid extracts from the symptomatic leaf sections were used for reverse transcription-PCR in order to confirm the existence of IYSV in the grass material. Primers specific to the IYSV small (S)-RNA-coded nucleocapsid (N) gene were employed (Evans et al., 2009). The study revealed that, the amplicon from the green foxtail (GenBank Accession No. FJ652594) samples had the highest nucleotide sequence identity (98%) with the equivalent region of an IYSV isolate from Jefferson County, according to nucleotide sequence examination and comparison with this known IYSV small RNA sequences. The study was reported as the first natural infection of *S. viridis* grasses by IYSV. Furthermore, it was also the first account of a *Tospovirus* infecting grass species, indicating a possible occurrence of grasses such as *S. viridis* serving as new reservoir host for IYSV (Evans et al., 2009). Plant viruses of economic concern such as SCMV and MaYMV have also been detected in *S. viridis*. An isolate of SCMV has been described with GenBank information (MN586598). SCMV infection significantly affects crop growth, which results in a marked drop in production and consequently serious economic losses (Viswanathan and Balamuralikrishnan, 2005).

Table 3. Viruses identified in *Setaria viridis*

virus names	abbrev		method (Sanger/HTS)	Genbank accession number	Geographical origin	Citation
Foxtail mosaic virus	FoMV		EM, serology	no sequence in GenBank	USA	Paulsen and Niblett, 1977
Barley yellow dwarf virus	BYDV		DAS-ELISA	no sequence in GenBank	USA	Remold, 2002
Thin paspalum asymtpomatic virus	TPAV		seedling inoculation	no sequence in GenBank	USA	Scheets, 2013
Iris yellow spot virus	IYSV		DAS-ELISA, RT-PCR	FJ652594	USA	Evans et al., 2009
Sugarcane mosaic virus	SCMV		Sanger dideoxy sequencing	MN586598	China	only GenBank
Maize yellow mosaic virus	MaYMV		Sanger dideoxy sequencing/RT-PCR	OP871831, OP871832	China	only GenBank

2.7.1.3. Virus infections in *Cynodon dactylon*

The weed *Cynodon dactylon* is a perennial grass. It belongs to the *Poaceae* family, and among a number of common names attributed to this weed species (Oudhia, 2003), it is popularly referred as the Bermudagrass. Bermudagrass has been categorized as a severe, primary weed with varying degrees of harm in most warm-climate regions in Africa, Asia, America, Australasia and southern Europe. It has been characterized as a major weed in Jordan and Turkey, and as a serious weed in Iran, Israel and Lebanon in the Middle East (Holm et al., 1979; Horowitz, 1996). *C. dactylon* like many grasses, demonstrates great tolerance, strong establishment and high spreading abilities in different environments (Linder et al., 2018).

A newly discovered plant virus from Bermudagrass in the United States, Bermudagrass latent virus (BGLV) is tentatively assigned to the family *Tombusviridae*, genus *Panicovirus* (Tahir et al., 2017). BGLV surveys conducted in Australia, which used a recently developed universal Panicovirus RT-PCR test for detection, revealed prevalent infection by this virus in a wide range of cultivars of Bermudagrass. The sequence amplicons all matched BGLV when subjected to search and BLAST analysis, as well as the identification of eight positive accessions outcome from samples of *C. dactylon* cultivars from New South Wales and Queensland of Australia (Tran et al., 2022) (Table 4). More interestingly about the study, BGLV was also diagnosed in two accessions of sterile hybrid Bermudagrass (with Genbank no. MZ671023 and MZ671027) that was sampled from different study locations in south-east Queensland. The sterile hybrid Bermudagrass was generated and originated from the United States of America (Hanna et al., 1997). Furthermore, the research raises the possibility of an autonomous virus acquisition from local sources subsequent to the grass's first introduction into Australia. The study representing the second recorded

incidence of BGLV after USA extends the gains of identifying more natural weed hosts of BGLV (Tran et al., 2022).

Wheat dwarf virus (WDV) is a *Mastrevirus* which are known to largely infect monocotyledonous plants. Based on its primary hosts, wheat and barley, the WDV disease was typically split into two distinct categories namely the wheat dwarf virus wheat (WDV-W) and wheat dwarf virus barley (WDV-B) strains (Schubert et al., 2007; Wu et al., 2015). In the phylogenetic analysis of wheat dwarf virus isolates from Iran, WDV-W strain sourced from *C. dactylon* weeds (GenBank accession no. KT958243) were utilized (Parizipour et al., 2017), emphasizing the significance of monocotyledonous grasses as natural hosts of especially the wheat strain of WDV. These grasses are mostly found to be present in cereal fields and could have significant impact on the epidemiology of WDV as they act as reservoirs of the virus (Ramsell et al., 2008;Wu et al., 2015).

Maize crops in most African maize growing countries have been periodically decimated by a disease known as maize streak disease (MSD), which is also caused by a *Mastrevirus.* The virus known as the maize streak virus (MSV) is the most recognized type of *Mastrevirus* (Shepherd et al., 2010). The study analyzing the abundance of *Mastreviruses* infecting cultivated and uncultivated plants revealed *C. dactylon* as possible host of isolates of MSV (GenBank accession no; OQ211437,OQ211434). Additionally, other Mastreviruses such as isolate of Eleusine indica–associated virus (EIAV) was also reported as host of *C. dactylon* (Claverie et al., 2023).

Monocot weeds such as *C. dactylon* gradually expands its host abilities as research reveals the presence of other recognized plant viruses in the weed. Genetic diversity studies of WMV and its prevalence in agricultural ecosystem reported WMV infection incidence in *C. dactylon* (Peláez et al., 2021). The presence of spartina mottle virus (SpMoV) in cordgrasses (spartina plants) was described and the first isolate obtained in Wales, England (Jones, 1980). Long after the description of this virus, it was only detected in *Spartina sp.*, as other means of viral transmission such as aphid species and mechanical inoculation to other hosts proved futile (Jones, 1980). The virus has since been isolated and reported in *Spartina* species from northern Germany (Götz et al, 2002; Rose et al., 2020) and from *C. dactylon* in Italy and Iran (Götz et al., 2002; Hosseini and Izadpanah, 2005; Hosseini et al., 2010), from which yielded also the first fragmentary genomic sequences (Götz et al., 2002). Lately, the full genomes have been sequenced (Rose et al., 2020; Thomas et al., 2021). By vegetative propagation, widely distributed weedy species such as *C. dactylon* could transmit SpMoV over large distances (Thomas et al., 2021).

Rice-growing countries have described incidence of rice tungro viruses (RTV) in rice crop fields. The virus has been detected and described in India with keen interest on possible alternative hosts and its insect vectors responsible for transmitting RTV on rice fields. In the research which sort to study the role of weed species and rice stubbles in rice-growing field in the persistence of this virus under natural conditions, it was discovered that RTV was only present on rice stubbles in the off-season. In addition, the viral vector favors rice as its principal food source but also grows well on some special weed species including the *C. dactylon*, when there are no rice crop plants nearby. This way, these weed species execute an essential role in the lifecycle of the vectors of the virus (Rao and John, 1974).

The detection of plant Rhabodoviruses in field studies could enhance the understanding of the epidemiology of these viruses in our crop fields. There are many viruses in the original *Rhabdoviridae* family, many of which are especially of agricultural significance and have the ability to infect a wide range of plants (Jackson et al., 2005). *C. dactylon* was identified as host of MIMV in Iran (Hortamani et al., 2018). The examination of an uncharacterized plant Rhabdovirus infecting Bermuda grass in South Africa revealed that, the identified virus shared close relatedness to Maize mosaic virus (MMV) and Taro vein chlorosis virus (TaVCV), both known plant viruses of the Nucleorhabdoviruses with the highest nucleotide sequence similarities. The virus was named Cynodon rhabdovirus of South Africa with the isolate deposited in the GenBank. (GenBank Accession no.EU650683) (Lamprecht et al., 2009). Moreover, a Rhabdovirus the Cynodon chlorotic streak virus (CCSV) that was found responsible for causing chlorotic streaks and stunting in maize was declared widespread in Bermuda grasses. More importantly, the virus was found present in maize crops growing in the locality of infected Bermuda grasses. Even though CCSV was initially discovered to infect maize, it seems to be more of a virus that affects Bermuda grasses with maize as its alternative host. CCSV resulted in chlorotic streak viral symptoms in Bermuda grasses in Morroco, and was reported to be extensively dispersed in the regions of the Mediterranean (Lockhart et al., 1985). Based on serological studies, Cynodon rhabdovirus was identified to be closely related to CCSV. The researchers even further proposed that, on the basis of their serological, morphological and phylogenic indications, Cynodon rhabodovirus is CCSV or could be a strain of CCSV, however the absence of sequence information for CCSV has hindered the establishment of this fact till date (Lamprecht et al., 2009).

In the study which presented serological and molecular evidence of the presence of GFLV in collected Bermuda grass samples, the discovery of GFLV in Bermuda grass by RT-PCR through the use of two GFLV-specific primer pairs confirmed the presence of a GFLV-like entity in Bermuda grasses of Iran. Near vineyards that had previously experienced GFLV infection, that is where the majority of these infected Bermuda grasses were sampled, indicating a possible viral transfer to these grasses. With the infection of sampled Bermuda grass with a grapevine isolate of GFLV, the study reported Bermuda grass as host for this virus and could be a source for the propagation of GFLV and other several plant viruses. The study further proposed that, since the discovery of a natural GFLV infection in Bermuda grass adds a new perspective to the epidemiology of the virus, managing fanleaf disease should take into account eliminating potential weedy reservoir sources of the virus. This is because weed strains of GFLV may exist close to vineyards (Izadpanah et al., 2003).

Table 4. Plant viruses reported as weed hosts of *Cynodon dactylon*

virus names	abbrev		method (Sanger/HTS)	Genbank accession number	Geographical origin	Citation
Bermuda grass latent virus	BGLV		Sanger dideoxy sequencing/Illumina/RT-PCR	MZ671022, MZ671024, MZ671025, MZ671026, MZ671028, OK258314, OK258317, OK258318	Australia	Tran et al., 2022
Wheat dwarf virus	WDV		Sanger dideoxy sequencing	KT958243	Iran	Parizipour et al., 2017
Maize streak virus	MSV		Sanger dideoxy sequencing	OQ211437, OQ211434	France	Claverie et al., 2023
Eleusine indica associated virus	EIAV		Sanger dideoxy sequencing	OQ211417	France	Claverie et al., 2023
Watermelon mosaic virus	WMV		Sanger dideoxy sequencing	MN814406	Spain	Peláez et al., 2021
Spartina mottle virus	SpMV		Immunoelectron microscopy and RT-PRC	AF491352	Italy	Götz et al., 2002
Spartina mottle virus	SpMV		Illumina	MW314143, MW314142	USA	Thomas et al., 2021
Rice tungro virus	RTV		Inoculation infected material	no sequence in GenBank	India	Rao and John, 1974
Maize Iranian mosaic virus	MIMV		Sanger dideoxy sequencing	MG242374	Iran	Hortamani et al., 2018
Cynodon rhabdovirus	CRV		Sanger dideoxy sequencing	EU650683	South Africa	Lamprecht, 2009
Cynodon chlorotic streak virus	CCSV		ELISA	no sequence in GenBank	Morocco	Lockhart et al., 1985
Grapevine fanleaf virus	GFLV		ELISA and RT-PCR	no sequence in GenBank	Iran	Izadpanah et al., 2003

2.7.1.4. Virus infection occurrences of *Sorghum halepense*

Johnsongrass (*Sorghum halepense*) is indigenous to the Mediterranean region of Europe and Africa, and it is extensively present in North America and southwestern Asia (Tutin et al., 1980; Warwick and Black, 1983). Due to its international introduction, the weed has expanded its boarders to be significant in warm temperate climates (Holm et al., 1991a). It is also a warm-season perennial grass that is among the most invasive and problematic weeds in the southern United States. Due to its detrimental impact on agricultural crop production, the weed is regarded as one of the most harmful weeds in the United States (Mueller et al., 1993). *S. halepense* belongs to the family *Poaceae*. One of the important plant families made up of common crops and weeds that serve as potential reservoir of inoculum is the *Poaceae* family. The grass family *Poaceae* is the plant family to which corn, sugarcane, sorghum and wheat also belong.

Maize dwarf mosaic virus (MDMV) could propagate mechanically through contaminated seeds or by feeding of insect vectors such as aphids. Aphids carry MDMV, which is spread by non-persistent means and possesses a positive-stranded RNA genome (Achon et al., 2012). Whilst maize is considered to be the main host of MDMV, the host range of the plant virus includes

Johnsongrass, which is a significant overwintering virus reservoir of MDMV (Ford and Tosic, 1972; Tosic et al., 1990). Examining the genetic diversity and population structure of MDMV from maize and Johnsongrass in eight distinct Spanish maize-growing regions revealed that, except for Andalucia these regions' high *S. halepense* prevalence confers a great potential for genetic variation due to the fact that MDMV infects this host for extended periods of time, occupying largely with the crop plants (Achon et al, 2011). The propensity of Johnsongrass as a common weedy grass to serve as a reservoir for this virus exacerbates possible viral transmission through infected weeds on crop fields (Gatton, 2015). As a result, it is critical to look out for Johnsongrass growing close to cornfields, especially if there are any telltale signs of MDMV infections, such as yellow or chlorotic leaf streaks (Byron et al., 2019). MDMV could be used in simultaneous gene expression and multi-gene silencing in crops, for example as demonstrated in maize by Xie et al. in 2021. Also in this study, RT-PCR was used to confirm that Ohio-collected Johnsongrass (*S. halepense*) was infected with MDMV. The cloned Johnsongrass-derived MDMV isolate, which was designated as MDMV OH-5, was fully sequenced and deposited as GenBank accession number MN615724.

Iranian johnsongrass mosaic virus (IJMV) is a pervasive plant virus of the *Potyviridae* family causing maize mosaic disease in Iran (Masumi et al., 2011). IJMV is clustered and subgrouped within the *Potyvirus* with plant viruses such as MDMV, Johnsongrass mosaic virus (JGMV), SCMV and Sorghum mosaic virus (SrMV) (Shukla et al., 1992; Berger et al., 2005; Masumi et al., 2011; Adams et al., 2012). The presence of these viruses have resulted in substantial yield losses in sugarcane, sorghum, maize, and other monocotyledonous plants (Chen et al., 2002). The Iranian case study provides interesting turns about host diversity of IJMV. Although in ecological context, Johnsongrass is considered a key natural host with recorded incidence of IJMV isolates in the GenBank(coat protein nucleotide sequences of accession no. KU746860 and KU746862), genome organization and phylogenetical studies has also revealed that IJMV potentially infects sugarcane and sorghum revealing its other plant hosts, and threats associated with IJMV occurrences in sugarcane cultivation and industry of Iran. The principal source of primary infection of maize, sorghum and sugarcane by IJMV could be attributed to Johnsongrass as a perennial grass host (Moradi et al., 2017).

Plant viruses of verified and unverified statuses have been identified in *S. halepense* and recorded in the GenBank. Examples of such viruses include a mastrevirus, WDV isolate identified in *S.*

halepense as host (Parizipour et al., 2017) and Sorghum arundinaceum associated virus(SAaV) from Ecuador. There are isolates of papaya leaf curl virus (PaLCuV) and tomato leaf curl Palampur virus (ToLCPalV) both of Indian origin, isolates of johnsongrass viruses(JVG) from Turkey and johnson chlorotic stripe mosaic virus (JCSMV) of Iran. In the study where plant samples from multiple farmed fodder and weed hosts were tested for the presence of maize stripe tenuivirus (MSpV), wheat and *S. halepense* showed positive ELISA reactions. The disease manifests as causing short panicles and chlorotic stripe, which is also a common occurrence in infected sorghum plants (Narayana and Muniyappa, 1995).

The maize chlorotic mottle virus (MCMV) was firstly identified in Peru and then spread to other countries such as central USA and China. It has also been detected in eastern Sub-Saharan Africa. On the condition pertaining to age of the plant upon infection, symptoms might range from a slight chlorotic mottling to yellowing, necrosis and plant death (Nault et al., 1979; Xie et al., 2011; Wangai et al., 2012). The genome of MCMV is a single-stranded positive sense RNA, and beetles and thrips are the primary vectors of viral transmission (Nault et al., 1978; Zhao et al., 2014). Sugarcane, maize and sorghum could all be infected by MCMV (Wang et al., 2014; Huang et al., 2016). A co-infection activity between MCMV and SCMV of the *Potyviridae* family results in lethal necrotic disease (LND) which is considered a synergistic or collaborative disease occurrence. Synergistic disease occurs when there is simultaneous infection of MCMV and another virus, most regularly a potyvirus either MDMV or SCMV (Niblett and Claflin, 1978). Kusia et al. in 2015 reported a similar occurrence, and to ascertain alternate hosts of MCMV and SCMV revealed that, isolates of MCMV and SCMV was also detected in samples of finger millet in the study regions of Kenya. Moreover, a number of plant species of monocot crops and other weedy grasses have been tested vulnerable to MCMV. To ascertain the presence of MCMV in Spain, weed grasses including *S. halepense* showing exclusive characteristic symptoms were sampled and investigated. Sequence amplified product and amplicon from *S. halepense* in the study shared highest identities with American isolates and African isolates respectively, indicating the first reportage of remarkable detection of MCMV in a perennial host *S. halepense* (Achon et al., 2017).

The non-coat protein Umbraviruses have been reported as infecting viruses which are mostly reliant on multiple simultaneous infections for plant-to-plant vector-borne transmission. The name represents the manner in which Umbraviruses rely on helper viruses to survive in the natural world (Ryabov and Taliansky, 2020). A complete genome sequencing and characterization led to the

discovery of two umbravirus-like associated RNAs (ulaRNAs) viral entities in maize (*Zea mays*) and *S. halepense* from Ecuador. For coherent expediency, the new ulaRNA from maize was referred to as maize umbra-like virus (MULV) and the one from johnsongrass was known as johnsongrass umbra-like virus (JgULV) (Quito-Avila et al., 2022).

Table 5. Plant viruses with reported occurrences of infections in *Sorghum halepense*

virus names	abbrev		method (Sanger/HTS)	Genbank accession number	Geographical origin	Citation
Maize dwarf mosaic virus	MDMV		PCR	FM883224, FM883214, FM883193, FM883174	Hungary	only GenBank
Maize dwarf mosaic virus	MDMV		Sanger dideoxy sequencing	MN615724	USA	Xie et al., 2021
Iranian johnsongrass mosaic virus	IJMV		Sanger dideoxy sequencing	KU746862, KU746860	Iran	Moradi et al., 2017
Sugarcane mosaic virus	SCMV		Sanger dideoxy sequencing	KX430773, KX430774	Iran	Moradi et al., 2017
Wheat dwarf virus	WDV		Sanger dideoxy sequencing	KT958235	Iran	Parizipour et al., 2017
Sorghum arundinaceum associated virus	SAaV		Illumina	PP461403	Ecuador	only GenBank
Papaya leaf curl virus	PaLCuV		Sanger dideoxy sequencing	MZ041266	India	only GenBank
Tomato leaf curl Palampur virus	ToLCPalV		Sanger dideoxy sequencing	MZ041256	India	only GenBank
Johnsongrass virus	JVG		Illumina	MW756211, MW756210	Turkey	only GenBank
Johnsongrass chlorotic stripe mosaic virus	JCSMV		Illumina	MT682309	Iran	only GenBank
Maize stripe tenuivirus	MSpV		ELISA	no sequence in GenBank	India	Narayana and Muniyappa, 1995
Maize chlorotic mottle virus	MCMV		Sanger dideoxy sequencing	KX824059, KX824060	Spain	Achon et al., 2017
Johnsongrass umbra-like virus 1	JgULV		Illumina	OM937760	Ecuador	Quito-Avila et al., 2022

2.7.2. Viruses of millet species and panicum grass hosts

Millets are a group of annual cereal grasses with minute grains that possess a number of unique botanical species. The foxtail (*Setaria italica*), proso (*Panicum miliaceum*), finger (*Eleusine coracana*) and pearl (*Pennisetum glancum*) millets are some of the most significant varieties. These different millet types have their distinct characteristics in terms of growing seasons, grain consistency, soil needs and sizes. Dryness and high temperatures could be tolerated by millets (Brown, 1999; Gomez and Gupta, 2003). Millets are members of the *Panicoideae* subfamily and *Paniceae* clan, which also includes all other millets (Morrison and Wrigley, 2004). Millets are widely consumed as traditional staple foods and are also used to make traditional beverages. Millets are cultivated in warm, tropical regions of the world including Africa, in Asia, Eastern and

Southern Europe. Millets are widely grown for fodder in nations like the United States, Australia, Brazil and South Africa. Their production is also expanding for usage in specialized foods. Additionally, pearl millet specifically is being produced in greater quantities for the production of chicken feed (Taylor et al., 2017).

However in recent times, it has been reported that, crop production increase and growth could compel millet to become precarious weed on crop fields. With the capacity to host plant viruses and serve as a reservoir, the plant could easily be dispersed and found in fields of wheat, maize and other crops (Pásztor et al., 2017; Pásztor et al., 2020). In the study of natural viral infections of weedy *P. miliaceum*, DAS-ELISA serological technique was used to investigate possible wheat viruses which could infect common millet in Hungary. The study led to the identification of important wheat and cereal viruses such as WSMV, WDV, barley stripe mosaic virus (BSMV) and BYDV in their significant infection rates. The study further revealed some multiple infection occurrences among some of these identified viruses. The outcome of the study revealed that, the spread of different cereal virus species can be significantly influenced by millet as a weed (Pásztor et al., 2017).

MaYMV is a possible novel *Polerovirus* which has been discovered from maize (*Zea mays*) crops in China and Brazil (Chen et al., 2016; Gonçalves et al., 2017). MaYMV has also been reported to infect grasses and sugarcane crops, with viral infection incidences of MaYMV reported from Africa (Yahaya et al., 2017). The first report of MaYMV in South Korea revealed an isolate of MaYMV infecting *P. miliaceum* and recorded in the GenBank (Lim et al., 2018). Proso millet and foxtail millet plants that showed yellow stripe symptoms in Korea were sampled from areas where there had been reports of RSV outbreak in rice. The diseased plants tested positive for RSV using an ELISA test, and the presence of the viral infection was validated with RT-PCR (Yoon et al., 2012). Plant viral infections in foxtail millet is a major concern in the growing region of China. Samples of foxtail millet variety exhibiting virus associated symptoms were diagnosed, where nucleotide BLAST search showed that sequences of the sampled foxtail millet revealed a significant close identity with BYSMV P gene from a wheat isolate (Shen et al., 2020). BYSMV is a member of the *Cytorhabdovirus* genus. It has been discovered infecting wheat and rice in China, but its first description has been linked to Italy (Almasi et al., 2010; Yan et al., 2015). The study further projected that, the detection of BYSMV on foxtail millet is crucial for developing a plan that will effectively monitor, prevent and manage BYSMV (Shen et al., 2020). BVG

possesses single stranded, positive sense RNA genome. The virus is associated with the *Polerovirus* genus in the *Solemoviridae* family. BVG has been detected in foxtail millet (*S. italica*) and proso millet (*P. miliaceum*) in Korea Republic (Park et al., 2017a; Park et al., 2017b), *P. miliaceum* samples in Hungary (Pásztor et al., 2020) and switchgrass (*Panicum virgatum*) from the Netherlands (Kumar et al., 2018). In addition, a novel *Polerovirus*, Panicum distortion mosaic virus has been investigated and identified in *P. milliaceum* in South Korea with reported GenBank record (Table 6).

Table 6. Some important cereal viruses detected in different species of millet and panicum grass hosts.

virus names	abbrev	Plant species	method (Sanger/HTS)	Genbank accession number	Geographical origin	Citation
Wheat streak mosaic virus	WSMV	*Panicum milaceum*	Small RNA HTS, Sanger sequencing	MT260879, MT780552, MT780553	Hungary	Pásztor et al., 2020
Barley stripe mosaic virus	BSMV	*Panicum milaceum*	DAS-ELISA	no sequence in GeneBank	Hungary	Pásztor et al., 2017
Barley yellow dwarf virus	BYDV	*Panicum milaceum*	DAS-ELISA	no sequence in GeneBank	Hungary	Pásztor et al., 2017
Maize yellow mosaic virus	MaYMV	*Panicum milaceum*	Sanger sequencing	MF622081	South Korea	Lim et al., 2018
Barley yellow striate mosaic virus	BYSMV	*Setaria italica*	High throughput sequencing, RT-PCR	MN434075, MN434076, MN434077	China	Shen et al., 2020
Barley yellow striate mosaic virus	BYSMV	*Panicum milaceum*	Small RNA HTS	MT260881, MT260882, MT260883, MT260884	Hungary	Pásztor et al., 2020
Barley virus G	BVG	*Panicum milaceum*	Small RNA HTS	MT260885	Hungary	Pásztor et al., 2020
Panicum distortion mosaic virus	PDMV	*Panicum milaceum*	PCR	LC424839	South Korea	only GenBank

2.8. Ways of viral transfer between the crop and the weed – role of the vectors

The role of insect vectors contributes a great deal to plant virus reservoir role of weeds. This is because in the case of most plant viruses, viral transmission is through insect vectors that carry viral pathogens across geographical distances as well as climatic variations in the ecological system (Ng and Falk, 2006). Crop production output is threatened by weeds reducing available nutrients to the crop plants and acting as reservoirs of vector-borne viral diseases (Vafaei and Mahmoodi, 2015). Weeds therefore enable the build-up of vector populations during the offseason, followed by a successive annexation into nearby crops and transmission of plant viruses as weeds function as oviposition host of vectors (Macharia et al., 2016).

The common insect vectors associated with plant viral infection and transmission includes thrips, aphids and whiteflies. Thrips are polyphagous insect vectors accountable for the transmission of *Tospoviruses* and other significant plant viruses of agricultural concern (Montero-Astúa et al., 2014). Thrips obtain and disperse the virus in a persistent, propagative manner, and the western flower thrip (*Frankliniella occidentalis*) is the most effective vector (Seepiban et al., 2015;

Rotenberg et al., 2015). Aphids are direct parasites to crops and as vectors of viral diseases (Warren and Schalau, 2014). The green peach aphid (*Myzus persicae*) has been the most destructive aphid species given its role in plant viral pathogen transmission in an all-pervasive manner (Przewodowska et al., 2015). Whiteflies are among the noxious agricultural insect vectors and are mostly typically found on the underside of leaves (Tosh and Brogan, 2015). The sweet potato whitefly (*Bemisia tabaci*) has the tendency to rapidly propagate, enhanced vector competency and insecticide resistance (Chen et al., 2015). It has been recounted that some vectors can spread viruses more efficiently from infected weeds to nearby crops than crop to crop transmission (Srinivasan et al., 2013). Studies have shown that some insect vectors have a strong affinity for weed hosts, improving vector prolificacy and prolonged existence (Srinivasan et al., 2013). Non-native plants and insects may also broaden their terrestrial avenues or habitats and settle in areas where they were previously absent. These species may serve as reservoir hosts or vectors, with the potential to spread epidemics among adjacent crops (Canto and Aranda, 2009).

2.9. The current call for immediate control practices against plant virus reservoir role of weeds on crop fields

The investigation of plant virus reservoir role of weeds is necessary to safeguard cultivated crops and improve food security. Indeed, gaining insight into the epidemiology and evolution of plant viruses requires awareness of their ecology by acquiring information about the changing aspects and genetic structure of viral populations in their various hosts. These activities are key in analyzing host specialization, identifying reservoirs and inoculums (Sacristán et al., 2004).

To effectively manage the occurrence, some of the key activities that can be conducted includes effective weed management procedures and the early detection of viruses in plant material, vectors or in natural reservoirs (Jeong et al., 2014; Clements et al., 2014). An efficient diagnostic process will enhance plant virus detection and reveal reservoir role weeds play on our crop fields during various stages of plant development. This would help to prevent continuous spread of viral infections and damage to crop plants from such viruses. Therefore, improved weed management systems and assessment methods are essential to evaluate the significance of weeds as virus reservoirs (Szabó et al., 2020).

Adoption of an integrated approach in weed management is vital in reducing the reservoir role weeds have in the transmission of plant viruses. Modern day weed management strategies should

effectively combine cultural, biological, physical, mechanical and chemical methods to control weeds on crop fields. Frequent scouting on crop fields for weed growth infestations and vector populations by farmers and producers would help identify and inform them of the immediate control mechanisms to adopt in tackling possible incidence of weeds serving as plant virus reservoirs on crop fields (Jones, 2000).

Integrated weed management strategies are necessary to design effective control plan in dealing with weeds on crop fields. The over-reliance of only one control method may come with its own challenges and may not prove to control weeds which may serve as reservoirs. In developing effective weed management strategies, it is important to examine the whole growing area, taking into consideration cropped and non-cropped areas, to enhance biodiversity and maximize natural regulatory mechanisms by means of on-farm husbandry practices and habitat management of landscape structures (Clements et al., 2014). Non-chemical weed control practices such as weed seeds removal, conservational tillage, use of certified or disease free planting materials are necessary in reducing the establishment of weeds on crop fields and also augment other weed management practices to achieve better results (Wei et al., 2010; Hossain et al., 2015; Lucas et al., 2017). To reduce weed seed bank in the soil, weeds must not be allowed to flower and shed viable seeds. Weed species that germinate early, usually just after establishment of the crop, produce the greatest number of seeds therefore preventing the establishment of such weed is ideal (Sweet et al., 2004).

The additional use of post-emergence herbicide may be required to control grass weeds and volunteer cereals. Alternatively, residual, pre-emergence herbicides may be used for broad-spectrum weed control (Copping and Naylor, 2002). Grasses and volunteer cereals significantly impede crop growth and development resulting in associated loss of quality of the crop through reservoir activities and contamination of the harvest by weed seeds (Rathke et al., 2005). It is important to reiterate that, the trend of incidence of over-reliance on herbicides is not the way to go as it may continue to increase the development of herbicide-resistant weeds on crop fields (Shaner, 2014). Management strategies that ensure integrated approaches to weed management would prove effective in limiting the role of weeds as reservoirs and ensure robust weed control program.

3. MATERIAL AND METHODS

3.1. Sample collection

Sample collection was carried out in August 2021 in Keszthely as the experimental location. Keszthely is a Hungarian city located on the Western shore of Lake Balaton. The city is known to be one of the important hub for Agriculture, tourism, culture and educational activities.

Symptomatic weed plants showing virus-specific symptoms were sampled at three different fields, US, U and BA to conduct deeper studies on the possible virus infections and their persistence (Figure 4).

Figure 4. Photos (Agyemang) and a map (Google) about the sample collection areas of Keszthely, Hungary **US**: "Újmajor susnyás" **U**: "Újmajor" and **BA**: Büdös árok.

In 2019, 2020, 2021 wheat, corn and wheat were grown on the US field, whilst potato, corn and corn were cultivated on the BA field respectively. No intense cultivation had taken place at the time of sampling at the experimental location of U. At US, where wheat was just harvested, *P. milliaceum*, *E. crus-galli*, *S. viridis* and *C. dactylon* were randomly sampled. At U, *S. halepense* weed plants were sampled and at BA, *P. milliaceum*, *E. crus-galli*, *S. viridis* and maize were randomly sampled (Table 7). Samples collected showed degree of viral symptoms and *P. milliaceum* was grown as a weed (Figure 5).

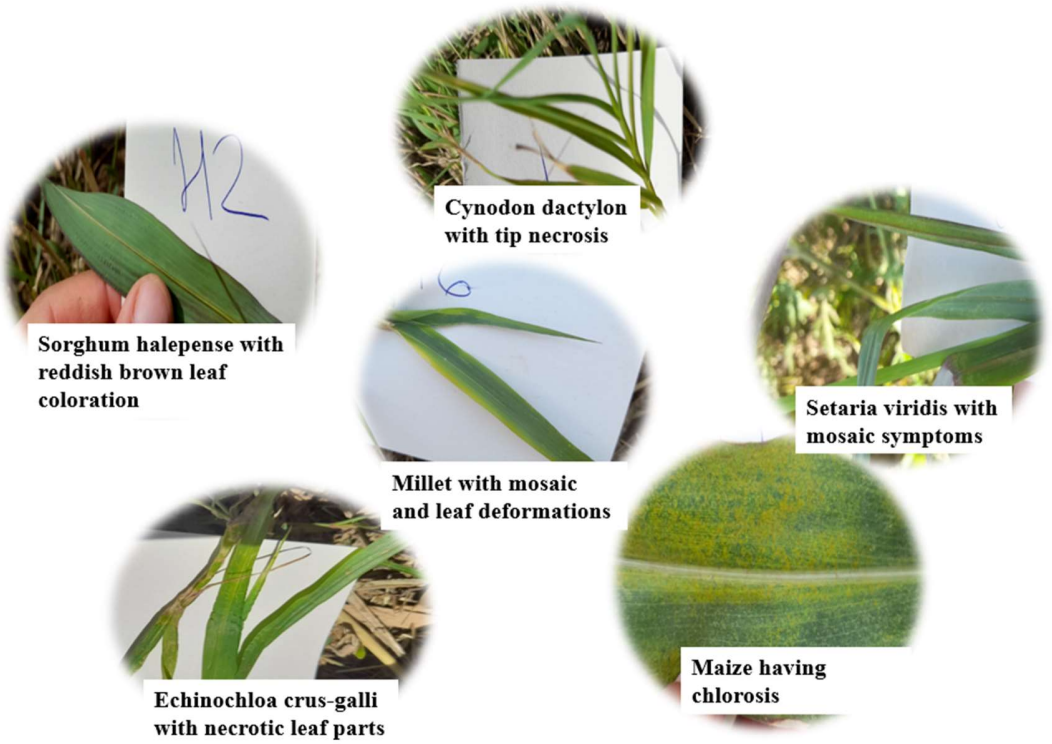

Figure 5. Symptoms on plant samples collected

Table 7. Sample collection fields and samples collected at each field

Újmajor susnyás (US)	**Újmajor (U)**	**Büdös árok (BA)**
Panicum milliaceum (millet)	*Sorghum halepense*	*Panicum milliaceum* (millet)
Echinochloa crus-galli		*Echinochloa crus-galli*
Setaria viridis		*Setaria viridis*
Cynodon dactylon		*Zea mays* (maize)

3.2. RNA extraction

Total RNA extraction was carried out using phenol-chloroform extraction method. The extraction buffer was prepared composed of 7 ml sterile water, 1 ml elution buffer (10xEB) and 2 ml Sodium Dodecyl Sulfate (10%SDS). In the initial step, the plant material was homogenized using mortar and pestle and 650 µl extraction buffer was added and transferred into labelled Eppendorf tubes containing 600 µl phenol and placed on ice. The samples were well vortexed and centrifuged in 15000 rpm for 5 minutes at room temperature.

In the next step, 600 µl phenol-chloroform:isoamylalcohol (24:1) was put into fresh labelled tubes and placed on ice. The supernatant after centrifugation were pipetted into the tubes containing phenol-chloroform:isoamilalcohol placed on ice, vortexed and centrifuged. This step was repeated with 600 µl chloroform. As a final step supernatant from this last separation were put on 20 µl 4M Na-acetate and 1ml 96% ethanol, prepared ahead in fresh labelled tubes on ice. This time the precipitated nucleic acid containing tubes were not vortexed but rather inverted several times and incubated on ice for 15 mins and centrifuged afterwards at 15000 rpm for 10 minutes. This helps to ensure that nucleic acid precipitates out well, and then ethanol was discarded to obtain the white precipitated total nucleic acid. It was then followed by the addition of 1 ml 70% alcohol, centrifuged for 5 mins in 15000 rpm at room temperature. Drops of ethanol were removed and pellet was air dried. The total nucleic acids (TNA) were dissolved in 30ul sterile water and gently vortexing for few seconds. Total nucleic acid solutions were stored at -70 ^{0}C. The final step involves the detection of total nucleic acid by separating aliquot on 1.2% agarose gel treated with a denaturating buffer (mixture of 2 µl sample, 3 µl water and 5 µl FDE) and heat denaturation (5 minutes at 65 ^{0}C).

3.3. Pool (mixture of total nucleic acid) preparation

To ensure enough quality data, RNAs from the same plants from the same location were combined. In some cases, samples of 2 plant species were also combined. Sterile Eppendorf tubes were labelled and placed on ice. 10 µl from all TNA extractions were pooled according to the species and locations. At US, 11 TNA of millet and 8 TNA of *E. crus-galli* were used for separate individual pools. 6 TNA from *S. viridis* together with 5 TNA from *C. dactylon* were pooled as one. For U, *S. halepense* were pooled from 5 TNA. From the sampling location of BA, 10 TNA were

used to prepare millet pool, and 5 maize TNA were also pooled separately. *E. crus-galli* and *S. viridis* were pooled together using 3 TNA from each. The final seven pools were used for the sRNA sequencing library preparation.

3.4. Small RNA library preparation

3.4.1. Isolation of sRNA fraction from total RNA

Gel isolation of small RNA fraction before library preparation is important for obtaining higher quality reads. For isolating small RNAs, 8% TBE polyacrylamide gel was prepared containing urea and pre-run the gel at 50 V for 15 mins. The samples were then loaded by pipetting 20 µl from the 7 redissolved TNA pools and added 20 µl FDE into a microcentrifuge tubes. FDE is not only a dye, but also contains formaldehyde which helps to denature the samples. The samples are denatured for 20 mins at 65 ^{0}C and spined briefly.

The wells of the gel were washed with 1×TBE and then the prepared samples (20 µl into two wells of the gel) are loaded. The gel was ran at a stable voltage of 100 V for 1 hr 30 mins, until the bromophenol blue dye reaches the bottom of the gel. It is imperative to ensure that this dye does not run out because small RNAs are located just above this level. The gel apparatus was dissembled and stained for 5 mins by soaking them in 60 ml 1×TBE containing 3µl ethidium bromide. Separate gels were used for every single TNA pools to eliminate cross contamination and then photos of the gels were taken with the Bio-Rad ChemiDoc Imaging system.

The small RNAs are located in the area above the bromophenol blue dye (about 15-30 nt). These portions were cut out from the gel and put the gel slices into 0.5 ml punctured tubes placed in 2 ml microcentrifuge tubes. The tubes were then centrifuged for 2 mins, 13000 rpm at room temperature to make sure that all of the gels have moved through the holes into the bottom of the 2 ml tubes. 0.3 M 500 µl NaCl was then added, and the gel pieces in the NaCl were gently shaked overnight at 4 ^{0}C elute RNAs. The elute RNAs were precipitated using 600 µl isopropanol. 1 µl glycoblue was added to make it easier to visualize the precipitant and incubated at -70 ^{0}C for 2 hours 30 minutes. The precipitated RNAs were centrifuged at full speed at 4 ^{0}C for 20 mins. The supernatant was carefully discarded and the intact pellet was washed twice with 1 ml 70% ethanol and carefully drained. The pellet was dried for 5 minutes and finally dissolved in 12 µl ultra pure water.

3.4.2. 3' Adapter ligation

Adapter ligation is a very important and crucial step of the library preparation. During this activity, DNA adapters with known sequences are ligated to the RNAs, making it possible to amplify and sequence them, using primers in the later steps. During this crucial process, working under the laminar flow is key to limit contamination and degradation of RNA by RNAses. The ligation mixture for each sample was prepared constituting 1 µl ligation buffer, 0.5 µl RNase inhibitor and 0.5 µl T4 RNA ligase in a sterile PCR tubes on ice, pipetted up and down severally and centrifuged to ensure a uniform mixture. To ligate 3' adapter, the thermocycler was preheated to 70 ^{0}C and 2.5 µl of the purified small RNA from each sample was pipetted into a sterile PCR tube, added 0.5 µl RNA 3'adapter and placed on ice. The reaction mixture was denatured at 70 ^{0}C for 2 minutes and immediately placed on ice after that. In the next step, the thermocycler was set to 28 ^{0}C. 2 µl of the ligation mixture was added to the denatured RNAs and the entire volume were gently mixed by pipetting and incubated at 28 ^{0}C for 1 hour. The 3'adapter ligation reaction was terminated with the addition of 0.5 µl ice-cold stop solution and mixed by pipetting up and down several times. The incubation was continued at 28 ^{0}C for 15 mins and finally placed the tubes on ice.

3.4.3. 5' Adapter ligation

To ligate the 5' adapter, the thermocycler was preheated to 70 ^{0}C and 0.5 µl RNA 5'adapter was pipetted into sterile PCR tubes, incubated at 70 ^{0}C for 2 mins and then placed on ice. The thermocycler was preheated to 28 ^{0}C . Next, 0.5 µl of 10 mM ATP and 0.5 µl T4 ligase were pipetted into separate sterile PCR tube and placed on ice. The total volume of the denatured 5'adapter was added to this mixture totaling 1.5 µl. The 5'adapter mixture was then added to the 3'adapter reaction tube and mixed thoroughly by pipetting. The total volume for 3'and 5'ligation reaction was 7 µl. The reaction tube was placed into the preheated thermocycler and incubated at 28 ^{0}C for 1hour, and then the 3'-5' adapter-ligation reaction was put on ice.

The reverse transcription mix was prepared each into 0.5 ml PCR tubes made up of 1.1 µl ultra pure water, 2.5 µl RT Buffer, 0.55 µl of 12.5 mM dNTP, 1.1 µl RNAase inhibitor and 1 µl of the Revert aid. In the next step, 5.5 µl from the reverse transcription mix was added to the denatured

RT-special primer and the 3'-5' ligated adapter product. The reaction product was then incubated for 1 hour at 50 ^{0}C.

3.4.4. cDNA synthesis

The cDNA synthesis was performed using the Revert Aid^{TM} First Strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) alongside random primers following the manufacturer's instructions. The RNA extracts of species pool which were seven in total, and the total nucleic acids of each plant were utilized as the templates for the cDNA activity.

With the use of Q5 DNA Polymerase (New England Biolabs, Ipswich, MA, UK), RT-PCR was conducted where primers amplify viral parts through the used annealing temperatures and cycling parameters set. GeneJET Gel Extraction Kit (Thermo Fisher Scientific) was employed in purifying the PCR products, followed by cloning into GeneJET (Thermo Fisher Scientific) and then, the clones were Sanger sequenced through a service order.

3.4.5. PCR for small RNA libries

PCR amplification was performed using a PCR reaction mixture made of 5 µl of 5x RT buffer, 10.75 µl ultra pure water, 0.5 µl of 10 mM dNTP, 1 µl forward primer (RP), 1 µl reverse primer (RPx) and 0.5 µl Phusion enzyme. The master mix is made up of 18.75 µl from the PCR reaction mix and 6.25 µl template that was prepared through the cDNA synthesis. In the next step, the reaction was denatured in a thermocycler and followed by cycling in the order presented in table 8 below and the amplified small RNA library is ready for purification.

Table 8. PCR parameters

PCR step	Temperature (^{0}C)	Duration used
Initial denaturation	98	30 s
Denaturation	98	10 s
Annealing	60	30 s
Elongation	72	15 s
Extension	72	10 mins
Number of cycles	**16**	

3.4.6. Purification of the small RNA library

Small RNA libraries were purified using an in-house protocol based on TruSeq small RNA library preparation kit (Illumina, San Diego, CA, USA). Two different size markers were used, a 20 bp DNA ladder and a 50 bp low-molecular-weight ladder which were placed in the two outermost positions, one on each side of the prepared gel. The amplified PCR product of 25 µl and 5 µl of 6x Orange DNA dye were loaded into the middle of the two consecutive wells of the gel and run at a stable voltage of 100V for 2 hours, as dye migrates to the bottom of the gel. The size marker and the amplified sRNA library were visualized on a UV transilluminator and the piece of gel that matches the desired size (140-160 nt) of the small RNA library were cut and placed in punctured 0.5 ml microcentrifuge tubes placed into 2 ml microcentrifuge tube. After eluting DNA, the tubes were gently shaked overnight at 4 ^{0}C with 0.5 M NaCl. The eluted DNA was separated from the gel on a mini coloumn. In the next step, 1ul GlycoBlue and 1ml 100% ethanol were added to the elute and incubated at -70 ^{0}C for 2 hours. The final step was centrifugation of the precipitate at full speed at 4 ^{0}C for 20 mins, followed by discarding the supernatant and then washing the intact pellet with 1 ml 70% cold ethanol. The pellet was dried in the speed vac machine for 5 mins and resuspended in 12 µl sterile 1x TE resuspension buffer. The small RNA sequencing library was stored at -70 ^{0}C and ready for sequencing. The pure small RNA libraries were sequenced using a single index on a HiScanSQ by UD-Genomed (Debrecen, Hungary) (50 bp, single-end sequencing).

3.5. Bioinformatic methods for sequence analysis

3.5.1. Bioinformatic analysis of the HTS reads

The obtained genomic sequence which was in FastQ files were subjected to bioinformatic analysis using the CLC Genomic workbench (Qiagen, Hilden, Germany). The CLC Genomic workbench is a powerful tool for analyzing and visualizing the data. In the CLC genomic workbench, quality control is conducted to reduce the occurrences of low-quality sequences. The size distribution of the sequenced reads were also checked (21, 22, 24nt long).

The generation of a non-redundant list is necessary to help keep one sequence read information from a sample at a time. For virus detection, CLC enabled the building of longer sequences from the sequence reads known as the contigs. The contigs were then Blasted to the reference genomes of known plant infecting viruses in the public database of the National Centre for Biotechnology Information (NCBI) (www.ncbi.nml.nih.gov), which reveals all possible presenting viruses in the investigated samples collected from the study area.

3.5.2. Phylogenetic analysis

From the workbench, multiple sequence alignments were conducted using Geneious prime and MUSCLE algorithm. The evolutionary history was inferred using the Jukes-Cantor model and the Neighbour-joining method. The trees were constructed using the best fit model for each alignment, and 1000 boot-strap replicates. The trees were drawn to scale, with branch lengths measured in the number of substitutions per site. For the outgroups, the closest relative of the virus was referred to in the tree's legend.

3.5.3. Analyzing sanger sequences

During the primary analysis of the sequence information, Chromas 2.6.5 was used. This program is a free trace viewer for simple DNA sequencing projects that do not require assembling of multiple sequences. Chromas provided the possibility of copying the sequence to the clipboard in FASTA format or plain text for further analysis in the CLC. It also provided insights into whether clear sequences were obtained or a mixture of them (Figure 6), and in the case of this study, where the sequences were mixed, further analysis was conducted using the CLC Genomic workbench.

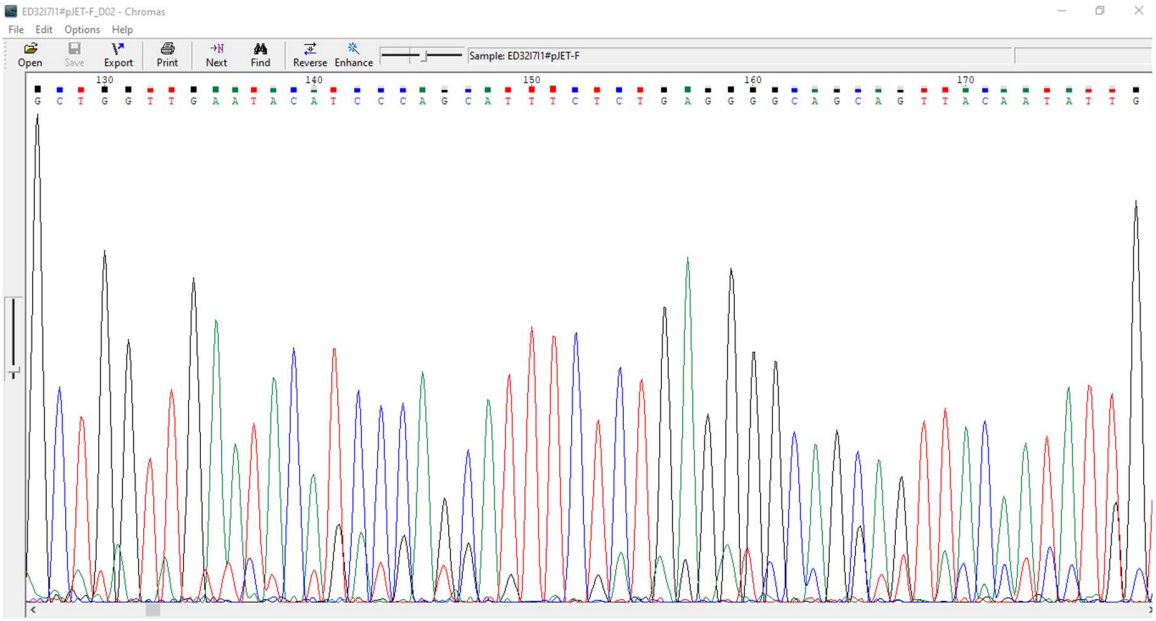

Figure 6. Chromatographic representation of sequence opened in Chromas 2.1

3.6. Validation of virus presence with RT-PCR

The presence of important viruses of interest in the plant samples from the results of the bioinformatic analysis were confirmed or validated by the use of virus specific primers (Table 9). The PCR mix for each pool of the plant samples were prepared with 5 µl of 5x buffer, 15.5 µl of MilliQ water, 1.25 µl forward primer, 1.25 µl reverse primer, 0.5 µl of 10 mN dNTP and 0.5 µl of Q5 Polymerase.

Table 9. PCR primers used for virus detection (Galbács et al., 2024)

Virus	Primer Name	Primer Sequence (5'-3')	Position on the reference genome	Reference genome
WSMV	WSMV_8499F	ATGTGCAAGATCAGACACCAGGC	8499-8520	NC_001886
	WSMV_9253R	TAGTTTCTACTGTGCTCACGCAAG	9253-9230	
BYSMV	BYSMV_158F	ATGGCAAAAGAAGATCATGGATTG	158-181	NC_028244
	BYSMV_1444R	CCTTTAGGAGAAGATCTGGTCAGC	1444-1421	
BVG	BVG_3508F	ATGAATACGGGAGGTAGAAATGG	3508-3530	NC_029906.1
	BVG_4115R	GATGCTCCGTCTACCTATTTCGG	4115-4093	
	BVG_215_F	ACTGTGCTGCCTCTTATGCTTC	215-237	
	BVG_5574R	TTGACTTGAGAACTGTCTGGC	5574-5554	
	BVG_1088_F	TCCTGACAACGCAGAACTCCG	1088-1101	
	BVG_4329_F	ACGATGAATCTTGGTCCAGTG	4329-4350	
	BVG_3367_R	GCTATCCAGTACTTGCAACTC	3367-3347	
	BVG_1790F	AGAACAACCGAAGGCGGCCATCG	1790-1812	
	BVG_2523R	GATCCAACAACGAGACACTCATG	2523-2501	
	BVG_700F	TATATATGCACAACGCTCTTG	700-720	
	BVG_1223R	CAGTGACCATCGAACCTGTAG	1223-1203	
ApGIV1	ApGlV1_439F	TATTTAGGATACGGTTGGACAC	439-460	OL472190
	ApGlV1_1323R	GTTGGCTGTACACTGAGATCGT	2303-2281	
	ApGlV1_1297F	TTCAGTGCATGTGCCAATTCAG	1297-1318	
	ApGlV1_2303R	GTACAGCAGGAGTGGAAGAGTCT	2303-2281	
LDV1	LDV1_1110F	TGCAGCTATGGTCGCTTACAC	1110-1130	OL472194
	LDV1_1856R	GAATATATCATTCCTTCCATTG	1877-1856	
	LDV1_5664F	AGGACCATCAGATGTGCAGGC	5564-5585	
	LDV1_6480R	TGTCCAGGTGATGACTGAGCAG	6501-6480	

In the next step, new sterile tubes were labelled and placed on ice in the lamina flow cabinet. From the cDNA template of each pooled plant sample, 1 µl of the cDNA was pipetted into labelled tubes, and 24 µl from the PCR mix were then added and centrifuged to obtain final volume of 25 µl. The samples were then loaded for PCR.

Table 10. PCR parameters set in the case of WSMV

PCR step	**Temperature (^{0}C)**	**Duration used**
Initial denaturation	98	30 s
Denaturation	98	10 s
Annealing	55	20 s
Elongation	72	60 sec
Extension	72	2 mins
Number of cycles	**40**	

3.6.1. Preparation and running of gels

After almost 2 hours to the end of PCR cycle, TBE agarose gel was prepared. During the preparation, 30 µl of agarose was measured into a glass tube with 1 µl of ethidium bromide pipetted into the agarose and the wells of the gels created. To run the gel, an amount of 4 µl of the DNA dye was mixed with the PCR product in the tubes. 5 µl of this mixed product was then finally transferred into the wells of the gel. The first well of the gel which is used as the DNA ladder was loaded with 3 µl of the marker dye. With the difference in position of the forward and reverse primers on the viral genome, the estimated size of the PCR product was recorded. The gel was finally run at 116 V for 30 minutes, and gel photo was then captured with the Bio-Rad ChemiDoc Imaging system.

3.6.2. Testing for viral presence in individual samples

The individual samples from libraries for which positive results was detected for all plant viruses under consideration (WSMV, BYSMV, BVG, Aphis glycine virus 1 (ApGV1) and Ljubljana dicistrovirus 1 (LDV1) were tested to investigate individuals which were singly positive for these viral infections. Complementary DNA (cDNAs) were prepared from all 56 samples. Using the WSMV, BYSMV, BVG, ApGV1 and LDV1 specific primers, the individuals samples were then tested for the presence of these plant viruses.

3.6.3. Purification of PCR product

Purification of PCR product was conducted using the Genejet gel extraction kit (Thermo Fisher Scientific). The PCR products were cut from gel and placed into a pre-weighed 1.5 ml tube. In the initial step, 60 µl of binding buffer was added for every 600 mg of agarose gel. The gel mixture was then incubated for 10 minutes at 65 ^{0}C until the gel slice was completely dissolved. To ensure that the gel was completely dissolved, the tube was inverted for few minutes to enhance melting process and then, the gel mixture was briefly vortexed.

In the next step, 800 µl of the solubilized gel solution was transferred to the GeneJet purification column and centrifuged for 1minute. The flow through was discarded and the column placed back into the collection tube. After this activity, 100 µl of binding buffer was then added to the purification column and centrifuged for a minute, followed by the addition of 700 µl wash buffer. After the flow through was discarded and the column placed back into the collection tube, the empty GeneJet purification column was then centrifuged for 5 minutes to completely remove

residual wash buffer. The purification column was then transferred into a new 1.5 mL microcentrifuge tube, and 23 µl of elution buffer was added into the center of the purification column and centrifuged for a minute. In the final step, the purification column was discarded and the purified DNA was stored at -20 ^{0}C.

3.7. Ligation and transformation

This procedure started with the preparation of the ligation mix constituting 5 µl of 2X reaction buffer, 2 µl MilliQ water, 0.5 µl pJET and an amount of 0.5 µl ligase. Using new labelled tubes, 8 µl of the MIX and 2 µl of the purified PCR product was pipetted into the tubes and incubated at room temperature for 15 minutes. In the next step, 14 ml ligation tubes were labelled and place on ice, and then 5 µl of the ligation mixture and 100 µl of competent cell solution was pipetted into the tubes and taken through the following steps:

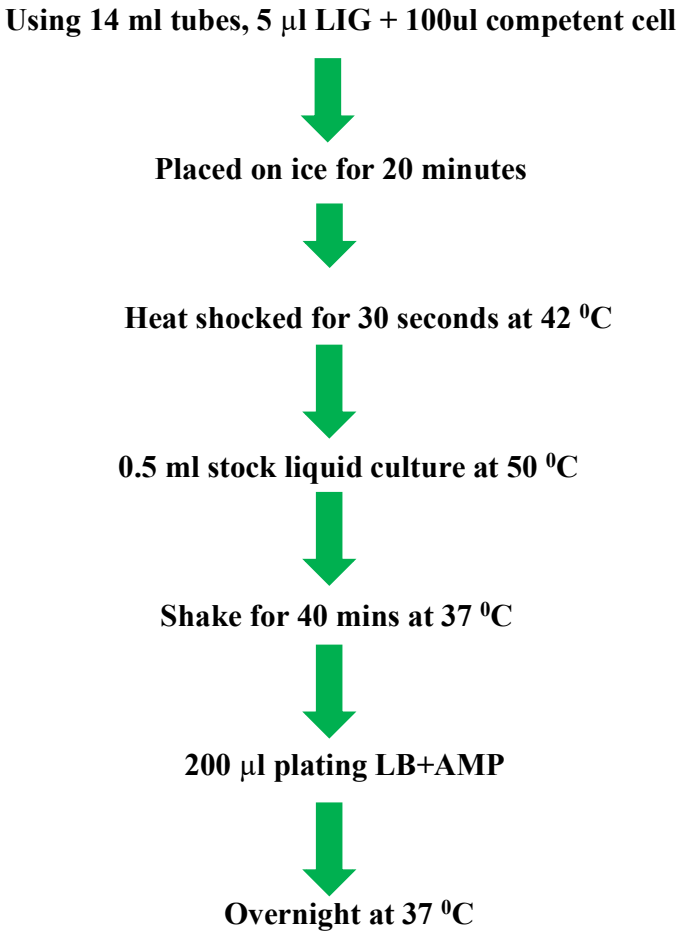

Figure 7. Diagrammatic presentation of the ligation steps

3.8. Plasmid DNA isolation

The initial steps involves cultivation and harvesting of bacterial cells by spinning tubes at high speed for 3 minutes. Cell lysis was undertaken by the addition of 250 µl A1 and A2 buffers and incubated at room temperature up to 5 minutes and then 300 µl A3 buffer was then added in the final lysis stage. In the quest to clarify the lysate, the tubes were incubated for 5 minutes at room temperature. The supernatant was then loaded into new labelled purification tubes to bind DNA and wash the silica membrane through the addition of 500 µl AW wash buffer and 600ul A4 buffer at room temperature. In the final step, the silica membrane was dried through centrifugation during the single washing step and 25 µl of AE buffer was then added and incubated at room temperature for a minute to elute DNA. A minipreparation mix consisting 2 µl Tango buffer, an enzyme Xho1 of 0.2 µl, 0.4 µl of Xbal and 5.4 µl of miliQ water was prepared. 8 µl of this mix together with 2 µl of the prepared plasmid was pipetted into a fresh tube constituting the digested plasmid. Both digested and undigested plasmid product were finally run through gel electrophoresis.

4. RESULTS AND DISCUSSIONS

4.1. Field assessment, visual inspection and weed surveys

The weed samples collected from the experimental fields of US, U and BA displayed a diverse array of symptoms characteristic of virus infection. Symptoms ranged from mosaics, leaf deformations, tip necrosis, reddish brown colorations, mottling, chlorosis, purple discoloration and spots of leaves.

Újmajor susnyás		Symptoms
millet	M1	Ldef, M, Chl
	M2	M, Ldef
	M3	M, Ldef, P: Purple
	M4	Mosaic symptoms
	M5	Mosaic, Mild chlorosis
	M6	Mosaic, deformation
	M7	M, Chl, N, Stu, Ldef
	M8	M, Chl
	M9	M, Chl, Ldef
	M10	M, Chl, TN
	M11	M, TN
Echinocloa crus-galli	E1	Ldef, mild mosaic symptoms
	E2	Ldef, stunting
	E3	Ldef, Chl, stunting
	E4	Ldef, MM, Chl, Stu
	E5	Ldef, Chl, Necrosis, stu, M
	E6	Ldef, Stu, Chl, M
	E7	Chl,M,Ldef
	E8	Ldef,Stu,N, Chl,M
Setaria viridis	S1	M, Ldef, N
	S2	vein necrosis,Ldef, Purple coloration
	S3	M, Ldef, P, Chl
	S4	Ldef, Chl, P
	S5	M, N, TN, Ldef
	S6	Ldef, P, TN, Chl
Cynodon dactylon	C1	Ldef, Mosaic, Mottle
	C2	Ldef, M, Mo, Chl, TN
	C3	Ldef, M, Mo, Chl, TN
	C4	Ldef, MM, Mo
	C5	Mild Mosaic, TN

Figure 8. Samples collected from "Újmajor susnyás" (US) with their associated viral symptoms Ldef: leaf deformations, M: Mosaic symptoms, Chl: Chlorosis, P: Purple colorations, N: Necrosis, Stu: Stunting, TN: Tip necrosis, MM: Mild mosaic and Mo: Mottling.

In the experimental location of US, samples of millet (*P. milleaceum*) collected showed symptoms such as leaf deformations, mosaic, chlorosis, necrosis, stunting of the plant, tip necrosis and purple colorations. *E. crus-galli* weed samples were having mild mosaic symptoms, leaf deformations, necrosis, stunting and chlorosis. The samples of *S. viridis* weeds exhibited purple coloration, vein and tip necrosis, leaf deformations and mosaic whilst samples of *C. dactylon* weeds had mottling, leaf deformations, mosaic, chlorosis and tip necrosis (Figure 8).

In location U, where weed samples of *S. halepense* were collected, samples revealed stunting of the plant, leaf deformations, mosaic, tip necrosis and some minor purple coloration (Figure 9).

Újmajor		Symptoms
Sorgum halepense	H1	M, Ldef, TN
	H2	M, P
	H3	M, Stu, Ldef
	H4	Ldef, M, Stu, P
	H5	M, Stu, P

Figure 9. Samples collected from "Újmajor" (U) with their associated viral symptoms M: Mosaic symptoms, Ldef: leaf deformations, TN: Tip necrosis, P: Purple colorations and Stu: Stunting.

The samples of *P. milleaceum* from BA also indicated symptoms of chlorosis, mosaic, leaf deformation, stunting of the plant, necrosis, mottling and some pale purple colorations. Samples of *E. crus-galli* from this study location exhibited mosaic, chlorosis, leaf deformations and plants were stunted whilst *S. viridis* weeds samples of BA were having mosaic symptoms, tip necrosis, chlorosis and leaf deformations. Interestingly, chlorotic leaves, some leaf deformations, mosaic and stunting were also observed in maize plants that were sampled from BA (Figure 10).

Büdös árok		Symptoms
millet	M2/1	M, Chl, Ldef
	M2/2	Stu, Chl, M, Ldef
	M2/3	Chl, N, Stu, Ldef
	M2/4	Stu, Chl, M
	M2/5	Chl, Ldef, M, N
	M2/6	Stu, Chl, M, TN
	M2/7	Chl, M, Ldef, P, N, Stu
	M2/8	M, Ldef
	M2/9	Stu, M, N
	M2/10	M, Chl, N, Mo
Echinocloa crus-galli	E2/1	Stu, Ldef, M
	E2/2	Ldef, Stu, Chl
	E2/3	M, Ldef
Setaria viridis	S2/1	Mosaic symptoms
	S2/2	M, Ldef, TN, Chl
	S2/3	Mosaic symptoms
maize	Ma1	Chl, M, Ldef
	Ma2	M, Stu, Chl
	Ma3	Chl, M, Stu
	Ma4	Stunning
	Ma5	Chl, Stu

Figure 10. Samples collected from "Büdös árok" (BA) with their associated viral symptoms M: Mosaic symptoms, Chl: Chlorosis, Ldef: leaf deformations, Stu: Stunting, N: Necrosis, P: Purple colorations, Mo: Mottling and TN: Tip necrosis.

4.2. Results of the sRNA HTS study of the weed populations of the experimental locations

The results of small RNAs high throughput sequencing revealed the viruses that were present in the investigated weed samples. The virus hits revealed the presence of WSMV, BYSMV, BVG, ApGlV1 and LDV1. After the 18–38 million sequenced reads were trimmed and quality controlled, 17–37 million redundant reads were maintained, which correspond to 1,5–4,2 million small RNA sequences (Table 11). Using the obtained non-redundant reads, 3103-20797 contigs could be assembled, and BLAST-ed to the reference genomes of known plant-infecting viruses.

Table 11. Statistics of the sequenced small RNA libraries of the investigated samples

Library code	Sequenced reads	Trimmed reads all (containing redundants)	Non-redundant reads	Number of contigs
1_M_US	21 453 088	21 273 768	3,351,469	11 377
2_ECG_US	23 965 784	23 623 757	2,497,935	12 366
3_SVCD_US	**38 236 832**	**37 392 543**	**4,198,026**	**20 797**
4_SH_U	**18 685 311**	**17 500 902**	**1,553,870**	3 586
5_M_BA	20 882 517	20 538 898	3,234,446	13 578
6_ECGSV_BA	32 030 185	31 197 132	2,408,431	8 461
7_Ma_BA	21 736 705	21 232 382	1,934,981	**3 103**

The contig hits with acceptable E-value (lower than 10^{-5}) revealed the presence of five viruses: WSMV (in 5_M_BA), BYSMV (in 5_M_BA), BVG (in 6_ECGSV_BA), ApGlV1 and LDV1 (both in the same library: 7_Ma_BA) were found (Table 12). Investigation of the distribution of the mapped viral reads to the viral genome showed that the viral genome was highly covered. Coverage was higher than 90% in the case of WSMV, BYSMV, BVG and ApGlV1, and was higher than 70% in the case of LDV1. Whilst in these libraries the number of the normalised redundant reads reached the set limit in the case of WSMV (128204), BYSMV (4446), BVG (212) and ApGlV1 (2359), in the case of LDV1 it was below this limit (87). Size distribution of the viral mapped reads in these libraries, in the case of WSMV, BYSMV and BVG indicated that most of them were 21nt long, whilst in the case of ApGlV1 and LDV1, however, most of the reads were 19-21nt long, where majority of them were 20nt long.

Table 12. Bioinformatic results indicating viruses present in the investigated samples

Library	virus		Wheat streak mosaic virus	Barley yellow striate mosaic virus	Barley virus G	Aphis glicines virus 1	Ljubjana dicistrovirus
	NCBI accession number of the genome what we used as a reference		NC_001886	NC_028244	NC_029906.1	OL472190	OL472194
1_M_US	Virus hit	number of contigs	2	0	0	0	0
		number of non-redundant reads	747	644	156	447	420
		number of redundant reads	1375	1140	299	809	689
		RPM	64	53	14	38	32
		coverage of the viral genome (%)	57.8	46	33.59	44	44
2_ECG_US	Virus hit	number of contigs	0	0	0	0	0
		number of non-redundant reads	461	615	222	349	518
		number of redundant reads	1384	1357	1056	703	1077
		RPM	58	57	44	29	45
		coverage of the viral genome (%)	46.4	48.5	41.85	42	54
3_SVCD_US	Virus hit	number of contigs	0	0	0	0	0
		number of non-redundant reads	896	1106	360	637	870
		number of redundant reads	2138	2315	748	1718	1904
		RPM	56	61	20	45	50
		coverage of the viral genome (%)	68.77	66.3	63.64	64	70
4_SH_U	Virus hit	number of contigs	2	0	0	0	0
		number of non-redundant reads	421	287	421	278	209
		number of redundant reads	1251	837	238	858	547
		RPM	58	45	13	46	29
		coverage of the viral genome (%)	39.77	41.7	25.69	30	29
5_M_BA	Virus hit	number of contigs	204	236	0	0	0
		number of non-redundant reads	62469	26322	173	945	816
		number of redundant reads	2743563	92931	214	1271	2164
		RPM	128204	4446	10	61	104
		coverage of the viral genome (%)	94.36	97.80	40.17	70	66
6_ECGSV_BA	Virus hit	number of contigs	6	0	18	0	0
		number of non-redundant reads	834	834	1556	275	580
		number of redundant reads	1825	1841	6772	1081	1753
		RPM	85	57	212	34	55
		coverage of the viral genome (%)	57.38	41.7	90.04	36	61
7_Ma_BA	Virus hit	number of contigs	4	0	0	91	1
		number of non-redundant reads	687	687	87	10830	1149
		number of redundant reads	1416	396	124	51285	1883
		RPM	66	18	6	2359	87
		coverage of the viral genome (%)	45.80	27.6	18.27	93	76

4.3. Validation of virus presence with RT-PCR

The validation of results of the sRNA HTS was conducted using an independent RT-PCR method. For the amplification, primers of Pasztor et al. (2020) study (in the case of WSMV, BYSMV and BVG) and newly designed ones (in the case of ApGlV1 and LDV1) were used. To avoid the possibility of failed priming, because of presenting genomes of different strains with slight differences during the primer design, sequences of the contigs and consensus sequences originating from the HTS were considered. The results of the sRNA HTS were successfully validated. Moreover, all of the viruses were detected in additional libraries: WSMV was found in 2_ECG_US and 3_SVCD_US; BYSMV was also detected in 3_SVCD_US; BVG in 1_M_US, 2_ECG_US

and 5_M_BA; ApGlV1 was detected in 4_SH_U and 6_ECGSV_BA; and LDV1 was identified in 3_SVCD_US, 5_M_BA and 6_ECGSV_BA according to RT-PCR results (Figure 11).

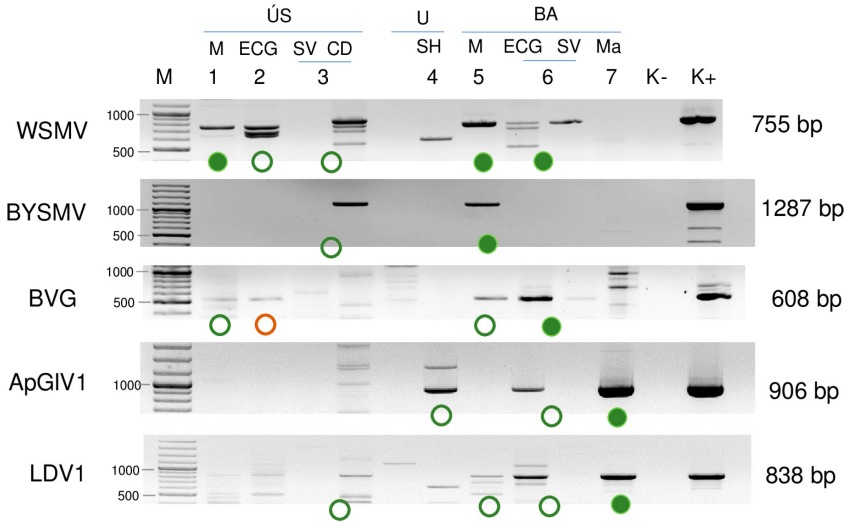

Figure 11. Virus diagnostics using RT-PCR and virus-specific primers. Circles indicate positive results, green when Sanger sequencing confirmed the presence of the virus, and red when Sanger sequencing did not confirm the RT-PCR result. Each library is represented only by one circle. Filled circled shows cases with the same sRNA HTS result. K- and K+ are the negative and positive controls. **M** – stands for a GenRuler 100bpPlus, used as a molecular marker (Galbács et al., 2024).

4.4. Testing of infections from individual plant samples with the five identified viruses of the study

To test how many individuals are infected with a particular virus in the pools, they were tested using RT-PCR and presented according to the viruses.

4.4.1. Detection and phylogenetic analysis of WSMV

Detection of the presence of WSMV in the species pools using RT-PCR revealed the infection of millet and *E. crus-galli* at the study locations of US and BA, *C. dactylon* weed samples from US and *S. viridis* from BA were also WSMV infected (Figure 12). Whilst all of the tested *C. dactylon* plants in US, all of the *E. crus-galli* and *S. viridis* plants at BA were infected, the infection rate was not 100% in the other cases: three millet samples out of 11, five *E. crus-galli* samples in US out of 8 and four millet samples in BA out of 10 were infected with WSMV (Figure 12).

By contrast, sRNA HTS did not detect the presence of WSMV in 2_ECG_US and 3_SVCD_US. In 1_M_US and 6_ECGSV_BA two and six WSMV-derived contigs have been detected, but the number of the normalised redundant reads was below the set threshold of 64 and 85 in 1_M_US and 6_ECGSV_BA, respectively (Table 12). In these two libraries the coverage of the WSMV genome was slightly below the set (60%) threshold, with the resulting 57.8 and 57.3, respectively (Table 12). The majority of the WSMV-derived reads in 2_M_BA and these two libraries were 21nt long, suggesting active antiviral silencing. Apart from the absence of contigs specific to WSMV, 2_ECG_US and 3_SVCD_US showed minimal coverage of the viral genome, a smeared size of small RNAs, and limited virus-derived sRNAs, all of which pointed to a possible low RNAi activity in these instances.

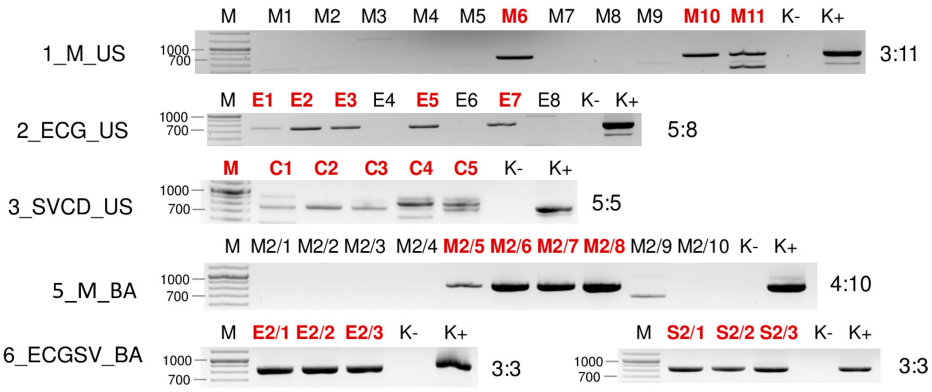

Figure 12. Virus diagnostics using RT-PCR to test the presence of WSMV in the sampled individuals amplifying a 755 bp long part of the WSMV genome using WSMV_8499F and WSMV_9253R primers. M—stands for a GenRuler 100 bpPlus, used as a molecular marker. K− and K+ are the negative and positive controls. Red indicates positive individuals. The infection rate of the plant species is also indicated (Galbács et al., 2024).

The PCR products sequenced that resulted from the species pool did not contain any single nucleotide polymorphism (SNPs), indicating that there was no variation in the sequence of the amplified viral portion, the rationale behind why one PCR product amplified from each species pool was cloned and sequenced. Despite sequencing WSMV variants in six cases, only two variants (PQ047238_2021_MUS and PQ047239_2021_MBA) were discovered. The sequence of the MUS variant, present in millet from US, which was also found in *E. crus-galli* of both locations

in US and BA, *C. dactylon* in US and *S. viridis* in BA, and the MBA variant present in millet at BA was 98.8% identical. They were also very similar and shared higher than 97% similarity with the variants sequenced in millet in 2019 (Pasztor et al., 2020). The variants sequenced during this study clustered into Clade B together with the previously sequenced European variants (Figure 13).

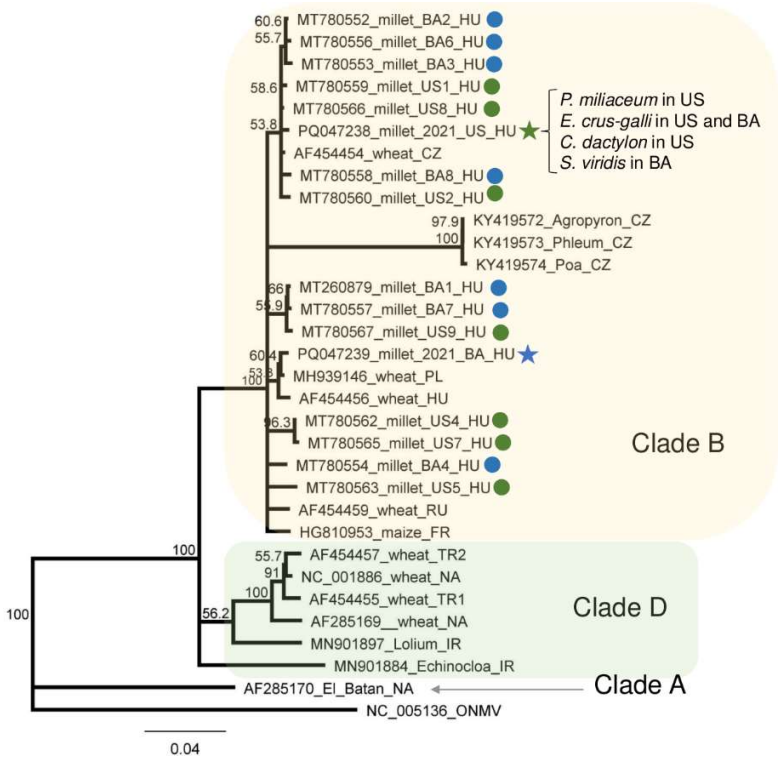

Figure 13. Phylogenetical analysis of the WSMV strains originating from BA and US. The phylogenetic tree was constructed based on the 755 nt long amplified and Sanger-sequenced, polyprotein-encoding (containing CP coding) part of the viral genome using the Neighbour-Joining analysis and the Jukes–Cantor model, with 1000x bootstrap replications. Bars represent 4% nucleotide diversity. Sequences originating from Pasztor et al. (2020) study are marked with circles, whilst sequences from this study are marked with stars. Green represents US, while blue represents BA. Sequences of the different strains are marked with their GenBank accession numbers, host species and countries of origin. HU—Hungary, CZ—Czechia; PL—Poland; RU—Russia; FR—France; TR—Turkey; NA—North America; IR—Iran. Green—US, blue—BA. ONMV—Oat necrotic mottle virus was used as an outgroup to root the tree (Galbács et al., 2024).

4.4.2. Detection and Phylogenetic analysis of BYSMV

The investigation of BYSMV in the species pools revealed the infection of *C. dactylon* in US and millet in BA. Whilst at BA three millets out of 10 were infected, testing individuals using RT-PCR showed that out of the tested five *C. dactylon* weed samples, three were infected at US (Figure 14).

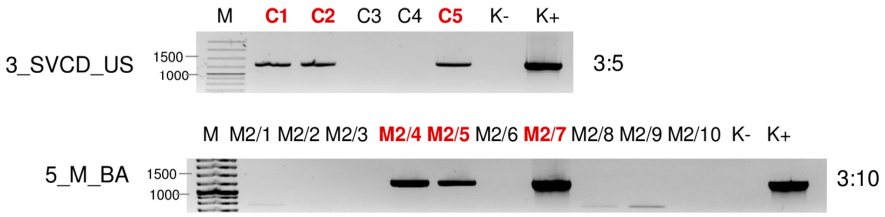

Figure 14**.** Virus diagnostics using RT-PCR to test the presence of BYSMV in the sampled individuals, amplifying a 1287 bp long part of the BYSMV genome using BYSMV_158F and BYSMV_1444R primers. M stands for a GenRuler 100 bpPlus, used as a molecular marker. K− and K+ are the negative and positive controls. Red indicates positive individuals. The infection rate of the plant species is also indicated (Galbács et al., 2024).

The detection of BYSMV infection in millet through the sRNA HTS revealed 236 virus-derived contigs, resulting in 4446 RPM. Moreover, 97.8% of the viral genomes were covered by small RNA reads. However, in 3_SVCD_US, there was no BYSMV-derived contig and the RPM was 61. The expression of BYSMV-originated small RNAs accounting for 66.3% of the viral genome was the only clue that BYSMV could exist in these plants (Table 12). Size distribution of the sRNA reads showed a peak at 21nt in the case of 5_M_BA, whilst in the case of 3_SVCD_US the majority of the BYSMV-derived reads were 24nt long, demonstrating that, the antiviral silencing was not very active. Sequences of the PCR amplified part prepared from the species pools show no differences and when sequenced as a clone turned out to be the same. This variant from the study was very similar and shared 99.78% similarity when clustered together with one of the variants found in 2019 (Pasztor et al., 2020) at the same place (Figure 15).

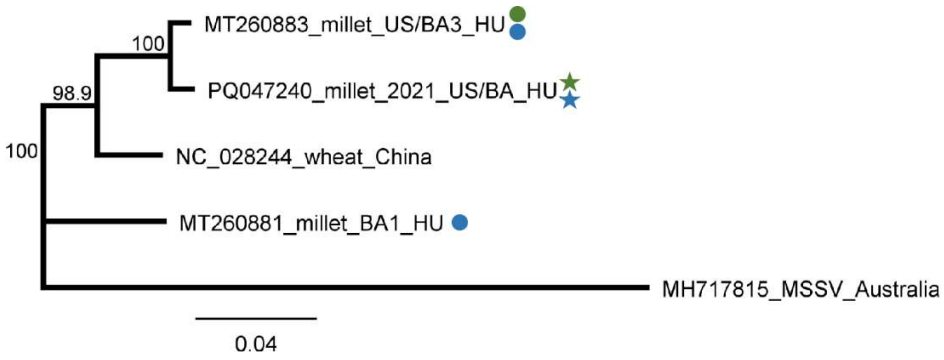

Figure 15. Phylogenetic analysis of the BYSMV strains originating from BA and US. The phylogenetic tree was constructed based on the 1287 nt long amplified and Sanger-sequenced, CP-coding part of the viral genome using Neighbour-Joining analysis and the Jukes–Cantor model, with 1000x bootstrap replications. Bars represent 4% nucleotide diversity. Sequences originating from Pasztor et al. (2020) study are marked with circles, whilst sequences from this study are marked with stars. Green represents US, while blue represents BA. Sequences of the different strains are marked with their GenBank accession numbers, host species and countries of origin. HU—Hungary. MSSV—maize sterile stunt virus (MSSV) was used as an outgroup to root the tree (Galbács et al., 2024).

4.4.3. Detection and phylogenetic analysis of BVG

Small RNA HTS identified BVG infection in *E. crus-galli* in BA, indicating this plant as a possible new host of BVG. This infection was validated using RT-PCR (Figure 11), but a PCR product at the expected size was also amplified from *E. crus-galli* in US and millet at both locations of US and BA. The PCR products when Sanger sequenced revealed that they originated from BVG with one exception. Investigating this sequence in the *E. crus-galli* sequences available in the GenBank did not give any hit, but it shows 98% similarity with other monocotyledonous species, upholding its background-origin. When testing individual plants of the positive pools and aiming to prevent false positive, new primers (BVG_707F and BVG_1223R) were created. RT-PCR of the individuals showed that three out of 11 in US and seven out of 10 millets in BA were infected whilst a sample out of three *E. crus-galli* was BVG infected (Figure 16).

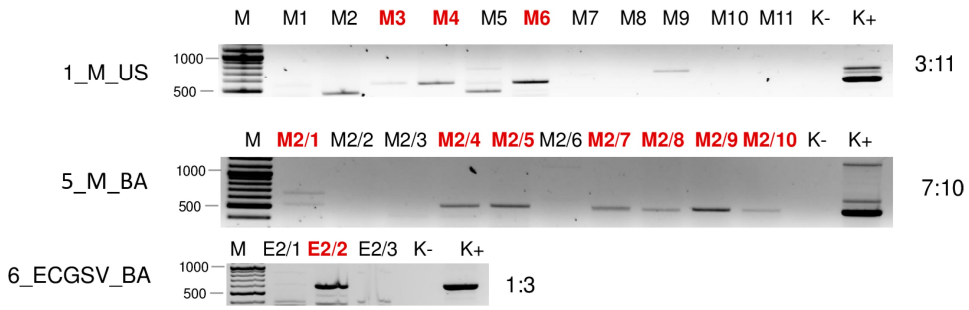

Figure 16**.** Virus diagnostics using RT-PCR to test the presence of BVG in the sampled individuals. amplifying a 523 bp long part of the BVG genome using BVG_700F and BVG_1223R in cases of 1_M_US and 5_M_BA and a 607 bp long part of the BVG genome using BVG_3508F and BVG_4115R primers in the case of 6_ECGSV_BA. M stands for a GenRuler 100 bpPlus, used as a molecular marker. K− and K+ are the negative and positive controls. Red indicates positive individuals. The infection rate of the plant species is also indicated (Galbács et al., 2024).

During the investigation of the virus BVG, the size distribution of the obtained small RNAs showed that in the case of 6_ECGSV_BA, the majority of the small RNAs were 21nt long, whilst in the case of 1_M_US and 5_M_BA, the majority of the small RNAs were 24nt and 21nt long. A nearly complete genome of the BVG strain in the *E. crus-galli* was amplified and sequenced. BVG variant sequenced on *E. crus-galli* is highly similar to the strains available in the GenBank, sequenced in different hosts at different locations (Figure 17a). It demonstrates the highest identity to a BVG variant sequenced in Great Britain from maize (98.7%) and in France from barley (98.3%). Sequence analysis of the smaller, 607 nt long part of the genome showed a similar, slightly unique trend (Figure 17b). The variants sequenced from millet in US, BA and *E. crus-galli* in BA were highly identical (higher than 99% identity) and were very analogous to the variant sequenced in 2019 (Pasztor et al., 2020). BVG variants were very similar, the variants sequenced in Europe showed higher than 97% identity, proposing a very conservative genome of the virus. The BVG variant from *E. crus-galli* clustered with the variant present in Great Britain, Slovenia and France. Although they were similar, the variants sequenced in 2019 (Pasztor et al., 2020) and in this study clustered distantly.

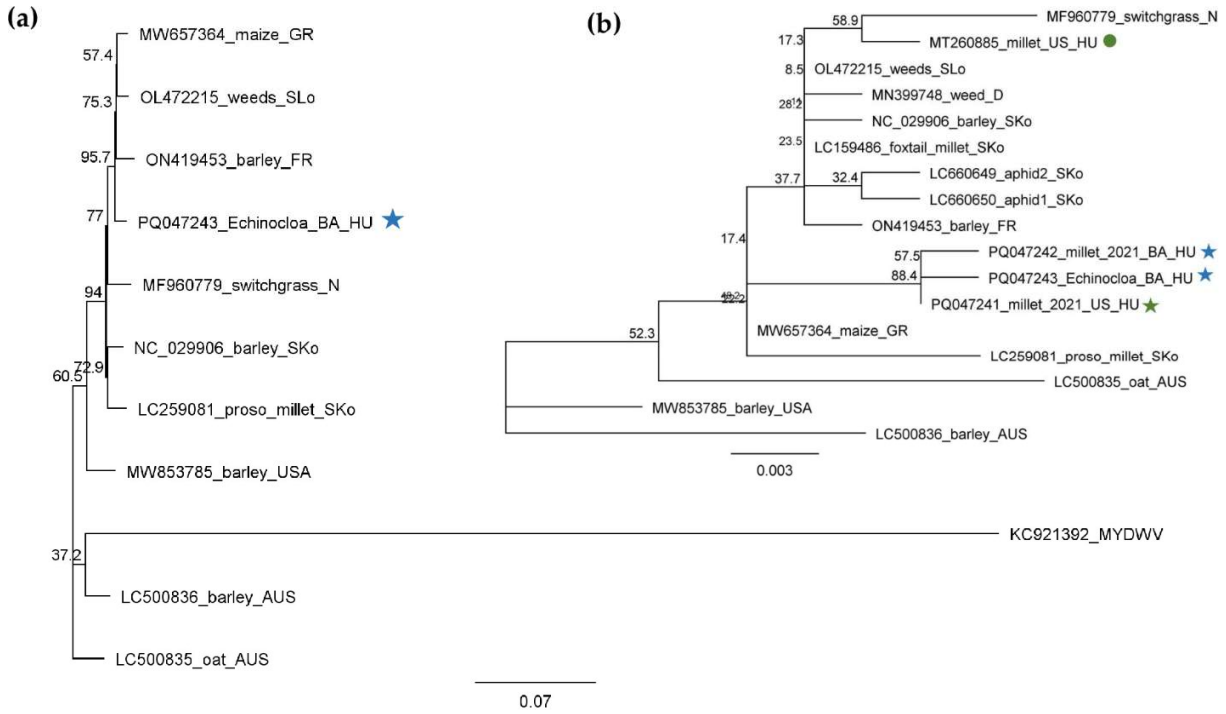

Figure 17. Phylogenetic analysis of the BVG strains originating from BA and US. The phylogenetic tree was constructed based on (a) nearly full (5362 nt long) BVG genomes and (b) the 607 bp long amplified and Sanger-sequenced, CP-coding part of the viral genome using the Neighbour-Joining analysis and the Jukes–Cantor model, with 1000x bootstrap replications. Bars represent (a) 7% and (b) 0.3% nucleotide diversity. Sequences originating from Pasztor et al. (2020) study are marked with circles, whilst sequences from this study are marked with stars. Green represents US, while blue represents BA. Sequences of the different strains are marked with their GenBank accession numbers, host species and countries of origin. GR—Great Britain; SLo—Slovenia; FR—France; HU—Hungary; N—the Netherlands; SKo—South Korea; AUS—Australia; D—Germany. MYDWV—Maize yellow dwarf virus was used as an outgroup to root the tree (Galbács et al., 2024).

4.4.4. Detection and phylogenetic analysis of ApGlV1

Small RNA HTS detected a hit for ApGlV1 in 7_Ma_BA, indicating the existence of 91contigs obtained from ApGlV1, 2359 RPM and 93% viral genome coverage (Table 12). The presence of this newly identified virus was validated using primers designed in line with the small RNA reads, covering the partial capsid protein-encoding region of the viral genome. Aside *Zea mays,* ApGlV1-specific PCR product was also amplified in 4_SH_U and 6_ECGSV_BA. Infection of the individual plants in each species pool was determined using RT-PCR, which showed that three *S. halepense* samples out of five at location U were infected. Two out of six samples of *E. crus-galli*

were infected and three samples out of five *Z. mays* in the pool from BA were infected with the virus (Figure 18).

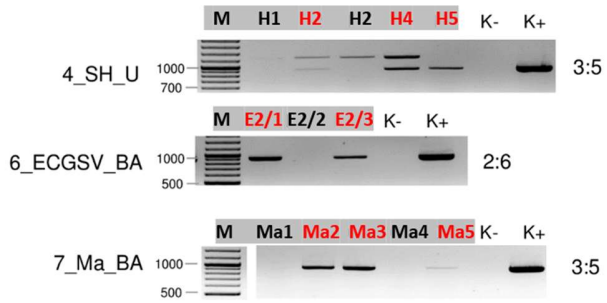

Figure 18. Virus diagnostics using RT-PCR to test the presence of ApGlV1 in the sampled individuals amplifying a 906 bp long part of the ApGlV1 genome using ApGlV1_439F and ApGlV1_1323R primers. M stands for a GenRuler 100 bpPlus, used as a molecular marker. K− and K+ are the negative positive control. Red indicates positive individuals. The infection rate of the plant species is also indicated.

Small RNA HTS exclusively detected ApGlV1 infection in maize, as the size distribution of the small RNA reads obtained from ApGlV1 revealed that they were primarily at length of 20nt. The reads in 4_SH_U had peaks at 21 and 24nt, whereas in 6_ECGSV_BA, they were 24nt long. The sequences of the cloned products showed no distinction between the strains present in the three different species. The Hungarian variant was very similar to other sequences, the sequence identity of all available strains was higher than 98.2%. Beside their high similarity, the variants clustered together according to their geographical origin, regardless of their plant or insect host, suggesting their on-site origin (Figure 19). The Hungarian variant however, clustered distantly with the sequence of ApGlV1 closest homologue: Tetranychus truncatus picorna-like virus 2 (TTPV2), which was employed as an outgroup. It is noteworthy that considering the amplified part, this virus is 92% highly identical to the ApGlV1 variants, and could be only a divergent strain of the same virus. This could also suggest that phylogenetically, the Hungarian variant is more connected to TTPlV2 than it is to the other variants, despite the fact that it is only 92% identical to the strain.

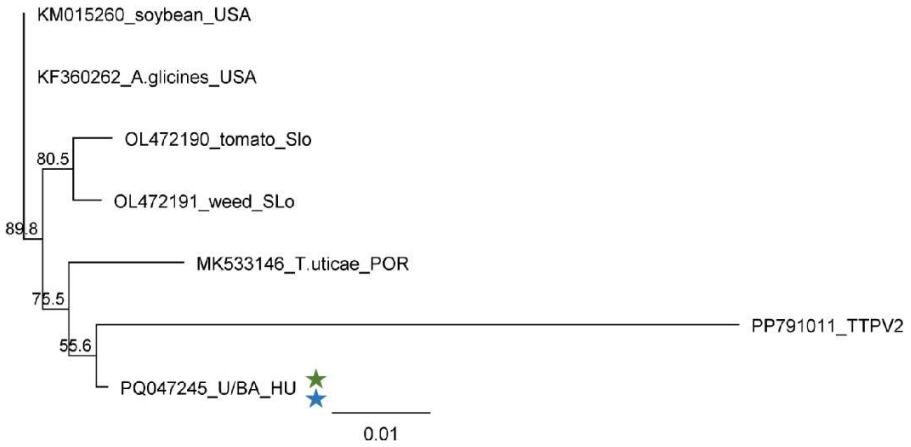

Figure 19. Phylogenetic analysis of the ApGlV1 strains originating from U and BA. The phylogenetic tree was constructed based on the 906 nt long amplified and Sanger-sequenced, VP4/VP3-coding part of the viral genome using the Neighbour-Joining analysis and the Jukes–Cantor model, with 1000x bootstrap replications. Bars represent 1% nucleotide diversity. Sequences originating from this study are indicated with stars. Green represents US, while blue represents BA. Sequences of the different strains are marked with GenBank accession numbers, host species and countries of origin. SLo—Slovenia; POR—Portugal; HU—Hungary. PP791011—Tetranychus truncatus picorna-like virus 2 (TTPV2) was used as an outgroup to root the tree (Galbács et al., 2024).

4.4.5. Detection and phylogenetic analysis of LDV1

Small RNA HTS revealed the presence of LDV1 in 7_Ma_BA. To probe further, LDV1-specific 816 bp products were amplified in three additional libraries utilizing an in-house designed primers. The libraries were 3_SVCD_US, 5_M_BA and 6_ECGSV_BA. LDV1 has not been described in any of these plant species, the rationale behind its further validation for the presence of this recently described virus in individuals of the species pools of this study. In the tested species, relatively high infection rates were recorded. Four *S. viridis* out of 5 in US, nine millet out of 10 at BA, three *E. crus-galli* out of 3 and four maize out of 5 emerged to be infected by LDV1 (Figure 20).

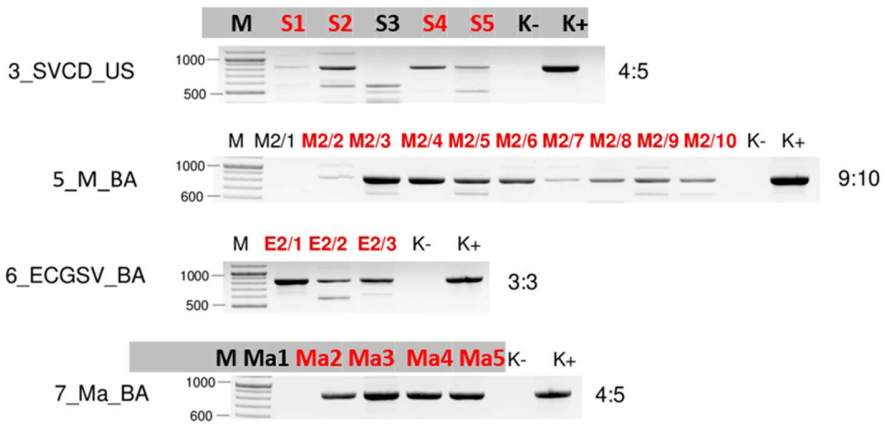

Figure 20**.** Virus diagnostics using RT-PCR to test the presence of LDV1 in the sampled individuals amplifying an 838 bp long part of the LDV1 genome using LDV1_5664F and LDV1_6480R primers. M stands for a GenRuler 100 bpPlus, used as a molecular marker. K− and K+ are the negative and positive controls. Red indicates positive individuals. The infection rate of the plant species is also indicated (Galbács et al., 2024).

The outcome from the cloned and sequenced LDV1 obtained amplified products found that they are identical in the case of *S. viridis* and *P. miliaceum*, but slightly distinct from the other two species. The Hungarian variants were 97.9 – 99.4% identical to each other whilst the Slovenian variant 95.9 – 97.8% identical. The variants found in BA in *E. crus-galli* and maize clustered together, proving their common origin (Figure 21).

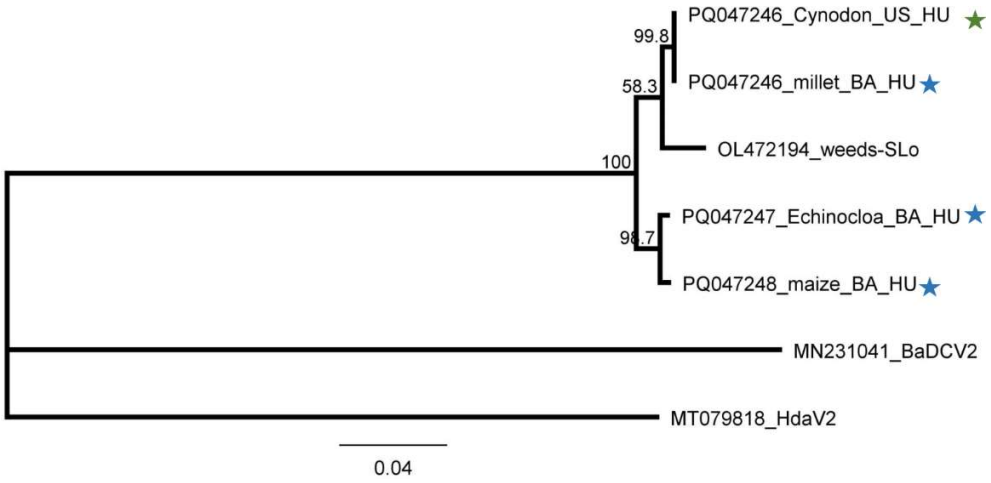

Figure 21. Phylogenetic analysis of the LDV1 strains originating from US and BA. The phylogenetic tree was constructed based on the 838 nt long amplified and the Sanger-sequenced part of the viral genome, encoding the end of the viral RdRP and the beginning of the CP, using the Neighbour-Joining analysis and the Jukes–Cantor model, with 1000x bootstrap replications. Bars represent 4% nucleotide diversity. Sequences originating from this study are indicated with stars. Green represents US, while blue represents BA. Sequences of the different strains are marked with their GenBank accession numbers, host species and countries of origin. SLo—Slovenia; HU—Hungary. MN231041—Bemisia-associated dicistrovirus 2 (BaDCV2) was used as an outgroup to root the tree (Galbács et al., 2024).

4.4.6. Infection rates, multiple and co-infections of the identified viruses

In this current study of plant virus diagnosis survey, where 56 plants showing virus-like symptoms were randomly sampled, SRNA HTS combined with RT-PCR revealed a high infection rate of the plants, revealing virus infection in 37 of them. Whilst 18 plants were infected with a single virus, 19 were infected with multiple viruses (eight, nine and two plants were infected with two, three and four viruses, respectively). The infection rate in US was 46% (14 plants out of 30), 60% in U (3 plants out of 5) and 95% at BA, where only one plant out of 21 was not infected with any of the viruses and 13 were coinfected. Whilst all the five viruses found in this study were detected in BA, only ApGlV1 was found in the sampled *S. halepense* plants in location U as WSMV, BYSMV, BVG and LDV1 were found in the study location of US.

4.4.7. Possible Viral Persistence of WSMV, BYSMV and BVG in two of the experimental locations

In a previous project of Pasztor et al. (2020) surveying two millet (*P. milliaceum*) populations, the study revealed the presence of WSMV, BYSMV and BVG in millet at US and BA. In this study, to investigate if these viruses could still be present and analyze its possible viral persistence (Table 13), the findings showed that in US and BA, WSMV was detected again in samples *P. milliaceum* (3:11) for US and (4:10) for BA, however, in a lower infection rate as compared to the study of Pasztor et al. (2020) who recorded higher rates and widespread of WSMV (7:10) in US and (9:10) infected *P. milliaceum* plants in BA. In US, *E. crus-galli* plants of (5:8) were WSMV infected while all *E. crus-galli* plants in the pool at BA were also infected. *C. dactylon* weeds of (5:11) were WSMV infected at US, and all samples of *S. viridis* in the pool at BA were significantly infected. In the case of BYSMV, the study revealed again the presence of this virus in *P. milliaceum* plants in BA (3:10). In US, the study revealed that *C. dactylon* weeds samples were also infected by BYSMV (3:11), indicating a possible new host of BYSMV. The study recorded an interesting outcome of BVG infecting *E. crus-galli* in BA (1:6).

Table 13. Virus persistence of WSMV, BYSMV and BVG in study locations of US and BA

Pasztor et al. (2020) US	***Panicum milliaceum* BA**	**In this study US**	***Panicum milliaceum* BA**	**New hosts identified in this study**
WSMV (7:10)	9:10	3:11	4:10	*Echinochloa crus-galli* (in both US and BA) *Cynodon dactylon* at US *Setaria viridis* detected at BA
BYSMV (3:10)	1:10	0:11	3:10	*Cynodon dactylon* weed of US
BVG (0:10)	1:10			*Echinochloa crus-galli* infected at BA

Plant virus persistence is a significance occurrence which needs to analyzed and addressed on crop fields. In its assessment whether in low or high infection rates, this activity could serve as the initiator and leads to the build up of plant viral populations in our crop fields. In the case of this study where these viruses have been detected in the same experimental locations year on after its description, coupled with identifying new hosts of these viruses, the study has helped to reveal this occurrence and the information provided could be employed when planning and organizing all-inclusive sustainable management practices against these viruses on the experimental fields.

4.5. Discussions of the results

The outcome of the field assessment of the study has reiterated that indeed, field assessment on crop fields is an important activity of any plant virus diagnosis program. In the field assessment study, researchers visit the experimental or crops fields to be able to have first-hand information about the possible occurrence of viral infections on the field through visual inspections and assessment. During the assessment, the collected samples enables deeper examination into the occurrence. Moreover, through this activity, sometimes the extent of damage caused at the time of assessment is also identified and analyzed as reported by Martinelli et al. (2015) who testified that, the monitoring, assessment and detection of pathogen in plants are essential to ease disease spread and facilitate effective management practices.

Virus infections in susceptible host plants mostly results in a series of physiological disorders that have undesirable consequences on the overall plant heath. The symptoms typical of virus infections are quite diverse and may appear on any part of the infected plants. The occurrence and severity of these symptoms could be attributed to factors such as time of infection, virus strain interactions of the virus, the host plant, the virus vector and the environment (Gergerich and Dolja 2006; Islam et al., 2020). Also, infections of different viruses of the same host could affect host range, transmission, virus accumulation and as a consequence, the existence and intensity of symptoms (Wintermantel et al., 2008).

Long before diagnostic tools and techniques were developed, viral symptoms played a key role in early identification of viral diseases. As a matter of fact, it is still relevant today because to evaluate virus effects, attention is often paid to symptoms such as characteristic discoloration, abnormal morphology, reddish brown, purple discoloration, chlorosis, mottling and necrosis. The early

detection of wild plant adapted plant viruses years ago was characterized by the identification of typical symptoms of diseases they caused in cultivated plants. The first attempts to monitor viruses in wild species were constrained by the lack of sensitive diagnostic methods. Moreover, researchers were interested only in plants with clear symptoms of the disease. Plant viral symptoms have played a significant role in early diagnosis of viral diseases on crop fields (Wren et al 2006). However, different viruses can produce similar symptoms or different strains of a virus can cause distinct symptoms in the same host. In some distinct cases, vector feeding patterns and abiotic or environmental factors could result in symptoms which may not correspond with infections occurrences. This notion was proven in this study where investigating the virus-specific symptoms on the plants did not coincide with their infection status. This is because symptomatic plants were sampled (Figure 8,9,10) but 19 of them turned out not infected. Plants infected with several viruses did not show stronger symptoms than the non-infected ones. That is why it is necessary to state that, whilst symptoms provide vital information on virus diseases, an accurate and effective plant virus diagnosis program does not involve only the symptoms. Also, adequate field experience is required when making a decision on symptomatology. Usually, it is necessary that visual inspection for symptoms in the field is done in conjunction with other confirmatory tests to ensure accurate diagnosis of virus infection (Bock, 1982).

Identifying weed species that can act as potential plant virus reservoirs can influence pest management during a given growing season, letting the grower to prioritize accordingly. It is worth the effort to scout for weeds growing on crop fields and in areas adjacent to the fields that are otherwise ignored. The prevalence of certain weeds and potential pathogens could also be managed through good agronomic and cultural practices on the fields, like the timing of planting, crop cultivar or variety and crop rotation schedule (Byron et al., 2019).

HTS method was first used to study plant viruses in 2009 (Adams et al., 2009). Since then, researchers have identified several RNA and DNA viruses using this method, some of which have not been described before (Barba et al., 2014; Roossinck et al., 2015). The common HTS method presently used for viral metagenomics is Illumina sequencing. In this study, small RNA HTS of the investigated samples revealed the presence of five viruses. Three of the hits: WSMV, BYSMV and BVG, were viruses found two years earlier at the same location. In the sRNA HTS-positive libraries the presence of these viruses were clear, and the high number of the viral reads were evenly distributed on the viral genome. Their size was dominantly 21nt, reflecting active antiviral

DICER (DCL2 and DCL4) activity (Pooggin, 2018). The presence of ApGlV1 and LDV1 was found only because a pipeline including recently described viruses from Slovenian rural samples (Rivarez et al., 2023) were used. In that study, sequences of 165 viral strains representing different viruses, originating from Slovenia have been determined and deposited into the GenBank allowing a search for their presence with a simple pipeline. The outcome of the RT-PCR validation revealed infection in more cases than the sRNA HTS itself (Table 14).

Table 14. Summary of the sRNA HTS and RT-PCR diagnostics. The infection rate of the plant species is also indicated (Galbács et al., 2024).

Library	test	WSMV	BYSMV	BVG	ApGlV1	LDV1
1_M_US	**sRNA HTS**	-	-	-	-	-
	RT-PCR	3:11	-	3:11	-	-
2_ECG_US	**sRNA HTS**	-	-	-	-	-
	RT-PCR	5:8	-	-	-	-
3_SVCD_US	**sRNA HTS**	-	-	-	-	-
	RT-PCR	5:11	3:11	-	-	4:11
4_SH_U	**sRNA HTS**	-	-	-	-	-
	RT-PCR	-	-	-	3:5	-
5_M_BA	**sRNA HTS**	+	+	-	-	-
	RT-PCR	4:10	3:10	7:10	-	9:10
6_ECGSV_BA	**sRNA HTS**	-	-	+	-	-
	RT-PCR	6:6	-	1:6	2:6	3:6
7_Ma_BA	**sRNA HTS**	-	-	-	+	+
	RT-PCR	-	-	-	3:5	4:5

During sRNA HTS diagnostics, the sRNA sequencing libraries were prepared from pools, containing a mixture of individual plant extracts. In this case, the RNA of the non-infected plant could dilute the vsiRNA concentration below the sensitivity level. However, the infection rate in the investigated cases was not low. Similar instances occurred in the case of grapevine rupestris stem pitting associated virus and grapevine virus T infecting grapevine when virus infection in grapevine was investigated (Czotter et al., 2018; Demian et al., 2020). In those cases, they

hypothesised that the antiviral RNA response has been weakened during long plant–virus coexistence leading to their latency and absence of strong host response, making sRNA HTS unable to detect thei virus infection. Similarly, this could take place in some of the current cases of this study. In addition, the type of the virus infection can change over time and is influenced by ecological factors, like changing climate and behaviour of the vectors (Elena et al., 2014). Moreover, virus infection happens through different phases. At the beginning the virus infection the host metabolism could be interfered but later can reach a persistent state, and become latent for a long time (Stobbe and Roossinck, 2016).

All individuals of *C. dactylon* in US and all three sampled *S. viridis* plants in BA were WSMV infected, while five (out of 8) and all three *E. crus-galli* were infected in US and BA, respectively. The sequence of the variants found in millet of US, *E. crus-galli*, *C. dactylon* and *S. viridis* were identical and clustered with several WSMV variants of 2019 (Pasztor et al., 2020). The sequence of WSMV variant sequenced from *E. crus-galli* in Iran is also available and clustered into Clade D, characteristics of the Asian (Turkey) variants, with another WSMV variant from the same country but from a *Lolium* host, suggesting that the variants do not show host specificity, but reflects their geographical origin. The variant present in millet of BA clustered distantly from the variants sequenced in 2019 (Pasztor et al., 2020), its closest neighbours were strains sequenced in Poland and Hungary, more than two decades ago (Rabenstein et al., 2004). At 5_M_BA sRNA HTS could successfully detect the infection of WSMV suggesting a new active infection. In contrast to the other cases, the amount of WSMV-derived siRNA was low suggesting a balanced virus titer in the plant without active antiviral silencing. In the millet plants in BA, the believe is that a new infection happened, whilst in the other plants, a lower-level background infection was maintained in the overwintering plants. However, the fact that WSMV has been found in different grasses supports our original hypothesis that they can help the virus to persist and have a virus reservoir role.

BYSMV has been identified in two hosts: millet and *C. dactylon* at two of the sampled locations. To the best of our knowledge, this is the first time to find *C. dactylon* as a host of this virus. SRNA detected BYSMV only in the 5_M_BA, where the number of BYSMV-derived sRNAs was high covering the entire genome. In contrast, BYSMV infection in 3_SVCD_US was missed using sRNA HTS. Although they covered the BYSMV genome, the low number of sRNA reads, matched

with their smeared size distribution, showing the persistent state of the infection in this case. The sequences of the variants were the same, very similar to one of the variants sequenced in 2019 (Pasztor et al., 2020) and have been found at both locations. At that time another variant, present at BA in two millet plants was also identified. It is possible that the infection with the strains originating from 2019 (Pasztor et al., 2020) was maintained persistently in US in overwintering roots of *C. dactylon*, not inducing a strong silencing signal in 2021, while the millet at BA was newly infected resulting in an acute infection, however, this theory should be investigated in the future. *C. dactylon* is an invasive weed, presence of a persistent form of BYSMV in it is alarming as this could serve as a constant source of new virus inoculum.

BVG has been identified to be able to infect *E. crus-galli*, in addition to millet as hosts. While sRNA HTS could detect the infection only in this latter case, a low infection rate was detected in millet at both locations. Although in 6_ECGSV_BA only one plant out of three was infected, the BVG genome was covered with virus-derived small RNAs, which were dominantly 21nt long, suggesting a strong antiviral RNAi in this case. Although several plants (three out of 11 in US and seven out of 10 millets at BA) were BVG infected, sRNA HTS could not detect its presence suggesting that the initial acute infection became persistent without inducing a strong RNAi. In this current study, three slightly different BVG variants were found, which clustered together suggesting their common origin. Their close identity to the variant sequenced in 2019 (Pasztor et al., 2020) at the same location could suggest the persistent presence of BVG in the millet population, but its new introduction is better supported because of its distant clustering. BVG could be present in several places for a long time as a latent infection, and perennial overwintering monocotyledonous weeds can play an important role in its maintenance. BVG has the potential to express P0 protein acting as a VSR in other *Poleroviruses* through destabilizing the antiviral Argonaute proteins (Csorba et al., 2010; Csorba et al., 2015).

In this study, ApGlV1 has been found to infect three different plant species: *S. halepense*, *E. crus-galli* and *Z. mays* at two locations. Only one variant of the virus was found, similar to other ApGlV1 sequences from insects or plants (soybean or tomato), suggesting a very high conservation of its genome. Infection with ApGlV1 seems to be persistent in *S. halepense* and *E. crus-galli*, but acute in the case of maize, reflected by the presence of a high number of vsiRNAs covering the entire genome.

The identification of ApGlV1 in weed samples of this study, and more interestingly in maize plants provides another dimension when it comes to the possible diversified host range of the virus. Most plant species (both crop plants and weed species) from which ApGIV1 has been described are dicotyledonous. The detection of ApGlV1 in monocotyledonous plant samples of this study reveals that, monocot plants could also serve as host of ApGlV1. In recent years, high throughput sequencing analysis has ignite the acceleration of finding new aphid viruses in more plant species (Chang et al., 2020; An et al., 2021).

LDV1, the recently found dicistrovirus, was detected in four plant species at both locations, describing *C. dactylon*, *P. miliaceum*, *E. crus-galli* and *Z. mays* a new host of the virus. We found three slightly different variants, suggesting its onsite evolution. The majority of the vsiRNAs were 21nt long in the case of the millet samples, characteristics for the acute phase of the infection with an active RNAi, while in the other species, the infection seemed to reach the persistent state. It is interesting to note, that the size distribution of the vsiRNAs showed maximum at 24nt in *C. dactylon* and *E. crus-galli*, while the majority of them was 20nt long in maize. LDV1 was found to infect plants, but being a dicistrovirus, that is usually present in insect vector species, it can be supposed that its presence and distribution was orchestrated by insect vector species.

After its original description in Slovenia, the identification of LDV1 weeds and maize samples of this study in Hungary is a significant findings, which might suggest a host range for this virus. Weeds may serve as natural reservoirs and alternative hosts for new crop-infecting viruses, as suggested by the discovery of these viruses in weed plants (Cooper and Jones, 2006). ApGlV1 and LDV1 have been described recently and there is no information about their possible impact on host fitness which question is also open for further research. The control of the aphids insect vectors, monitoring virus diversity and evolutionary changes of widespread viruses could help anticipate emergence of plant viral strains, thus inhibiting disease outbreaks (McLeish et al., 2021).

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Detection of viruses in plant material and natural reservoirs is essential to ensure safe and sustainable agriculture. In the study of plant virus diagnosis of weeds on crop fields and the investigation of their role as virus reservoir, the field assessment and visual inspection activity led to the identification of viral associated symptoms ranging from mosaics to spots of leaves on samples collected from the study locations from which small RNA HTS combined with RT-PCR analysis were conducted.

The results of bioinformatics analysis of small RNA HTS revealed virus hit list of the investigated samples which were collected from the experimental locations of US, U and BA. Viruses that were recorded to be present in the weed samples collected were WSMV, BYSMV, BVG, ApG1V1 and LDV1.

Cereal viruses WSMV, BYSMV and BVG were detected in the study location of BA. The study also enabled the confirmation of the presence of WSMV in *E. crus-galli* weeds and millet at US and BA, *C. dactylon* at US and *S. viridis* at BA. BYSMV was found present in weed samples of *C. dactylon* in US and first time in this study, *E. crus-galli* is reported as new host of the polerovirus BVG. Alongside acute infections, several cases of persistent infections were recorded, suggesting possibilities for virus reservoir roles of these plants.

Spectacular variations were observed between the infection rates of the sampled locations. In the study location of US was found infected with three viruses: WSMV, BVG and ApGlV1. At this place, wheat and maize were cultivated alternatively and in the year of sampling, wheat was grown, and were just harvested before the sample collection. In U, where no intensive cultivation is carried out, we only sampled an established population of *S. halepense*, which was only infected by ApGlV1. BA was highly infected with viruses and we found coinfection of the plants in several cases. Here maize was cultivated and we also found viruses in the crop. This place is just at the edge of a little creek where several plants are widely grown on its bank, offering a more humid place and alternative shelter for the vectors.

The study further reports the presence of ApGlV1 and LDV1 in Hungary. These are newly described viruses from Slovenia whose presence have not been reported in Hungary. The study

revealed *S. halepense*, *E. crus-galli* and *Z. mays* as new hosts of ApGlV1 whilst LDV1 was detected in different hosts including *C. dactylon* and *P. milliaceum*.

In the study of possible viral persistence maintained by these plant viruses a year on after their description in US and BA, the results of this study showed that, WSMV was found to be present again in *P. milliaceum* from US and BA, but in a lower infection rate. Factors such as nutritional behavior, viral transmission mediated by insect vectors, dietary preferences and population dynamics could influence the final outcome of viral infections. This study is a step towards expanding research in plant virus diagnosis of weeds in cultivated crops fields, to help provide information that would enhance protecting crops and improving yields.

Globalization coupled with the possible effects of climate change also facilitate spread of viruses and their vectors, thereby altering the diagnostic landscape. It is important to note that, with the changing adaptability, characteristic dynamic nature of plant pathogenic viruses and the reservoir role of surrounding plants, it is necessary to optimize protocols and establish better routine assays and techniques that reveals all presenting plant viruses in an investigated sample and also meeting the changing needs of emerging viruses and their relationships.

5.2. Recommendations

Weeds could host more viruses as it is currently described and could to serve as fundamental sources of virus discoveries in the future for a while. Though this study has described a number of plant viruses detected in monocotyledonous weeds including the identification of new ones in the studied region, plant virus diagnosis of dicotyledonous weeds on crop fields could be recommended.

Our understanding of the details of fluxes of viruses from crops to weeds and from weeds to crops is far from complete, as our knowledge on virus reservoir role of weeds is still improving. More future research in this area are highly recommended.

6. NEW SCIENTIFIC RESULTS

On the account of the results obtained from the experiments conducted in line with the present research topic, the following points highlight the new scientific results of the study.

1. We confirmed that WSMV infects *Cynodon dactylon* and *Setaria viridis* and provided partial sequence of the infecting WSMV strain.
2. We first described *Cynodon dactylon* as a new host of BYSMV and *Echinochloa crus-galli* as a new identified host of BVG.
3. We first described the presence of ApGlV1 in Hungary. *Sorghum halepense*, *Echinochloa crus-galli* and maize have been identified as new potential hosts of the virus.
4. We detected Ljubljana dicistrovirus 1 first time in Hungary, and identified *Setaria viridis*, *Echinochloa crus-galli*, *Panicum milliaceum* and *Zea mays* as its new potential hosts.

7. SUMMARY

The dissertation covers plant virus diagnosis of weeds on crop fields and investigation of their role as virus reservoirs, focusing on monocotyledonous weeds. Monocot weeds pose a greater challenge for crops because they occupy comparable ecological niches to cereal vegetation. Cereal crops such as maize, wheat and rice are among the widely produced crops worldwide. The presence of monocot weeds in agricultural fields poses a risk to these cereal crops. They could play as reservoirs of pathogenic viruses on crop fields, their rapid growth and prolific seed production can lead to dense populations and out-competing of the cereal crops and indirectly mount disease pressures.

In a previous study examining virome of millet plants as weeds in field crops using small RNA HTS, the presence of three viruses was detected. The viruses were wheat streak mosaic virus (WSMV), which was known to be present in Hungary and two others, barley yellow striate mosaic virus (BYSMV) and barley virus G (BVG), which were not known to be present in Hungary (Pasztor et al., 2020). Based on these findings, the authors hypothesized that monocotyledonous weeds may play a significant role in the persistence and spread of plant viruses.

To further investigate this in the summer of 2021, a total of 56 plant leaf samples were collected from millet plants (*P. milliaceum*) grown as weed, maize and four monocotyledonous weed species (*C. dactylon*, *E. crus-galli*, *S. halepense* and *S. viridis*) from sampling areas of US, U and BA in Keszthely. The collected plants showed viral associated symptoms. Nucleic acids were purified from the samples and small RNAs were isolated. Small RNA sequencing libraries were prepared based on the sampling areas and sequenced on Illumina platform. Sequences were analyzed using bioinformatic pipelines. FastQ files of the sequenced libraries were analyzed using CLC Genomic software package. The results of the virus hit list were confirmed using RT-PCR.

The bioinformatic results of the study revealed virus hit list of WSMV, BYSMV, BVG, ApGlV1 and LDV1 present in the investigated samples of the study.

The RT-PCR results of the study showed that samples of *E. crus-galli* and *S. viridis* tested positive for WSMV. The results further indicated that, weed samples of *C. dactylon* were infected by BYSMV in US, whilst BVG was first time reported in samples of *E. crus-galli* from BA. The

research results proved that WSMV, BYSMV and BVG were detected in the study location of BA. The study also enabled the validation of the occurrence of WSMV and BYSMV in the study area of US. After the description of WSMV, BYSMV and BVG in the sampling area of US and BA (Pasztor et al., 2020), the results of the current study confirmed that, WSMV was still found present in millet samples from US and BA.

The research reports the identification of two new viruses originally described from Slovenia and has not yet been described in Hungary; the ApGlV1 and LDV1. Interestingly these two viruses were found present in maize plants and *E. crus-galli* weeds sampled from BA. In addition, weed samples of *S. halepense* from the study location of U were infected with ApGlV1 whilst *S. viridis* and *P. milliaceum* were infected with LDV1 in the study location of US and BA respectively. The results of the research suggest that monocotyledonous weed plants could assist plant viruses to persist and act as virus reservoirs in cultivated crops.

8. REFERENCES

Achon, M.A., Alonso-Dueñas, N., Serrano, L. 2011. Maize dwarf mosaic virus diversity in the Johnsongrass native reservoir and in maize: evidence of geographical, host and temporal differentiation. Plant pathology, 60(2), pp. 369-377.

Achon, M.A., Larranaga, A., Alonso-Duenas, N. 2012. The population genetics of maize dwarf mosaic virus in Spain. Archives of virology 157, pp. 2377-2382.

Achon, M.A., Serrano, L., Clemente-Orta, G., Sossai, S. 2017. First report of Maize chlorotic mottle virus on a perennial host, Sorghum halepense, and maize in Spain. Plant Disease, 101(2), pp. 393-393.

Adams, I. P., Glover, R. H., Monger, W.A., Mumford, R., Jackeviciene, E., Navalinskiene, M. 2009. Next-generation sequencing and metagenomics analysis: A universal diagnostic tool in plant virology. Molecular Plant Pathology, 10, 537–545.

Adams, M.J., Zerbini, F.M., French, R., Rabenstein, F., Stenger, D.C., Valkonen, J.P.T. 2012. Virus Taxonomy: 9th report of the International Committee on the Taxonomy of Viruses, ed. By A.M.Q. King, M.J. Adams, E.B. Carstens (Elsevier Academic Press, San Diego, 2012), pp. 1069–1089.

Alexander, H.M., Mauck, K.E., Whitfield, A.E., Garrett, K.A., Malmstrom, C.M. 2014. Plant–virus interactions and the agroecological interface. European Journal of Plant Pathology, 138: 529-547.

Almasi, R., Afsharifar, A., Niazi, A., Pakdel, A., Izadpanah, K. 2010. Analysis of the complete nucleotide sequence of the polymerase gene of barley yellow striate mosaic virus- iranian isolate. Journal of Phytopathology, 158, 351–356.

An, X., Zhang, W., Ye, C., Smagghe, G., Wang, J.J., Niu, J. 2021. Discovery of a widespread presence bunyavirus that may have symbiont-like relationships with different species of aphids. Insect Science, pp. 1–6. doi: 10.1111/1744-7917.12989.

Banttari, E.E., Goodwin, P.H. 1985. Detection of potato viruses S, X, and Y by enzyme-linked immunosorbent assay on nitrocellulose membranes (dot-ELISA). Plant Disease 69, pp. 202–205.

Barba, M., Czosnek, H., Hadidi, A. 2014. Historical perspective, development and applications of next-generation sequencing in plant virology. Viruses, 6, 106–136.

Berger, P.H., Adams, M.J., Barnett, O.W., Brunt, A.A., Hammond, J., Hill, J.H., Jordan, R.L., Kashiwazaki, S., Rybicki, E., Spence, D.C. Stenger, S.T. Ohki, I. Uyeda, A. van Zaayen, J. Valkonen, H.J. Vetten. 2005 in Virus taxonomy, 8th report of the ICTV, ed. by C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A. Ball (Elsevier Academic Press, San Diego).

Bernardo, P., Charles-Dominique, T., Barakat, M., Ortet, P., Fernandez, E., Filloux, D., Hartnady, P., Rebelo, T.A., Cousins, S.R., Mesleard, F., Cohez, D. 2018. Geometagenomics illuminates the impact of agriculture on the distribution and prevalence of plant viruses at the ecosystem scale. The ISME journal, 12(1), pp.173-184.

Biel, S.S., Nitsche, A., Siegert, W., Ozel, M., Gelderblom, H.R. 2004. Detection of human polyomaviruses in urine from bone marrow transplant patients: a comparison of electron microscopy with PCR. Clinical Chemistry 50, pp. 306–312.

Biswas, K., Jhabarmal, J., Tarafdar, A. 2016. Detection of plant viruses for their management: Recent trends trends plant pathogen interaction. In Plant Pathogen Interaction: Recent Trends; Mitra, R., Barman, A., Eds.; Sharma Pubs & Distributors: New Delhi, India, pp. 71–88. ISBN 9789382310051.

Bock, K. R. 1982. The identification and partial characterisation of plant viruses in the tropics. International Journal of Pest Management, 28(4), 399-411.

Brown, H.R. 1999. C4 Plants and humanity. In: Sage, R.F., Monson, R.K. (Eds.), C4 Plant Biology. Academic Press, San Diego, CA, pp. 473–508.

Byron, M., Treadwell, D.D., Dittmar, P.J. 2019. Weeds as Reservoirs of Plant Pathogens Affecting Economically Important Crops: HS1335, 9/2019. EDIS, 2019 (5), pp.7-7.

Canto, T., Aranda, M. A., Fereres, A. 2009. Climate Change Effects on Physiology and Population Processes of Hosts and Vectors That Influence the Spread of Hemipteran-Borne Plant Viruses. Global Change Biology, 15 (8), 1884–1894. https://doi.org/10.1111/j.1365-2486.2008.01820.x.

Cao, M., Du, P., Wang, X., Yu, Y.Q., Qiu, Y.H., Li, W., Gal-On, A., Zhou, C., Li, Y., Ding, S.W. 2014. Virus infection triggers widespread silencing of host genes by a distinct class of endogenous siRNAs in Arabidopsis. Proceedings of the National Academy of Sciences, 111(40), pp. 14613-14618. doi: 10.1073/pnas.1407131111.

Cao, Q., Xu, W. Y., Gao, Q., Jiang, Z. H., Liu, S. Y., Fang, X. D., Gao, D. M., Wang, Y., Wang, X. B. 2018. Transmission characteristics of barley yellow striate mosaic virus in its planthopper vector Laodelphax striatellus. Frontiers in Microbiology 9, 1419.

Chalupniková, J., Kundu, J.K., Singh, K., Bartaková, P., Beoni, E. 2017. Wheat streak mosaic virus: incidence in field crops, potential reservoir within grass species and uptake in winter wheat cultivars. Journal of Integrative Agriculture, 16(3), pp. 523-531.

Chang, T.Y., Guo, M.M., Zhang, W., Niu, J.Z., Wang, J.J. 2020. First report of a mesonivirus and its derived small RNAs in an aphid species Aphis citricidus (Hemiptera: Aphididae), implying viral infection activity. Journal of Insect Science 20, 14.

Chao, S., Wang, H., Zhang, S., Chen, G., Mao, C., Hu, Y., Yu, F., Wang, S., Lv, L., Chen, L. Feng, G. 2022. Novel RNA viruses discovered in weeds in Rice Fields. Viruses, 14(11), p. 2489. https://doi.org/10.3390/v14112489.

Chen, J., Chen, J., Adams, M.J. 2002. Characterisation of potyviruses from sugarcane and maize in China. Archives of Virology, 147, pp. 1237-1246.

Chen, S., Jiang, G., Wu, J., Liu, Y., Qian, Y., Zhou, X. 2016. Characterization of a novel polerovirus infecting maize in China. Viruses, 8(5), p. 120.

Chen, W., Hasegawa, D., Arumuganathan, K., Simmons, A., Wintermantel, W., Fei, Z., Ling, K.S. 2015. Estimation of the Whitefly Bemisia Tabaci Genome Size Based on K-Mer and Flow Cytometric Analyses. Insects, 6 (3), 704–715. https://doi.org/10.3390/insects6030704.

Choi, Y.M., Lee, S.H., Ryu, G.H. 1989. Studies on the host range of rice black-streaked dwarf virus. Research Reports of the Rural Development Administration, Crop Protection, Korea Republic, 31(1), pp. 14-18.

Clark, M.F., Adams, A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34, pp. 475–483.

Clark, A. J., Perry, K. L. 2002. Transmissibility of field isolates of soybean viruses by Aphis glycines. Plant Disease 86, pp. 1219-1222.

Claverie, S., Hoareau, M., Chéhida, S.B., Filloux, D., Varsani, A., Roumagnac, P., Martin, D.P., Lett, J.M., Lefeuvre, P. 2023. Metagenomics reveals the structure of Mastrevirus–host interaction network within an agro-ecosystem. Virus Evolution, 9(2), p.vead043.

Clements, D. R., DiTommaso, A., Hyvönen, T. 2014. Ecology and Management of Weeds in a Changing Climate. In Recent Advances in Weed Management; Chauhan, B. S., Mahajan, G., Eds.; Springer New York: New York, NY, pp 13–37. https://doi.org/10.1007/978-1-4939-1019-9_2.

Conti, M. 1969. Investigations on a bullet-shaped virus of cereals isolated in Italy from planthoppers. Journal of Phytopathology 66, 275–279. doi: 10.1111/j.1439-0434.1969.tb02437.x.

Cooper I, Jones, R.A.C. 2006. Wild plants and viruses: under-investigated ecosys-tems. Advances in Virus Research 67, pp. 1–47.

Copping, L. G., Naylor, R. E. L. 2002. Herbicide discovery. Weed Management Handbook.

Coutts, B.A., Kehoe, M.A., Webster, C.G., Wylie, S.J. and Jones, R.A.C. 2011. Zucchini yellow mosaic virus: biological properties, detection procedures and comparison of coat protein gene sequences. Archives of Virology 156, 2119–2131 (119).

Crowther, J.R. 2001. The ELISA guidebook. Totowa, NJ: Humana. p. 421.

Csorba, T., Kontra, L., Burgyán, J. 2015.viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. Virology, 479-480, 85-103, doi: https://doi.org/10.1016/j.virol.2015.02.028.

Csorba, T., Lózsa, R., Hutvágner, G., Burgyán, J. 2010. Polerovirus protein P0 prevents the assembly of small RNA-containing RISC complexes and leads to degradation of ARGONAUTE1. The Plant Journal, 62, 463-472, doi:https://doi.org/10.1111/j.1365-313X.2010.04163.x.

Czotter, N., Molnar, J., Szabó, E., Demian, E., Kontra, L., Baksa, I., Szittya, G., Kocsis, L., Deak, T., Bisztray, G., Tusnady, G.E. 2018. NGS of virus-derived small RNAs as a diagnostic method used to determine viromes of Hungarian vineyards. Frontiers in Microbiology, 9, p. 122. doi:10.3389/fmicb.2018.00122.

Defelice, M.S. 2002. Green Foxtail, Setaria viridis (L.) P. Beauv. Weed Technology 16: 253–257.

Delwart, E.L. 2007. Viral metagenomics, Reviews in Medical Virology vol. 17, no. 2, pp. 115–131. https://doi.org/10.1002/rmv.532.

Demian, E., Jaksa-Czotter, N., Molnar, J., Tusnady, G.E., Kocsis, L., Varallyay, E. 2020. Grapevine rootstocks can be a source of infection with non-regulated viruses. European Journal of Plant Pathology, 156, 897-912, doi:10.1007/s10658-020-01942-w.

Di, D.P., Zhang, Y.L., Yan, C., Yan, T., Zhang, A.H., Yang, F., Cao, X.L., Li, D.W., Lu, Y.G., Wang, X.B., Miao, H.Q. 2014. First report of Barley yellow striate mosaic virus on wheat in China. Plant Disease, 98(10), pp. 1450-1450. . doi: 10.1094/PDIS-06-14-0579-PDN.

Douglas, B. J., T. A. Gordon, I. N. Morrison, M. G. Maw. 1985. The biology of Canadian weeds. 70. Setaria viridis (L.) Beauv. Can. Journal of Plant Sciences 65, pp. 669–690.

Drab, T., Svobodov a, E., Ripl, J., Jarosova, J., Rabenstein, F., Melcher, U. and Kundu, J.K. 2014. SYBR Green I based RT-qPCR assays for the detection of RNA viruses of cereals and grasses. Crop Pasture Science 65, 1323–1328 (115).

Du, Z.Q., Li, L., Liu, L., Wang, X.F., Zhou, G. 2007. Evaluation of aphid transmission abilities and vector transmission phenotypes of barley yellow dwarf viruses in China. Journal of Plant Pathology. Jul 1, pp. 251-9.

Dunoyer, P., Voinnet, O. 2005. The complex interplay between plant viruses and host RNA-silencing pathways. Current Opinion in Plant Biology 8, pp. 415–423.

Elbeaino, T., Digiaro, M., Mielke-Ehret, N., Muehlbach, H.P., Martelli, G.P. 2018. ICTV Virus Taxonomy Profile: Fimoviridae. Journal of General Virology 99, pp. 1478–1479.

Elena, S. F. 2011. Evolutionary constraints on emergence of plant RNA viruses. In C. Caranta, M. A. Aranda, M. Tepfer, & J. J. López-Moya (Eds.), Recent Advances in Plant Virology (pp. 283-300). United Kingdom: Caister Academic Press.

Elena, S. F., Fraile, A. 2014. García-Arenal, F. Evolution and Emergence of Plant Viruses. Advances in virus research, 88, 161–191.

Erickson, A., Falk, B. 2022. Barley virus G (Barley virus G).

Erickson, A., Falk, B.W. 2021. First report of barley virus G in the United States and California. Plant Disease, 105(10):3312. DOI:10.1094/PDIS-03-21-0478-PDN.

Evans, C.K., Bag, S., Frank, E., Reeve, J., Ransom, C., Drost, D., Pappu, H.R. 2009. Green foxtail (Setaria viridis), a naturally infected grass host of Iris yellow spot virus in Utah. Plant Disease, 93(6), pp. 670-670.

Fang, X., Qi, Y. 2016. RNAi in plants: an argonaute-centered view. Plant Cell 28, 272–285. doi: 10.1105/tpc.15.00920.

Fargette, D., Konaté, G., Fauquet, C., Muller, E., Peterschmitt, M., Thresh, J. M. 2006. Molecular Ecology and Emergence of Tropical Plant Viruses. Annual Review of Phytopathology 44 (1), pp. 235–260. https://doi.org/10.1146/annurev.phyto.44.120705.104644.

Ford, R.E., Tosic, M. 1972. New hosts of maize dwarf mosaic virus and sugarcane mosaic virus and a comparative host range study of viruses infecting corn. Journal of Phytopathology 75(4), pp. 15–48. https://doi.org/10.1111/j.1439-0434.1972.tb02627.x.

Forsius, M., Anttila, S., Arvola, L., Bergström, I., Hakola, H., Heikkinen, H.I., Helenius, J., Hyvärinen, M., Jylhä, K., Karjalainen, J., Keskinen, T. 2013. Impacts and adaptation options of climate change on ecosystem services in Finland: a model based study. Current Opinion in Environmental Sustainability, 5 (1), pp. 26-40.

French, R., Stenger, D.C. 2003. Evolution of Wheat streak mosaic virus: dynamics of population growth within plants may explain limited variation. Annual Review of Phytopathology 41, 199-214 (118).

French, R., Stenger, D. C. 2002. Wheat streak mosaic virus. AAB Descriptions of Plant Viruses, No. 293 (110).

Gadiou, S., Kudela, O., Ripl, J., Rabenstein, F., Kundu, J.K., Glasa, M. 2009. An amino acid deletion in Wheat streak mosaic virus capsid protein distinguishes a homogeneous group of European isolates and facilitates their specific detection. Plant Disease 93, pp. 1209–1213.

Galbács, Z. N., Agyemang, E. D., Pásztor, G., Takács, A. P., Várallyay, É. 2024. Viromes of Monocotyledonous Weeds Growing in Crop Fields Reveal Infection by Several Viruses Suggesting Their Virus Reservoir Role. Plants, 13(18), 2664.

Garcia-Arenal, F., Fraile, A., Malpica, J.M. 2001. Variability and genetic structure of plant virus populations. Annual Review of Phytopathology 39, pp. 157–186.

Gatton, H. A. 2015. Crop Profile for Sweet Corn in Virginia. IPM Crop Profiles, Virginia Polytechnic Institute and State University.

Gavrili, V., Lotos, L., Mollov, D., Grinstead, S., Tsialtas, I.T., Katis, N.I., Maliogka, V.I. 2021. First report of Barley virus G infecting corn in Greece. Journal of Plant Pathology, 103 (14):1331. DOI: 10.1007/s42161-021-00903-4.

Gent, D.H., du Toit, L.J., Fichtner, S.F., Mohan, S.K., Pappu, H.R., Schwartz, H.F. 2006. Iris yellow spot virus: an emerging threat to onion bulb and seed production. Plant disease, 90 (12), pp.1468-1480.

Gergerich, R.C., Dolja, V.V. 2006. Introduction to Plant Viruses, the Invisible Foe. Plant Health Instrument.

Ghorbani A., Izadpanah K., Dietzgen, R. 2017. Complete sequence and corrected annotation of the genome of maize Iranian mosaic virus. Archives of Virology DOI 10,1007/s00705-017-3646-0.

Gibbs, A. J.; Ohshima, K.; Phillips, M. J.; Gibbs, M. J. 2008. The Prehistory of Potyviruses: Their Initial Radiation Was during the Dawn of Agriculture. PLoS ONE, 3 (6), e2523. https://doi.org/10.1371/journal.pone.0002523.

Gomez, M.I. Gupta, S.C. 2003. Millets, in Caballero BBT-E of FS and N, 2nd edition. Academic Press, Oxford, pp. 3974–3979.

Gonçalves, M.C., Godinho, M., Alves-Freitas, D.M.T., Varsani, A., Ribeiro, S.G. 2017. First report of maize yellow mosaic virus infecting maize in Brazil. Plant disease, 101(12), p. 2156.

Götz, R., Huth, W., Lesemann, D.E., Maiss, E. 2002. Molecular and serological relationships of Spartina mottle virus (SpMV) strains from Spartina spec. and from Cynodon dactylon to other members of the Potyviridae. Archives of Virology, 147, 379–391.

Hadi, B.A.R., Langham, M.A.C., Osborne, L., Tilmon, K.J. 2011. Wheat streak mosaic virus on wheat: biology and management. Journal of Integrated Pest Management 2, 1–5 (112).

Hanna, W., Carrow, R., Powell, A. 1997. Registration of 'Tift 94' bermudagrass. Crop Science, 37.

Helmy, M., Awad, M., Mosa, K.A. 2016. Limited resources of genome sequencing in developing countries: Challenges and solutions. Applied and Translational Genomics 9, 15–19.

Hillman, B.I., Cai, G. 2013. The family Narnaviridae: simplest of RNA viruses. Advances in virus research, 86, pp.149-176.

Holm, L. G., Plucknett, D. L., Pancho, J. V., Herberger, J. P. 1991a. The World's Worst Weeds. Distribution and Biology. Krieger Publishing Company.

Holm, L. G., Plucknett, D. L., Pancho, J. V., Herberger, J. P. 1991b. The World's Worst Weeds: Distribution and Biology. Malabar, FL: Krieger Publishing. 609 p.

Holm, L., Pancho, J.V., Herberger, J.E., Plucknett, D.L. 1979. A Geographical Atlas of World Weeds. Wiley-lnterscience Publication, New York, NY.

Horowitz, M. 1996. Bermudagrass (Cynodon dactylon): A history of the weed and its control in Israel. Phytoparasitica, 24, pp.305-320.

Hortamani, M., Massah, A., Talebi, M., Izadpanah, K. 2018. Genetic diversity and phylogenetic analysis of Maize Iranian mosaic virus isolates based on geographical distribution, host and type of symptoms. Iranian Journal of Plant Pathology, 54(1).

Hossain, M. M., Begum, M., Hossain, M. M., Begum, M. 2015. Soil Weed Seed Bank: Importance and Management for Sustainable Crop Production- A Review. https://doi.org/10.22004/AG.ECON.235284.

Hosseini, A., Izadpanah, K. 2005. Biological, serological and physicochemical properties of Bermudagrass filamentous viruses from Iran. Parasitica, 61, 55–59.

Hosseini, A., Koohi Habibi, M., Izadpanah, K., Mosahebi, G.H., RubiesAutonell, C., Ratti, C. 2010. Characterization of a fila-mentous virus from Bermudagrass and its molecular, serological and biological comparison with Spartina mottle virus. Archives of Virology 155,1675–1680.

Hsu, C. L., Hoepting, C. A., Fuchs, M.,Smith, E. A., Nault, B. A. 2011. Sources of Iris Yellow Spot Virus in New York. Plant Disease 95 (6), 735–743. https://doi.org/10.1094/PDIS-05-10-0353.

Huang, J., Wen, G.S., Li, M.J., Sun, C.C., Sun, Y., Zhao, M.F. He, Y.Q. 2016. First report of Maize chlorotic mottle virus naturally infecting sorghum and coix seed in China. Plant Disease, 100(9), pp.1955-1955.

Hullé, M., Cœur d'Acier, A., Bankhead-Dronnet, S., Harrington, R. 2010. Aphids in the face of global changes. Revolutionary Biology , 333, 497–503.

ICTV, 2021. Virus taxonomy 2021 release, genus: Polerovirus. International Committee on Taxonomy of Viruses (ICTV). https://talk. ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/solemoviridae/1692/genus-polerovirus.

Ilbagi H., Citir A., Kara A., Uysal M. 2018. Poaceae Weed Hosts of Yellow dwarf viruses (YDVs) in the Trakya Region of Turkey. Ekin J. 4(2): 8-19, 2018.

Islam, W., Noman, A., Naveed, H., Alamri, S.A., Hashem, M., Huang, Z., Chen, H.Y.H. 2020. Plant-insect vector-virus interactions under environmental change. Science of the Total Environment 701, 135044.

Ivanov, K.I., Eskelin, K., Lõhmus, A., Mäkinen, K. 2014. Molecular and cellular mechanisms underlying potyvirus infection. Journal of General Virology 95, 1415–1429.

Izadpanah K., Ahmadi A., Jafari S. A., Parvin S. 1983a. Maize rough dwarf in Fars. Iranian Journal of Plant Pathology 19: 25-29.

Izadpanah K., Ahmadi A., Parvin S., Jafari S. A. 1983b. Transmission, particle size and additional hosts of the rhabdovirus causing maize mosaic in Shiraz, Iran. Phytopathology Zeitschrift 107: 283-288.

Izadpanah, K., Ebrahim-Nesbat, F., Afsharifar, A. 1991. Barley yellow striate mosaic virus as the cause of a major disease of wheat and millet in Iran. Journal of Phytopathology 131, 290–296. doi: 10.1111/j.1439-0434.1991.tb01199.x.

Izadpanah, K., Zaki-Aghl, M., Zhang, Y.P., Daubert, S.D., Rowhani, A. 2003. Bermuda grass as a potential reservoir host for Grapevine fanleaf virus. Plant Disease 87(10), pp.1179-1182.

Jackson, A. O., Dietzgen, R. G., Goodin, M. M., Bragg, J. N., Deng, M. 2005. Biology of plant rhabdoviruses. Annual Review of Phytopathology, 43, 623–660.

Jeong, J.J., Ju, H.-J., Noh, J. 2014. A Review of Detection Methods for the Plant Viruses. Research in Plant Disease, 20 (3), 173–181. https://doi.org/10.5423/RPD.2014.20.3.173.

Jo, Y.H., Bae, J.Y., Kim, S.M., Choi, H.S., Lee, B.C., Cho, W.K. 2018. Barley RNA viromes in six different geographical regions in Korea. Scientific Reports, 8(1):13237. https://www.nature.com/articles/s41598-018-31671-4.pdf.

Jones, P. 1980. Leaf mottling of Spartina species caused by a newly recognised virus, Spartina mottle virus. Annals of Applied Biology, 94, 77–81.

Jones, R. 2000. The Distribution, Density and Economic Impact of Weeds in the Australian Annual Winter Cropping Systems; CRC for Weed Management Systems: Glen Osmond, S. Aust.

Jones, S., Baizan-Edge, A., Macfarlane, S., Torrance, L. 2017. Viral Diagnostics in Plants Using Next Generation Sequencing: Computational Analysis in Practice. Frontiers in Plant Science 8, 1770.

Karikari, T. 2015. Bioinformatics in Africa: The Rise of Ghana? PLOS Computational Biology, 11, e1004308.

Kegler, H., Kontzog, H. G., Richter, J. 1989. "On the ecology of tobacco rattle virus." 91-93.

Khan, S., Jan, A.T., Aquil, B., Haq, Q.M.R. 2011. Coat protein gene based characterization of cucumber mosaic virus isolates infecting banana in India. Journal of Phytological Research 3(2):94–101.

Kiedrowicz, A., Kuczynski, L., Laska, A., Lewandowski, M., Proctor, H., Skoracka, A. 2017a. Dispersal strategies in passively spreading phytophagous mites. In: ASAB Easter Conference 2017, Liverpool, 5–7 April 2017. Abstract book, p. 10. The Association for the Study of Animal Behaviour, University of Liverpool, Liverpool.(111).

Kil, E.J., Byun, H.S., Hwang, H., Lee, K.Y., Choi, H.S., Kim, C.S., Lee, S. 2021. Tomato yellow leaf curl virus infection in a monocotyledonous weed (Eleusine indica). The Plant Pathology Journal 37(6), p.641.

Kreuze, J.F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., Simon R. 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. Virology 388(1):1–7.

Kumar, L.M., Foster, J.A., McFarland, C., Malapi-Wight, M. 2018. First report of Barley virus G in switchgrass (Panicum vir-gatum). Plant disease, 102(2), pp.466-466. DOI: 10.1094/PDIS-09-17-1390-PDN.

Kusia, E.S., Subramanian, S., Nyasani, J.O., Khamis, F., Villinger, J., Ateka, E.M. Pappu, H.R. 2015. First report of lethal necrosis disease associated with co-infection of finger millet with Maize chlorotic mottle virus and Sugarcane mosaic virus in Kenya. Plant Disease 99(6), pp. 899-900.

Lacombe, S., Bangratz, M., Ta, H.A., Nguyen, T.D., Gantet, P., Brugidou, C. 2008. Optimized RNA-Silencing Strategies for Rice Ragged Stunt Virus Resistance in Rice. Plants (Basel). Sep 24;10(10):2008. doi: 10.3390/plants10102008. PMID: 34685817; PMCID: PMC8540896.

Lacroix, C., Renner, K., Cole, E., Seabloom, E.W., Borer, E.T., Malmstrom, C.M. 2016. Methodological Guidelines for Accurate Detection of Viruses in Wild Plant Species. Applied and Environmental Microbiology 82, 1966–1975.

Lamprecht, R.L., Pietersen, G., Kasdorf, G.G.F., Nel, L.H. 2009. Characterisation of a proposed Nucleorhabdovirus new to South Africa. European journal of plant pathology, 123, pp. 105-110.

Latourrette, K., Holste, N.M., Garcia-Ruiz, H. 2021. Polerovirus genomic variation. Virus Evolution, 7(2):1-18. DOI: 10.1093/ve/veab102.

Lázár, J., Bisztray, G.D. 2011. Virus and virus-like diseases of grapevine in Hungary. International Journal of Horticultural Science, 17(3), pp. 25-36.

Lim, S., Yoon, Y., Jang, Y.W., Bae, D.H., Kim, B.S., Maharjan, R., Yi, H., Bae, S., Lee, Y.H., Lee, B.C., Park, C.Y. 2018. First report of Maize yellow mosaic virus infecting Panicum miliaceum and Sorghum bicolor in South Korea. Plant disease, 102(3), pp. 689-689.

Linder, H.P., Lehmann, C.E., Archibald, S., Osborne, C.P. and Richardson, D.M. 2018. Global grass (*Poaceae*) success underpinned by traits facilitating colonization, persistence and habitat transformation. Biological Reviews, 93(2), pp. 1125-1144. https://doi.org/10.1111/BRV.12388.

Lockhart, B.E.L., Khaless, N., Maataoui, M.E. and Lastra, R. 1985. "Cynodon chlorotic streak virus, a previously undescribed plant rhabdovirus infecting Bermuda grass and maize in the Mediterranean area." 1094-1098.

López, M.M., Llop, P., Olmos, A., Marco-Noales, E., Cambra, M., Bertolini, E. 2009. Are molecular tools solving the challenges posed by detection of plant pathogenic bacteria and viruses? Current Issues in Molecular Biology, 11, 13–46.

Lucas, P., Ratnadass, A., Deguine, J.P. 2017. Moving from integrated pest management to agroecological crop protection. 1st edition.; Springer Berlin Heidelberg: New York, NY.

MacDiarmid, R., Rodoni, B., Melcher, U., Ochoa-Corona, F. Roossinck, M. 2013. Biosecurity implications of new technology and discovery in plant virus research. PLoS pathogens, *9*(8), p.e1003337. https://doi.org/10.1371/journal.ppat.1003337.

Macharia, I., Backhouse, D., Wu, S.B., Ateka, E. M. 2016. Weed Species in Tomato Production and Their Role as Alternate Hosts of Tomato Spotted Wilt Virus and Its Vector Frankliniella Occidentalis: Weeds as Reservoir of TSWV. Annals of Applied Biology, 169 (2), 224–235. https://doi.org/10.1111/aab.12297.

Madeley, C.R. 1997. Electron microscopy and viral diagnosis. Journal of Clinical Pathology 50, pp. 454–456.

Makkouk, K. M., Kumari, S. G., Ghulam, W., Attar, N. 2004. First record of Barley yellow striate mosaic virus affecting wheat summer-nurseries in Syria. Plant Disease 88, 83. doi: 10.1094/PDIS.2004.88.1.83A.

Makkouk, K.M., Kumari, S.G., van Leur, J.A.G., Jones, R.A.C. 2014. Control of plant virus diseases in cool-season grain legume crops. In Advances in Virus Research; Loebenstein, G., Katis, N., Eds.; Academic Press Inc.: Cambridge, MA, USA, Volume 90, pp. 207–253.

Malmstrom, C.M., Shu, R.J. 2004. Multiplexed RT-PCR for streamlined detection and separation of barley and cereal yellow dwarf viruses. Journal of Virological Methods, 120(1): 69-78. DOI: 10.1016/j.jviromet.2004.04.005.

Malmstrom, C.M., Alexander, H.M. 2016. Effects of crop viruses on wild plants. Current Opinion in Virology 19, 30–36.

Martinelli, F., Scalenghe, R., Davino, S., Panno, S., Scuderi, G., Ruisi, P., Dandekar, A. M. 2015. Advanced methods of plant disease detection. A review. Agronomy for Sustainable Development, 35, 1-25.

Marwal, A., Sahu, A. K., Gaur, R. K. 2014. Transmission and host interaction of Geminivirus in weeds. In Plant Virus–Host Interaction (pp. 143-161). Academic Press.

Massah A., Izadpanah K., Afsharifar A. R.,Winter S. 2008. Analysis of nucleotide sequence of Iranian maize mosaic virus confirms its identity as a distinct nucleorhabdovirus. Archives of Virology 153:1041-1047.

Massawe, D.P., Stewart, L.R., Kamatenesi, J., Asiimwe, T., Redinbaugh, M.G. 2018. Complete sequence and diversity of a maize-associated Polerovirus in East Africa. Virus Genes, 54, 432–437.

Masumi, M., Zare, A., Izadpanah, K. 2011. Biological, serological and molecular comparisons of potyviruses infecting poaceous plants in Iran.

McKirdy, S. J., Jones, R. A. C., Nutter Jr, F. W. 2002. Quantification of yield losses caused by Barley yellow dwarf virus in wheat and oats. Plant Disease, 86(7), 769-773.

McLeish, M.J., Fraile, A., García-Arenal, F. 2021. Population genomics of plant viruses: the ecology and evolution of virus emergence. Phytopathology®.111:32–9.

McMechan, A.J., Hein, G.L. 2016. Planting date and variety selection for management of viruses transmitted by the wheat curl mite (Acari: Eriophyidae). Journal of Economic Entomology 109, 70–77.

Mehmood, Z., Ashiq, M., Noorka, I. R., Ali, A., Tabasum, S., Iqbal, M. S. 2014. Chemical control of monocot weeds in wheat (Triticum aestivum L.). American Journal of Plant Sciences, 2014.

Michel, A.P., Mittapalli, O., Mian, R.M.A. 2011. Evolution of Soybean Aphid Biotypes: Understanding and Managing Virulence to Host-Plant Resistance; InTech: West Palm Beach, FL, USA, pp. 355–372.

Mitrofanova, I., Zakubanskiy, A.V., Mitrofanova, O.V. 2018. Viruses infecting main ornamental plants: An overview. Ornamental Horticulture 24, 95–102.

Mlotshwa, B.C., Mwesigwa, S., Mboowa, G., Williams, L., Retshabile, G., Kekitiinwa, A., Wayengera, M., Kyobe, S., Brown, C.W., Hanchard, N.A., Mardon, G. 2017. The collaborative African genomics network training program: a trainee perspective on training the next generation of African scientists. Genetics in Medicine, 19 (7), pp.826-833.

Montero-Astúa, M., Rotenberg, D., Leach-Kieffaber, A., Schneweis, B. A., Park, S., Park, J. K., German, T. L., Whitfield, A. E. 2014. Disruption of Vector Transmission by a Plant-Expressed Viral Glycoprotein. MPMI, 27 (3), 296–304. https://doi.org/10.1094/MPMI-09-13-0287-FI.

Moradi, Z., Mehrvar, M., Nazifi, E., Zakiaghl, M. 2017. Iranian johnsongrass mosaic virus: the complete genome sequence, molecular and biological characterization, and comparison of coat protein gene sequences. Virus Genes, 53, pp.77-88.

Morrison, L.A., Wrigley, C. 2004. Taxonomic classification of grain species. Wrigley, C., Corke, H., Walker, C.E. (Eds.), Encylopedia of Grain Science, vol. 3, Elsevier, Oxford, pp. 271–280.

Mueller, J. P., Lewis, W. M., Green, J. T., Burns J. C. 1993."Yield and Quality of Silage Corn as Altered by Johnsongrass Infesta-tion." Agronomy Journal 85 (1): 49–52. https://doi.org/10.2134/agronj1993.00021962008500010010x.

Mulenga, R.M., Miano, D.W., Kaimoyo, E., Akello, J., Nzuve, F.M., Al Rwahnih, M., Chikoti, P.C., Chiona, M., Simulundu, E., Alabi, O.J. 2020. First report of southern bean mosaic virus infecting common bean in Zambia. Plant disease, 104(6), pp.1880-1880.

Nancarrow, N., Aftab, M., Zheng, L., Maina, S., Freeman, A., Rodoni, B., Spackman, M., Trębicki, P., 2019b. First report of barley virus G in Australia. Plant Disease, 103(7):1799-1800. DOI: 10.1094/PDIS-01-19-0166-PDN.

Nancarrow, N., Maina, S., Zheng, L., Aftab, M., Freeman, A., Kinoti, W.M., Rodoni, B., Trębicki, P., 2019a. Coding-complete sequences of barley virus G isolates from Australia, obtained from a 34-year-old and a 1-year-old sample. Microbiology Resource Announcements, 8(47):e01292-19. https://mra.asm.org/content/8/47/e01292-19.

Narayana, Y. D., Muniyappa, V. 1995. "Survey, symptomatology and detection of an isolate of maize stripe virus on sorghum in Karnataka."171-176.

Nault, L.R., Gordon, D.T., Gingery, R.E., Bradfute, O.E., Castillo Loayza, J. 1979. Identification of maize viruses and mollicutes and their potential insect vectors in Peru. Phytopathology 69(8), pp.824-828.

Nault, L.R., Styer, W.E., Coffey, M.E., Gordon, D.T., Negi, L.S., Niblett, C.L. 1978. Transmission of maize chlorotic mottle virus by chrysomelid beetles. Phytopathology 68(7), pp. 1071-1074.

Navia, D., de Mendonca, R.S., Skoracka, A., Szydło, W., Knihinicki, D., Hein, G.L., da Silva Pereira, P.R., Truol, G., Lau, D. 2013. Wheat curl mite, Aceria tosichella, and transmitted viruses: an expanding pest complex affecting cereal crops. Experimental and Applied Acarology 59, 95–143 (113).

Ng, J. C., Falk, B. W. 2006. Virus-Vector Interactions Mediating Nonpersistent and Semipersistent Transmission of Plant Viruses. Annual Review of Phytopathology 44, 183–212.

Nhlapo, T., Rees, D., Odeny, D., Mulabisana, J., Rey, M. 2018a. Viral metagenomics reveals sweet potato virus diversity in the Eastern and Western Cape provinces of South Africa. South African Journal of Botany, 117, 256–267.

Nhlapo, T.F., Mulabisana, J.M., Odeny, D.A., Rey, M.E.C., Rees, D.J.G. 2018b. First Report of Sweet potato badnavirus A and Sweet potato badnavirus B in South Africa. Plant Disease, 102, 1865.

Niblett, C., Claflin, L. 1978. Plant disease reporter–Corn lethal necrosis-A new virus disease of corn in Kansas. The Plant Disease Bulletin, 62(1–6), p.15. Vol. 62, No. 1.

Nicaise, V. 2014. Crop immunity against viruses: Outcomes and future challenges. Frontiers in Plant Science 5, 660.

Nordenstedt, N., Marcenaro, D., Chilagane, D., Mwaipopo, B., Rajamäki, M.L., Nchimbi-Msolla, S., Njau, P.J.R., Mbanzibwa, D., Valkonen, J.P.T. 2017. Pathogenic seedborne viruses are rare but Phaseolus vulgaris endornaviruses are common in bean varieties grown in Nicaragua and Tanzania. PLoS ONE, 12, e0178242.

Oh, J., Park, C.Y., Min, H.G., Lee, H.K., Yeom, Y.A., Yoon, Y., Lee, S.H. 2017. First report of barley virus G in foxtail millet (*Setaria italica*) in Korea. Plant Disease, 101(6):1061-1062. DOI: 10.1094/PDIS-01-17-0036-PDN.

Otim-Napea, G.W., Sserubombwe, W.S., Alicai, T., Thresh, J.M. 2003. Plant virus diseases in sub-Saharan Africa: impact, challenges, and the need for global action. In: Plant virology in Sub-Saharan Africa, proceedings of a conference organized by IITA. J.d'A. Hughes and J. Odu (eds). International Institute of Tropical Agriculture. Ibadan, Nigeria. Pp 229-311.

Oudhia, P. 2003. Traditional medicinal knowledge about herb Doobi (cynodon dactylon) in Chhatisgarh, India. http://www. Botanical . com \ column_poudhia/III_doobi.html.

Pallett, D. W., Ho, T., Cooper, I., Wang, H. 2010. Detection of Cereal Yellow Dwarf Virus Using Small Interfering RNAs and Enhanced Infection Rate with Cocksfoot Streak Virus in Wild Cocksfoot Grass (Dactylis Glomerata). Journal of Virological Methods, 168 (1–2), 223–227. https://doi.org/10.1016/j.jviromet.2010.06.003.

Parizipour, M.H.G., Schubert, J., Behjatnia, S.A.A., Afsharifar, A., Habekuß, A., Wu, B. 2017. Phylogenetic analysis of Wheat dwarf virus isolates from Iran. Virus genes, 53, pp.266-274.

Park, C.Y., Min, H.G., Lee, H.K., Yeom, Y.A., Oh, J., Kim, B.S., Bae, D.H., Yoon, Y.N., Lee, S.H. 2017. Occurrence of viruses infecting foxtail millet (Setaria italica) in South Korea. Research in Plant Disease, 23(1), pp.69-74.

Park, C.Y., Oh, J.H., Min, H.G., Lee, H.K. and Lee, S.H. 2017a. First report of barley virus G in proso millet (Panicum miliaceum) in korea. Plant Disease, 101(2), pp.393-393.

Park, C.Y., Min, H.G., Oh, J., Kim, B.S., Lim, S., Yoon, Y., Lee, S.H. 2017b. First complete genome sequence of barley virus G identified from proso millet (Panicum miliaceum) in South Korea. Genome Announcements, 5(29):e00523-17. DOI: 10.1128/genomea.00523-17.

Pasztor, G., Galbacs N., Z., Kossuth, T., Demian, E., Nadasy, E., Takacs, A. P., Varallyay, E. 2020. Millet Could Be Both a Weed and Serve as a Virus Reservoir in Crop Fields. Plants, 9 (8), 954. https://doi.org/10.3390/plants9080954.

Pásztor, G., Szabó, R., Takács, A., Henézi, Á., Nádasy, E. 2017. The natural viral infections of the weedy Panicum miliaceum (L.). Columella Journal of Agriculture and Environmental Sciences 4, 35–38.

Paulsen, A. Q., Niblett, C. L. 1977 Purification and properties of foxtail mosaic virus. Phytopathology, 67(1), 346-1.

Peláez, A., McLeish, M.J., Paswan, R.R., Dubay, B., Fraile, A., García-Arenal, F. 2021. Ecological fitting is the forerunner to diversification in a plant virus with broad host range. Journal of evolutionary biology, 34(12), pp.1917-1931.

Perry, K. L., Kolb, F. L., Sammons, B., Lawson, C., Cisar, G., Ohm, H. 2000. Yield effects of barley yellow dwarf virus in soft red winter wheat. Phytopathology, 90(9), 1043-1048.

Pike, K. S. 1990. A review of barley yellow dwarf virus grain losses. World Perspectives on Barley Yellow Dwarf Virus. 6-11 July 1987, İtaly. 356-359.

Pooggin, M. M. 2016. "Role of small RNAs in virus host interaction," in PlantVirus Interactions - Molecular Biology, Intra- and Intercellular Transport, ed. T. Klejnow (Berlin: Springer), 161–189.

Pooggin, M.M. 2018. Small RNA-Omics for Plant Virus Identification, Virome Reconstruction, and Antiviral Defense Characterization. Frontiers in Microbiology., 9, 2779.

Prajapat, R., Marwal, A., Gaur, R. K. 2014. Begomovirus Associated with Alternative Host Weeds: A Critical Appraisal. Archives of Phytopathology and Plant Protection, 47 (2), 157–170.

Price, J. A., Workneh, F., Evett, S. R., Jones, D. C., Arthur, J., and Rush, C. M. 2010. Effects of Wheat streak mosaic virus on root development and water-use efficiency of hard red winter wheat. Plant Dis. 94:766-770. (106).

Przewodowska, A., Zacharzewska, B., Chołuj, J., Treder, K. 2015. A One-Step, Real-Time Reverse Transcription Loopmediated Isothermal Amplification Assay to Detect Potato Virus Y. American Journal of Potato Research, 92 (3), 303–311. https://doi.org/10.1007/s12230-015-9430-3.

Quito-Avila, D.F., Reyes-Proaño, E.G., Mendoza, A., Margaria, P., Menzel, W., Bera, S., Simon, A.E. 2022. Two new umbravi-rus-like associated RNAs (ulaRNAs) discovered in maize and johnsongrass from Ecuador. Archives of Virology, 167(10), pp.2093-2098.

Rabenstein, F., Stenger, D.C., French, R. 2004. Genus tritimovirus. In: Lapierre H, Signoret P A, eds., Viruses and Virus Diseases of Poaceae (Gramineae). INRA, France. pp. 398–402.

Rabieifaradonbeh, S., Afsharifar, A., Finetti-Sialer, M. M. 2021. Molecular and functional characterization of the barley yellow striate mosaic virus genes encoding phosphoprotein, P3, P6 and P9. European Journal of Plant Pathology, 161, 107-121.

Ragsdale, D. W., Voegtlin, D. J., O'Neil, R. J. 2004. Soybean aphid biology in North America. Annals of the Entomological Society of America 97, pp. 204-208.

Ramsell, J.N.E., Lemmetty, A., Jonasson, J., Andersson, A., Sigvald, R., Kvarnheden, A. 2008. Sequence analyses of Wheat dwarf virus isolates from different hosts reveal low genetic diversity within the wheat strain. Plant pathology 57(5), pp.834-841.

Rao, R.D.V.J.P., John, V.T. 1974. Alternate host of rice tungro virus and its vector. Plant disease reporter 58(9), pp.856-860.

Rathke, G.W., Christen, O., Diepenbrock, W. 2005. Effects of Nitrogen Source and Rate on Productivity and Quality of Winter Oilseed Rape (Brassica Napus L.) Grown in Different Crop Rotations. Field Crops Research, 94 (2–3), 103–113. https://doi.org/10.1016/j.fcr.2004.11.010.

Remold, S.K. 2002. Unapparent virus infection and host fitness in three weedy grass species. Journal of Ecology, pp.967-977.

Richet, C., Kraberger, S., Filloux, D., Bernardo, P., Harkins, G.W., Martin, D.P., Roumagnac, P., Varsani, A. 2019. Novel circular DNA viruses associated with Apiaceae and Poaceae from South Africa and New Zealand. Archives of Virology, 164, 237–242.

Rivarez, M.P.S., Pecman, A., Bačnik, K., Maksimović, O., Vučurović, A., Seljak, G., Mehle, N., Gutiérrez-Aguirre, I., Ravnikar, M. and Kutnjak, D. 2023. In-depth study of tomato and weed viromes reveals undiscovered plant virus diversity in an agroecosystem. Microbiome, 11(1), p.60.

Rochon, D., Rubino, L., Russo, M., Martelli, G.P., Lommel, S. 2012. Tombusviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus taxonomy-Ninth Report of the International Committee on Taxonomy of Viruses. Academic Press, London, pp 1111–1138.

Rodenburg, J., Meinke, H., Johnson, D.E. 2011. Challenges for weed management in African rice systems in a changing climate. The Journal of Agricultural Science. Aug;149 (4):427-35.

Roossinck, M.J., Martin, D.P., Roumagnac, P. 2015. Plant Virus Metagenomics: Advances in Virus Discovery. Phytopathology 105, 716–727.

Rose, H., Menzel, W., Knierim, D., Rabenstein, F., Maiss, E. 2020. Complete genome sequence of a German isolate of Spartina mottle virus supports its classification as a member of the proposed genus “Sparmovirus” within the family Potyviridae. Archives of Virology, 165, 2385–2388.

Rosida, N., Senoaji, W., Ibrahim, E., Kuswinanti, T. 2023. Molecular detection of rice tungro bacilliform virus (RTBV) on weed host in South Sulawesi. In IOP Conference Series: Earth and Environmental Science April, (Vol. 1160, No. 1, p. 012054). IOP Publishing.

Rotenberg, D., Jacobson, A. L., Schneweis, D. J., Whitfield, A. E. 2015. Thrips Transmission of Tospoviruses. Current Opinion in Virology, 15, 80–89. https://doi.org/10.1016/j.coviro.2015.08.003.

Ryabov, E.V., Taliansky, M.E. 2020. Umbraviruses (Calvusvirinae, Tombusviridae). Reference module in life sciences. Elsevier, Amsterdam.

Sacristán, S., Fraile, A., García-Arenal, F. 2004. Population Dynamics of Cucumber Mosaic Virus in Melon Crops and in Weeds in Central Spain. Phytopathology 94 (9), 992–998.

Sánchez-Sánchez, H., Henry, M., Cárdenas-Soriano, E., AlvizoVillasana, H. F. 2001. Identification of Wheat streak mosaic virus and its vector Aceria tosichella in Mexico. Plant Disease 85:13-17 (107).

Scheets, K., 2013. Infectious transcripts of an asymptomatic panicovirus identified from a metagenomic survey. Virus research 176(1-2), pp.161-168.

Schubert, J., Habekuß, A., Kazmaier, K., Jeske, H. 2007. Surveying cereal-infecting geminiviruses in Germany—diagnostics and direct sequencing using rolling circle amplification. Virus research 127(1), pp.61-70.

Schubert, J., Ziegler, A., Rabenstein, F. 2015. First detection of Wheat streak mosaic virus in Germany: molecular and biological characteristics. Archives of Virology 160, 1761–1766.

Seepiban, C., Charoenvilaisiri, S., Kumpoosiri, M., Bhunchoth, A., Chatchawankanphanich, O., Gajanandana, O. 2015. Development of a Protocol for the Identification of Tospoviruses and Thrips Species in Individual Thrips. Journal of Virological Methods, 222, 206–213. https://doi.org/10.1016/j.jviromet.2015.06.017.

Shaner, D. L. 2014. Lessons Learned From the History of Herbicide Resistance. Weed sci., 62 (2), 427–431. https://doi.org/10.1614/WS-D-13-00109.1.

Shen, H.R., Dong, Z.P., Wang, Y.F., Quan, J.Z., Bai, H., Li, Z.Y. 2020. First report of dwarf disease in foxtail millet (Setaria italica) caused by Barley Yellow Striate Mosaic Virus in China. Plant disease, 104(4), pp.1262-1262.

Shepherd, D.N., Martin, D.P., Van Der Walt, E., Dent, K., Varsani, A., Rybicki, E.P. 2010. Maize streak virus: an old and complex 'emerging'pathogen. Molecular plant pathology, 11(1), pp.1-12.

Shukla, D.D., Frenkel, M.J., McKern, N.M., Ward, C.W., Jilka, J., Tosic, M., Ford, R.E. 1992. Present status of the sugarcane mosaic subgroup of potyviruses. Springer, Vienna. pp. 363-373.

Singh, K., Kundu, J.K. 2017. Variations in Wheat streak mosaic virus coat protein sequence among crop and non-crop hosts. Crop and Pasture Science 68, 328–336. (116).

Singh, K., Wegulo, S. N., Skoracka, A., & Kundu, J. K. 2018. Wheat streak mosaic virus: a century old virus with rising importance worldwide. Molecular Plant Pathology, *19*(9), 2193-2206.

Srinivasan, R., Alvarez, J. M., Cervantes, F. 2013. The Effect of an Alternate Weed Host, Hairy Nightshade, Solanum Sarrachoides (Sendtner) on Green Peach Aphid Distribution and Potato Leafroll Virus Incidence in Potato Fields of the Pacific Northwest. Crop Protection, 46, 52–56. https://doi.org/10.1016/j.cropro.2012.12.015.

Stenger, D. C., French, R. 2009. Wheat streak mosaic virus genotypes introduced to Argentina are closely related to isolates from the American Pacific Northwest and Australia. Archives of Virology 154, 331-336.(109).

Stobbe, A., Roossinck, M.J. 2016. Plant virus diversity and evolution, in Current Research Topics in Plant Virology, Wang, A. and Zhou, X., Eds., Cham: Springer, pp. 241–250. https://doi.org/10.1007/978-3-319-32919-2_8.

Stobbe, A.H., Roossinck, M.J. 2014. Plant virus meta-genomics: what we know and why we need to know more, Frontiers in Plant Science vol. 5, art. 150. https://doi.org/10.3389/fpls.2014.00150.

Stukenbrock, E.H., McDonald, B.A. 2008. The origins of plant pathogens in agro-ecosystems, Annual Review of Phytopathology vol. 46, no. 1, pp. 75–100. https://doi.org/10.1146/annurev.phyto.010708.154114.

Susi, H., Filloux, D., Frilander, M.J., Roumagnac, P., Laine, A.L. 2019. Diverse and variable virus communities in wild plant populations revealed by metagenomic tools. PeerJ, 7, 6140.

Suzuki, N., Sasaya, T., Choi, I.R. 2015. Editorial: Viruses threatening stable production of cereal crops. Frontiers in Microbiology 6, 470.

Sweet, J., Simpson, E., Law, J., Lutman, P.J.W., Berry, K.J., Payne, R.W., Champion, G.T., May, M.J., Walker, K., Wightman, P., Lainsbury, M. 2004. Botanical and rotational implications of genetically modified herbicide tolerance in winter oilseed rape and sugar beet (BRIGHT Project)(H-GCA Project Report No. 353), pp 242.

Szabó, A.K., Várallyay, É., Demian, E., Hegyi, A., Galbács, Z. N., Kiss, J., Bálint, J., Loxdale, H. D., Balog, A. 2020. Local Aphid Species Infestation on Invasive Weeds Affects Virus Infection of Nearest Crops Under Different Management Systems – A Preliminary Study. Frontiers in Plant Science 11, 684. https://doi.org/10.3389/fpls.2020.00684.

Tahir, M.N., Lockhart, B., Grinstead, S., Mollov, D. 2017. Characterization and complete genome sequence of a panicovirus from Ber-mudagrass by high-throughput sequencing. Archives of Virology 162, 1099–1102. doi: 10.1007/s00705-016-3165-4.

Taylor, John R.N., Joseph Awika, eds. 2017. Gluten-free ancient grains: cereals, pseudocereals, and legumes: sustainable, Nutritious, and health-promoting foods for the 21st century. Woodhead publishing.

Thomas, J. A., Hein, G. L. 2003. Influence of volunteer wheat plant condition on movement of the wheat curl mite, Aceria tosichella, in winter wheat. Experimental and Applied Acarology 31, 253-268.(117).

Thomas, J.E., Raymond, M., Tran, N.T., Crew, K.S., Teo, A.C., Geering, A.D. 2021. Complete genome sequences and properties of Spartina mottle virus isolates from hybrid Bermudagrass (Cynodon dactylon× Cynodon transvaalensis). Plant Pathology, 70(5), pp.1062-1071.

Tolin, S.A., Fayad, A. 2016. Virus diseases of tropical vegetable crops and their management. In Integrated Pest Management of Tropical Vegetable Crops; Muniappan, R., Heinrichs, E.A., Eds.; Springer: Amsterdam, The Netherlands, pp. 41–76. ISBN 9789402409246.

Torrance, L. 1998. Developments in serological methods to detect and identify plant viruses. Plant Cell, Tissue and Organ Culture 52, 27–32.

Tosh, C. R., Brogan, B. 2015. Control of Tomato Whiteflies Using the Confusion Effect of Plant Odours. Agronomy for Sustainable Development 35 (1), 183–193. https://doi.org/10.1007/s13593-014-0219-4.

Tosic, M., Ford, R.E., Shukla, D.D., Jilka, J. 1990. Differentiation of sugarcane, maize dwarf, Johnsongrass, and sorghum mosaic viruses based on reactions of oat and some sorghum cultivars. Plant Disease 74(8), 549–52. https://doi.org/10.1094/PD-74-0549.

Tran, N.T., Teo, A.C., Crew, K.S., Thomas, J.E., Campbell, P.R., Geering, A.D. 2022. Bermudagrass latent virus in Australia: genome sequence, sequence variation, and new hosts. Archives of Virology, 167(5), pp.1317-1323.

Tutin, T. G.; Heywood, V. H.; Burges, N. A. 1980. Flora Europaea. Vol. 5: Alismataceae to Orchidaceae (Monocotyledones). Cambridge, UK: University Press, p 452.

Umer, M., Liu, J., You, H., Xu, C., Dong, K., Luo, N., Kong, L., Li, X., Hong, N., Wang, G., Fan, X. 2019. Genomic, morphological and biological traits of the viruses infecting major fruit trees. Viruses, 11(6), p.515.

Vafaei, S. H., Mahmoodi, M. 2015. Distribution and Partial Properties of Three Viruses Infecting Cucumber in West Iran and Their Reservoir Weed Hosts. Archives of Phytopathology and Plant Protection, 48 (6), 519–536.

Várallyay, É., Gábor, G., Burgyán, J. 2012. Virus induced gene silencing of Mlo genes induces powdery mildew resistance in Triticum aestivum. Archives of Virology 157:1345–1350.

Viswanathan, R., Balamuralikrishnan, M. 2005. Impact of mosaic infection on growth and yield of sugarcane. Sugar Tech, 7, 61.

Walia J. J., Willemsen A., Elci E., Caglayan K., Falk B. W., Rubio L. 2014. Genetic variation and possible mechanisms driving the evolution of worldwide fig mosaic virus isolates. Phytopathology 104:108-114.

Walker, P. J., Blasdell, K. R., Calisher, C. H., Dietzgen, R. G., Kondo, H., Kurath, G., Longdon, B., Stone, D. M., Tesh, R. B., Tordo, N., Vasilakis, N., Whitfield, A. E. 2018. ICTV Report Consortium. ICTV report consortium ICTV virus taxonomy profile: Rhabdoviridae. Journal of General Virology, 99(4), 447–448.

Walls, J., III, Rajotte, E., Rosa, C. 2019. The past, present, and future of barley yellow dwarf management. Agriculture 9(1), 23. DOI:10.3390/agriculture9010023.

Wamaitha, M.J., Nigam, D., Maina, S., Stomeo, F., Wangai, A., Njuguna, J.N., Holton, T.A., Wanjala, B.W., Wamalwa, M., Lucas, T., Djikeng, A., Garcia-Ruiz, H. 2018. Metagenomic analysis of viruses associated with maize lethal necrosis in Kenya. Virology Journal, 15(90). https://virologyj.biomedcentral.com/articles/10.1186/s12985-018-0999-2.

Wang, Q., Zhou, X.P., Wu, J.X. 2014. First report of Maize chlorotic mottle virus infecting sugarcane (Saccharum officinar-um). Plant Disease 98(4), pp.572-572.

Wang, S. Y., Boa, X. Z., Chen, R. L., Zhai, B. P. 1996. Study on the effects of the population dynamics of soybean aphid on both growth and yield of soybean. Soybean Science 15, 243-247.

Wangai, A.W., Redinbaugh, M. G., Kinuya, Z. M., Miano, D. W., Leley, P. K., Kasina, M., Mahuku, G., Scheets, K., Jeffers, D. 2012. First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. Plant Disease 96, 1582.

Warren, P. L., Schalau, J. 2014. Aphids. The University of Arizona - College of Agriculture and Life Sciences - Cooperative Extension, AZ1635. http://extension.arizona.edu/pubs/az1635-2014.

Warwick, S. I., Black, L. D. 1983. "The Biology of Canadian Weeds.: 61 Sorghum halepense (L.) Pers." Canadian Journal of Plant Science , 63 (4): 997–1014. https://doi.org/10.4141/cjps83-125.

Webster, C.G., Wylie, S.J., Jones, M.G.K. 2004. Diagnosis of plant viral pathogens. Current Science 86 (12), 1604–1607.

Wei, D., Liping, C., Zhijun, M., Guangwei, W., Ruirui, Z. 2010. Review of non-chemical weed management for green agriculture. International Journal of Agricultural and Biological Engineering, 3(4), pp.52-60.

Wintermantel, W.M., Cortez, A.A., Anchieta, A.G., Gulati-Sakhuja, A., Hladky, L.L. 2008. Co-Infection by Two Criniviruses Alters Accumulation of Each Virus in a Host-Specific Manner and Influences Efficiency of Virus Transmission. Phytopathology, 98, 1340–1345.

Workneh, F., Price, J. A., Jones, D. C., and Rush, C. M. 2010. Wheat streak mosaic: A classic case of plant disease impact on soil water content and crop water-use efficiency. Plant Disease 94, 771-774 (108).

Wren, J.D., Roossinck, M.J., Nelson, R.S., Scheets, K., Palmer, M.W., Melcher, U. 2006. Plant virus biodiversity and ecology. PLoS biology, 4(3), p.e80. https://doi.org/10.1371/journal.pbio.0040080.

Wu, B., Shang, X., Schubert, J., Habekuß, A., Elena, S.F., Wang, X. 2015. Global-scale computational analysis of genomic sequences reveals the recombination pattern and coevolution dynamics of cereal-infecting geminiviruses. Scientific reports, 5 (1), p.8153.

Wu, Z., Schenk-Hamlin, D., Zhan, W., Ragsdale, D. W., and Heimpel, G. E. 2004. The soybean aphid in China: A historical review. Annals of the Entomological Society of America 97:209-218.

Wylie, S.J., Adams, M., Chalam, C., Kreuze, J., López-Moya, J.J., Ohshima, K., Praveen, S., Rabenstein, F., Stenger, D., Wang, A., Zerbini, F.M. 2017. ICTV virus taxonomy profile: Potyviridae. Journal of General Virology, 98(3), pp.352-354.

Xie, L., Zhang, J., Wang, Q., Meng, C., Hong, J., Zhou, X. 2011. Characterization of maize chlorotic mottle virus associated with maize lethal necrosis disease in China. Journal of Phytopathology, 159(3), pp.191-193.

Xie, W., Marty, D.M., Xu, J., Khatri, N., Willie, K., Moraes, W.B., Stewart, L.R. 2021. Simultaneous gene expression and multi-gene silencing in Zea mays using maize dwarf mosaic virus. BMC plant biology, 21(1), p.208.

Yadav, N., Khurana, S.M.P. 2016. Plant virus detection and diagnosis: Progress and challenges. In Frontier Discoveries and Innovations in Interdisciplinary Microbiology; Shukla, P., Ed.; Springer: New Delhi, India; pp. 97–132.

Yahaya, A., Al Rwahnih, M., Dangora, D.B., Gregg, L., Alegbejo, M.D., Lava Kumar, P., Alabi, O.J. 2017. First report of maize yellow mosaic virus infecting sugarcane (Saccharum spp.) and itch grass (Rottboellia cochinchinensis) in Nigeria. Plant Disease 101(7), pp.1335-1335.

Yan, J., Uyedau, I., Kimura, I., Shikata, E., Chen, C.C., Chen, M.J. 1994. Echinochloa ragged stunt virus belongs to the same genus as rice ragged stunt virus. Japanese Journal of Phytopathology, 60(5), pp.613-616.

Yan, T., Zhu, J.R., Di, D., Gao, Q., Zhang, Y., Zhang, A., Yan, C., Miao, H., Wang, X.B. 2015. Characterization of the complete genome of Barley yellow striate mosaic virus reveals a nested gene encoding a small hydrophobic protein. Virology, 478, pp.112-122. doi: 10.1016/j.virol.2014.12.042.

Yatskievych, G. 1999. Steyermarks's Flora of Missouri. Volume 1. St. Louis, MO: Missouri Botanical Garden Press. 991 p.

Yoon, Y., Jung, J., Lee, B., Lee, Y., Lee, J., Kim, H., Bae, S., Nam, M., Lee, K. and Yago, J. 2012. First report of rice stripe virus of proso millet in Korea. Plant Disease 96(1), pp.150-150.

Yoon, Y.N., Lee, B.C., Jung, J.H., Kim, J.I., Hwang, J.B., Kim, C.S., Hong, S.J., Kang, H.W., Song, S.B., Hong, Y.G., Park, S.T. 2009. New Alternate Host of Rice stripe virus-'Deulmuksae'. Research in Plant Disease 15(2), pp.63-67.

Zablocki, O., Adriaenssens, E.M., Cowan, D. 2016. Diversity and ecology of viruses in hyperarid desert soils, Applied and Environmental Microbiology, vol. 82, pp. 770–777. https://doi.org/10.1128/AEM.02651-15.

Zhao, F.M., Lim, S., Yoo, R.H., Igori, D., Kim, S.M., Kwak, D.Y., Kim, S.L., Lee, B.C., Moon, J.S. 2016. The complete genomic sequence of a tentative new polerovirus identified in barley in South Korea. Archives of Virology, 161(7):2047-2050. DOI: 10.1007/s00705-016-2881-0.

Zhao, M., Ho, H., Wu, Y., He, Y., Li, M. 2014. Western Flower Thrips (Frankliniella occidentalis) Transmits Maize Chlorotic Mottle Virus. Journal of Phytopathology, 162(7-8), pp.532-536.

9. SCIENTIFIC PUBLICATIONS OF THE AUTHOR

9.1. Papers published in peer-reviewed and impact factor Journals

- Zsuzsanna, N. Galbács#, **Evans Duah Agyemang**#, György Pásztor, András Péter Takács, Éva Várallyay. Virome of monocotyledonous weeds growing at crop fields revealed infection with several viruses and suggests their virus reservoir role. *Plants*, *13*(18), 2664. https://doi.org/10.3390/plants13182664.
- Salamon, Pal, Zsuzsanna Nagyne-Galbacs, Emese Demian, Adam Achs, Peter Alaxin, Lukáš Predajňa, **Evans Duah Agyemang**, Francesco Desiderio, Andras Peter Takacs, Wulf Menzel, Dijana Škorić, Miroslav Glasa, Eva Varallyay. *Clematis vitalba* Is a Natural Host of the Novel Ilarvirus, Prunus Virus I. *Viruses* 15, no. 9 (2023): 1964. https://doi.org/10.3390/v15091964.
- Ofosu, Rita, **Evans Duah Agyemang**, Adrienn Márton, György Pásztor, János Taller, and Gabriella Kazinczi. "Herbicide Resistance: Managing Weeds in a Changing World." *Agronomy* 13, no. 6 (2023): 1595. https://doi.org/10.3390/agronomy13061595.
- **Evans, Duah Agyemang**; Martine, Lihwa Nsongoma ; György, Pásztor ; András, Takács. Detection of viral infections in a Hungarian vineyard. *Georgikon For Agriculture*: A Multidisciplinary Journal in Agricultural Sciences 26 : 2 pp. 83-96. , 14 p. (2022). HU ISSN 0239 1260.
- Simon Okwangan; Kelvin Kiprop; **Evans, Duah Agyemang**; Rita Ofosu; György, Pásztor; András, Takács. Identification and occurrence of potential phytopathogenic fungi infecting seeds of invasive allelopathic dicot weeds. *Georgikon For Agriculture*: A Multidisciplinary Journal in Agricultural Sciences 28, Sppl.2 : pp. 139-144 (2024). HU ISSN 0239 1260.
- **Evans, Duah Agyemang**; Molla, Gereme Taye ; Ari, Kurniawati ; András, Takács. Environmental Problems Associated with Small Scale Mining in Africa: Ghana's Perspective. *Georgikon for Agriculture*: A Multidisciplinary Journal in Agricultural Sciences 25 : 1 pp. 2-17. , 16 p. (2021). HU ISSN 0239 1260.
- RABILU, Sahilu Ahmad ; **AGYEMANG, Evans Duah** ; FARKAS, Bernadett. Antifungal activity of Salvia officinalis subsp. lavandulifolia and Salvia officinalis subsp. major aqueous extracts against Botrytis cinerea. *Journal Of Central European Agriculture* 22 : 2 pp. 420-428. , 9 p. (2021). https://doi.org/10.5513/JCEA01/22.2.3104.

9.2. Chapter in a Book Publication

- Takács, A. ; Szabó, R. ; Henézi, Á. ; Nádasy, E. ; **Agyemang, E. D**. ; Pásztor, Gy. Natural virus infections of weedy? (*Panicum miliaceum* L.) In: Tarcali, Gábor; Kövics, György; Radócz, László (eds.) Növényorvos képzés Debrecenben Debrecen, Hungary : University of Debrecen Faculty of Agricultural and Food Sciences and Environmental Management Institute of Plant Protection, Printart-Press Kft. (2021) 424 p. pp. 212-222.

9.3. List of Scientific talks presented in Conferences

- **Evans Duah Agyemang**, Pasztor Gyorgy, Takács András Péter, Zsuzsanna Nagyne Galbacs and Várallyay Éva. Potential role of monocotyledonous weeds as plant virus reservoirs from three selected Hungarian crop fields. *11th International Plant Protection Symposium*, University of Debrecen, Hungary. October 15. 2024.
- **Evans Duah Agyemang**, Pasztor Gyorgy, Takács András Péter, Zsuzsanna Nagyne Galbacs and Várallyay Éva. Investigating the potential role of monocotyledonous weeds as plant virus reservoirs in Crop fields. *Georgikon Days Scientific Conference*, MATE Georgikon campus, Keszthely, Hungary. May.17-18. 2024.
- **Evans Duah Agyemang**, Pasztor Gyorgy, Zsuzsanna Nagyne Galbacs, Takács András Péter and Várallyay Éva. Possible plant virus reservoir role of millet and common weeds at crop fields using small RNA HTS. *World congress on Plant Pathology and Plant Biotechnology* (WCPPPB-23), Washington DC, USA- online conference 2023. November 16.
- **Evans Duah Agyemang**, Pasztor Gyorgy, Zsuzsanna Nagyne Galbacs, Tackacs Andras Peter and Varallyay Eva. Virological studies into the plant virus reservoir of millet and some common crop field weeds using small RNA HTS. *XXXII. Keszthely Plant Protection Forum*. MATE Georgikon Campus Plant Protection Institute, 01.18-20. 2023.
- **Agyemang Evans Duah**, Pasztor Gyorgy, Zsuzsanna Nagyne Galbacs, Tackacs Andras Peter and Varallyay Eva. Plant virus reservoir role; A virological examination of millet and some common weeds at crop fields using small RNA HTS. *I Magyar Agrartudomanyi Doktoranduszok Szimpoziuma* (MADSZ) 2023. Absztraktkotet pp.59. ISBN 978-615-6457-18-9.

- **Evans Duah Agyemang**, Pasztor Gyorgy, Zsuzsanna Nagyne Galbacs, Tackacs Andras Peter and Varallyay Eva. The Contribution of plant virus diagnosis in ensuring food security; A study into the Possible plant viral reservoir of weeds on crop fields. *Georgikon 225 Days and LXIV Georgikon Scientific Conference*. MATE Georikon campus, Keszthely, 11.17-18. 2022.
- **Agyemang, Evans Duah**; György, Pasztor; Galbacs Nagyne, Zsuzsanna; Andras Takacs, Peter; Varallyay, Eva. Endemic weeds could serve as possible viral reservoir on crop fields. *DOSZ Conference (XXIV Tavaszi Szel)*, Pecs Egyetem, 6th-8th May 2022.
- **Evans, Duah Agyemang** ; Francesco, Desiderio ; Emese, Demian ; Andras, Takacs ; Pal, Salamon ; Eva, Varallyay. Putative Ilarvirus found in *Clematis vitalba* showing virus-like symptoms. In: Molnár, Dániel; Molnár, Dóra (eds.) *XXIV. Tavaszi Szél Konferencia* May 28-30, 2021.
- **Evans, Duah Agyemang** ; Francesco, Desiderio ; Emese, Demian ; Andras, Takacs ; Pal, Salamon ; Eva, Varallyay. Searching for the causative agent of a viral-like symptom in Clematis vitalba. In: Fiatal RNS Kutatók Fóruma 2021 - online konferencia. *Forum for Young RNA Investigators* 2021 - online conference. (March, 2021) 8 p. pp. 2-2.

9.4. List of Posters presented in Conferences

- **Agyemang, Evans Duah**; György, Pasztor; Galbacs Nagyne, Zsuzsanna; Andras Takacs, Peter; Varallyay, Eva. Possible viral reservoir role of investigated endemic weeds on crop fields. *FIBOK2022* - MATE GBI, GÖDÖLLŐ, 2022. ÁPRILIS 11-12.
- **Evans Duah Agyemang**, Pasztor Gyorgy, Zsuzsanna Nagyne Galbacs, Tackacs Andras Peter and Varallyay Eva. Investigation of the possible plant viral reservoir role of weeds on crop fields. *International Advances in Plant Virology Conference* 2022. Association of Applied Biologists (AAB). Ljubljana, Slovenia, 10.5-7.2022.
- Francesco, Desiderio ; **Evans, Duah Agyemang** ; Andras, Takacs ; Eva, Varallyay. Prunus virus F is present in Hungarian sour cherries. *Hungarian Molecular Life Sciences*, November 5-7 (2021) pp 239. Conference proceedings.
- **Evans, Duah Agyemang** ; Francesco, Desiderio ; Emese, Demian ; Andras, Takacs ; Pal, Salamon ; Eva, Varallyay. Symptom on *Clematis vitalba* could be a reason for an infection with Prunus virus I. *Hungarian Molecular Life Sciences*, November 5-7 (2021) pp 240. Conference proceedings.

10. ACKNOWLEDGEMENTS

I am humbly thrilled to recognize everyone who supported me in the build-up of my scientific disposition. Glory and Thanks be to God for the finalization of this dissertation. I would like to thank the Hungarian government/Tempus Public Foundation in collaboration with the Government of Ghana through the Ghana Scholarship Secretariat for granting me a Stipendium Hungaricum scholarship to continue my Ph.D. study.

My heartfelt gratitude goes to my mentors and role model, the 21st Century educationist, the Honorable Minister of Education of Ghana, Dr Yaw Osei Adwutwum and Mrs Selina Adutwum for their counsel, guidance and support throughout my tertiary educational pursuits.

I am grateful to my kind supervisors Dr. habil. András Peter Takács and Dr. Éva Várallyay whom I referred to as my Hungarian parents for their supervision and assistance, valuable guidance and critical advice. I appreciate all their contributions of time, ideas, experience, comments, corrections and encouragement. Without them, I would not be able to have achieve this goal.

I would like to thank all my colleague PhD students, colleagues of the Plant Protection Institute, my Ghanaian sister Rita, Bence and Administrator Éva. To the members of the Plant virus Diagnostic Group in Godöllő especially Dr. Nagyné Galbács Zsuzsanna, I appreciate them deeply for their helpful assistance and suggestions during my research work, and for providing a friendly atmosphere which makes it easy to adapt, and possible to solve the smaller or complex research problems in the lab. To my very good friend Erzsike who was very helpful to me during the beginning of my labs, thank you. I know you would be very happy to hear that I finally completed my PhD program.

Special thanks also to the head of Doctoral school Prof. Anda Angéla for her assistants, suggestions and encouragement. Many thanks also to Mrs. Beáta Bődör and Ms. Adrienn Soós for managing many administrative processes during my study.

Finally, I owe so much thanks to my family especially my parents Mr. Faris Agyemang Duah and Mrs. Grace Adusei for their care and support in every step of my life. Very special thanks to my dear wife Angela, for all her support and love during the study years. She always helps and motivates me to continue my study.