

BIOTECHNOLOGY IN THE BATTLE AGAINST POTYVIRUSES: A REVIEW OF DISEASE MANAGEMENT IN SOLANACEAE

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ABSTRACT

Some Solanaceae members are among the most important agricultural commodities cultivated globally. Climate change poses a threat of increased pathogen infections in solanaceous plants, with viruses (especially potyviruses) being among the most difficult to control. Over the past few decades, various methods have been developed, starting with conventional methods for the prevention and control of viral infections. The presence of virus resistance genes in some cultivars has encouraged traditional and molecular breeding, and most recently, genetic engineering and genome editing. The latter two methods have significantly improved specificity while decreasing the time required. However, genetically engineered crops are still highly regulated, if not opposed, in many countries. In this context, genome editing, and particularly the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 method (CRISPR/Cas9), is considered versatile, highly specific, and relatively fast. Although genome editing in chili has not yet been widely studied, existing literature suggests its potential. In this review, we discuss available methods for controlling potyvirus infection in solanaceous crops and propose that genome editing be routinely used as part of efforts to develop resistant cultivars.

Key words: CRISPR/Cas9, genome editing, molecular tools, viral infection, virus prevention, virus removal

INTRODUCTION

The Solanaceae family, which includes both perennial trees and herbaceous annuals, comprises approximately 4,000 plant species with diverse characteristics (Gebhardt 2016). The Solanaceae family has undergone significant diversification. This process is supported by whole-genome duplications and gene duplication, which have contributed to the family's complexity and adaptability to dynamic environments (Huang et al. 2023). Despite their diversity, only a few members – potato, eggplant, pepper, and tobacco – are crucial to global food security and are cultivated on approximately 28 million hectares worldwide (Motti 2021).

Solanaceae plants provide essential nutrients for humans, and some are used in nutraceuticals (Añibarro-Ortega et al. 2022). These crops are not only staples in many diets but also contribute significantly to the economy. Tomato production exceeds 3 million tons, with an estimated value of approximately 1 billion dollars, while chili pepper production in Mexico exceeds 2 million tons, also valued at over 1 billion dollars (Barchenger et al. 2018; Barraza et al. 2020).

Several threats affect the yield and quality of Solanaceae. The most important, particularly those posed by potyviruses, include the risk of economic losses and threats to food security (Hañčinský et al. 2020).

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The virus can cause widespread damage, leading to potential losses in global crop production. In Uganda, potyvirus infection contributes to a reduction in potato yield of approximately 85.1–87.2% (Byarugaba et al. 2020). In Switzerland, economic losses due to potyvirus in seed and ware production have been estimated at 2000 and 200 CHF per hectare, respectively (Dupuis et al. 2024). Tuber yield losses caused by a single potyvirus can exceed 80% when combined with other viruses. *Potato virus X* (PVX) is widespread worldwide, causing 10–40% losses in single infections and is particularly damaging when combined with *Potato virus Y* (PVY) or *Potato virus A* (PVA). Potato tuber yield losses of up to 80% are caused by synergistic interactions between potyviruses (Campos & Ortiz 2019). Potyviruses also contribute to the decline in tobacco yields (Trojak-Goluch 2024).

To mitigate the potyvirus threat to solanaceous crops, an integrated management strategy combining conventional and advanced techniques is essential. Regular monitoring and early detection of potyviruses are needed for effective management (Tatineni & Hein 2023). The spread of potyviruses can be minimized through crop rotation, intercropping, and sanitation. These practices alter the life cycles of the virus and its vectors, thereby reducing infection rates (Gilbert & Parker 2023). Controlling aphid populations, which are the primary carriers of potyviruses, is essential. This can be achieved through careful application of insecticides, especially during the early stages of plant growth when they are most susceptible to colonization (Arogundade et al. 2020). However, these methods have significant limitations, as plant viruses are constantly changing. They also face challenges posed by modern farming practices and climate change (Niraula & Fondong 2021; Sharma et al. 2024). Traditional methods often struggle to keep up with rapid changes and the emergence of new viral strains. This requires the development of more effective and better-coordinated management strategies (Meena et al. 2024). The development of biotechnology in the management of plant viruses has greatly transformed the way we control them (Saikia et al. 2022).

Recent biotechnological innovations have allowed the development of promising strategies to combat these pathogens. These advances include genetic engineering, molecular diagnostics, and new antiviral agent delivery systems. Together, they improve our ability to effectively detect, prevent, and manage viral plant diseases. This paper reviews the potential of biotechnology in managing potyviruses, particularly in the Solanaceae family.

POTYVIRUS OVERVIEW

Potyviruses are a group of RNA viruses that infect plants. They belong to the genus *Potyvirus* and the family Potyviridae (Yang et al. 2021). Potyviruses have rod-shaped, flexible filamentous particles that contain a single-stranded, positive-sense RNA genome (Abd El-Aziz 2020a). Potyviruses can infect a wide range of plants in the Solanaceae family. For instance, *Potato virus V* (PVV) and *Potato virus T* (PVT) can affect potatoes, tomatoes, and peppers. They cause symptoms such as mosaic patterns, yellow lesions, and dead areas in the veins (Melgarejo et al. 2004). Many potyviruses, such as those affecting groundnuts and potatoes, cause mosaic and mottling patterns on leaves. They are characterized by light and dark green patches on leaves, which can affect photosynthesis and plant vigor (Zalat et al. 2024). Leaf vein lightening, in which the veins become translucent, is another symptom observed with potyvirus infections. This can progress to vein banding, where the veins appear darker than the surrounding tissue (Tolin 2008). Infected plants often exhibit leaf deformation and stunted growth. This is particularly evident with *Papaya ringspot virus* and PVY infections, where leaves may become crinkled or shoestring-like, and overall plant growth is reduced (Desbiez & Lecoq 2021). Some potyviruses, such as *Zucchini yellow mosaic virus* (ZYMV), can cause necrotic lesions and blistering on leaves and fruit, severely affecting the quality and marketability of the produce. In crops such as papaya and squash, potyvirus infections can lead to fruit deformation with bumps and ringspot symptoms, affecting both yield and quality (Abd El-Aziz 2020b).

Potyvirus virions are long (700–750 nm), flexuous filaments, comprising a helical assembly of coat protein (CP) subunits bound to an 8–11 kb single, positive-sense single-stranded RNA genome (+ssRNA) (Zamora et al. 2017; Inoue-Nagata et al. 2022). For instance, the PVV genome is approximately 9,851 nucleotides long, with a 5'-NTR, a single open reading frame, and a 3'-NTR, longer than those of other related potyviruses, such as PVY and PVA (Oruetxebarria et al. 2000). The RNA genome encodes a large polyprotein, which is subsequently cleaved into functional proteins. The 6K2 PVA protein has been identified as a host-specific determinant of viral movement and symptom induction, emphasizing the role of specific viral proteins in host interactions (Spetz 2003). The potyvirus RNA genome also contains a specialized protein, the viral genome-linked protein (VPg), which covalently binds to its 5' end (Tavert-Roudet et al. 2017). This protein can interact with eukaryotic translation initiation factor 4E (*eIF4E*) of the host plant to form the VPg-eIF4E complex, initiating translation of the viral genomic RNA into two polyproteins (ORF I and II). These polyproteins were then processed by 3 viral proteinases (P1-pro, HC-pro, and Nia-pro) into 11 mature viral proteins (P3, P3N-PIPO, 6K1, CI, 6K2, VPg, Nia-Pro, Nib, and CP). These viral proteins are essential for viral replication, movement, and vector transmission (Revers & García 2015; Nigam et al. 2019).

The function and position of each protein in the potyvirus genome (monopartite, linear, positive-stranded ssRNA of 8–10 kb) are shown in Figure 1. Potyvirus RNA has a VPg protein at the 5' end and a poly-A tail at the 3' end. It functions as both the genome and mRNA for ORF I, which is translated by the VPg-eIF4E complex. The resulting polyprotein are processed into 10 viral proteins by 3 autoproteolytic proteinases (P1-pro, HC-pro, and Nia-pro). Polymerase slippage in P3 ORF initiates the synthesis of ORF II, which, following translation and proteolysis, leads to the formation of the P3N-PIPO protein (Olsper et al. 2015; Rodamilans et al. 2015).

Studies have shown limited genetic variability among European PVV isolates, whereas South American isolates exhibit greater variability. This suggests distinct strain groups in these regions (Spetz 2003). Potyvirus virulence varies with the plant cultivar and virus strain (Arogundade et al. 2020). Furthermore, coinfection of potyvirus with other viruses has been reported to accentuate disease symptoms in various plants, including chili pepper (Arogundade et al. 2012; Damiri 2014). Many factors influence the spread and prevalence of potyvirus, including host abundance, environmental conditions, and agricultural practices. Potyvirus prevalence and incidence factors are summarized in Table 1.

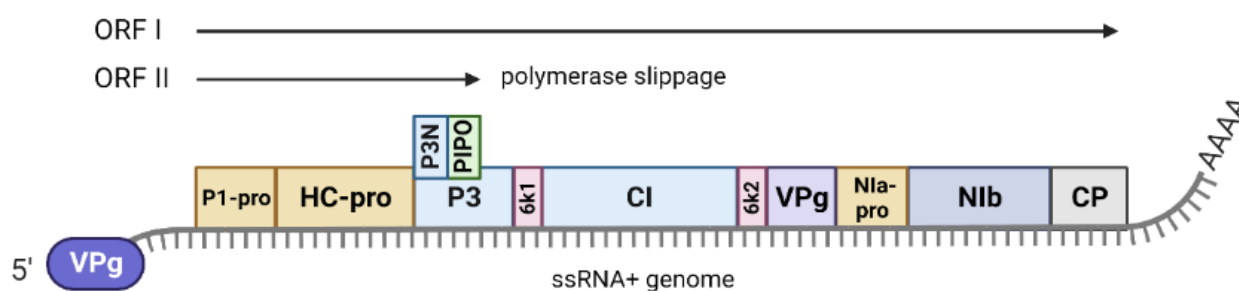


Figure 1. Transcriptional slippage in Potyviridae (adapted and synthesized from Olsper et al. 2015 and Rodamilans et al. 2015)

Gene products: VPg – viral genome-linked protein (for translation, virus movement, and replication); P1-pro – protein 1 protease (a modulator of replication, and host adaptation); HC-Pro – helper component protease (for RNA silencing suppression, aphid transmission, protease, and viral yield enhancement); P3 – virus replication and movement; P3N-PIPO – cell-to-cell movement; 6K1 and 6K2 – formation of replication vesicles; CI – cylindrical inclusion protein (RNA helicase, replication, and virus movement); Nia-Pro – nuclear inclusion A protease; Nib – nuclear inclusion B (for RNA-dependent RNA polymerase); and CP – coat protein (virus movement, virion formation, and aphid transmission)

Table 1. The key aspects of potyvirus dispersal and prevalence

Factor	Incidence	Reference
Host abundance and diversity	In wild ecosystems, such as the riparian forests in Spain, the presence of potyviruses such as <i>Iberian hop mosaic virus</i> (IbHMV) is strongly influenced by the number of host plants and the number of extant species. These factors also influence genetic diversity and selective pressure on the virus population. This supports the idea that ecological factors play a role in virus spread and evolution.	Rodríguez-Nevado et al. 2020
Climatic conditions	Environmental factors such as temperature, humidity, and rainfall significantly influence the risk of infection and the genetic diversity of potyviruses. For example, in the evergreen oak forests of the Iberian Peninsula, the presence of the <i>Mediterranean ruda virus</i> (MeRV) is shaped by host density and environmental conditions. These factors influence the genetic diversity of the virus and the selective pressure it has encountered.	Rodríguez-Nevado et al. 2017
Agricultural practices and human activities	In Venezuela, the presence of potyviruses in pepper plants has been linked to factors such as wastewater use, uncertified seeds, and improper use of agricultural chemicals.	Rodríguez-Román et al. 2023
Trade networks	The movement of agricultural products through trade networks can contribute to the spread of potyviruses across regions. This is evidenced by the widespread distribution of viruses such as <i>Potato virus Y</i> (PVY) in Colombia and the Samara region of Russia, where different strains of the virus have been detected in potato crops.	Ishkin et al. 2023; Riascos Chica et al. 2018
Viral evolution and genetic diversity	Potyviruses exhibit significant genetic diversity, often driven by recombination, as seen in <i>Zucchini yellow mosaic virus</i> (ZYMV) in Pakistan. This leads to the emergence of new virus strains, posing challenges for crop management. The genetic diversity of potyviruses is also evident in their phylogenetic relationships. In Turkey, <i>Tobacco etch virus</i> (TEV) has distinct phylogenetic lineages. These lineages are closely related to isolates from other regions, indicating the ability of the virus to spread across different areas.	Ahsan et al. 2023; Arpacı 2019

POTYVIRUS DISEASE MANAGEMENT

A variety of strategies have been implemented to prevent potyvirus infection and spread. Prevention of plant viral diseases includes phytosanitary, cultural, chemical, and biological methods, as well as the use of natural host resistance (Jones & Naidu 2019). These virus management strategies can be modified and implemented in chili pepper plantations. Virus-infected plants cannot be treated directly with chemicals, but their spread can be regulated by controlling the ongoing infection (Melgarejo et al. 2004). Virus control methods have evolved from conventional techniques to more advanced methods, such as the generation of transgenic organisms. Current and prospective methods for managing potyvirus diseases are divided into three categories: (1) prevention and control, (2) *in vitro* virus elimination, and (3) resistant plant cultivars.

POTYVIRUS PREVENTION AND CONTROL

Preventing and controlling the spread of virus infection on plantations are the simplest countermeasures in the fight against the potyvirus outbreak.

Phytosanitary, cultural, chemical, and biological methods have been developed and implemented to prevent or control the spread of plant viruses. All these methods aim to eliminate the source of the virus (infected seeds, propagules, plants, aphids, etc.) or inhibit its spread. Integrating diverse prevention and control methods can provide robust, economically feasible, environmentally sustainable, and socially acceptable solutions to virus outbreaks. To implement a comprehensive approach for managing potyvirus disease, it is important to consider local factors such as farming practices, climate, and the practicality of each method (Jones & Naidu 2019). The advantages and disadvantages of potyvirus prevention and control methods are summarized in Table 2.

Control of potyvirus diseases involves a range of strategies aimed at reducing the incidence and impact of potyviruses on crops. These strategies include phytosanitary and cultural practices, chemical, biological, and nanotechnology methods. Phytosanitary measures include quarantine, crop hygiene, and the use of virus-free planting material. The goal is to prevent the introduction and spread of potyviruses. Quarantine and the use of certified virus-free seeds effectively prevent the introduction of potyviruses into new areas.

This is especially important because some potyviruses, such as *Pea seed-borne mosaic virus*, can have 100% seed transmission efficiency (Shanmugam et al. 2024). The use of rigorous hygiene practices and the removal of infected plants, along with phytosanitary measures, can help limit the spread of potyviruses within and between fields (Gadhav et al. 2020). This can be combined with other control strategies, such as the use of resistant cultivars and vector control, to improve overall effectiveness. In resource-limited regions such as sub-Saharan Africa, this combined approach can be particularly helpful (Thresh 2003), although implementation can be challenging. And once infection occurs, it may prove insufficient. There is also a risk of resistance developing in insect vector pathogens (Thresh 2003).

Techniques such as crop rotation, sanitation, and the use of certified virus-free seeds help reduce the initial viral inoculum. These methods are environmentally friendly and can be incorporated into current farming practices without major extra costs (Mackenzie et al. 2021). Although effective, these methods must be strictly followed. They might not be sufficient in areas with a high risk of virus infection or where vectors are frequently present (Mackenzie et al. 2021).

The use of insecticides and mineral oils can effectively reduce the number of aphid vectors, thereby limiting virus transmission. These methods work well in areas with high vector activity. However, insecticides use is associated with environmental issues, such as pesticide resistance and impact on nontarget species. Moreover, the timing and frequency of application are crucial to effectiveness, which can be costly and require more labor (Mackenzie et al. 2021; Shanmugam et al. 2024).

Bio-based approaches often include genetic resistance and the use of viral vectors. Traditional methods of potyvirus control involve breeding for genetic resistance. This process includes introducing resistance genes into various cultivars. Developing resistant cultivars is a sustainable way to achieve long-term control of potyviruses. The use of NLR genes and other genetic tools can improve crop resistance and reduce reliance on chemical treatments (insecticides and repellents) (Hong et al. 2024). However, this process is often time-consuming and expensive (Yang et al. 2021). Potyviruses are spread mainly by aphids, and their spread is short-lived. Controlling aphid populations is an important part of potyvirus control (Ali 2020). Specific strategies include the use of insecticides, biocontrol agents, and cultural practices that limit aphid habitats. This also includes weed control (Kil et al. 2021).

Table 2. Current and prospective potyvirus disease prevention and control in Solanaceae

Method	Advantage	Disadvantage	Reference
Phytosanitary (hygiene routine, selection of healthy plants, sterilization and disinfection of media and tools)	tackles internal or external virus sources, easy to use, inexpensive, supports integrated pest management	effective only against early spread and labor intensive	Gadhav et al. 2020, Jones & Naidu 2019, Shanmugam et al. 2024, Thresh 2003
Cultural (planting or farming methods intended to block or minimize potyvirus spread)	can diminish early and late spread, inexpensive	the need to predict peak vector flights and optimal sowing dates	Jones & Naidu 2019, Mackenzie et al. 2021
Chemical (using chemicals to repel, kill, or prevent the growth of aphid vectors)	addresses early or late spread	may harm beneficial insects, promote insecticide-resistant pests, and leave toxic residues	Jones & Naidu 2019, Manjunatha et al. 2022
Biological (cross-protection, biopesticide, natural predator, parasite or pathogen of vector)	suitable for managing epidemics caused by insect-transmitted viruses	the need to predict the best moment to release the biological control agent	Jones & Naidu 2019
Nanotechnology (nanoparticle delivery system, nanophytovirology)	biocompatibility and biodegradability	requires further research, concerns about potential toxicity and environmental impact	Elsharkawy 2024, Marwal & Gaur 2021

Nanotechnology methods use nanoparticles to deliver antiviral agents or improve plant defense mechanisms. Researchers have used nanoparticles such as carbon dots and mesoporous silica to deliver double-stranded RNA (dsRNA) to plants. This process triggers RNA interference (RNAi) pathways, which boost plant defenses against viruses such as *Turnip mosaic virus* (TuMV) (Zarrabi et al. 2026). This method demonstrated improved efficiency in lowering viral levels and delaying symptom onset compared to naked dsRNA. Researchers have examined glycyrrhizic acid ammonium salt and salicylic acid nanoparticles for their ability to manage *Potato leafroll virus* (PLRV) by changing certain plant defense pathways. These nanoparticles can increase the expression of genes involved in plant defense, offering a promising option for managing the virus (Shoala et al. 2021). This new field focuses on the use of nanotechnology to manage plant viruses. Nanomaterials can interact with viruses and host plants in unique ways, improving the delivery and stability of antiviral agents, such as dsRNA. This method shows promise for developing sustainable and effective virus management strategies (Elsharkawy 2024). Nanoparticles can serve as biostimulants, drug carriers, and diagnostic tools, providing a multifaceted approach to virus management. However, challenges remain, such as determining optimal nanoparticle concentrations and application intervals. On the other hand, nanotechnology-based approaches require further research to fully understand their mechanisms and long-term effects on the environment and human health (Marwal & Gaur 2021).

IN VITRO VIRUS ELIMINATION

In vitro virus elimination techniques offer significant advantages for managing potyvirus diseases, particularly in crops such as potatoes, where viral infections can severely impact yield and quality. These methods, which include thermotherapy, chemotherapy, and cryotherapy, aim to produce virus-free planting material, thereby enhancing crop productivity and facilitating the safe exchange of germplasm (Naik & Buckseth 2018).

Virus elimination from plants is mostly based on *in vitro* culture combined with thermotherapy, chemotherapy, or cryotherapy (Naik & Buckseth 2018).

Thermotherapy involves the application of heat for a specific period to kill pathogens with minimal side effects on the host plant. In general, the higher the temperature and longer the exposure time, the greater the frequency of virus eradication, as high temperatures interfere with the synthesis of ssRNA and dsRNA (Lal et al. 2015). Thermotherapy is typically applied at 35–42°C for 4–6 weeks on the target plant, depending on the virus type and target plant species (Wang et al. 2018). Although increasing the temperature and duration of thermotherapy can enhance virus eradication, this method can induce high-temperature stress in the plant (Wahid et al. 2007).

Chemotherapy involves the use of chemical compounds to eradicate viruses in plant tissues. Several antiviral compounds function to control viral diseases in plants. In general, the use of exogenous antiviral compounds inhibits viral RNA synthesis (Xia et al. 2006; Zhao et al. 2006). Several chemical compounds, such as salicylic acid and ribavirin (Kidulile et al. 2018), and antiviral drugs, such as inosine monophosphate dehydrogenase inhibitors (IMPDH), S-adenosylhomocysteine hydrolase inhibitors, and neuraminidase inhibitors (NA) (Panattoni et al. 2013), have been shown to effectively eradicate viruses infecting plants.

In cryotherapy, plant pathogens such as viruses, phytoplasmas, and bacteria are exposed to liquid nitrogen for extended periods. This prevents metabolic reactions requiring heat from occurring (Vieira et al. 2015). This treatment can kill virus-infected cells while allowing healthy, virus-free cells to survive and develop into pathogen-free plants (Wang & Valkonen 2008; Smith et al. 2017).

The effectiveness of *in vitro* virus removal can vary depending on several factors. These include the type of virus, the plant cultivar, and the specific combination of methods used. For instance, the effectiveness of chemotherapy alone can range from 20% to 100%, depending on the virus and the conditions (Bettoni et al. 2022). Combining chemotherapy with cryotherapy has proven effective in eliminating multiple viruses. In some cases, this method has achieved up to 100% virus elimination (Bettoni et al. 2022). Methods for eliminating viruses from *in vitro* plant cultures can be complex and resource-intensive, requiring specialized equipment and expertise.

The need for precise control of factors such as temperature and chemical concentrations further complicates matters (Gong et al. 2019).

These methods are vital for producing virus-free planting material, which is essential for sustainable crop production and breeding programs. Virus-free plants produce higher yields and better-quality. Studies have shown that virus-free potato plants produce more microtuber growth (Bettoni et al. 2022). *In vitro* virus elimination helps ensure the safe transport of plant material across borders. This process is crucial for breeding and conservation programs. Despite high success rates, there is still a risk of not eliminating the virus. This can lead to a recurrence of the virus. This requires thorough, long-term testing after eradication, using methods such as ELISA and RT-PCR, to confirm that plants are virus-free (Gong et al. 2019).

DEVELOPMENT A RESISTANT CULTIVAR USING BIOTECHNOLOGY ADVANCEMENT

The development of resistant Solanaceae cultivars to potyvirus diseases is an important area of research, as these viruses significantly affect the economic value of crops such as potatoes and tomatoes. Various strategies are in use, including hybridization with resistant parents, genetic engineering such as RNA interference, and modern genome editing techniques using the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) method. These approaches aim to enhance the durability and spectrum of resistance in cultivars, addressing the challenges posed by virus specificity and the potential for evolving virus strains to overcome resistance. The advantages and disadvantages of several *in vitro* virus elimination methods are summarized in Table 3.

Plants have evolved multilayered defense systems against viral infections, including RNA silencing, RNA stability regulation, ubiquitin-mediated proteolysis, autophagy, the hypersensitive response (HR), other resistance (R) gene-mediated responses, and systemic acquired resistance (Fang et al. 2021). Several studies have identified the natural resistance of Solanaceae to potyviruses and their associated resistance locus (Table 4). Cultivars such as *Capsicum annuum* Avelar and *C. chinense* PI159236 have been shown to exhibit varying levels of resistance to several, but not all, types of potyviruses. The genetic resistance factors identified in these cultivars demonstrate efficacy not only against them but also against other distantly related viruses that infect chili, such as *Tobamovirus* (Murphy et al. 1998; Yoon et al. 2020). This indicates that the factors or genes responsible for chili potyvirus are not universal but may affect a wide range of viruses. This also suggests that distantly related chili viruses may use similar infection mechanisms.

The severity of viral infection depends on the resources provided by the host plant. Susceptibility (S) genes, such as *eIF4E*, play a role in viral replication. Loss or modification of these genes can lead to incompatible interactions and the development of plant resistant traits (Mäkinen 2020). In potyvirus infections, the VPg protein binds to the 5' end of the viral RNA and interacts with *eIF4E*, which is important for initiating translation of the viral genome and successful infection (Eskelin et al. 2011; Contreras-Paredes et al. 2013; Bastet et al. 2017). The *eIF4E* gene family in chili peppers includes *eIF4E1*, *eIF4E2*, and *eIF(iso)4E* (Moury et al. 2020). Some variants of these genes, such as *pvr1*, *pvr2*, and *pvr6*, are unable to efficiently interact with potyvirus VPg, resulting in a tolerant or resistant phenotype. Studies have shown that *pvr1*, *pvr2*, and some *pvr2* variants are allelic and encode *eIF4E1*, while *pvr6* encodes *eIF(iso)4E* (Kang et al. 2005; Ruffel et al. 2006; Rubio et al. 2008).

Table 3. Current and prospective development of plants cultivar

Method	Advantage	Disadvantage
Natural host resistance	several resistant cultivars	resistance-breaking virus strains are usually able to overcome these cultivars
Genetic engineering such as RNAi	possibility of achieving extreme resistance	difficult to gain approval; resistance-breaking virus strains can overcome them
Genome editing: potyvirus susceptibility gene knockout or modifications to mimic resistant genotype	more widely accepted than transgenic approaches	resistance-breaking virus strains can overcome them

Table 4. Several types of potyviruses and natural resistance genes of Solanaceae plants

Target virus	Resistance locus	Cultivars	Reference
PepMoV, TEV, and PVY	<i>pvr1</i>	<i>Capsicum chinense</i> PI159236 and PI152225	Yoon et al. 2020
PVY and TEV	<i>pvr2</i>	<i>Capsicum annuum</i> Yolo RP10, Yolo Y PI264281, SC46252, and Florida VR2	Rubio et al. 2008
PepMoV and TEV	<i>pvr3</i>	<i>Capsicum annuum</i> Avelar	Murphy et al. 1998
PepMoV, PepSMV, and PVY	<i>Pvr4</i>	<i>Capsicum annuum</i> CM334	Kim et al. 2015
PVY	<i>pvr5</i>	<i>Capsicum annuum</i> CM334 and Serrano Criollo de Morelos	Caranta et al. 1996
PVMV	<i>pvr6</i>	<i>Capsicum annuum</i> Perennial	Ruffel et al. 2006
PepMoV and PVY	<i>Pvr7</i>	<i>Capsicum chinense</i> PI159236	Liu et al. 2016; Venkatesh et al. 2018
PepMoV	<i>Pvr9</i>	<i>Capsicum annuum</i> CM334	Tran et al. 2015
PepYMV	unknown	<i>Capsicum annuum</i> PIM-025	Rezende et al. 2020
PVY and TEV	<i>pot-1</i>	<i>Solanum habrochaites</i> PI247087	Moury et al. 2014; Parrella et al. 2002
PVYo, PVYC, PVYN and PVYNTN	<i>Rysto</i>	<i>Solanum tuberosum</i> Group Phureja	Torrance et al. 2020
PVYa, PVYb, PVA, and PVV	<i>Rysto</i>	<i>Solanum stoloniferum</i>	Solomon-Blackburn & Barker 2001
PVA	<i>Raadg</i>	<i>Solanum tuberosum</i> ssp. <i>andigena</i>	Solomon-Blackburn & Barker 2001
PVY and PVA	<i>Nydm5</i>	<i>Solanum demissum</i>	Solomon-Blackburn & Barker 2001

Dominant R genes in chili peppers provide resistance to potyviruses. These genes encode R proteins that recognize pathogen effectors or avirulence factors, leading to the activation of a hypersensitive response (HR) or extreme resistance (ER) (Baebler et al. 2020; Fang et al. 2021). R proteins typically consist of a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region (Ellis et al. 2000). NBS domain hydrolyzes ATP and triggers resistance signaling (Tameling et al. 2002; Rairdan & Moffett 2006), while the LRR domain recognizes Avr factors (Ellis et al. 2007). NBS-LRR proteins can be categorized into two groups: TIR-NBS-LRR proteins with Toll/interleukin-1 receptor (TIR) homology and CC-NBS-LRR proteins with an N-terminal coiled-coil (CC) motif (Dangl & Jones 2001). Dominant resistance genes, such as *Pvr4*, *Pvr7*, and

Pvr9, confer HR to potyviruses in chili peppers and encode CC-NBS-LRR R proteins (Tran et al. 2015; Kim et al. 2017). Recent research has revealed that *Pvr4* and *Pvr7* are allelic (Venkatesh et al. 2018). A comparison of the molecular mechanisms between S-gene and R-gene-mediated resistance is shown in Figure 2. S-gene-mediated resistance is a recessive resistance involving incompatible interactions between host translation initiation factors (*eIF4Es*) and the viral protein VPg, resulting in suppressed viral protein production and viral multiplication. R-gene-mediated resistance, on the other hand, is a dominant resistance involving recognition of avirulent viral proteins by host R proteins, leading to the activation of a hypersensitive or extreme response. Such responses can limit virus movement within the plant or directly inhibit its replication.

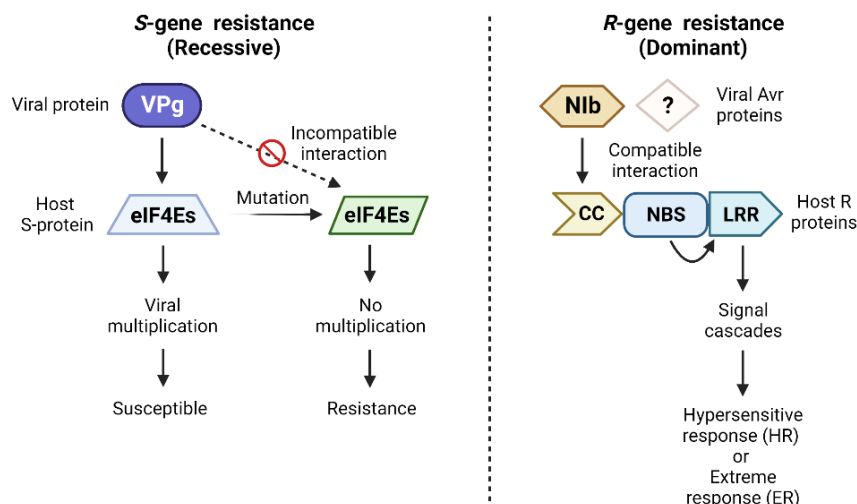


Figure 2. Two contrasting molecular mechanisms of host-mediated susceptibility and resistance to potyvirus infection in chili
 VPg – viral genome-linked protein; *eIF4E* – eukaryotic translation initiation factor 4E; NIb – nuclear inclusion B; CC – coiled-coil motif; NBS – nucleotide-binding site; LRR – leucine-rich repeat

RNAI AS A GENETIC ENGINEERING TOOL

The transgenic approach is a method in which a foreign gene is introduced into a target organism (Gu et al. 2021). This produces much more robust and stable genetically modified organism than the nontransgenic approach. Most Solanaceae plants are considered recalcitrant to genetic engineering, especially since *Agrobacterium* is the most commonly used approach for introducing foreign genes into plant cells (Wolf et al. 2001). Promising methods such as RNA interference (RNAi) (Schmitt-Keichinger 2019) and CRISPR/Cas13 (Bayoumi & Munir 2021) have the potential to drive further advances and breakthroughs. RNAi is a natural mechanism for regulating gene expression and plays an important role in defense against viral pathogens in many eukaryotic organisms, including plants (Worrall et al. 2019). RNAi works by recognizing target RNA via base complementation and inducing its degradation (Prasad Babu et al. 2019). RNAi remains one of the most reliable and widely used techniques for controlling gene expression, and to enhance plant resistance, RNAi can be used to silence host S-gene expression (Pecot et al. 2011) or inhibit viral gene expression (Worrall et al. 2019). Silencing the *eIF4E* gene family using RNAi technology has been shown to confer broad-spectrum resistance to potyviruses (Schmitt-Keichinger 2019;

Gao et al. 2020). The potential of RNAi-mediated viral interference is well established, as numerous studies have successfully demonstrated the ability to confer potyvirus resistance in plants (Cruz & Aragão 2014; Montes et al. 2014; Guo et al. 2015; Yang et al. 2018). A study by Yoon et al. (2021) showed that targeting both the mid NIb and HC-Pro parts of PepMoV, an important member of the Potyviridae, resulted in the greatest inhibition of virus replication in the model plant *Nicotiana benthamiana*.

CRISPR/Cas9 INCREASING PLANT RESISTANCE TO VIRUSES

CRISPR/Cas9 is the most widely used method due to its simplicity and ease of manipulation (Anzalone et al. 2020). CRISPR/Cas9 was developed as an RNA-guided endonuclease (RGEN) for precise and programmable genome editing (Jinek et al. 2012). The CRISPR/Cas9 genome editing technique works by inducing double-strand breaks (DSBs) in the target DNA. In the target eukaryotic cell, the DSB induces DNA repair through the non-homologous end joining (NHEJ) pathway. This results in indel mutation (gene knockout) or homology-directed repair (HDR), leading to integration of the template DNA (transgene) into the genome (Staahl et al. 2017; Gu et al. 2021).

The genes encoding the *eIF4E* protein represent attractive targets for genetic engineering to develop potyvirus-resistant Solanaceae plant cultivars. As mentioned before, the *eIF4Es* gene constitutes a multigene family. Due to the redundancy of *eIF4E* genes, knocking out (KO) a single gene from the family is likely to confer resistance to some potyvirus strains without significantly impairing plant growth (Kim et al. 2017). This approach has been tested to induce potyvirus resistance in *Arabidopsis thaliana* (Pyott et al. 2016) and *Cucumis sativa* (Chandrasekaran et al. 2016). However, these tests were limited to partial resistance and were often easily overcome by potyviruses evolving to recruit other members of the *eIF4E* gene family (Gallois et al. 2010; Chandrasekaran et al. 2016). Furthermore, several Potyviridae species frequently select one of the *eIF4E* isoforms, and plants carrying the knockout allele for an isoform remain susceptible to the virus by utilizing the other *eIF4E* isoform (Bastet et al. 2017).

The CRISPR system has also been used to edit specific DNA bases. Base-editing CRISPR uses a modified CRISPR/Cas that lacks double-strand break-inducing activity and combines it with the ssDNA deaminase enzyme. There are three types of base-editing CRISPR: cytosine base editors (CBEs), adenine base editors (ABEs), and dual base editors (DBEs). As their names suggest, CBEs catalyze the conversion of C–G base pairs to T–A base pairs, ABEs catalyze the conversion of A–T base pairs to G–C base pairs, and DBEs combine the functions of both editors in a single system (Grünwald et al. 2020; Zhang et al. 2020). To date, more than 12 base-editing CRISPR/Cas variants have been identified, differing in efficiency, sequence preferences, and activity windows. The choice of base-editing CRISPR/Cas can be made based on objectives, target sequence criteria, and using decision trees.

Base-editing CRISPR/Cas is currently extensively used for editing plant genomes. Several studies have demonstrated the use of base editors to induce herbicide resistance in rice and marker-free

tomato (Shimatani et al. 2017) and resistance to *Clover yellow vein virus* (CIYVV) in *Arabidopsis* (Bastet et al. 2019). This has typically been carried out by editing a susceptibility gene or factors to mimic resistance genotypes identified in nature or *de novo*. Resistance genotypes are alleles of host susceptibility factors that are incompatible with supporting viral infections. This was previously done to mimic the CIYVV-resistant *eIF4E1* gene in *Arabidopsis thaliana* (Worrall et al. 2019). A disadvantage of using base-editing CRISPR to confer viral resistance in plants is that it relies heavily on knowledge of resistant variants or polymorphisms in host susceptibility genes. However, base editing provides a major advantage over the indel-inducing CRISPR: base-edited plants are resistant to one virus and are less likely to be susceptible to another (Zafirov et al. 2021).

CONCLUSIONS

Potyviridae species pose a significant threat to global solanaceous crop production, and effective control measures are vital to protect crop yields and food security. Various approaches have been used, including traditional methods, resistance-oriented breeding, genetic engineering, and the CRISPR/Cas9 genome editing system. The CRISPR/Cas genome editing technique holds great promise for enhancing the resistance of Solanaceae plants to viruses and addressing production challenges. Compared to conventional methods and breeding, which are time-consuming, CRISPR/Cas editing enables precise modifications of viral resistance genes. However, acceptance and regulation of genetically modified crops differ across countries, emphasizing the need for further research and careful consideration of regulatory frameworks. Full implementation of CRISPR/Cas editing in chili pepper breeding could alleviate the impact of potyviruses on Solanaceae production and ensure a sustainable supply of this widely consumed spice.

Author contribution statement

RH – A – Research concept and design, B – Collection and/or assembly of data, D – Writing the article
 AP – A – Research concept and design, B – Collection and/or assembly of data, D – Writing the article
 HN – B – Collection and/or assembly of data, D – Writing the article
 JT – A – Research concept and design, B – Collection and/or assembly of data, D – Writing the article
 FR – B – Collection and/or assembly of data, D – Writing the article
 YP – B – Collection and/or assembly of data, D – Writing the article
 KM – A – Research concept and design, E – Critical revision of the article, F – Final approval of the article

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Conflict of interest

The authors declare no competing interests.

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