Current Strategies for Management of Plant Viruses and Future erspectives: Enhancing Crop Health, Yield and Productivity

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ABSTRACT

Plant viruses pose significant threats to agricultural productivity and food security worldwide. This article explores various management strategies employed to overcome the impact of plant viruses on crops, with a focus on enhancing crop health and productivity. It underscores the need for proactive management strategies to minimize yield losses, reduce disease spread, and maintain sustainable crop production through preventive measures such as Conventional and Non- conventional approaches by highlighting the key methods such as the use of resistant cultivars, cultural practices, vector control, thermos, electro, and chemotherapy, along with CRISPR, RNAi, and nanotechnology. This review article provides an overview of the current management approaches employed for controlling plant viruses.

Keywords: Plant viruses. Preventive measures. Cultural practices. Nanotechnology. CRISPR. RNAi. AI.

1. INTRODUCTION

Plant viruses pose significant challenges to global agriculture, impacting crop health, reducing productivity, and threatening food security. In plants, among all the disease-causing pathogens, viruses are the most harmful, as they cause about 40% of total crop losses. Globally, more than twenty-five families of plant viruses are known to infect a variety of crop species, leading to higher economic losses [1]**.**The highest impact occurs with emerging diseases infected with DNA or RNA viruses, which are transmitted by vectors and are defined by a rapid increase in disease incidence, geographical range, and pathogenicity. There are many factors that drive the emergence of viruses: a) Areas which are totally based on monocrops with low genetic range and excessive plant density, that are prone to pathogens and pests; b) Plant material like germplasm and live plants which enables viruses movements via vectors to new areas and environments; c) Change in climate and humidity, which also affect the distribution vicinity of hosts and vectors

simultaneously impacting the prevalence and spread of plant viruses; d) the potential of viruses for speedy mutation, evolution, and adaptation contributing to the challenges in controlling and treating viral infections [2-4].

In today's scenario, curing virus-infected plants is not easy at all, unlike bacteria or fungi, which can be easily treated with antibacterial or antifungal agents or biocontrol agents, respectively. So, disease management strategies play a vital role in preventing viruses from entering plants. Specific tools for virus diagnostics and identification are used for setting up and evaluating disease management. By understanding the modes of transmission, infection mechanisms, and factors influencing virus spread, effective control measures can be implemented. This article provides a comprehensive review of the management of plant viruses, highlighting conventional and non-conventional approaches and emerging strategies. Conventional strategies such as cultural practices, sanitation, vector control, chemical control, and quarantine measures continue to play crucial roles in plant virus management. However, the development of resistant crop varieties through natural resistance, breeding, and genetic engineering approaches has shown great promise. Furthermore, the emergence of new technologies such as RNA interference (RNAi), genome editing, nanotechnology applications, and the utilization of biocontrol agents offer exciting avenues for future virus management. Integrating these technologies with approaches like integrated pest management (IPM), and artificial intelligence (AI) applications can provide more effective and sustainable solutions. Managing plant viruses is of utmost importance to protect agricultural systems and sustain crop productivity by employing integrated approaches and adopting preventive measures, utilizing diagnostic tools, incorporating genetic resistance, and implementing appropriate cultural practices, the detrimental impact of plant viruses on crop health and productivity can be mitigated.

2. DISEASE MANAGEMENT OF PLANT VIRUSES

Eradication of viruses from diseased plants is difficult due to their speedy mutation, evolution and adaptation for efficient and sturdy manage, it's important to remember the genetic variety and evolution of virus populations and feature unique, fast, and reliable diagnostic approaches.

Here are some commonly used strategies for plant virus disease management that can be broadly categorized into conventional and non-conventional methods.**(Figure 1.)**

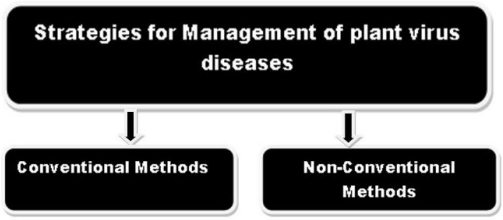

Fig.1. Strategies for management of plant viruses

CONVENTIONAL METHODS

Conventional strategies typically involve the use of Virus-free plant propagation through in vitro techniques coupled with various therapies like thermotherapy, chemotherapy, and electrotherapy, Cultural practices, pest control, and host resistance have been successfully employed for plant virus management. **(Figure 2.)**

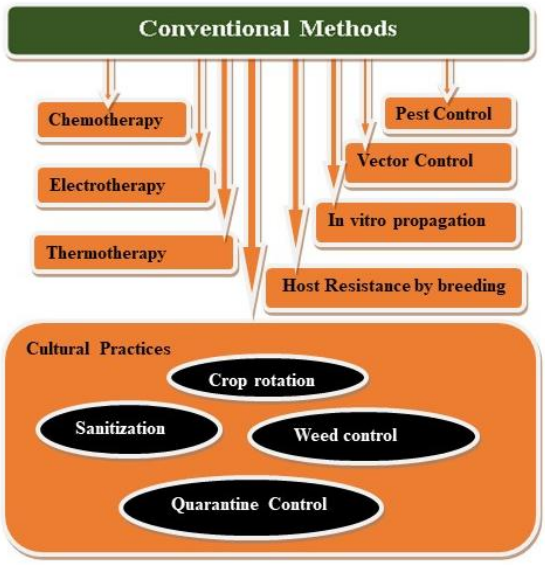

Figure 2.

Fig.2. Conventional Methods for management of plant viruses.

In vitro propagation:

In vitro propagation involves the growth and development of plant tissues in a controlled laboratory environment, typically on nutrient media. Meristematic tissues, such as shoot apical meristems or axillary buds, are used as starting materials due to their high regeneration potential. The advantage of in vitro propagation is that it allows the production of a large number of plants in a short period of time while maintaining virus-free statusMost commercially cultivated orchid plants are generally infected with cymbidium mosaic virus (CyMV) and odontoglossum ringspot virus (ORSV). Two methods were used in order to generate virus-free plants: meristem culture and thin section culture with chemotherapy. Meristems (0.10 mm to 1.00 mm) were excised from infected axillary shoots of an infected monopodial orchid hybrid (*Mokara* Char Kuan 'Pink') and cultured in modified Vacin and Went medium. Only larger meristem explants survived and the regenerated plantlets remained virus-infected[5].

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Chemotherapy & Thermotherapy:

Chemotherapy is based on antiviral drugs such as Ribavirin to inhibit or disrupt specific steps of the virus life cycle, such as nucleoside analogues, which inhibit replication, and protease inhibitors, which prevent protein processing[6].Thermotherapy involves subjecting infected plant materials or explants to elevated temperatures, typically ranging from 37°C to 40°Cthermotherapy should inactivate viruses by using viral RNA breakage, viral particle disruption or coat protein rupture. Previous studies have shown that exposing plants to thermotherapy can be effective for ACLSV, ASGV, ApMV (*Apple mosaic virus*), and ASPV eradication in diseased apple cultivars [7].

Table 1. Chemotherapy was employed to eliminate some viruses.

S.No.	Crops	Virus susceptibility	References
1.	Potato Virus	*Potato virus S*(PVS), *Potato virus A*(PVA) and *Potato virus M* (PVM)	[8]
2.	Vitis vinifera	*Grapevine Fanleaf Virus* (GFLV)	[9]
3.	Cassava	*Cassava Brown Streak Virus*(CBSV).	[10]

Electrotherapy:

Electrotherapy, also known as electrofusion or electroporation, involves the application of an electric current to plant tissues. This technique disrupts the cell membranes and allows the introduction of antiviral agents or other therapeutic molecules into the cells. Electrotherapy has been explored for the control of systemic plant viruses by introducing antiviral RNA molecules or other nucleic acids into infected tissues. Electrotherapy was successful in eliminating *Potato virus X*PVX[11].

Cultural Practices:

Crop rotation: Rotating crops can help break the disease cycle by reducing the buildup of viral pathogens in the soil. Different crops act as hosts for different viruses, preventing the continuous

presence of the same virus by adjusting planting density to minimize contact between plants, and implementing irrigation methods that avoid splashing water and potential virus spread[12].

Sanitation: Proper sanitation measures, such as removing infected plant debris, can reduce the source of inoculum and prevent the spread of viruses.Practicing good sanitation measures can help manage viral diseases. Additionally, cleaning tools and equipment to prevent mechanical transmission and using virus-free planting material are important sanitation practices[13].The sanitated plants must be evaluated and confirmed to be virus-free with very sensitive techniques such as real-time qPCR. This approach has been assayed recently with a plant virus, *Tobacco Mosaic Virus* (TMV), resulting in a loss of viral infectivity[14].

Weed control: Weeds can act as reservoirs for plant viruses, serving as hosts for viral pathogens and potential vectors, so effective weed management can help reduce the spread of viruses by mechanical, chemical, or cultural methods.

Table 2.Weed plants which can be infected by viruses.

S.No.	Weed species	Viruses' susceptibility	References
1.	Dandelion (*Taraxacum officinale*)	*Dandelion Yellow Mosaic Virus, Dandelion Curly Top Virus.*	[15]
2.	Common chickweed (*Stellaria media*)	*Chickweed Yellows Virus*	[16]
3.	Purslane (*Portulaca oleracea*)	*Cucumber Mosaic Virus*	[17]
4.	Broadleaf plantain (*Plantago major*)	*Broadleaf Plantain Mottle Virus, Cucumber Mosaic Virus*	[18]
5.	Pigweed (*Amaranthus spp.)*	*Pigweed Mosaic Virus*	[19]

Host Resistance:Developing and utilising resistant plant varieties is an effective strategy for virus disease management. Breeding programs aim to introduce genetic resistance to specific

viruses into crop plants, making them less susceptible to infection or reducing the severity of symptoms.Breeding for resistance to plant viruses can involve selecting natural resistance genes or introducing them through genetic engineering. Genetic modification techniques, such as transgenic approaches, can be used to introduce specific resistance genes into susceptible plant species. For example, genetically engineered crops with resistance to *Papaya ringspot virus* (PRSV) have been successfully developed by [20].

Vector Control:maximum plant viruses are transmitted with the aid of arthropod vectors, especially aphids, whiteflies, and thrips. Translocation of begomoviruses via whitefly causes *Tomato Yellow Leaf Curl Virus* (TYLCV) infection, which is a major threat to tomato crops. Peppers, cucurbits, eggplants, and beans are also infected with begomoviruses transmitted by vectors. Hence, Controlling the insect vectors responsible for transmitting viruses can significantly reduce disease spread[21].

NON-CONVENTIONAL METHODS

Non-conventional methods for plant virus management involve innovative approaches that go beyond traditional cultural practices and chemical control. Here are some strategies for the disease management of plant viruses. **(Figure 3.)**

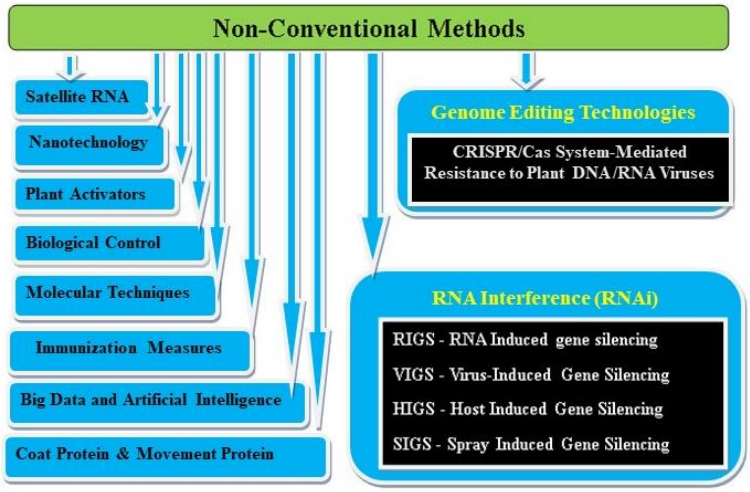

Figure 3.

Fig.3. Non-Conventional Methods for management of plant viruses.

Biological Control:

Beneficial microorganisms, such as bacteria, fungi, and viruses, can be used as biocontrol agents to suppress plant viruses. They can either directly inhibit viral replication or induce systemic resistance in plants against viruses[22]. Use of parasitic wasps has been successful in controlling aphid vectors of plant viruses, biopesticides are used to control insect vectors or directly inhibit viral replication in plants. Baculoviruses and entomopathogenic fungi have shown potential as biocontrol agents for vector management[23].

Plant Activators:

Plant activators are compounds that induce systemic acquired resistance (SAR) in plants, making them more resistant to viral infections. They activate the plant's immune response and strengthen natural defence mechanisms [30].

RNA Interference (RNAi):

RNAi-based strategies involve the introduction of small RNA molecules that target viral RNA, thereby triggering sequence-specific degradation of the viral genetic material. This can effectively reduce viral replication and the development of symptoms. RNAi has been successfully used to confer resistance to various plant viruses, including *Tomato yellow leaf curl virus* and *Potato virus Y* (PVY)[30].

Table 3. Biocontrol agents used to suppress plant viruses.

S.No.	Crops	Virus susceptibility	Biocontrol agent	vectors	References
01	Tomato	*Tomato yellow leaf curl virus (TYLCV)*	*Encarsiaformosa*	Whitefly	[24]
02	Potato	*Potato virus Y (PVY)*	*Aphidiuservi*	Aphids	[25]
03	Citrus	*Citrus tristeza virus (CTV)*	*Tamarixiaradiata*	Asian citrus psyllid	[26]
04	Cucumber	*Cucumber mosaic virus (CMV)*	*Aphidiuscolemani*	Aphids	[22]
05	Pepper	*Pepper mild mottle virus (PMMoV)*	*Oriusinsidiosus*	Thrips	[27]
06	Bean	*Bean common mosaic virus (BCMV)*	*Aphidoletesaphidimyza*	Aphids	[28]
07	Cotton	*Cotton leaf curl virus (CLCuV)*	*Trichogrammachilonis*	Cotton leafhopper	[29]

08	Wheat	*Wheat streak mosaic virus (WSMV)*	*Coccinellaseptempunctata*	Aphids	[30]

RNA Induced gene silencing:

RNA Induced gene silencing: Gene silencing is triggered by small RNA (sRNA) molecules. sRNAs are small interfering RNA (siRNA) and microRNA (miRNA). The silencing process is initiated when long dsRNAs are cleaved into small fragments (21–25 nts) of sRNAs with the help of the Dicer (DCL) enzyme inside the cytoplasm [31, 32]**.**Now these sRNA duplexes unwind themselves and are loaded into the RISC, where the stable strand is selected as the guide strand and the second strand is the passenger strand, which is later discarded. Guide strands integrate into the RISC and activate it. An essential member of the RISC is the Argonaut (AGO) protein. After loading the guide strand, the AGO protein, by its endo-nucleolytic action, mediates the repression or degradation of the targeted mRNA [33, 34]**.**Cleavage of targeted mRNA is initiated 10–12 nt away from the region's centre, recognized by the guide sRNA[32].The mechanism is accomplished by amplifying sRNA molecules through RNA-dependent RNA polymerase (RDRs) enzymes. RDRs produced double-stranded RNA, which was further cleaved and processed by DCLs and continued in the next round of RNA silencing[35].

Virus-Induced Gene Silencing (VIGS):

Virus-Induced Gene Silencing (VIGS): In VIGS, the viral genome is manipulated by deleting the disease-causing genes, and then cloning can be performed for the modification of the viral genome cDNA into a binary vector. Viruses that lack gene silencing or weak suppressors are potential targets as VIGS vectors[36, 37]**.** The specific silenced gene is cloned into the MCS of the binary vector The recombinant virus then enters the plant cells through an Agrobacterium-mediated transformation or DNA bombardment into the host cells. Once the recombinant virus enters the plant cell, RNA-dependent RNA polymerase (RdRp) transcribes the viral RNA and transgene[38].Double-stranded RNAs (dsRNAs) are generated and further cleaved by Dicer into 21–25 nts long siRNA now these siRNAs further loaded into the RISC that targeted the complementary DNA[37]. The *Tobacco mosaic virus* (TMV), *Tomato golden mosaic virus*

(TGMV), and *Potato virus X* (PVX) belong to the first generation of the VIGS vector system, which causes the short-term silencing of endogenous gene expression and leaf chlorosis[39].

Host-Induced Gene Silencing (HIGS):

It is based on the plant's natural immune system, which utilizes RNA-induced silencing to defend against the viral infection. HIGS is further advanced to VIGS, which silences the pathogenic genes inside plants by targeting the specific genes of the pathogen inside the host plant[40, 41].In HIGS, transgenic plants are generated by introducing an inverted repeat sequence inside the plant genome. Double-stranded RNAs are produced as small RNAs inside the transgenic plants, introduced either through Agrobacterium or VIGS.The improvement of efficient, resistant, and polycistronic miRNA and the fusion of multiple genes in hairpin RNA are efficient and successful [42]. Infections towards the *wheat streak mosaic virus* (WSMV) have been decreased using a coat protein and a full-period viral replicase (Nib) gene[43].

Spray Induced Gene Silencing (SIGS):

It is an advanced RNA silencing strategy for disease control. It is used for monocot and dicot pathogen infections and has been used for crop protection based on findings that plant pathogens can uptake dsRNA, which is applied externally[44, 45]. This dsRNA then silences the targeted pathogen genes, which is critical for disease improvement. SIGS is an eco-friendly and advanced strategy for pathogen control at pre-harvesting and post-harvesting stages and offers fewer off-target effects. The topical application of dsRNA confers resistance against *Alfalfa mosaic virus* (AMV), *Pepper mild mottle virus* (PMMoV), and *Tobacco etches virus* (TEV). SIGS had been performed by the low-pressure spraying of siRNAs, which was previously reported to fail GFP silencing. However, the problem was overcome by spraying siRNA at high pressure, which successfully silenced the GFP. Further investigation is required to optimize the high-pressure spraying technology against targeted tissues[46].

Genome Editing Technologies:

Techniques such as CRISPR-Cas9 play major role in modifications of plant genome, including editing of viral susceptibility genes. Thisapproachshowspromiseindevelopingvirus-tolerantcropsbydisruptingessentialviralgenesorhostsusceptibilityfactors.**(Figure 4.)**

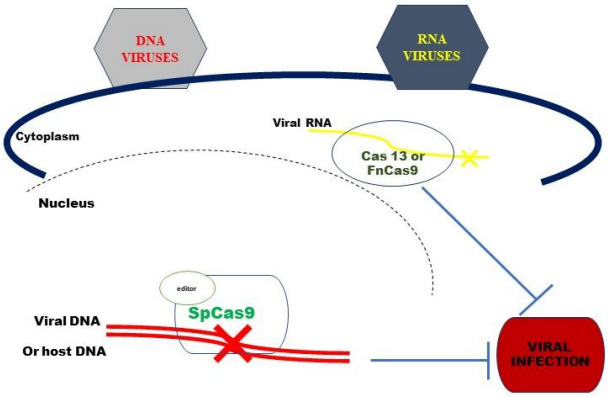

Figure 4.

Fig.4. Schematic representation of class 2 CRISPR/Cas systems to confer resistance to plant viruses. After plant viruses enter plant cells, the viral genome is uncoated and transcribed, or translated, with the help of host factors. Plant viruses then multiply their genomes in the nucleus or cytoplasm. The viral genome DNA or RNA can be targeted, destroyed, or interfered with by CRISPR/Cas9 or CRISPR/Cas13 (or FnCas9) systems in the nucleus or in the cytoplasm, respectively, which inhibits viral infection. In addition, the mutation or deficiency in host susceptibility factors edited by the CRISPR/Cas9 system also perturbs viral infection. However, some new viral variants might be generated as by-products when CRISPR/Cas systems edit the viral genome, which might increase the risk of viral evolution.

CRISPR/Cas System-Mediated Resistance to Plant DNA Viruses: Geminiviridae and Caulimoviridae are two major destructive plant DNA virus families that contain 485 species with single-stranded DNA (ssDNA) genomes and 85 species with double-stranded DNA (dsDNA) genomes. Before the emergence of the CRISPR/Cas systems, zinc finger nucleases (ZFNs) and

transcription activator-like effector nucleases (TALENs) were applied to manipulate host and viral DNAs in plants. ZFN- and TALEN-mediated resistance to several geminivirus, including *Tomato Yellow Leaf Curl China virus* (TYLCCV) and *Tobacco Curly Shoot Virus* (TbCSV), by targeting the viral genomic replication-associated region has been reported[47, 48]. CRISPR/Cas9 constructs with single guide RNAs (sgRNAs) targeting the viral replication-associated region, or intergenic region (IR), have exhibited effective DNA interference and conferred viral resistance against *Beet Severe Curly Top Virus*(BSCTV), *Cotton Leaf Curl Multan Virus*(CLCuMuV), and *Bean Yellow Dwarf Virus* (BeYDV) in transgenic Nicotianabenthamiana or Arabidopsis thaliana plants[49, 50]. Targeting the *Tomato Yellow Leaf Curl Virus*(TYLCV) genome with Cas9-single guide RNA at the sequences encoding the coat protein (CP) or replicase (Rep) resulted in efficient virus interference, as evidenced by the low accumulation of the TYLCV. DNA genome in the transgenic tomato and N. benthamiana plants CRISPR/Cas9-triggered mutations in the open reading frame (ORF), but not the geminivirus IR, are capable of replication and whole-body movement, thus circumventing the CRISPR/Cas9 machinery. Using this system via a single sgRNA targeting the conserved stem-loop sequence of the origin of replication in the intergenic region of TYLCV, we were also able to confer broad-spectrum resistance to other geminiviruses in plants, including the monocomponentgeminivirus, beet curly top virus (BCTV), and the bicomponentgeminivirusMeMV[51]. The CRISPR/Cas9 system combined with sgRNAs targeting the motility protein (MP) or CP regions conferred resistance to wheat dwarf virus (WDV) and banana streak virus (BSV), respectively [52].In addition, CRISPR/Cas9-mediated immunity has been recently utilized to defend against plant dsDNA viruses. A. thaliana transgenic plants consistently expressing both Cas9 protein and sgRNAs targeting the CP region of *cauliflower mosaic virus* (CaMV) in the Caulimoviridae family conferred effective resistance to this species[53].

CRISPR/Cas System-Mediated Resistance to Plant RNA Viruses:RNA targeting and editing CRISPR-associated proteins were Cas9, derived from Francisellanovicida (FnCas9), and Cas13a (formerly called C2c2), from Leptotrichiashahii (LshCas13a) it is the first Cas13 ortholog for programmable RNA-targeting activities, which expanded the application of CRISPR/Cas systems from DNA to RNA[54]. LshCas13a are programmed to cleave ssRNA viruses in plants by targeting viral RNA shows resistance towards TMV, *Rice Stripe Mosaic Virus* (RSMV), and *Southern Rice Black-Streaked Dwarf Virus*(SRBSDV) in transgenic tobacco and rice harbouring.

By targeting the P3-, NIb-, or CP-coding sequences in the *potato virus Y* (PVY) genomic region, this CRISPR/Cas13a system also showed its effectiveness in interfering with and inhibiting PVY infection[55]. Researchers found that Cas13d showed great advantages over Cas13a, Cas13b, or other Cas13 variants when it was used to interfere with TuMV infection by targeting the GFP, CP, or HC-Pro regions in the TuMV-GFP genome[56].

Nanotechnology:

Nanotechnology has emerged as a promising field for the management of plant viruses. It involves the manipulation and application of materials at the nanoscale level to achieve desired outcomes. Several approaches utilizing nanotechnology have been explored for the detection, prevention, and treatment of plant virus infections. Nanoparticles can be used to deliver antiviral agents or RNA molecules to plants, targeting viral replication and reducing viral load[57].

Nanosensors for virus detection:Nanosensors can be used for the rapid and sensitive detection of plant viruses. These sensors are designed to recognize specific viral components or nucleic acids. For instance, gold nanoparticles functionalized with viral-specific antibodies or aptamers can be used to detect and quantify virus particles in plant samples[48].

Nanocarriers for targeted delivery:Nanoparticles can serve as effective carriers for delivering antiviral agents to plant cells. Functionalized nanoparticles can be loaded with antiviral compounds or siRNA molecules targeting viral genes. These nanoparticles can be designed to specifically target infected cells or tissues, thereby reducing off-target effects[58].

Nanovaccines for plant protection:Nanotechnology offers a platform for developing nanovaccines against plant viruses. Nanoparticles can be engineered to display viral antigens, mimicking the virus's structure. These nanovaccines stimulate the plant's immune system, leading to enhanced resistance against viral infections. Carbon nanotubes, liposomes, and virus-like particles are examples of nanocarriers used for nanovaccines delivery[59].

Nanoparticle-mediated RNA interference (RNAi):RNAi-based approaches can be used to silence viral genes and inhibit viral replication. Nanoparticles can be utilized to deliver small interfering RNA (siRNA) molecules into plant cells, thereby triggering RNAi-mediated antiviral

responses. Various nanoparticles, such as liposomes, carbon nanotubes, and dendrimers, have been investigated for efficient siRNA delivery[60].

Artificial Intelligence (AI):

The integration of big data and artificial intelligence (AI) has significant potential for managing plant viruses through predictive modelling and precision control strategies.Predictive modelling: AI techniques, including machine learning and data mining, can analyse historical and real-time data on environmental factors, host plant characteristics, and virus spread patterns to develop predictive models.These models can forecast virus outbreaks, identify high-risk areas, and guide targeted surveillance and management strategies by proactively predicting virus spread, farmers can implement timely preventive measures. Precision control strategies: big data and AI enable the development of precision control strategies for managing plant viruses. By integrating data from various sources, including weather data, crop growth parameters, and disease records, AI algorithms can optimize the timing and dosage of interventions such as pesticide applications or cultural practices. This approach helps minimize the use of chemical inputs and reduces the risk of resistance development[61].

CONCLUSION

The management of plant viruses is a critical aspect of protecting agricultural crops and ensuring global food security. Both conventional and non-conventional methods have been employed to mitigate the impact of viral diseases on plants. Conventional methods such as crop rotation, sanitation, vector control, and resistant varieties have proven effective in reducing virus transmission and minimizing crop damage. Non-conventional methods, including cross-protection, RNA interference, and plant vaccines, offer innovative approaches to enhance plant resistance and control viral infections. These methods have shown promise in providing long-term solutions by targeting the viruses directly or inducing plant immunity. Furthermore, emerging technologies have opened up new avenues for plant virus management. Genome editing techniques like CRISPR-Cas9 allow precise modifications to plant genomes, enabling the development of virus-resistant crops. Next-generation sequencing facilitates rapid and accurate virus detection, while nanotechnology offers novel delivery systems for antiviral agents. Remote

sensing combined with artificial intelligence allows for early detection and monitoring of virus-induced changes in crop health.

It is crucial to continue research and development in plant virus management to stay ahead of evolving viral strains and their vectors. Collaboration between scientists, farmers, and policymakers is essential to implementing effective management strategies, promoting awareness, and developing sustainable agricultural practices. It is necessary to continue research and development in these areas to enhance plant virus management practices, reduce the impact of plant viruses on crop production, and safeguard global food security.

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