A systematic review of conventional and advanced approaches for the control of plant viruses

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ABSTRACT

Viruses are the obligatory intracellular parasites infecting microbes, plants, animals, and humans. They are dead outside host cell but can take-over the host’s cell machinery as soon as they are into it. Several studies on inhibitor compounds have been done for animal viruses including those that are affecting humans, but there is inadequacy in terms of research and literature for plant viruses that are responsible for losses in crop yield and quality loss all across the globe. This could be focal point to study plant viruses, their transmission and pathogenicity, and to establish widely used, effective, and advanced approaches for their control. The purpose of this review is to discuss various approaches to control plant viruses that have been developed and applied to combat plant viral infections. We have divided these approaches into two categories conventional (meristem-tip culture, cryotherapy, thermotherapy, and chemotherapy) and advanced (nucleic acid-based approaches like RNA Silencing, cross-protection, transgenic plants, gene pyramiding, and protein-protein interaction). Moreover, we have discussed and compared the principles, methodologies, advantages, and disadvantages of each technique. The approaches have been explored to promote their application in best suited way on various plants to control viral diseases and to improve food crops quality with increase in production.

1. INTRODUCTION

Viruses are obligatory intracellular parasites whose replication and pathogenicity strictly depends on their host cell machineries [1]. Viruses have caused significant damage to plants, livestock, and human health and are still the most prominent threat to any living beings. Moreover, a number of wild plants are always found to be surrounded by viruses [2,3]. Tobacco mosaic virus (TMV) was the first plant virus to be studied, which is responsible for the mosaic disease in Tobacco [4]. According to the International Committee for the taxonomy of viruses, there are about 900 species of plant viruses [5] and studies indicate many more new plant viruses are yet to be discovered [3].

Most of the viruses that cause damage to cultivated plants are acute, i.e., they bring about a dreadful infection for a short-time period, but in case of wild plants, a vast number of viruses have a persistent lifecycle that is they continue with their plant hosts machinery [2]. These viral infections in plants result in substantial damage to the crop production and quality. Plants infected with TMV, Papaya ring spot virus, Potato virus Y, etc. show symptoms such as leaf distortion and yellowing, whole plant stunting, and abnormality in flowers and fruits. In agricultural field, the worldwide estimated cost yield losses due to plant viruses are more than $30 billion annually [6] [Table 1].

Thus, it is imperative to study these viruses and develop and deploy strategies to curb plant viral diseases. The plant virus control techniques either employ transgenic technology or utilize the natural resistance observed in some plants. The available plant virus control approaches can be broadly classified as conventional and advanced approaches, which we discussed elaborately in this review. Moreover, we highlighted the drawbacks of conventional methods and congregated the successes and failures of the techniques used in conventional approach and emphasized the use of advanced methods over conventional approach while targeting plant viruses.
Table 1: Estimated cost of crop damage and crop loss per year by plant viruses across the globe.

| Location                  | Crop infected       | Estimated cost of crop damage by viruses per year | Crop loss per year | Virus responsible     | References |
|---------------------------|---------------------|---------------------------------------------------|--------------------|------------------------|------------|
| Africa, India, Srilanka   | Cassava crop        | 25 million tons                                    | Rice affecting culture virus | Cassava mosaic begomovirus | [7-9]      |
| US                        | Potato              | $ 100 million                                     | 200 million trees    | Potato leafroll polerovirus | [6,10]     |
| UK                        | Potato              | $ 30–50 million                                   |                    | Potato leafroll polerovirus | [6,10]     |
| UK                        | Barley, Oats, Rice, Wheat, Maize | $ 13.93 million                                  |                    | Barley yellow dwarf luteovirus | [11]       |
| South-East Asia           | Rice                | $ 1.5 billion                                     |                    | Rice affecting culture virus | [12-14]    |
| Togo, Ghana, Nigeria      | Cacoa Trees         |                                                  |                    | Cacao swollen shoot | [15]       |
| Worldwide                 | Citrus Trees        |                                                  |                    | Citrus triteza closterovirus | [16,17]    |

2. CONVENTIONAL METHODS

2.1. Meristem-Tip Culture

The meristem tip culture is performed by cutting out of organized shoot apex from mother plant for subsequent in vitro culture which confirmed to be the most active technique to eliminate phloem-associated viruses [18]. It began in meristem tips of Nasturtium (Tropaeolum majus) by the formation of rooted plants [19]. In several cases, meristem tip culture proved to be effective in removing plant viruses such as eliminating Sugarcane yellow leaf virus in sugarcane [20], Peanut stripe virus (PStV) in patchouli plants [21], and Piper yellow mottle virus (PYMoV) with 84% success rate in black pepper plants [22]. The advantage of this technique includes working with small explants devoid of pathogenic organisms taken from mother plant for the in vitro culture. The other advantage is the inherent genetic stability of the technique [23,24]. Disadvantages include expensiveness, acclimatization, variability, production scheduling, and contamination.

2.2. Chemotherapy

Anti-viral compounds are useful to control plant viral diseases. Chemical compounds such as ribavirin (RBV) (virazole), azidothymidine, and 2-thiouracil [25] and some antiviral drugs such as inosine monophosphate dehydrogenase (IMPDH) inhibitors, S-adenosylhomocysteine hydrolase inhibitors, and neuraminidase (NA) inhibitors [26] are generally used in chemotherapy. These compounds and drugs enter the plant during soaking process and prevent viral replication [27]. Grapevine leafroll-associated virus-1 and -3 (GLRaV-1 and 3) have been eradicated from plants by using selective chemotherapy. Specifically, IMPDH inhibitors were more active against GLRaV-1, whereas NA or purine biosynthesis inhibitor was found to be more effective against GLRaV-3 [28]. Grapevine rupestris stem pitting-associated virus (GRSPav) has been eradicated by using the antiviral IMPDH inhibitors, tiazofurin, RBV, and mycophenolic acid with exposure to in vitro chemotherapy [29]. Prior to meristem tip culture, chemotherapy was used and it resulted in complete elimination of Lily symptomless virus [30]. Although they incur some disadvantages as they all have different modes of action [31,32] and are not effective ex vitro even at higher concentration [30], this method proved to be successful in many instances (Table 2).

2.3. Cryotherapy

Researchers use saline solutions containing crushed ice at a temperature of −18° to −24°C for the treatment of human tumors (breast, cervical, and skin), which also help in decreasing pain [33]. Similarly in plant cryotherapy, pathogens such as viruses, phytoplasma, and bacteria are exposed to low temperature (−196°C) for a prolonged time, which successfully eradicates virus complexes resulting in virus-free plants with high frequency as compared to meristem tip culture [Table 2] [27,48]. It does not allow the occurrence of thermally directed metabolic reactions. It has been found that three Closteroviridae viruses who cause leafroll disease in grapevine are eradicated by vitrification (using dehydrating material) based cryotherapy of buds of contaminated clones [54]. Advantages of cryotherapy include treatment of large numbers of plantlets and the technique is applicable independent of shoot tip size, whereas major disadvantage includes large consumption of certain gases like Argon and Nitrogen [55].

2.4. Thermotherapy

In thermotherapy, heat treatment is given for a particular time, which kills the conserved pathogen with little effect on host. Heat is applied mainly by water, air, or vapor [56]. Temperature used for this technique is 52°C–55°C for 10–30 minutes. It has been found that increasing temperature significantly reduces the virus-related diseases as it disrupts viral ssRNA and dsRNA synthesis [27]. Exposure of tubers to 37°C for 4 days followed by 34°C for 3 days up to two weeks has the highest survival rate of 50% among the infected plants [57]. It has been found that thermotherapy becomes more effective when applied with other conventional therapies, e.g., Apple chlorotic leaf spot virus, Apple stem grooving virus, and Apple stem pitting virus, which infect apple plant, have been eradicated with the help of thermotherapy at various temperatures.
with chemotherapy at different concentrations of RBV [58]. Chemotherapy along with thermotherapy is used to eliminate Arabis mosaic virus (ArMV), Prunus necrotic ringspot virus (PNRSV), and ArMV + PNRSV from rose infected plants with a success rate of 63.33%, 90.09%, and 85.18%, respectively [53]. (Table 2)
3. ADVANCED METHODS

3.1. RNA Interference (RNAi)-Mediated Response and Applications

RNAi is a technique in which a dsRNA is used to silence definite functions of a gene that is useful to protect the host organism against viruses and unfamiliar nucleic acids [59]. This mechanism is illustrated by different names in different organisms such as quelling, post-transcriptional gene silencing, and RNA interference in fungi, plants, and animals, respectively [60]. RNA Silencing is a diverse technique [61], first reported in *Petunia hybrid* [62] and was the first antiviral mechanism used against RNA viruses [63].

RNA Silencing is an innate antiviral defense mechanism initiated by dsRNA [62]. RNA viruses are both activators as well as targets of RNA Silencing [64]. The excess of leftover RNA is changed to dsRNA by RdRP (RNA dependent RNA polymerase), thus activating RNA silencing [65,64].

Majority of the plant viruses have ds secondary structure elements in their RNA genome and produce dsRNA intermediates by viral RdRPs during replication. Then, virus-derived small RNAs are produced by RNA silencing system with the help of dsRNA intermediates (VsRNAs). VsRNAs integration in RISC (RNA-induced silencing complex) leads to the sequence-specific degradation of viral genome and initiation of mobile-silencing signal, which proliferates via plasmodesmata between cells and over very large distances via a relay-amplification process associating host RdRPs [66]. This triggers the RNA silencing process in uninfected cells and is prominently liable for the plant recovery process. Immune responses induced by gene silencing are exceptionally unique and specific to the pathogen and it is generally approved that RNAi is classified to plant adaptive immunity [67,63].

Current studies have shown that maximum success rate of RNA silencing is seen against RNA viruses and rarely against DNA viruses. One such example is Gemini virus, which is a ssDNA virus in which RNA silencing mechanism was used to target its genome via bombarding with hpRNA construct having promoter sequence of Gemini virus, *Vigna mungo* yellow mosaic virus (VMYMV), under the regulation of 35S promoter. With this strategy, a large number of plants completely retrieved from VMYMV infection [68,69].

Since RNA silencing-mediated resistance deals with many of the interactions between various factors, such as sequence similarities, selection of target, pathogen titer, and surrounding temperature, it becomes challenging to accurately conclude the efficacy [70]. This is one of the major limitations of RNA Silencing-mediated resistance. Therefore, more scientific research is needed for the evaluation of resistance efficiency in the crop field and also to unveil the limitations in peculiar cases. However, the most important advantage of RNAi over other alternative techniques is that a normal cellular response is activated by dsRNA leading to an extremely specific RNA degradation and increasing gene silencing efficiency in numerous RNAi models at cellular level [71]. It is also a precise, efficient, rapid, and stable technique as compared to the anti-sense technology for the gene expression [72].

3.1.1. RNAi-based approaches for controlling insect vectors transmitting plant viruses

The mechanism of RNAi has been reviewed in approximately thirty insect species from different orders of class Insecta [73]. Two approaches used for silencing insect vectors are silencing that led to hindrance with the transmission and the other one is suppression of the target genes that leads to death and thus declining the insect population. RNAi has been applied to various insects that serve as vectors of plant viruses such as Aphids that are responsible for transmitting 28% of all the plant viruses including Turnip mosaic virus (TuMV), Cereal yellow dwarf virus (CYDV), and Barley yellow dwarf viruses (BYDV). The approach has been applied for Planthoppers transmitting approximately 3% of all plant viruses such as Phytoreovirus, Nucleo-rhabdovirus, etc. [74] and other insects including whiteflies, leafhoppers, beetles, and thrips as well [75].

3.2. Cross Protection

Prevention of infection by a similar virus called secondary virus or another isolate of the same virus on the basis of prior infection with primary virus is known as cross protection. This strategy was first reported in 1929 for TMV [76]. Although, by definition cross-protection is a natural process where resistance of a plant to one virus strain is induced by systemic infection with a second [77], RNA mediated cross-protection is functionally equivalent to post-transcriptional gene silencing [78]. There are several hypothesis explaining how primary virus infection prevents secondary infection such as encapsidation or the prevention of encapsidation of the RNA of second strain by the primary strain coat-protein (CP), competing for factors crucial for replication among different strains of viruses, and lastly the limitation of the replication sites by primary strain [77,79]. Among all the hypotheses put forth, RNA-mediated and CP-mediated cross protection are extensively acknowledged. Transgenic plants expressing TMV-CP are the focal point of CP-mediated cross protection and thus resistant to TMV infection [80]. This method has a drawback that CP-deficient viruses and viroids can confer cross protection. Thus, RNA-mediated cross protection was preferred to justify the cross protection method not only for DNA and RNA virus but also for viroids [77]. Both sap-transmissible and non-sap-transmissible viruses such as Potato virus X (PVX) and Potato leaf roll virus (PLRV), respectively, as well as DNA viruses and RNA viruses have been successfully managed by Cross Protection [77,79]. A new model for viral cross-protection along with super-infection exclusion is successfully applied to control Turnip crinkle virus (TCV) [81]. Sour Oranges in Florida that were severely affected by Citrus tristeza virus (CTV) have also been controlled using this approach [82].

3.3. Transgenics in Viral Containment

Loss of productivity of crops due to viral damage is massive. Control measures available to date are inadequate and costly. The application of genetic engineering and plant transformation methods has enabled up the possibility of introduction of resistant gene to several crop species [83]. Transgenic technique had been applied in crops like tomato, potato, rice, legumes, cucurbits,
and other crops, where viral infection is a serious menace [84]. Transgenic plant research is based on plant transformation which is of two-type plant transformation using Agrobacterium as a biological vector (e.g., in maize and rice) and the other one is direct gene transfer method which involves insertion of foreign DNA into host cells through electrical, chemical, or physical methods. Single genes were included in the first generation of transgenic crops toward enhancement of traits. More recently, transgenic approach has proved beneficial in crop modification evoking new genes into plants that are essential for plant growth, metabolism, stress tolerance, and pathogen control [85]. Among all of them, the most widely used technique is involving Agrobacterium tumefaciens due to its natural DNA transfer capacity. It is an efficient technique with less complexity of host specificity and cell culture constraint [86] but limitations have been reported in some plant species like grape and maize where Agrobacterium infection leads to tissue necrosis [87,88] though it can be overcome by the development of specific plant cell culture procedures and defining inoculation and co-cultivation conditions [89,90]. The regeneration competency and the effectiveness of Agrobacterium transformation are dependent on the factors like plant genotype, selection of bacterial strain, external conditions during the pre-culture and co-cultivation. Direct gene transfer transformation techniques for DNA delivery are independent of species, cell culture constraint, and genotype for DNA delivery, but their efficiency is affected due to change in target cell. Moreover, in maximum cases, their utility in transgenic plants development is dependent on the regeneration ability of the targeted cells. It has been found that transgenic maize plants are available with resistance to Maize streak virus (MSV) by expression of a defective form of a viral gene involved in viral replications [91]. Another application is on transgenic rice plants with the introduced RNAi construct targeting the Rice dwarf virus factor for Pns VI (viroplasm associated macromolecule and movement protein), P8 (major outer capsid), and Pns12 (viroplasm associated protein), which were nearly proof against RDV infection [92].

Transgenic plants provide advantages like higher yield, improving shelf life (tomato), increasing nutritional quality (yellow or golden rice, canola oil) [93], production of therapeutics drugs (potato and banana), insect resistance (Bt-cotton), herbicide resistance (tomato, potato, tobacco, and cotton), virus resistance (tobacco, potato, rice, and papaya), reduced environmental impact (heat, cold, and drought), which ultimately lead to economical benefits [94].

However, transgenic plants have their own set of problems. They may induce the development of super weeds and other environmental risk expansion of new allergens and toxins to traditional foods and cause antibiotic resistance by introducing new strains of viruses into the food chain [95]. It has been commonly observed that serious potential risk could result from recombination between a viral transgene mRNA and the genomic RNA of a non-target virus. It appeared in cucumoviruses that similar population of recombinant viruses show up in transgenic plants expressing a CMV CP gene contaminated by another cucumovirus and equal non-transgenic ones infected at the same time with two cucumoviruses [96].

3.4. Gene Pyramiding

Gene pyramiding involves production of durable resistance by stacking of multiple genes resulting in the simultaneous expression of multiple genes in a variety. It has gained importance because it enhances the capability of plant breeding directed toward the production of genetic stocks and accurate development of broad spectrum resistance potential. Gene pyramiding success is based on various important parameters like number of genes to be transferred, number of genotype selected in each breeding generation, the distance between the target genes and flanking markers, and germplasm nature. Advanced tools like micro arrays, DNA chips, and SNPs are very helpful in improving the evaluation of the functions of gene via genome wide experimental approaches. Gene pyramiding holds high resistance against biotic and abiotic stresses in crops; however, the disadvantage is the development of pyramid lines, which is a time consuming and expensive issue in addition to the epistatic effect [97]. In an individual study, though it has been shown that mosaic strategy (set of resistance genes when deployed individually in regional mosaics instead of being stacked into a single plant cultivar) often outperforms pyramiding strategy in some agricultural landscapes [98].

3.5. Protein–Protein Interaction Studies and Applications

Plant viruses exploit cellular factors in infected cells for their replication and to establish systemic infections. Proteomics methods or tools that are used to identify host protein interactions give considerable knowledge about viral protein functions. They can also reveal about unknown protein functions through interaction connections. Viral host interactome data also provide insights for function of interacting proteins [99,100].

Protein interactions have been found in tobacco and Arabidopsis where Alfalfa mosaic virus (AMV) is able to establish a compatible interaction with the hosts. Moreover, the coat protein (CP) of AMV interacts directly with transcription factor (TF) ILR3 of both the species. ILR3 is a basic helix-loop-helix (bHLH) family member of TFs, which has been shown to regulate NEET in Arabidopsis, a critical protein in plant development, senescence, iron metabolism, and reactive oxygen species (ROS) homeostasis [101].

3.5.1. Yeast-hybrid system

Yeast-hybrid system has been used in mapping protein–protein interactions on a global level [102]. In plants, this has been used enormously for analysis of known interactions, isolating new interacting partners, and also in study of various processes in which protein–protein interactions are involved such as floral development [103], self-incompatibility mechanisms [104], the circadian clock [105], plant disease resistance, and phyto-hormone signaling [106]. This system has helped in the analysis of interacting transcription factors illuminating different control levels in plants development [107].

One of the examples of application of Y2H has been observed for Lolium latent virus (LoLV) infecting Nicotiana benthamiana leaf tissue. The information deduced from protein interaction studies have reduced the level of viral RNA in young leaves compared with levels in control plants suggesting an inhibition of virus
movement. Silencing of target interaction had no obvious effect on plant phenotype but is able to interfere with LoLV infection, opening the way for a new strategy for virus infection control [108].

3.5.2. Protein microarray

Protein microarray or Protein chip is a cost effective, solid-phase assay method, used in protein–protein interactions detection [109]. It is a highly sensitive and high throughput method requiring very minimal reagent sample. Successful application of protein microarray has been observed where an array of approximately 5,000 Saccharomyces cerevisiae proteins were screened to identify proteins that could preferentially bind a small RNA hairpin attached with a clamped adenine motif (CAM). A CAM is required for the replication of Brome Mosaic Virus (BMV), a plant-infecting RNA virus that can replicate in S. cerevisiae. Several hits were selected for further characterization in N. benthamiana. Pseudouridine Synthase 4 (Pus4) and the Actin Patch Protein 1 (App1) modestly reduced BMV genomic plus-strand RNA accumulation, but dramatically inhibited BMV systemic spread in plants. Pus4 also prevented the encapsidation of a BMV RNA in plants and the reassembly of BMV virions in vitro. These results demonstrate the feasibility of using proteome arrays to identify specific RNA-binding proteins for antiviral activities [110] [Table 3].

4. CONCLUSIONS

Undoubtedly, genetic engineering of crop plants for virus resistance is a fundamental biotechnological tool, which can be used to decrease the crop production losses due to viral diseases in our country as well as across the globe. Most of the viruses have been identified, and cloning as well as molecular characterization of their genomic components is at advanced stages. Engineering techniques for functional genomics must be harnessed to understand the interaction at molecular level between viruses, the resistant and susceptible plants leading to pathogenesis or resistance. Various advanced plant virus control approaches discussed in the review can be utilized according to the available resources and can be employed as anti-viral defense arrangements in plants. The aim is the improvement of high-health nursery material with agriculture potential with low running cost and the growth of virus free plant at a high frequency. The complicated interactions between host and virus have been underlined by recent evidence, such as gene silencing and silencing-suppressor proteins, leading to new tools and improved antiviral therapies.

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Table 3: Virus inhibition through various advanced methods.

| Methods          | Plant affected | Virus inhibited | References |
|------------------|----------------|-----------------|------------|
| RNA-interference | Rice           | Rice dwarf virus (RDV) | [111]       |
|                  | Soybean        | Soybean dwarf virus (ShDV) | [112]       |
|                  | Mustard        | Turnip mosaic virus (TuMV) | [113]       |
|                  | Cereal crops   | Cereal yellow dwarf virus (CYDVi) | [114]       |
|                  | Cereal crops   | Barley yellow dwarf viruses (BYDV) | [114]       |
| Grapevines       |                | Grapevine fanleaf virus (GFLV) | [115]       |
| Sour oranges     | Citrus tristeza virus (CTV) | [116]       |
| Cucumber         | Zucchini yellow mosaic virus (ZYMV) | [116]       |
| Cross-protection | Papaya         | Papaya ringspot virus (PRSV) | [117]       |
| Pepper           | Potato virus X (PVX), Potato leaf roll virus (PLRV) | [116]       |
| Arabidopsis      | Turnip crinkle virus (TCV) | [81]         |
| Tobacco          | Potato virus A (PVA), Potato virus Y (PVY) | [118-120]   |
| Transgenics      | Sweetpotato    | Sweetpotato chlorotic stunt virus (SPCSV), Sweetpotato feathery mottle virus (SPFMV) | [121]       |
| Tobacco          | Grapevine virus A (GVA) | [122]       |
| Winter Barley crops | Barley yellow mosaic virus (BaYMV) | [123]       |
| Gene pyramiding  | Oilseed pump   | Zucchini yellow mosaic virus (ZYMV) | [124]       |
| Tobacco          | Tobacco mosaic virus (TMV), Bamboo mosaic virus (BMV) | [97]         |
| Rice             | Rice yellow mottle virus (RYMV) | [97]         |
| Protein–protein interaction | Tobacco | pepper mild mottle virus (PMMoV) | [97]         |
| Arabidopsis      | Turnip mosaic virus (TuMV), Tobacco mosaic virus (TMV) |           |
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