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Review Article

THE WHO'S WHO OF PLANT VIRUSES: A COGNITIVE APPROACH

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ABSTRACT

Viral diseases in plants pose a serious threat to the plant production. Plant viruses are among the major factors that affect productivity and cause vast economic losses. Across the globe plant viruses persist to be a major threat to vegetation of fruits, vegetables, ornamental crops, etc. and their prevention and control are the major areas of concern. Over the past century, efforts have been put to understand the underlying mechanism of defense against plant viruses and reasons for this intercellular invasion. Resistance to plant viruses can be offered by several means such as conventional breeding methods, chemotherapy, thermotherapy and plant biotechnological interventions (plant tissue culture technology and production of transgenic plants). The present work is an attempt to understand the biology and mechanism of plant viral infections, the dangers posed by them and all possible alternatives for prevention.

Keywords: Classification, Morphology, Transmission, Virus-free plant production, Genetic engineering.

INTRODUCTION

Plant viruses are obligate intracellular parasites lacking molecular machinery making them unable to replicate without a host. Virus particles are immobile outside the infected host; relying on other organisms or the environment for their dissemination. These are metastable macromolecular assemblies of a nucleic acid core (5-40%) enclosed within a protein coat known as a capsid (60-95%). The capsid is made up of one or few proteins (capsomeres: Coded by viral genome) that form repeating units which assemble around the genome to protect it from enzymatic degradation inside the host cell [1]. Viral genome codes for only a few structural proteins (besides non-structural regulatory proteins are involved in virus replication). Capsids are formed as single or double protein shells and consist of only one or a few structural protein species and multiple protein copies self-assemble to form the continuous three-dimensional capsid structure. The coat proteins play an important role in almost every step of the viral infection cycle, including virus delivery into the plant cell, disassembly of virus particles, viral RNA translation, viral genome replication, assembly of progeny virus, movement in the plant, activation or suppression of host defense and transmission of the virus to healthy plants.

The viral nucleic acid surrounded by protein subunits is called a nucleocapsid. A fully assembled infectious virus is called a virion, which may either be a nucleocapsid alone or a nucleocapsid with additional components such as a lipid envelope (located either externally or underneath the capsid) an enzyme or other structural proteins.

The virions are responsible for a myriad of plant diseases. The diseases, however, not necessarily result in plant death. Infected plants might show a range of symptoms such as leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortions (e.g. curling) and/or other growth distortions *viz*. stunting of the whole plant, abnormalities in flower or fruit formation, deformed growth, bark scaling, etc. These abnormalities ultimately result in massive damage to the plant and plant products. Although the crop plants suffer from loss of grains or other edible parts, ornamental plants bear a failure of quality flower production and forest species exhibit a marked degradation of timber and fruit quality. Virus infection, therefore, is responsible for huge losses in crop quality and yield across the entire world. What intensifies the complications of viral infections is recognition and reporting of viral diseases. Most of the times symptoms of viral infection are mistaken for nutrient deficiency or pest attack resulting in a delay in the actual detection of infection. With the advent of recent biotechnological interventions there has been a dramatic improvement in viral detection in plants. However, the problem of virus-eradication remains uncured to a large extent. As the world faces the challenge to feed an exponentially rising population, the havocs caused by plant viruses are a major problem to solve giving an impetus for extensive research in the area. Dedicated studies have been carried out to develop systems for producing virus-resistant plants after understanding the cellular and molecular biology of viral pathogens, viral vectors and mechanism of plant-virus interactions. The study area has now extended where plant viruses; have been used as model systems for enhanced gene expression in plants.

Although a lot of studies have been done on plant viruses, in most cases only specific aspects have been discussed. The present review aims to compile all possible information on varied facets of plant viruses that shall help plant biologists understand and utilize the best possible techniques to combat the giant of plant viral infection.

NOMENCLATURE

Viruses are difficult to classify and therefore are given descriptive names based on the type of symptom and disease they cause in the first host in which they were discovered *viz*., tobacco ring spot, watermelon mosaic, barley yellow dwarf, potato mop top, citrus tristeza, sugar beet curly top etc. (Table 1). Many of these viruses also infect plants of other species. For example, tobacco ring spot virus causes bud blight in soybeans; maize dwarf mosaic infects sorghum, Sudan grass, sugarcane, and Johnson grass in addition to corn, but it still retains its original name.

Table 1: Some common plant viruses

Crop	Virus
Bean	Bean common mosaic virus
Brassica	Turnip mosaic virus
Capsicum	CMV, PV Y
Carrot	Carrot virus Y
Celery	Celery mosaic virus
Lettuce	Lettuce mosaic virus
Sweet corn	Johnson grass mosaic virus
Sweet potato	Sweet potato feathery mottle virus

PV: Potato virus, CMV: Cucumber mosaic virus

CLASSIFICATION

Plant viruses can be classified on the basis of morphology (size and shape), genome organization and mode of transmission.

Morphology

Virus particles are extremely small, therefore, for determination of their shape and size transmission electron microscopy is widely used. Based on the assembly of capsids, plant viruses have been divided into two morphological groups rod-shaped and spiral shaped.

Rod shaped viruses (roughly elongated) are more common and vary in diameter (3-25 nm), length (150-2000 nm), packaging of subunits, pitch of the helix, and flexibility of the particle [2]. Here the nucleic acid core is highly ordered i.e. it assumes the same helical conformation as the proteinaceous capsid. The modal length of particles is determined from measurements of many particles in an electron micrograph, which shows whether the particles are straight or flexuous (bent or curved). Tobacco mosaic virus (TMV) is the best-characterized rod-shaped virus with a helical symmetry in which both the nucleic acid core and protein subunits are arranged in a helix. The coat protein is intimately associated with the genetic material so that the caspid encloses it in the groove created when the subunits assemble together in the particle.

Contrastingly a roughly spherical (more spherically icosahedral) virus has been identified. An icosahedron has 20 equilateral triangles arranged around the face of a sphere. It is defined by having 2, 3 and 5-fold axis of symmetry. The symmetrical shell encloses the nucleic acid-containing core. The advantages of this symmetry are that triangulating a dome into 20 is the best way of producing a shell of equivalently bonded identical structures and it is the minimum free energy structure. Spherical plant viruses occur singly or in pairs and vary in diameter from 20 to 70 nm. The smallest isometric viruses have a capsid with 60 protein subunits so they have three subunits associated with each of the 20 faces.

Another known variant are bacilliform viruses that have proportions resembling those of bacteria in the genus *Bacillus*. They may or may not be surrounded by an envelope. The most common example of non-enveloped bacilliform viruses is alfalfa mosaic virus, which also has an isometric particle virus [3-5].

Genome organization

Plant viruses contain genomic nucleic acid molecules which can be mono-, bi-, tri- or multi- partite and can comprise of any one of the four types of genetic material: Single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) (Table 2). Single-stranded virus genomes may be: Positive (+) sense, i.e. of the same polarity (nucleotide sequence) as mRNA; negative (−) sense or ambisense - A mixture of the two. The genomes of nearly all plant viruses are made of RNA and approximately 65% of these genomes are composed of ssRNA that is of the same (positive-sense) polarity as the messenger RNAs of the cell and are termed as (+) ssRNA [6].

The positive sense RNA genomes play multiple roles in the infection cycle directing virus specific protein synthesis from their genes and acting as mRNA; acting as templates for transcription into negative sense RNA copies which are the starting point of all subsequent stages of virus genome replication; acting as template for subgenomic RNA synthesis and regulating gene expression. The viral RNA molecules of individual viruses have different structures at their 3' and 5' end. The 5' untranslated region (UTR) is of small length, constituted by few nucleotides and can bear any of the three structures: Methylated cap-like structure, genome linked proteins (viral protein genome) or di- and tri-phosphates, whereas the 3' UTR is a long and can carry tRNA like structure or poly(A) tail [7].

In some cases the genome needed for infection is divided between two or more segments which may be encapsidated in the same particle or in separate particles (multicomponent) and can even have associated satellite RNAs [8,9]. The genome organization and sequence similarities of the non-structural proteins, in particular of their RNA-dependent RNA polymerases (RdRp) and helicases, show that most plant RNA viruses are genetically related and appear to have possible evolutionary links with some animal RNA viruses [10,11].

Satellite viruses are infectious particles that are capable of infecting bacteria, plants, fungi, and animals. They code for their own protein capsid, however they rely on a helper virus (HV) in order to replicate. Satellite viruses cause plant diseases by interfering with specific plant gene activity. In some instances, plant disease development is dependent upon the presence of both the HV and its satellite. While satellite viruses alter the infectious symptoms caused by their HV, they do not influence or disrupt viral replication in the HV.

Due to this enormous variation in the very nature of the genetic material of viruses, the reproductive cycles and lifestyles of different viruses are often very distinct from each other.

Mode of transmission

Loss of plants by viral infection is well-understood. However, the plant cells have a robust cell wall that is difficult for viruses to breach to cause infection. To invade the plant, virus penetrates its outer protective layer. Therefore, plants that have been damaged by the weather, pruning, or vectors are more susceptible to a virus attack. Plant viruses are generally spread by two common mechanisms: horizontal transmission and vertical transmission.

Horizontal transmission

This method occurs when the plant virus is transmitted as a result of an external source. For initial entry into a plant cell they require a wound, which can occur naturally, by agronomic or horticultural practices, mechanically, infections by fungus, nematode, parasites or by vectors such as insects.

Insect vectors

Insect transmission is the most widespread means of virus transmission in the field. In 1930s, Watson and Roberts proposed modes of virus transmission by insects. Approximately 80% of the plant viruses depend on insect vectors for transmission, and plant viruses demonstrate a high level of specificity for the group of insects that may transmit them. The important arthropod vectors of plant viruses are; four families of homopterans (aphids, whiteflies, leafhoppers, and delphacid planthoppers), thrips, chrysomellid beetles, and, among the acarines, the eriophyid mites [12].

Insects in the order homoptera are well adapted to their role as vectors by their capacity to pierce the epidermis and delicately deposit the virus in the cytoplasm without risking the integrity of the plant cell. More than 380 viruses from 27 plant virus genera are transmitted by the homoptera. Two systems of terminology have been devised to describe and group homopteran-borne plant viruses. One is based on persistence of transmissible virus in the vector and the other on the route of virus transport in the vector. The two systems are combined to create the four transmission groups, *viz.*; (1) the non-persistently transmitted, stylet-borne viruses; (2) the semi-persistently transmitted, foregut-borne viruses; (3) the persistently transmitted, circulative viruses; and (4) the persistently transmitted, propagative viruses. These have been classified as non-persistent, semi-persistent, and persistent, depending on the length of the period the vector can harbor infectious particles, which can range from minutes to hours (non-persistent) to days (semipersistent) and to live-time and even inheritance by the insect progeny (persistent) [13-15]. The non-persistent and semipersistent viruses specifically associate with the epicuticle that lines the stylets (mouthparts) or foreguts of their vectors, respectively, and were often referred to as stylet-borne or foregut-borne viruses. The cuticle (including the lining of the mouthparts and foregut) is shed during each molt, and therefore any acquired virus is also lost. Collectively, all of these viruses have been referred to as non-circulative [13,16,17] (Table 3). Persistent viruses also referred to as circulative viruses and can be further divided into propagative viruses, which replicate in their

Table 2: Classification of some plant viruses

Morphology	Genome organization	Family	Type
Filamentous	1 ssRNA	Potyviridae	PV Y
	1 or 2 ssRNA	Closterviridae	Beet yellow virus
Bacilliform	(−) ssRNA	Rhabdoviridae	Lettuce necrosis yellow virus
Membranous circular particle		Bunyaviridae	Tomato spotted wilt virus
Isometric	1 ss(+) RNA	Sequiviridae	Rice tungro spherical virus
		Tombusviridae	Carnation ring spot virus
		Luteoviridae	Potao leafroll virus
	2 ss(+) RNA	Comoviridae	Cowpea mosaic virus
	3 ss(+) RNA	Bromoviridae	Cucumber mosaic virus
	dsRNA	Reoviridae	Fiji disease virus
		Partitiviridae	Lettuce big-vein virus
	dsDNA	Caulimoviridae	Cauliflower mosaic virus
	(+) ssDNA	Geminiviridae	Beet curly top virus
	ssRNA (RT)	Pseudoviridae	Retrotransposons
List of some important plant virus to which family has not been assigned			
Rod shaped particles	1 ssRNA	-	Tobacco mosaic virus
	2 ssRNAs	-	Tobacco rattle virus
	3 ssRNAs	-	Potato mop-top virus
Filamentous	1 ssRNA	-	Carnation latent virus
Isometric	1 ssRNA	-	Carrot mottle virus
	2 ssRNAs	-	Raspberry bushy dwarf virus
Bacilliform	3 ssRNAs	-	Ourmia melon virus

ssRNA: Single-stranded RNA, ssDNA: Single-stranded DNA, PV: Potato virus

arthropod vector in addition to their plant hosts, and non-propagative viruses, which replicate only in their plant hosts.

After elucidating the route of transmission two principal modes of transmission have emerged: Circulative or internal, where the virus crosses body barriers and enters the circulatory system of the insect and accumulates inside the salivary glands and non-circulative or external, where the virus remains attached to the cuticle (cuticle-borne) of the insect and does not cross body barriers (Fig. 1) [18].

In circulative transmission, viruses move from the foregut further to the mid- and hindgut, from where they are transported to the hemolymph and further to the salivary gland, from where they are released into the plant tissue during feeding [19-22]. Non-circulative viruses are transmitted by aphids during intracellular stylet penetration [23-25].

The molecular and physiological basis for virus-vector interactions that regulate the transmission are not well-understood. Environmental or abiotic factors also play a role in determining virus-vector interactions, but in general these factors seem to influence the efficiency of the interaction rather than to determine the ability of the interaction to take place.

Mechanical transmission

Mechanical transmission occurs when a plant comes in contact with other plant and leaves rub together or by humans interferences like tools/hands/clothing. It involves the introduction of infective virus or biologically active virus into a suitable site in the living cells through wounds or abrasions in the plant surface. Spreading viruses by mechanical method is generally used for experimental purposes under laboratory conditions- also known as Sap inoculation. Despite the ease of mechanical transmission under experimental conditions, transfer of virus from one host to another without the intervention of a vector is not common in nature. In nature, only a few viruses are disseminated by contact: TMV, potato virus X (PVX), PV S (PVS), Andean potato mottle virus, Andean potato latent virus and potato spindle tuber viroid. These viruses can contaminate structures, tools, soil debris and wounding of a host plant allowing contact of the tissue with a source of virus can lead to infection [28]. Cucumber and PV are some of the most common viruses spread by sap.

Vertical transmission

In vertical transmission, the virus is inherited from a parent. This type of transmission occurs in both asexual and sexual reproduction.

Table 3: Some important crop viruses spread by aphids in a non-persistent manner

Crop	Virus
Bean	Bean common mosaic virus
Brassicas	Turnip mosaic virus
Capsicum	CMV, PV Y
Carrot	Carrot virus Y
Celery	Celery mosaic virus
Lettuce	Lettuce mosaic virus
Sweet corn	Johnson grass mosaic virus
Sweetpotato	Sweetpotato feathery mottle virus

PV: Potato virus, CMV: Cucumber mosaic virus

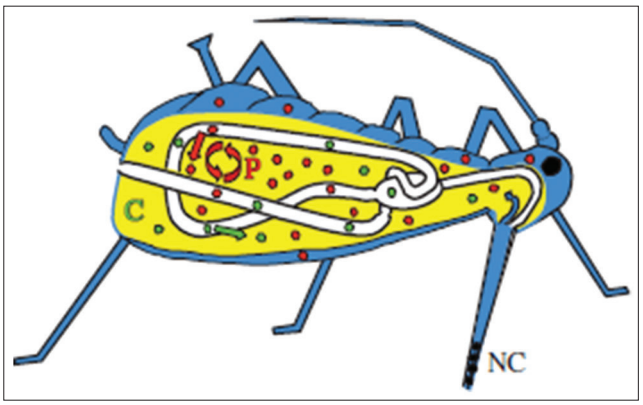

Fig. 1: Schematic representation of modes of transmission by vectors. Viruses transmitted in a non-circulative (NC) manner (black) are restricted to the mouth parts. Viruses transmitted in a circulative (C) manner (green) enter the hemocoel (yellow) by endocytosis-exocytosis from the gut, and usually enter the salivary glands from the hemolymph. Viruses transmitted in a propagative (P) manner (red) replicate in different organs of the vectors and may enter the salivary glands either from the hemolymph or from other connecting tissues, e.g. the nervous system or trachea [26,27]

In asexual reproductive methods such as vegetative propagation, the offspring develops from and are genetically identical to a single plant.

When the new plants develop from the stems, roots, bulbs, etc. of the parent plant, the virus is passed along to the developing plants. In sexual reproduction, viral transmission occurs as a result of seed infection.

Seeds

Seeds can sometimes carry virus infection because of external contamination or by an infection of the embryo's living tissues. Host plants show a high degree of protection possessed by embryos of seeds against invasion by viruses that affect the mother plant [29-31]. Despite this protection, an appreciable number of viruses have been found to pass from one generation to the next through the medium of the seed. This leads to new crops breaking out in disease, which is at first only local in circulation. However, infection can spread to the rest of the crop by mechanical means. Approximately, 18% of plant viruses are seed transmitted in one or more host [32]. The most common type of seed transmission the viruses are found within the tissues of the embryo. The developing embryo can become infected either prior to fertilization by infection of gametes or by direct invasion after fertilization.

However transmission via seeds is rare due to the inability of most viruses to infect mother cells of infected plants together with the inability of viruses to infect the developing embryo because of the lack of plasmodesmatal connection with the endosperm. Pea seed-borne mosaic virus is a seed-transmitted virus in pea and other legumes which invades pea embryos early in development [33].

Grafting

Grafting or vegetative propagation is a means of increasing vegetation. Grafting is considered to be a universal method for transmitting viruses because systemic viruses can be transmitted by grafting. Graft transmission of viruses to susceptible host plants is indicated when the virus strain is not readily or not at all mechanically transmissible. Viruses can also develop and multiply from contaminated buds, cuttings and rootstocks. It is, therefore, necessary to use only certified virus-free grafting or budding stock. Grafting is particularly useful for transmission of phloem-restricted viruses that cannot be transmitted mechanically and viruses whose vectors remain unknown, and for detecting viruses found in low concentrations.

Molecular biology of plant viral replication inside host cell

Once inside the host cell, virus controls the cellular machinery. DsDNA viruses typically must enter the host cell's nucleus before they can replicate. ssRNA viruses, however replicate mainly in the host cell's cytoplasm. At the cellular level, when a virus infects a plant, the first stage is known as adsorption where virus binds to the outer cell membrane or plasma lemma of the host cell. The protein coat is removed from the genome either outside or inside the cell. Next, the viral genome replicates using the host's cellular machinery. If the genome is RNA; it acts as mRNA, using the ribosomes from the cytoplasm, cellular transfer RNAs and amino acids to translate the first of the virus-encoded genes into the replication associated protein(s) (Fig. 2). The host's nucleus or membranes are associated with the synthesis of new virus RNA by the RdRp or replicase (Rep) enzyme, which copies the viral RNA to form a double-stranded replicative form; in turn this directs the formation of new virions. The newly synthesized RNA includes the gene for the virus coat protein which is also translated in the host cell. DNA genomes of viruses are transcribed to mRNA before virus replication commences. Assembly of the genome and the coat protein to produce new virus particles then completes the replication cycle. In the end newly produced viruses are expelled from the host cell.

Production of virus-free plants

Fungal and bacterial diseases can be controlled by application of fungicides and antibiotics. Contrastingly, viruses and viroids generally do not respond to such chemical control. As a result, understanding physiology and molecular biology of plant viruses became imperative to design strategies for control of plant viral infection. Of the several methods tried only a few techniques have proved fruitful, which

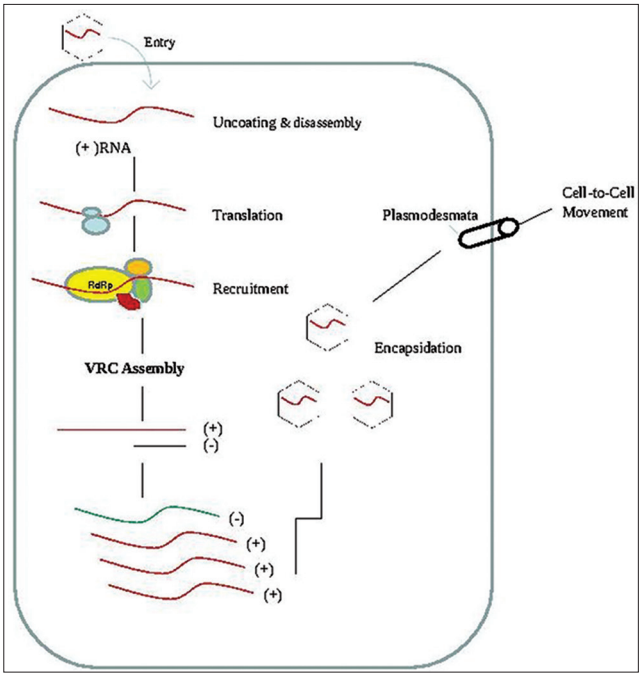

Fig. 2: Pictorial representation of the various stages of plant (+) RNA virus replication. First the positive-sense RNA ((+) RNA) viruses enter plant cells through wounds. When the virus is inside the cell, the (+) RNA genome is released into the cytosol. The uncoated viral RNA is used by host ribosomes to translate replication-associated proteins. The resulting viral replication proteins then recruit the (+) RNA to subcellular membrane compartments, where functional viral replication complexes (VRCs) are assembled. The viral (−) RNA is synthesized by active VRC and the (−) RNA is used as a template to synthesize large amounts of (+) RNA progeny asymmetrically. The new (+) RNAs are released from the VRCs, whereas the (−) RNA is retained. The released (+) RNAs start a new cycle of translation and replication, become encapsidated, and then exit the cells or move to neighboring cells through plasmodesmata

unfortunately are not applicable in all cases. The potential of the virus to form new species/rapid mutation rate intensify the complications in controlling them. However, the major techniques utilized for production of virus-free plants can be summarized as under.

Tissue culture techniques

The viral diseases in plants transfer easily and lower the quality and yield of the plants, and it is very difficult to treat and cure the virus infected plants. In some crops, it has become possible to produce virus free plants through tissue culture at the commercial level. This is done by regenerating plants from cultured tissues derived from meristems, callus culture [34,35], protoplast culture [36] and chemical treatment of the media e.g. addition of cytokinins suppressed the multiplication of certain viruses [37].

Among the culture techniques, meristem-tip culture is the most reliable method for virus and other pathogen elimination. Viruses have been eliminated from a number of economically important plant species, which has resulted in a significant increase in the yield and production.

Meristem tip culture

Tissue culture is an excellent tool for multiplying, maintaining, storing and distributing plants. *In-vitro* propagation of apical meristems is an important part of virus-elimination therapy for improving the health of plant collections. Meristem culture is a unique technique to free plants from various pathogens including viruses, viroides, mycoplasma, bacteria and fungi. Apical meristem is a dome of actively

dividing cells located at the apex of shoots and roots. Plantlets derived from meristem-tip culture usually retain the genetic characteristics of mother plants. Many important plants contain systemic viruses, which substantially reduce their potential yield and quality. It is, therefore, important to produce virus free stock plant [38]. The main reason behind the using the meristem tip for virus eradication is that the terminal region of the shoot meristem, above the zone of vascular differentiation, is unlikely to contain pathogenic particles. Meristem-tip culture is, therefore, the most widely applicable approach for virus elimination. The explant of small size (1 mm) i.e. meristem tip is preferred for *in vitro* culture. Meristem tip is excised in aseptic conditions and cultured on nutrient medium. The inoculated tubes are incubated properly in light, and dark regime (24±1°C in dark conditions for 3 days, and then under standard illuminated conditions is preferable). The meristem-tip technique can be linked with heat therapy, antiviral and chemotherapeutic agents to enhance its effectualness of disease elimination to quarantine regulations [39,40]. The first demonstration of the elimination of viruses was seen from Dahlia using meristem culture [41]. Since then, the use of meristem culture to obtain virus-free ornamental plants has been widely used (Table 4).

Table 4: Application of shoot-tip culture in plant virus elimination

Species	Virus	Reference
Alstromeria sp.	Almv	[42]
C. morifolium	Mixed infection by CMV, TAV	[43]
D. gratianopolotanus	Carmv, CLV, potyviruses	[44]
Lilium sp.	LSV	[45]

C. morifolium: Chrysanthemum morifolium, Almv: Alstroemeria mosaic virus, D. gratianopolotanus: Dianthus gratianopolotanus, TAV: Tomato aspermy virus, Carmv: Carnation mottle virus, CLV: Carnation latent virus, LSV: Lily symptomless virus, CMV: Cucumber mosaic virus

Table 5: Successful examples of plant virus elimination by thermotherapy

Host	Target virus	Temperature range
Chrysanthemum	Chrysanthemum B virus	35-38°C
Carnation	Carnation ringspot virus	35-40°C
Gooseberry	Gooseberry vein banding virus	35°C
Potato	PV Y, S, X	33-38°C

PV: Potato virus

Cryotherapy

Prolonged exposure to a low temperature (−196°C) followed by shoot tip culture has proved quite successful in virus elimination. Cryotherapy of shoot tips can result in virus-free plants at a high frequency. Thermotherapy had other effects, which together with subsequent cryotherapy resulted in virus elimination. When used for cryotherapy, conditions are selected to allow survival of only a limited number of the least differentiated cells and to eliminate a large proportion of virus-infected tissues. Therefore, cryotherapy can result in virus-free regenerants with a much greater frequency than what is typically obtained with a conventional meristem culture [46]. Cryotherapy has successfully eliminated pathogens (viruses and bacteria) from numerous plant species: *Solanum tuberosum*, *Ipomea batatas*, *Vitis vinif-era*, *Citrus* spp., *Rubus idaeus*, *Musa* spp. Healthy plants can be regenerated from the surviving pathogen-free meristematic tissue [47]. The method facilitates treatment of large numbers of samples and is independent of shoot tip size. It has the potential to replace more traditional methods like meristem culture.

Thermotherapy

Heat treatment is used in those plants in which viruses cannot be eradicated just by meristem tip culture alone. It was originally applied by Kassanis in 1949 to eliminate viruses from plant tissue [48]. Since then, thermotherapy has been extensively used for elimination of different viruses from various plants. Growing host plants at higher temperatures significantly reduces replication of many plant viruses by disrupting viral ssRNA and dsRNA synthesis as the union of the protein subunits that protect the nucleic acid of the virus becomes weaker causing temporal fissures to appear, allowing the attack of nucleases, which inactivate the virus and decreases its concentration Thermal inactivation point differs for different viruses. Mostly used temperature range is 50-52°C with exposition about 10-30 minutes. In the case this method is applied on whole plants, lower temperatures have to be used (32-4°C) with the exposition about 4-30 days. Since high temperature can inhibit virus replication and movement, thermotherapy combined with meristem culture can greatly improve virus elimination efficiency by augmenting the virus-free region of treated shoot tips. Thermotherapy along with meristem culture has been used to raise virus-free carnation, narcissus, chrysanthemum and others (Table 5) [49].

Chemotherapy

Plant chemotherapy has been defined as the control of plant disease by compound that through their effect upon the host or pathogen after it has entered the plant. The word "chemotherapy" simply means the cure of existing disease by mean of chemical. Usage has narrowed the term to mean control of disease by chemicals, which are introduced into the plant [50]. It is being used as an alternate approach for *in vitro* virus elimination in plants by supplementing the nutrient medium with a chemical of a known ability to prevent virus replication. Incorporation of antiviral compounds into explant and meristem culture media has resulted in a higher percentage of virus-free progeny plants originating from virus-infected explant or meristem donor plants.

To be effective chemotherapeutic compound a chemical must show one of the following properties; (i) kill the pathogen as it enters the host, (ii) rid the host of an established pathogen or, (iii) mitigates disease. Various chemotherapeutic compounds are in use *viz.*, ribavirin (vira-zole), acycloguanosine, azidothymidine, and 2-thiouracil [51]. Addition of 100 mg/L of virazole to the meristem culture media has been successful for the eradication of PVS, PVX, and PVY, but not of potato leaf roll virus [52]. The efficiency of virazole in suppressing virus diseases was dependent on its concentration, the host species, and the infected tissue treated [53].

Genetic engineering

With an insight about the plant-viral interaction during pathogenesis, genetic transformation has opened a plethora of possibilities of genetic engineering toward controlling plant virus diseases. Depending upon the source of gene used there are two approaches for developing genetically engineered resistance, *viz*., pathogen-derived resistance (PDR) and non-PDR (Table 6). The various strategies used in both the approaches can be briefed as under.

Strategies for PDR

This approach is based on using pathogenic virus as a source of gene. Strategies for PDR are divided into those that require the production of proteins and those that require only the accumulation of viral nucleic acid sequences. Virus-resistant transgenics have been developed in many the details of which are summarized below.

Coat protein mediated resistance (CPMR)

The use of viral CP as a transgene for producing virus resistant plants is one of the most spectacular successes achieved in plant biotechnology and was first reported in the TMV-tobacco model system [54]. It can affect single or multiple stages *viz.* virus disassembly, replication, cell-to-cell and systemic movement stages. Considerable research has suggested that CPMR results from the tendency of the transgenically expressed CP to form aggregates. In some cases, mutation is done in the transgenically expressed CP, which increases the in inter-subunit interactions and consequently leads to elevated level of virus resistance [55,56]. In case of TMV, the transgenically expressed CP subunits recoat the disassembled viral RNA hence limiting them for translation [57].

Table 6: Genetic transformation studies pertaining to virus-tolerant plants

Mechanism utilized	Study plant	Target virus	Reference
Coat protein	*N. tobaccum*	TMV	[74]
Movement protein	*Potato* sp.	PV M, PV S	[75]
Rep gene	*N. tobaccum*	TMV	[76]
Satellite RNA	*N. tobaccum*	CMV	[77]
DI nucleic acid	*N. benthamiana*	Artichoke mottled crinkle virus	[68]
PTGS	*N. clevelandii*	Tomato black ring neopvirus	[78]
Disease resistance gene	*Capsicum* sps.	Pepper mild mosaic virus	[79]
RIP	*N. benthamiana*	Artichoke mottled crinkle virus	[80]
Protease inhibitors	*O. sativa*	Tobacco etch virus	[81]
Plantibodies	*N. benthamiana*	Artichoke mottled crinkle virus	[82]
SAR	*N. tobacum*	TMV	[83]
Secondary metabolite pathway	*N. tobaccum*	TMV	[84]

TMV: Tobacco mosaic virus, *N. tobaccum: Nicotiana tobaccum, N. benthamiana: Nicotiana benthamiana, N. clevelandii: Nicotinana clevelandii, O. sativa: Oryza sativa*, PV: Potato virus, DI: Defective interfering, PTGS: Post transcriptional gene silencing, RIP: Ribosomal-inactivating proteins, SAR: Systemic acquired resistance

Movement proteins (MP)

MP enable the movement of plant viruses systemically and locally [58] by change in the gating function of plasmodesmata. The resistance is believed to be based on the antagonism between wild-type viruses encoded MP and the performed dysfunctional MP to bind to the plasmodesmatal sites [59,60]. The resistance so conferred is successful against distantly related or unrelated viruses, signifying functional conservation of this protein among several viruses [61].

Rep-MR

Genes encoding the Rep proteins can confer resistance to infection, which is limited to the virus strain from which the gene sequence was obtained. The exact mechanisms involved in Rep-MR are still unknown [62], but it is anticipated that protein produced by the transgene interferes with the function of the Rep produced by the virus, perhaps by binding to host factors or virus proteins that regulate replication and virus gene expression [63]. Even though, this kind of resistance remains limited to a narrow range of viruses but the resistance generated by the use of Rep sequences is very rigid as high level of infection can be resisted by the transgenic plant.

Satellite RNA

This strategy exploits the utilization of satellite RNA, which is dependent on its HV for replication, movement, encapsidation and transmission. The resistance is conferred as a result of the competition between the satellite RNAs and their helper viral RNAs for replication [64]. The co-replication satellite RNAs is known to suppress the replication of HV genome, which ultimately affects its accumulation in the host species. This is paralleled by reduction in the disease induced by HV. The mechanism behind sat-RNA mediated resistance may be attributed to the reduction in accumulation of the HV and its long distance movement and down-regulation of replication [65].

Defective interfering (DI) viral nucleic acids

Several plant DNA viruses produce significant quantities of deleted versions of their DNA in the infected plant, which are called DI DNA, because of their inhibitory effect on the HV. Resistance is believed to be by competition for essential viral and host factors, thereby reducing levels of HV. Similarly, DI RNAs are truncated forms of a wild-type virus that accumulate with some natural virus infections to upgrade viral symptoms [66]. Expression of cloned forms of this DIs in transgenic plants could yield a potent form of pathogen-derived protection. Although native DI RNAs have been identified only in a limited number of plant viruses [67] synthetic DI RNAs can be constructed by deleting specific genes. Not many studies have been carried out to predict, which regions of the genes can be deleted to yield synthetic DI molecules that effectively interfere with replication of their parental viruses [68].

Strategies for non-PDR

Transforming plants with resistance genes derived from host plant or any other non-pathogenic source has also evolved as a potent technique in developing virus-tolerant plants. Following are the major strategies used for the purpose.

RNAi

Also known as post-transcriptional gene silencing (PTGS) or RNA silencing. The mechanism is responsible for degradation of any unwanted, excess, foreign or aberrant RNA. When utilized for viral RNA degradation, the mechanism is better known as virus induced gene silencing. This involves a dsRNA, spread within the organism from a localized initiating area, getting degraded into small interfering RNAs (siRNAs) by action of RdRp, RNA helicases, RNAse and proteins containing PAZ and Piwi domains. It has been identified that PTGS participates in the mechanism for plant resistance to viruses. Introduction of transgenes constitutively expressing part of the genome of the virus can lead to resistance of the plant to infection by this virus [69]. Plants are classified as immune where the transgene undergoes PTGS prior to infection, while plants where the transgene undergoes PTGS after infection are said to show recovery [70]. Both resistant plants and plants that exhibit recovery are immune to secondary infection by the same virus or by another recombinant virus carrying part of the genome of the first virus.

However, many viruses have developed strategies to counteract PTGS and infect the plants. An example is inducing endogenous suppressors of PTGS by viruses. Consequently, biochemical steps of PTGS need to be understood in order to select the appropriate gene for engineering viral resistance.

Plant disease resistance genes

In some plants, disease resistance genes (R) against crops plants viruses have been identified. The hypothesis is that R genes in the host exist in the matching system with avirulence genes (avr genes) in pathogens. R genes code for proteins that act as sensors for corresponding avr elicitors and initiate signaling cascades for expression of defense-related genes. It is the hypersensitive reaction between the products of R and avr genes results in arresting pathogen spread by localized cell death [71,72].

Ribosomal-inactivating proteins (RIPs)

Antiviral proteins (or RIP) in some plants inhibit viral protein translation by catalytically removing a specific adenine base from 28S ribosomal RNA without affecting endogenous 28S RNA [73]. Specific genes coding for such proteins are used as transgenes.

Protease inhibitors from plants

Plants exhibiting cysteine protease inhibitors might show resistance to viruses that essentially require a cysteine protease activity for their

growth and propagation. The idea of exploring genes coding such proteins has been tested and successfully implemented in some cases.

Antiviral plantibodies

The concept is to raise monoclonal antibodies against viruses and the gene for the same cloned and expressed in the desired plant. The transgenic plans so developed have exhibited lower virus accumulation and reduced incidence of infection.

Systemic acquired resistance (SAR)

Refers to the use of genes coding for catalase enzyme (a salicylic acid-binding protein) in antisense orientation. Salicylic acid accumulation is associated with SAR in plants after a viral infection. Transgenic plants with antisense catalase showed a severe reduction in catalase activity, hence, higher accumulation of Salicylic acid and consequently enhanced resistance to viral infection.

Secondary metabolite pathways

The idea is to target metabolic pathways in viral pathogenesis. Here antisense RNA for a particular gene (coding a particular enzyme of a biochemical pathway in virus) when expressed in plants enzyme results in the interruption of the pathway. Although the transformed plants show abnormalities, resistance against virus is also exhibited.

Development of transgenic plants with virus resistance has its own share of limitations and controversies. High level of viral gene variability is a major challenge to the biotechnologists. Lack of proofreading activity of viral Rep and high recombination rates of viral genomes during an infection process accounts for significant variability in viruses. To develop a system for producing resistant species against a virus, therefore, is not an easy task. Efficient data collection pertaining to virus genotypes, degree of diversity, population structure, variations due to vector behavior, etc. is essential before designing a transgene.

To add to it, the biological risks remain allied with transgenic techniques. There are probable risks of recombination between viral-derived transgenes and non-target virus, changes in host-range (due to encapsidation of genome of non-target virus with transgenically expressed coat proteins). Although field trials have not indicated any such abnormality, yet the potential risks cannot be overlooked and sufficient care must be ensured in designing gene constructs.

Virus indexing

Virus indexing is the testing of plants for the presence or absence of viruses. Even though, the plant material undergoes various treatments favoring virus eradication it has been observed that only a fraction of cultures yield virus free plants necessitating the need to test each plant for specific viruses before using them as mother plant for production of virus free stock. As some viruses do not have a prompt reappearance period it is required to test several times during the first 18 months of plant growth and the plant, which consistently gives a negative result is termed as "virus-tested" and released commercially.

The basic test for virus indexing is a visual assessment where the leaves and stems are examined for any visible symptom of the virus. But as the symptoms may take a prolonged period to develop more responsive tests are vital. One of the effective methods being used on a commercial scale for virus indexing is sap transmission test also known as bioassay or infectivity test [85,86]. Cell sap from the test plant (i.e. plant to be tested for the presence of the virus) is obtained after grinding the leaves in 0.5 M phosphate buffer. Filtered leaf sap (extract) is put on the indicator plant (i.e. a plant susceptible to a specific virus or group of viruses). If the indicator plant develops symptoms, the leaf extract contains viral particles and if no symptoms develop it indicates that the plant is free from viruses. This biological assay is reasonably accurate, but too slow and difficult, but to use for screening a large number of plants. It is also not effective in case of latent viruses. Therefore, alternate techniques involving electron microscope, use of indicator plants, serology or a combination of both have been developed for virus detection in plant tissues. The most rapid and reliable serological test among the several techniques is enzyme-linked immunosorbent assay. In this method antibodies against the viral coat protein are prepared and with the use of a small amount of antiserum test is performed in simple equipments. Many of the agricultural crops are routinely indexed by the above-mentioned methods before their commercial release.

CONCLUSION

The most vital reason for comprehensive surveys of viruses affecting plants in the crop-growing areas of the developing world is the need to be aware of factors limiting crop yields in these areas. Plant viruses cause devastating diseases on plants and threaten food security. The loss is in terms of yield and quality of flowers and fruits, vigor and longevity of productive life and reduction in clonal propagation. Viruses are majorly transmitted by vector organisms that feed on plants. Although in many instances virus infections are suspected, these infections are never satisfactorily identified and in many other cases it is not known whether crops are infected. However, fungal and bacterial diseases can be controlled by application of fungicides and bactericides, the control of viral disease pose a serious problem. Traditional methods used for virus eradication were thermotherapy, cryotherapy and chemotherapy. Tissue culture techniques, alone and in combination with the traditional methods, has proved to be immensely successful and widely accepted method for virus elimination. Another practical approach to combat viral diseases is by using biotechnological interventions such as genetic engineering of crop plants. The knowledge about plant- virus interaction and the possibility of genetic transformation in many crop plants has widened the horizon for production of transgenic plants as an efficient strategy for exhibiting the wide-spectrum resistance or tolerance to the virus. To assess the reliability of any of the virus elimination strategy, it is essential to follow the step of virus indexing. The entire information about the virus biology, transmission and resistance can be extensively used to eradicate the viruses leading to an optimized crop yield and facilitate the movement of plant materials across international boundaries.

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