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Recent Advances in Virus-host Coevolution and Protective Mechanisms against Plant Viruses

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Authors' contributions

This work was carried out in collaboration between all authors. Author AS reviewed the literatures and drafted the manuscript. Author MZ checked the manuscript and participate in the coordination of the project. Author SA co-ordinate the study and gave the opinion in the manuscript. Author MME participated in the design and coordination of the project and reviewed the final manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

In plant pathology, the study of the interaction between the plant host and the viral pathogen has been a very active area of research in the last few decades. The infection process of a plant pathogen usually begins with the exchange of molecular signals. With particular emphasis on plant virus evolution, and focusing on quantitative and population genetics, plant virus-host interactions and coevolution allow understanding of the major factors favoring disease emergence. The

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exploitation of viral interaction phenomena will improve established genetic engineering strategies for viral cross-protection in plants. Also, the study of plant immunity against viral infection, both innate and adaptive (e.g. RNA silencing), has helped in the development of resistant varieties to several plant viruses based on genetic engineering. This review summarizes the recent advances in plant-virus interactions and co-evolution as well as current developments in resistant crop investigations.

Keywords: Plant-virus interactions; virus evolution; plant immunity; antiviral resistance; cross-protection; genetic engineering.

1. INTODUCTION

Plant infection by viruses causes physiological disorders responsible for plant diseases. This causes economic and agronomic significant losses in many crops. In 2012, the International Committee on Taxonomy of Viruses reported 92 genera of plant viruses, of which 82 were assigned to 21 different families [1]. In the last few decades, the molecular dialogue between plant hosts and their viral parasites was highlighted as a major breakthrough, providing new strategies that are directly exploitable in crop development programs. Nevertheless, the spread of crop viruses has increased dramatically in recent years. As long as there is no chemical capable of providing curative control against plant viruses, only preventive control can be considered.

Using recent knowledge in genomics and bioinformatics tools, researchers have been able to study in depth the genome of many plant viruses, the relationships between viruses and their plant hosts, and the mechanisms of virus evolution and virus-host co-evolution. Plant viruses are a convenient model system for studying viral evolution. Despite increasing efforts devoted to modeling the molecular evolution of many viral populations, such as Potyvirus [2,3,4], Carlavirus [5], Begomovirus [6,7] and Potexvirus [8] (Hasiów-Jaroszewska et al. 2011), there have been few attempts to study adaptive evolution, which could bring important information about the nature of the interaction between the host and the virus. One of the reasons for considering plant viruses as an appropriate model is that they allow for the use of infectious *in vitro*-generated viral RNAs as an inoculum for host organisms and permit controlled studies in multiple, genetically similar, intact hosts [9,10].

Another hallmark of virus evolution is the findings of viral sequences integrated into the plant genome. Some endogenous viruses could be benign components of plant genomes; others could potentially be pathogenic or, on the contrary, contribute to viral immunity [11]. Moreover, RNA viruses replicate with extremely high mutation rates due to the low fidelity of RNA-dependent RNA polymerases and reverse transcriptases. Mutation, recombination, short replicative cycles and high copy yields contribute to the generation of complex distributions of closely related mutant genomes able to adapt to fluctuating environments; these are termed quasispecies. Also, the host environment may be associated with the diversity observed in quasispecies. One biological implication of viral quasispecies, of great sanitary impact, consists in the emergence of resistant mutants after antiviral treatment. Plant virus systems are used as *in vivo* experimental model for understanding the evolution of viruses subjected to chemical mutagenesis. One of the most widely studied is tobacco mosaic virus (TMV) [12].

Additionally, the number of viral variants present in a single host cell, and even more the multiplicity of infection (MOI) are rarely experimentally studied in plant viruses. Yet, it is a fascinating and promising generic question in terms of the biology and evolution of viruses, since the MOI is responsible for important processes such as intracellular competition, functional complementation and recombination. Indeed, there may be a variety of control mechanisms for the entry of these infectious units in the cell, limiting them to a small number, or even exclusion mechanisms that do not allow the co-existence of two infectious units in the same cell [13].

During the process of infection, plants employ various strategies to combat viral infections. Understanding how plants defend themselves from pathogens is essential in order to protect crops and develop highly disease-resistant plant species. While cross-protection has been intensively studied for decades since the discovery of this phenomenon by McKinney in

the 1920s [14], less is known about antiviral mechanisms based on genetic tools such as PAMP-triggered immunity (PTI), effector-triggered immunity (ETI) [15], or gene silencing and how they could be the source of novel plant resistance mechanisms in the field.

Most economically important crops are susceptible to viruses. Plant viruses manifest a wide variety of pathogenicity that requires individual management strategies. The use of a resistant cultivar, against either two or more types of pathogen species, called broad spectrum resistance (BSR), remains the best and cheapest control strategy in managing viral diseases. In addition, several in-depth reviews and books on the multiplication of RNA plant viruses, viral quasispecies, genetic diversity, and plant-virus interactions based on proteomic analysis have been published elsewhere [16,17,18].

With these considerations in mind, this review will focus on giving a summary of recent advances plant-virus interactions and co-evolution, as well as to indicate current developments in important strategies of combining modern molecular and physiological techniques with phytopathology for future investigations in resistant crop varieties.

2. PLANT VIRUS INFECTION

Plant viruses are obligate parasites that enter the plant cell passively through mechanical wounds caused by environmental factors or by vectors, due to lack of plant virus receptors. The next phase of virus infection proceeds in the cytoplasm after capsid disruption [19].

During the infection process, plant viruses interact with various host factors essential for their accumulation, translation, replication, cell–to-cell and long-distance movement and utilize plant proteins, normally involved in host-specific activities, for their own purposes. Considerable progress has been made in recent years toward understanding some of those virus-host interactions [20,21,22].

Most of plant viruses have an RNA genome that consists of single stranded RNA (ssRNA). A part of these genomes are positive-sense polarity in the same sense of orientation of the messenger RNAs of the cell, the other part uses ssRNAs of negative polarity. The accumulation of progeny of both DNA and RNA plant viruses involves translation and replication of viral sequences. The ability of a virus to interact with a host depends on the formation of a functional heterocomplexes between host and virus proteins [23].

Viral RNAs share characteristics with host cell mRNAs, but display a variety of structures at their 5’ and 3’ends that differentiate them from cellular mRNAs. The interaction of host and viral factors at both ends 3’ and 5’ and sometimes along the viral RNA is mediated by RNA-RNA interactions or through the recruitment of host translation initiation factors especially the eukaryotic translation initiation factor 4G (eIF4G) [24].

In addition, virus-host interactions during DNA virus accumulation have been studied. An NAC domain protein of tomato (*Solanum lycopersicum*) interacting with a viral replication enhancer (RE) protein of Geminivirus was identified and proposed to participate in viral replication [25].

Moreover, an interaction between NAC protein of *Arabidopsis thaliana* and an RNA virus coat protein is necessary during a host resistance response [26]. This type of host proteins present different functions during DNA and RNA virus infection, thus making the virus-host interaction process more complex.

Cell-to-cell transport occurs through plasmodesmata (PD) connecting adjacent cells in plant tissues and involves virus proteins named movement proteins (MPs). Viruses reveal different movement strategies, but all of them are the result of virus factors and host components interactions [27]. A 30 kDa protein of Tobacco mosaic virus (TMV) was the first identified viral movement protein. Its functions have been clarified lately [28], including targeting of viral RNA to plasmodesmata and increasing PD pore size (SEL, size exclusion limit) [29] to allow trafficking of ribonucleoprotein complex (RNP) formed between the TMV MP and the infectious RNA [30,31]. The PD-targeted MP transport and associated viral RNA occurs by diffusion in the endoplasmatic reticulum (ER) membranes [32].

In the case of rhabdoviruses, the infection stage constitutes a challenge compared to other viruses, which are not able to move between cells because the diameter of plant plasmodesmata is smaller than virus particles [33]. The transit of nucleocapsid throught

plasmodesmata is facilitated by MPs, expressed by the additionel genes of plant-infecting rhabdoviruses such as gene 3, positioned between the P and M genes. It encodes proteins that are required for cell-to-cell movement, called sc4 for sonchus yellow net virus (SYNV) [34] and P3 for rice yellow stunt virus (RYSV) [35]. These proteins were categorized in the "30K" superfamily of viral MPs [28].

Unlike Tobamovirus and Rhabdovirus, members of Potexvirus need capsid protein for cell-to-cell transport [36]. Proteins coded by triple gene block (TGB) are also required for cell-to-cell transport of many plant virus genera [37,38].

Recently, a possible relation between virus accumulation and cell-to-cell movement was identified. Host eukaryotic translation factors eIF4E and eIF(iso)4E, which are involved in potyviruses accumulation and replication, were also shown to participate in virus cell-to-cell movement [39].

For tospoviruses, investigations are underway by Wintermantel et al. to evaluate the efficiency of transmission and resistance of lettuce against Impatiens necrotic spot virus (INSV) and Tomato spotted wilt virus (TSWV) using comparative effect of thrips transmission and mechanical inoculation. Previous studies showed that sequential mechanical inoculation with tospovirus results in loss of infectivity, if the virus does not pass through the triphs vector in which it also replicate. In addition, repeated mechanical passages cause loss in thrips transmissibility of TSWV. This phenomenon is associated with the accumulation of defective haplotypes in the population that show specific mutations in M RNA segment, which are not transmissible by thrips [40].

3. GENETIC EVOLUTION OF PLANT VIRUSES

3.1 Multiplicity of Infection

The MOI is defined as the average number of genomes that infect and penetrate a cell. MOI is a term commonly used in cell culture, as a theoretical value, which determines the number of infectious units available in the environment per cell present in the same medium. It does not necessarily correspond to the real number of infectious units penetrating and multiplying in each cell. The number of infectious units that effectively infects each cell can be considered as the true MOI; this is often not considered, nor compared with the theoretical MOI in cell culture. The parameter of interest is precisely the real value of the MOI under conditions of "natural" infection in a multicellular host [13].

For plant viruses, it is a key parameter in the evolution of viruses, as it governs essential processes such as genetic exchange between genomes, the intensity of selection among viral genes, epistatic interactions, and the evolutionary aspect of multipartite viruses. This trait has been studied and estimated for TMV in its systemic host *Nicotiana benthamiana* [41] and cauliflower mosaic virus (CaMV) [13].

In a recent study, MOI levels were estimated based on the frequency of cells infected with two different TMV genotypes, discriminated using green or red fluorescent proteins (GFP and RFP). The MOI was high, but it changed during the progression of infection, reducing from an initial level of about six to a final one to two, with most infection cycles occurring at higher MOI levels. The decreasing MOI can be explained by mechanisms limiting superinfection and/or by genotype competition within co-infected cells, which was shown to occur in double infected tobacco protoplasts [41]. The same was observed for Turnip mosaic virus, co-infection of cells by lineages originating in different primary foci is severely limited by the fast-acting mechanism of supeinfection exclusion [42].

A reasonable hypothesis explaining the presence or absence of bottlenecks would be the regulation of the MOI of cells by several genomes of the viral population. Indeed, it has been shown that the viral genomes of plum pox virus (PPV), responsible for Sharka disease, cannot secondarily invade *Prunus* tissues that have already been infected by a closely related genome. This generates bottlenecks, thus preventing extensive mixing of genetic variants within a host [43].

3.2 Viral Quasispecies

In general, RNA virus replication is characterized by high mutation rates, short replication times and high yields [44]. Also, several RNA viruses have genetically variable but closely related populations, called viral quasispecies, which act as a unit of selection and interact with the host species [12].

The quasispecies cloud size (quasispecies diversity level) may depend on adaptability and host range. In a prior study, Schneider *et al.* were limited to a single host, *Nicotiana benthamiana*, for direct comparison of quasispecies cloud size using three alpha-like RNA viruses, TMV, cucumber mosaic virus (CMV), and cowpea chlorotic mottle virus (CCMV). Yet, they indicated that evolutionarily related viruses have very different levels of diversity that correlated with their relative host range sizes. Furthermore, the quasispecies cloud size remained constant through 10 consecutives passages, i.e., all individuals had a similar average genetic distance from the consensus sequence [45].

Moreover, in a following study done by the authors cited above, it was shown that quasispecies cloud sizes are not constant; rather, they vary with different plant hosts and in changing environments. First, these differences may be due to selection pressure or bottlenecks imposed by the host-virus infection cycle. Second, this work proposed the possibility that different hosts may accelerate or decelerate the rate of viral evolution by allowing or denying high levels of diversity in viral populations [10].

Quasispecies diversity in viruses has been described previously as a mechanism to escape host resistance responses [46,47] or as a reservoir to maintain variants with selective advantages in other environments [48]. This diversity has been correlated with the ability to infect numerous hosts [45]. Nevertheless, excessive diversity can generate complications if the virus is exposed to recurrent bottlenecks.

3.3 Viral Bottlenecks

Most mutations are deleterious. Frequent bottlenecks can lead to the rapid loss of fitness known as Muller's ratchet [44,49]. A study has shown that the population diversity of marked CMV mutants decreased significantly and stochastically when the population moved from inoculated tobacco leaves to primary systemic leaves and reduced further as the systemic infection progressed, providing clear evidence of a genetic bottleneck [50]. Furthermore, the severity of bottlenecks varies and may depend on the structure of the minor veins and plasmodesmata of individual hosts [51].

Other studies have shown that in multicomponent RNA viruses, the function can be responsible for multiple differences in the genetic structures of different genomic segments. Populations of tomato chlorosis virus (ToCV) have a heterogeneous and complex genetic structure that depends on the RNA segment considered; i.e., it is more complex for RNA1 (encoding replication-associated proteins) than for RNA2 (encoding encapsidation, movement, and insect transmission) [52].

3.4 Genetic Adaptation

The rate of genetic adaptation of organisms depends on the degree of mutation, the population size and the range of selection, mainly during population bottlenecks. A single nucleotide substitution may facilitate virus adaptation to the host's translation machinery due to a correlation between viral amino acid codons and those used by the host plant [53]. A study on turnip mosaic virus mutations genomes revealed that several nucleotide sites may be involved in adaptation to *Raphanus sativus* [54].

For pathogens that affect multiple hosts, adaptation is a key factor in determining the probability and the severity of emergent disease outbreaks and can be used as a tool for the preservation of genetic diversity both in host and pathogen species. Tobacco etch potyvirus (TEV) and four natural hosts were used in an experimental evolution study that revealed the strong adaptive potential of TEV to new hosts without severe evolutionary limitations. The analysis of genome consensus sequences of the evolved lineages established that all mutations shared between lineages were host-specific [55].

Recent research has provided evidence of adaptation by plant viruses to host plants. Such adaptation may alter host–plant phenotypes in ways that facilitate transmission by arthropod vectors [56].

In addition, Gutiérrez et al. reported that vector physiology and behavior can be influenced by plant virus by increasing virus transmission either directly or via modification of the host plant [57].

There have been an important interest toward studying endogenous viral elements, which are knowledgeable in animals and much less well in plants. This aroused the interest of researchers who have carried out several independent studies on endogenous viral sequences in diverse plant species [11]. The sequences which have been found inserted into plant chromosomes were derived from both groups of plant DNA viruses, i.e. the single-stranded DNA

geminiviruses [58] and pararetroviruses with double-stranded DNA [59,60].

Recently, researchers at National Institute of Agronomic Research (INRA) Versailles-Grignon (France) and French Agricultural Research Centre for International Development (CIRAD) described a new kind of virus belonging to caulimovirus family (Caulimoviridae) called "Florendovirus" whose members have colonized the genomes of a large diversity of flowering plants during evolution. The genome of the grapevine (*Vitis vinifera*) was first identified and revealed previously unknown viral sequence elements. The assembly of these endogenous sequences allowed for reconstituting the viral genome, whose genetic composition is close to that of the Caulimoviridae [61]. Previously, Francois et al found that the micropropagation using in vitro tissue culture of banana (*Musa balbisiana*) triggers the activation of infectious endogenous sequences of Banana streak virus (eBSV) [62].

4. PLANT DEFENSE

4.1 Plant Resistance Mechanisms

Faced with viral invasion, plants protect themselves by a variety of complementary mechanisms in terms of defense timing, location, and targeting virus-derived molecules, i.e. the viral genome or viral proteins. There are four known defense mechanisms in plants against viruses. i) Dominant resistance relies on interactions between R gene products and viral avirulence (avr) gene products through the establishment of the so-called “gene-for-gene” interaction; these gene products belong to the nucleotide binding site-leucine-rich repeat (NBS-LRR) class. ii) Recessive resistance, which corresponds to the absence of appropriate host factors that are required for the virus cycle; this is a type of non-inducible resistance, as it is passive and effective throughout plant colonization. It confers resistance at the infection step and requires the cellular factor of interest. iii) RNA interference (RNAi), also called gene silencing, targets viral nucleic acids. Once established after a few days, the effectiveness of this defense mechanism increases and spreads to the whole plant through a relay-amplification process. iv) Hormone-mediated resistance against viral pathogens is mediated by salicylic acid (SA) and methyl-salicylate (Me-SA) in systemic acquired resistance (SAR) [63].

Furthermore, plant defenses always play important roles in the interaction between viruses and their vectors. The population growth of arthropod vector species may be affected positively, negatively or neutrally, when feeding on virus-infected host plants [64,65,66]. Some studies reported that the mechanism of how plant viruses modify the interaction of plant and its vector is SA-mediated [67,68].

4.2 Plant Immunity

There are two types of immunity in plants: i) innate immunity, which relies on cellular actors already present before to the infection, and ii) adaptive immunity (e.g., gene silencing), where defense responses are acquired following an infection and are adapted to the pathogen.

For a long time, researchers believed that the frontline of defense against RNA virus infection was provided by the nonsense-mediated mRNA decay (NMD) system. The NMD system is known as a cellular quality control and regulatory system for RNA that degrades aberrant transcripts, including incorrectly fabricated and non-functional messenger RNA molecules in cells [69,70]. The viral RNA genome has some similarities with incorrect messenger RNA. This system ensures that the genome of certain RNA viruses is broken down, thereby preventing viruses from replicating in host cells [71].

Recently, researchers at ETH Zurich discovered a new form of innate immune defense during their work with human cell culture and a model virus, the Semliki forest virus. In an extensive screening process, the scientists turned off individual genes inside host cells; they discovered that the cells were more susceptible to infection by the virus if the genes of the NMD system were turned off [72].

The same mechanism has been verified in plants by Garcia and colleagues in 2014. Their research was carried out using *Arabidopsis thaliana* and potato virus X (PVX) [73]. However, the mechanism is not 100% effective, because if this were the case, RNA viruses would not exist at all. Instead, viruses have evolved ways to avoid or actively suppress NMD. The NMD mechanism probably contributed to shaping the genomes of RNA viruses and the evolution of viruses into what we see today.

Other concepts that have been described as defense mechanisms in plant innate immunity

are PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI).

4.2.1 Pattern-triggered immunity

Plants are able to recognize conserved molecules, i.e. pathogen-associated molecular patterns (PAMPs), as a first line of defense. These elicitors are recognized by pattern recognition receptors (PRRs), including receptor-like kinases (RLKs) and receptor-like proteins, which are located on the surface of plant cells [15,74].

In a plant virus, a PAMP is identified as a non-conserved molecule, and the primary plant defense is thought to be based mainly on RNA silencing [75]. Endogenous silencing pathways generate 21-24 nt small (s)RNAs, micro (mi)RNAs and short interfering (si)RNAs that repress genes post-transcriptionally and/or transcriptionally. Four distinct Dicer-like (DCL) proteins, which normally produce endogenous miRNAs and siRNAs, are responsible for the biogenesis of viral siRNAs in infected plants. PTI-based innate responses may contribute to antiviral defense [76].

4.2.2 Effector-triggered immunity

To counteract PTI and establish robust infection in susceptible hosts, pathogens deploy effector proteins (virulence factors) in the plant host cell. In specific cases, plants have evolved resistance (R) genes that mediate the intracellular recognition of effector proteins, which results in ETI. Most plant antiviral R genes encode nucleotide binding site leucine-rich repeat (NBS-LRR) proteins that mediate resistance through the specific (direct or indirect) recognition of a virus avirulence (Avr) factor, and, as a result, trigger the hypersensitivity response (HR) and programmed cell death (PCD) in virus-resistant hosts [77]. In some cases, viral Avr proteins also function as silencing suppressors.

4.2.3 Zigzag model

The interaction between plant defense systems and its suppression by pathogens has been described as a “zigzag model” by Jones and Dangl [15], also called an “arms race” between plant and pathogens. Counterstrategies developed by successful viruses rely on specific pathogen effector/virulence factors that support pathogen growth by suppressing PTI, which results in effector-triggered susceptibility (ETS). In order to overcome the action of effectors, plants have evolved ETI. This evolutionary arms race between the host and the pathogen occurs with multiple rounds of ETS followed by ETI.

4.3 RNA Silencing

RNA silencing is a simple form of adaptive immunity. It is a mechanism that regulates gene expression and chromatin and destroys invasive nucleic acids such as transgenes and viruses. This evolutionarily conserved mechanism is sequence-dependent [78]. RNA silencing is a collective term for homology dependent RNA-based mechanisms directed by small RNAs. Silencing is activated during the production of small double-stranded RNA, leading to RNA degradation of the transgene and the homologous endogenous gene [79]. Small RNAs are known to trigger silencing by initiating the assembly of protein–RNA complexes. This assembly inhibits the expression of specific target genes by downregulating their transcription levels, their mRNA stability, or the translation of their mRNAs into protein [80]. The best studied silencing trigger is double stranded RNA (dsRNA). In addition, siRNA can also be generated via *de novo* synthesis by host RNA polymerases, such as RDRP2 in *Arabidopsis*, using single-stranded RNA transcripts [81,82].

Analogous to the zigzag model, viruses have evolved diverse mechanisms to avoid silencing-mediated resistance. Silencing suppressors target RNAi pathways at different points and through diverse mechanisms, including impaired siRNA biogenesis, defective siRNA incorporation into the RNA-induced cytoplasmic silencing complex (RISC), degradation of argonaute proteins (AGOs), trapping of sRNAs, and the suppression of RNAi amplification (reviewed by Bologna and Voinnet, [83]; Li et al. [84]. In turn, plants have developed specific defenses against RNA silencing suppression by pathogens, thus far providing another illustration of the never-ending molecular arms race between plant pathogens and their hosts [85,86,63].

In other words, innate (PTI and ETI) and adaptive (gene silencing) antiviral immunities act in a complementary way to fight off the pathogen. However, viruses neutralize this dual defense by effectors that suppress PTI and ETI innate responses and RNA silencing to launch successful infection [75,76].

5. CROSS-PROTECTION

It has been 50 years since researchers discovered the phenomenon of cross-protection. A plant inoculated with a mild virus, with weak infectivity, prevents the multiplication of a subsequent challenge of the same virus or closely related virus which is more virulent. This phenomenon is similar to vaccination in humans and animals and has been used on a large scale in cases where no resistant plants are available. Cross-protection may offer an alternative strategy to reducing economic losses in crops [87,88,89].

Cross-protection has been applied to control various viral diseases such as *tobacco mosaic virus* [90], zucchini yellow mosaic virus [91], and papaya ringspot virus [92]. Tristeza disease, caused by citrus tristeza virus (CTV), is currently controlled by mild CTV isolates [93].

Recently, the cross-protection phenomenon was examined between pathotypes of pepino mosaic virus (PepMV) representing the Chilean 2 genotype. Prior inoculation with PepMV-P22, a mild Polish strain, conferred protection against two other severe strains, PepMV-P19 and PepMV-P5-IY, and reduced symptom severity [94]. Moreover, another study showed that enhanced symptom severity can occur when the mild and challenge PepMV isolates belong to different genotypes (EU or LP). This may be due to synergism between different PepMV genotypes or recombinants resulting during co-infection [95]. When a plant is co-infected by diverse viral genomes, competition may lead to decreased fitness of individual genotypes in comparison with their fitness in single infections [96].

Several hypotheses have been suggested to explain the mechanism underlying cross-protection: i) the translation of the primary virus may prevent the transcription of the incoming viral nucleic acid, ii) the production of genome length RNA could be inhibited even if the challenge virus is replicated, iii) prevention of cell-to-cell movement [97], and iv) pre-activation by the protective virus of the RISC with small interfering RNA (siRNA) [98,92].

Cross-protection can be mediated by protein or by RNA or a combination of mechanisms. For example, a recent study showed that unencapsidated viral RNA did not confer protection against challenge inoculations by a related virus. Superinfection exclusion by CTV required the production of a specific viral protein, the p33 protein, as the absence of functional p33 protein completely eliminated the ability of the virus to exclude superinfection by the same or a closely related virus. Curiously, the protein seemed to work only in a homology-dependent manner [99].

In addition, identifying effective attenuated viruses for each virus of economic importance might be very laborious. A potential solution came recently from the development of engineered vaccines based on viral vectors carrying a genomic fragment of the virulent virus of interest [100,101,102,103].

6. ENGINEERING RESISTANCE

Plant disease resistance is crucial to the reliable production of food. Most economically important crops such as tomato, potato, and wheat are susceptible to viruses. Natural disease resistance carried by plants has not always guaranteed their durable protection against viral disease for the entire productive stage of the crop. The use of broad spectrum resistant (BSR) cultivars remains the best option in virus disease management.

6.1 Breeding and Marker-assisted Selection

Conventional methods of developing broad-spectrum resistance employ traditional breeding for resistance genes [104]. Employing multiplex parental material, containing three or four copies of dominant resistance genes, has been conducted with limited success due to interspecific barriers and genetic variation between crossed resistant host cultivars and resistant wild relatives. This has been overcome with somatic hybridization through the fusion of protoplasts to transfer resistance to potato virus Y (PVY), PVX, potato leaf roll virus (PLRV), and CTV from wild relatives [105,106].

Another approach that has proven to provide more durable and broad spectrum resistance is called marker-assisted selection (MAS). This approach uses gene-specific targeted transfer and pyramiding of resistance loci into an elite cultivar [107,108]. Pyramiding of Rsv resistance genes from soybean is a promising method for creating durable and broad spectrum resistance to all strains of soybean mosaic virus (SMV) [109]. However, gene pyramiding via

conventional breeding has some limitations like losing or affecting important traits over the desired quality or the inevitable transfer of undesirable traits, including toxins and allergens. Traditional breeding for resistance is not sufficient to arm potential crops with high levels of resistance that would maximize yield and quality [110].

Plant transformation or genetic engineering has provided a way to avoid the limitations of conventional breeding. A specific gene is transferred and its expression is directed to the appropriate time or tissue, contributing to the achievement of new desired traits for virus resistance [111].

6.2 Pathogen Derived Resistance

The pathogen-derived resistance (PDR) approach has been adopted by many plant virus resistance researchers. It consists of introducing a sequence of the viral genome into the host plant [112,113].

6.2.1 Protein-mediated resistance

Coat protein-mediated resistance (CPMR) has been widely used [97,114,115]. CPMR involves the interaction between the transgenic CP and the CP of the challenging virus. The efficiency of CPMR depends on the viral infection cycle [116].

Other viral transgenes including movement and replicase proteins have been used in plant resistance engineering. Replicase protein-mediated resistance (RPMR) using RNA-dependent RNA-polymerase (RdRp) to confer resistance to RNA viruses was first reported for TMV [117], then assessed for CMV and rice yellow mottle virus (RYMV) [118,119]. However, this type of resistance is very specific and limited to similar viral strains. For single-stranded DNA viruses, induced resistance takes advantage of viral replication (Rep) associated proteins that interact with host polymerases [120]. Expression of the tomato yellow leaf curl Sardinia virus (TYLCSV) C1 gene encoding Rep confers resistance to the homologous virus in *Nicotiana benthamiana* and tomato plants by repressing C1 translation and TYLCSV replication [121].

In addition, movement proteins have been utilized to engineer virus resistance in plants by producing transgenic plants with modified genes of the movement of proteins. Resistance is based on the competition between the preformed dysfunctional movement proteins and wild type virus encoded movement proteins to bind to plasmodesmatal sites. The first description of movement protein-mediated resistance (MPMR) was shown in tobacco plants [122].

6.2.2 RNA-mediated resistance

Further investigations have shown that protein-mediated resistance level is not directly related to the viral protein expression level, because high virus resistance levels could remain in transgenic lines that do not express any viral CP. These non-expressing transgenic plants are still resistant because the expressed CP mRNA triggers post-transcriptional gene silencing (PTGS) and provides RNA-mediated resistance to the virus through the siRNA pathway [123]. Based on differences in their biogenesis, two types of sRNAs have been identified, siRNA and miRNA.

The siRNAs produced are then incorporated into the RISC, which guides the cleavage of target RNAs [124]. However, this type of resistance appears to be effective only against related viral sequences. An attempt was undertaken based on the mechanism of PTGS using multiple gene inserts in a single construct. A single chimeric hairpin RNA construct containing viral 4 N gene segments, which is the main constituent of the nucleocapsid from tomato spotted wilt virus (TSWV), groundnut ringspot virus (GRSV), tomato chlorotic spot virus (TCSV), and watermelon silver mottle virus (WSMoV) was arranged as inverted repeats. Transgenic plants expressing this construct displayed broad-spectrum resistance against tospoviruses [125]. A similar strategy was used in developing multiple resistance to Prunus fruit viruses [126].

The second type of sRNA is miRNA; these are single-stranded RNAs about 21 nt in length, generated from the processing of longer miRNA precursors (pre-miRNA) by Dicer [127]. Recently, the miRNA pathway was shown to downregulate endogenous gene expression in plants. This pathway has been used to design artificial miRNAs (amiRNAs) whose expression in transgenic plants can confer resistance against plant viruses [128,129]. This new anti-viral approach, which has the benefit of reducing possible bio-safety risks associated with protein- and RNA-based strategies, is a first step to designing environmentally friendly virus resistance in transgenic crops [130].

As a strategy for controlling virus diseases, the expression of RNA satellites (Sat-RNAs) in transgenic plants confers resistance to some extent. Sat-RNAs are small parasitic RNAs associated with some plant viruses and are frequently able to modify the symptoms induced by their helper virus [131]. Small sat-RNAs (194 to 400 bases) do not encode proteins [131] and consequently are entirely dependent on virus association for replication and spread in plants. Symptom attenuation (resistance) mediated by sat-RNAs is widely attributed to the inhibition of helper virus replication due to competition for limited replication factors between the helper virus genome and the sat-RNA [132,133].

Induced resistance using sat-RNA has been intensively used against CMV [134,135]. However, the expression of sat-RNAs in transgenic plants may not be strong enough to protect plants from natural viral infections. Transgenic plants expressing both sat-RNA and the coat protein of CMV exhibit enhanced resistance to the virus [136]. Sat-RNA drastically reduces the replication of the viral genome, particularly in Solanaceous hosts and such plants are poor of aphid vector populations [137].

6.3 Antibody-mediated Resistance

Antibody-based resistance is a novel strategy for producing transgenic resistant plants. Decades ago, it was shown that polyclonal and monoclonal antibodies can specifically bind to the surface of pathogens such as viruses, bacteria and selected fungi, and result in neutralization. This method has been improved recently by the development of recombinant antibodies (rAbs). Crop resistance can be engineered by the expression of specific antibodies, fragments of antibodies or antibody fusion proteins. This approach has allowed for the engineering of efficient rAbs targeting almost any molecule. It has been confirmed that the expression of functional pathogen-specific rAbs in plants confers effective protection against pathogens. The efficacy of the antibody-based resistance approach for plant viruses has been shown and its application to other plant pathogens is becoming widely used. However, the successful use of antibodies to generate plant pathogen resistance relies on appropriate target selection, careful antibody design, stability, efficient antibody expression, and expression the appropriate cellular compartments [138,139].

Recently, a study generated a model that simulates the complex dynamic interaction between Begomovirus genetics and adaptability to certain plants. This model indicates that the main reason for epidemic outbursts and the global spread of the disease can be found in the patterns of inter-species interactions, many of which are human-induced. In particular, the extensive use of resistant cultivars results triggers aggressive virus adaptability through accelerated mutation. It appears that the only simple option would be to develop more diverse and less concentrated cropping patterns, both in terms of crop land extent and in time [140].

6.4 Chemical-mediated Induced Resistance

Resistance in plants to numerous viruses can be induced by several types of synthetic compounds applied by injection, spraying into leave or absorption through petiole or through roots. Such chemicals reported as effective inducers include Salicylic acid (SA) [141].

Extensive reports indicate that SA-induced defenses are important in regulating both anti-herbivore (insect) and anti-pathogen (virus) defense responses [68]. For example, Abe et al. [142] showed that TSWV infection increased SA contents and induced SA-regulated gene expression in *Arabidopsis*. Rodriguez-Saona et al. [143] demonstrated that the SA-mediated defense responses are effective against both pathogens and aphids in tomato, because TMV infection reduces plant susceptibility to aphids in wild-type tomato but not in SA-deficient transgenic plants.

However, few diseases are currently controlled commercially by the mechanism of chemical-mediated induced resistance.

7. CONCLUSIONS

Plant viruses are critical problems to several agricultural and horticultural crops around the world. These pathogens depend on the cellular machinery of their plant hosts. Several aspects of plant-virus interactions happen during the stages of virus infection process both in virus translation and/or replication and in viral cell-to-cell movement.

During the progression of plant-virus coevolution, both have evolved features to battle each other; the plant is equipped with sophisticated and rapidly mounted defense mechanisms, while the virus has developed counterstrategies to

overcome those defenses. Understanding the forces that control viral quasispecies may lead to novel ways of predicting the emergence of new viral pathogens and resistance-breaking variants, and provide an overview of the structures of other organism populations. Furthermore, a detailed analysis of viral variability within hosts is needed to manage strategies of disease control, since quasispecies are reservoirs of viral variants that can potentially emerge with increased virulence or altered tropism.

As long as there are no direct methods to control viruses, as with other plant pathogens, current strategies rely on indirect measures for the management of viral diseases. Until the past few decades, classical breeding was considered an effective means to generate resistant plants, but this is costly and difficult; strong genes are rare and when known they do not always confer durable resistance. This occurs especially when breeding with single-gene is employed; because pathogens frequently overcome this host resistance over time due to the emergence of new plant pathogen races. During the breeding process between plant varieties or species, not only do transferred genes confer the desired strength, but also sometimes affect undesirable genes present on neighboring loci. In addition, features such as crop quality and quantity may be compromised.

Recent advances in molecular biotechnology have made it possible to obtain and to modify genes that are useful for generating disease-resistant crops. A management strategy based on cross-protection can only be successful in areas where one genotype is dominant, otherwise co-infection may result in the emergence of virus variants with new traits after recombination between the protective and challenge isolates. In addition, identifying effective attenuated viruses for each virus of economic importance might be very arduous. Besides, for perennial crop, like citrus tree, the cross protection ability of mild strain appears to be lost after a few years, and severe strains may affect the mild strain-free area because the distribution of mild protecting strain within trees is only partial.

In contrast, numerous strategies, including pathogen-derived expression of sequences or anti-pathogenic agents, have been established to engineer upgraded pathogen resistance in transgenic plants. Transgenic plants expressing the RNA silencing pathway have been shown to efficiently resist viral invasion. It seems that this pathway represents the most specialized molecular strategy that plants use to combat viruses. The expression of sat-RNAs in transgenic plants confers some measure of resistance that may not be strong enough to protect plants from natural virus infections.

However, the risks of biotechnology on the environment and food security related to culture and trade in these transgenic crops are concrete, and it is necessary to define appropriate policies and strategies to protect against adverse effects. Engineering methods that aim to overcome the risks associated with transgenic plants have become more important; these approaches are based on inducing viral RNA silencing without altering the plant genome. In addition, the exploitation of changes in mRNA, cellular metabolites, and protein, once virus invasion has occurred, will help to improve our understanding of plant-virus interactions. In the near future, this will contribute to improving the efficiency of current strategies and allow the establishment of new approaches in favor of sustainable socio-economic development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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