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Review Article

Viruses as a Bridge to Synthetic Genomes in Plant Biotechnology

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Abstract

Thanks to recent developments in genome engineering, it is now possible to make targeted changes to DNA sequences in plant cells, resulting in plants with desirable characteristics. The efficiency with which sequence-specific nucleases and repair templates are delivered to plant cells is crucial for effective gene targeting. Particle bombardment and Agrobacterium-mediated transformation are two methods that do this; however, they each change just a small percentage of cells in the tissues being treated. Vectors based on viruses that can replicate on their own have recently been shown to be an effective method of delivering GE reagents to plants. Gene targeting rates in model plants (*Nicotiana benthamiana*) and crops have been shown to be high for both DNA and RNA viruses (Shan.S. 2017). The use of plant viruses as vectors for the heterologous expression of genes dates back more than 30 years. Different approaches have been taken to using them since then, but what has consistently propelled viral vector use is the three-stage convergence of technology and virology tools that we will explore below. Firstly, the development of molecular biology and reverse genetics allowed for the cloning and manipulation of viral genomes to express genes of interest. The second is due to the convenience and extensive application of virus-induced gene silencing made possible by the discovery of RNA silencing and the development of high-throughput sequencing tools (Cody et al., 2019). Transient RNA and DNA replicons based on viruses provide a number of advantages over transgenic gene expression, including speed and ease of development and the potential for widespread application in a wide range of plant species. While the instability of the foreign gene in the viral genome can be problematic, these features are especially useful when rapidly increasing gene expression is a priority. Gene replacement, gene insertion, epitope presentation, the use of virus-controlled gene expression cassettes, and complementation are only some of the methods that have been tried and evaluated for foreign gene expression in various virus-based vectors (Herman B. et al. 1996). The study of host gene activities in plants and the delivery of short RNAs to insects have both benefited from the use of virus-induced gene silencing, which is RNA-mediated and activated through homology-dependent RNA degradation mechanisms (Peter A. et al. 2020).

Introduction

Genome engineering (GE) involves targeted genetic manipulation of living organisms. GE technologies may benefit many creatures, including plants (Schaeffer and Nakata, 2016). GE improves gene disruption, correction, and insertion by modifying a specific sequence on a chromosome (Songstad et al., 2017). Choosing vectors that distribute GE chemicals in a systematic manner is a major difficulty in plant genome engineering. Plant viruses have long been utilised as vectors for industrial protein synthesis (Rybicki, 2009). Viral genomes are good vectors due to their efficient machinery and extensive genome structure. Autonomous virus-based vectors deliver GE reagents to plant cells. RNA viruses include the monocot Wheat streak mosaic virus (WSMV), the dicot Barley stripe mosaic virus (BSMV) (Lee et al., 2012), and the tricot Tobacco rattle virus (TRV). Single-stranded (ss) DNA viruses like geminiviruses have

been used as vectors for several crops. These viruses can carry heterologous coding sequences and express proteins in wheat, barley, corn, oats, and rye (Choi et al., 2000). GE technology has encouraged scientists to use viral vectors to efficiently transport GE reagents to plant cells. Plant viruses as heterologous protein overexpression tools have intrigued virologists for at least 30 years (Siegel A. 1985). Since viral vectors were invented, much research has concentrated on finding and improving the best viruses and designing heterologous protein synthesis systems. This has been covered in prior reviews (Gleba Y, et al., 2007) and is briefly discussed here to provide context. Rather, we reflect on three aspects of relatively recent viral vector technology development that were not, and could not have been, predicted more than two decades ago (Scholthof HB, et al. 1996): (a) the ongoing exploration of diverse virus-derived gene expression tools and adaptation of delivery methods to greatly enhance gene expression levels; (b) viral vector applications in plant functional genetics (e.g., RNA silencing) specifically on nonmodel (e.g., not Arabidopsis) plants. Although some applications involve transgenic techniques, the following sections focus on viruses as independent temporary delivery vehicles. In a broad perspective, the expanded availability of various plant virus-derived tools over the past thirty years has shifted the exploitation of viruses and their components from plant virology novelty instruments to the workbench of nearly every plant molecular biologist. Within this context, it is the continual conceptual development of plant viral gene vectors enabled by contemporary breakthroughs in important technological developments that constitutes a consistent thread throughout the history of viruses as biotechnological instruments.

Vector

A vector is a vehicle (such as a plasmid) used to transport genetic material, such as DNA sequences, from one creature to another. There are two main ways in which vectors carry out their roles: the first is as transcription vectors. Transfection into a target cell is possible with transcription vectors but not translation; thus, while they can be duplicated, they cannot express their genetic material. Expression vectors are often plasmids or viruses modified for use in cellular gene expression; in contrast, transcription vectors are employed to amplify their insert before being expressed. Specifically, the vector can be used to introduce a gene into a target cell and then use that cell's protein-synthesis machinery to make the protein encoded by that gene. The most typical expression vector (Fig. 1).

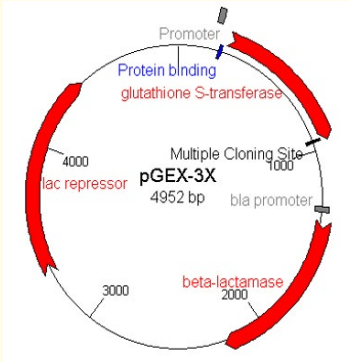

Figure 1: Source: Plasmid map of the pGEX-3X cloning vector. creative Commons Attribution-Share Alike 1.0 Generic

Well-designed expression vectors and their goals are used worldwide, is efficient protein production, which may be achieved by producing a lot of stable messenger RNA, which may be translated into protein.

Use of multiple virus vectors (Fig. 2) (a) Therapeutic protein production in plants as an alternative scale-up production platform (b) VLPs or epitope-displaying viral vaccines (c) Plants guard against human and animal infections by producing AMPs, lysins, and bacteriocins. Pathogens are less likely to colonise AMP-expressing plants. (d) Virus-induced gene silencing (VIGS) can be used in reverse genetics to determine gene function or to inhibit the expression of a specific gene (or genes) to influence phenotype. (e) Virus-induced gene editing (VIGE) virus vectors carry single-guide RNA (sgRNA) co-delivered with Cas9 or inserted into transgenic

Cas9 plants. VIGE vectors can alter, insert, or overexpress genes. (f) Pest control via generating RNA interference (RNAi) in pests or pathogens RNAi is caused by virus vectors that contain essential pest/pathogen genes. Pest/pathogen uptake of short interfering RNA (siRNA) following feeding or infection leads to silencing of target genes in nematodes, insects, or fungi and subsequently increased mortality and/or growth inhibition. (g) Virus-induced flowering (VIF) helps in quickening breeding operations by overexpressing the flowering locus T in woody or recalcitrant plants. (h) Virus vector nanoparticles (VNPs) are exploited as instruments in nanomedicine and as nanopesticides. Imaging, diagnostics, and cancer or illness treatment use VNPs. VNPs like empty cowpea mosaic virus (eCPMV) can be targeted to mouse tumors, loaded with siRNAs to silence target genes in mammals, and used as pesticide carriers in difficult systems like soil-borne pest control. VPNs can carry pesticides like abamectin to kill nematodes and other soil microbes.

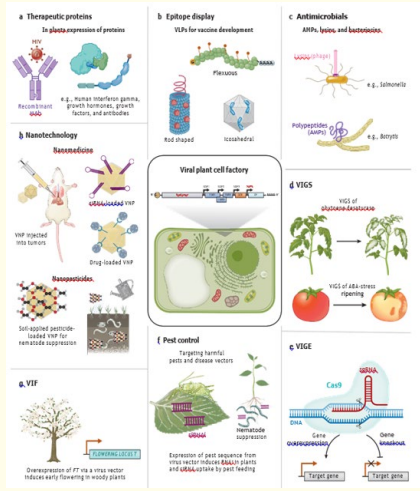

Figure 2: Source: Annu. Rev. Virol. 2020.7:513-535. Downloaded from www.annualreviews.org Access provided by 110.224.253.155 on 12/01/22.

What is synthetic biology?

A viral vector is a laboratory-engineered virus that can be used to deliver foreign DNA to cells. To achieve the desired result, simply swap out the relevant genes (for instance, insulin production in the case of diabetics). The viral genes responsible for genome integration into the host genome must survive the operation.

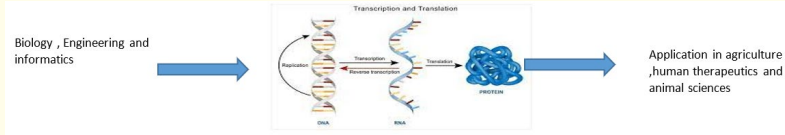

Figure 3: Synthetic biology and its application.

Three predominant factors led to the eventual wider use of viral vectors outside of the virology field: advances in plant functional genomics are.

a) The discovery of post-transcriptional RNA silencing (PTGS).

b) The development of modern sequencing tools-initially, the modernization of Sanger sequencing followed by massively parallel high throughput (next generation) sequencing.

c) Virus-Induced CRISPR-Cas9-Mediated Editing.

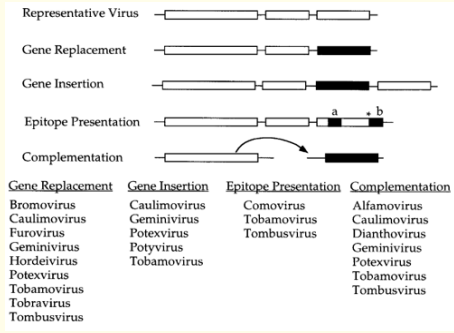

Figure 4: Comparison of strategies used to express foreign genes (black box) from different viruses as discussed in the text or references therein. White boxes indicate viral genes. The epitope presentation method (*a*) involves translational fusion of a small sequence inside the coat protein gene or (*b*) translational read through of an amber stop codon (*) at the 3r end.

Gene Replacement Vectors

Many researchers have investigated building virus vectors by replacing viral genes with foreign genes to lessen the risk of genome size alterations caused by foreign gene insertion. The deleted viral gene must be non-essential, which precludes replacing replication and movement genes. Insect transmission factor genes may be replaced. Some plant viruses can infect hosts systemically without the coat protein gene. Given these restrictions and possibilities, the first effective attempts to establish plant viruses' gene vector potential involve replacing insect transmission or coat protein genes. Isometric Viruses PARARETROVIRUSES Currently, there are two identified groups of double-stranded DNA plant viruses: the spherical calicivirus and the bacilliform badnavirus. These viruses belong to the pararetrovirus supergroup of animal and plant viruses whose encapsidated DNA replicates via reverse transcription of a terminally redundant full-length RNA transcript. In the early 1980s, various investigators postulated that CaMV might be employed as a gene vector for foreign DNA expression (Gronenborn B et al. 1981). Subsequently, Brisson et al. switched the insect transmission gene of CaMV (gene II) with a bacterial dihydrofolate reductase (dhfr) gene and found that this gene gave systemic resistance against methotrexate in conjunction with replication and propagation of the vector. The recombinant virus can encapsidate and migrate because the dhfr gene is only 240 bp. Previous findings showed that CaMV amplifies only short DNA segments of 250 bp. Since those initial attempts, the CaMV vector has expressed numerous small genes, but packaging or other undiscovered structural constraints appear to limit the size of foreign genes that can be maintained. Caulimovirus genome molecular recombination activities rapidly delete foreign inserts, limiting the gene vector's potential. CaMV and kindred caulimoviruses' narrow host range may limit their utility as vectors to a few plant species. Bacilliform badnaviruses like rice tungro, which may infect monocotyledonous plants, may have more vector potential. GEMINIVIRUSES Geminiviruses are characterised by twin particles that encapsulate single-stranded DNA whose replication occurs in the nucleus via a double-stranded DNA intermediate that remains infectious when cloned. Monopartite geminiviruses like maize streak virus (MSV) have a single genomic DNA, while bipartite ones like tomato golden mosaic virus (TGMV) have a segmented genome.

Gene Insertion Vectors

Isometric viruses Even though a gene may not be necessary for infection under experimental conditions, all viral genes certainly

contribute to the fitness of the virus. Therefore, replacement tactics for foreign gene expression may undermine critical or advantageous indigenous viral gene expression, making them less appealing. Gene insertions or modifications that leave viral genes untouched have been examined for foreign gene expression to avoid such harmful effects. Foreign genes placed into MSV's short intercistronic region are expressed, but they only spread systemically once vector molecules revert to wild type. These findings show monopartite geminiviruses may have limited gene expression vector capacity in entire plants. However, insertion of the herbicide resistance gene bar in MSV's short intergenic region gives inoculated leaves resistance to Basta. Viral Rods TOBAMOVIRUSES Several rod-shaped monopartite viruses have tried foreign gene insertions or additions instead of gene replacement. TMV was initially used to create an infectious clone with a duplicated subgenomic coat protein mRNA promoter. This promoter expressed the CAT gene between the 30-kDa cell-to-cell migration gene and the native coat protein gene. Homologous recombination between the duplicated promoter regions rapidly lost the CAT gene insert. After inserting a subgenomic RNA promoter from related tobamoviruses, TMV vectors became more stable. Reduced sequence relatedness reduces recombination events between the two subgenomic promoters, making them a more stable vector to express foreign genes throughout the plant. POTEXVIRUSES For full plant production of PVX foreign genes, duplication of a homologous coat protein subgenomic promoter works well. A duplicated promoter was placed between the triple gene block and the original subgenomic promoter upstream of the PVX coat protein gene to enable systemic infection (Chapman S., Hills et al. 1992). The GUS gene, or jellyfish GFP, downstream of the duplicated promoter sequence increased reporter gene production throughout the plant. Virus vectors are stable. Foreign genes cannot be introduced between viral genes because potyviruses use cis-regulated proteolytic polyprotein processing. However, insertional fusion with existing genes or the inclusion of adequate nearby proteolytic cleavage sites allows effective protein processing and release of the foreign gene product together with the important viral proteins.

Epitope Presentation

The translational fusion of tiny foreign peptides to viral proteins is a significant technique with potential biological applications that was just developed. The insertion sites are selected so that the resultant peptides extend outward from the viral particle (Figure 4). Therefore, these viruses can be extracted from infected plants and utilised as epitope presentation vehicles for the production of specific antibodies against short peptides. For internal peptide fusions, the change cannot interfere with normal functions. Alternately, foreign gene segments can be fused to the 30-terminus of a viral gene in a way that permits the production of both the fusion product and the native viral protein to prevent interference with normal gene functions. The success of epitope presentation tactics is contingent on an atomic-level understanding of virus structure, which is only available for a restricted number of viruses. Consequently, examples of epitope presentation tactics include testing with viruses with well-defined structures, such as TMV, CPMV, and TBSV. Utilizing terminal protein fusions to express foreign peptides has been effective for TMV (Hamamoto H, 1993). In this method, a translational read-through sequence was copied and placed into the replicase gene. Downstream of the read-through sequence, a short foreign gene producing the 12-amino acid peptide angiotensin-I-converting enzyme inhibitor was introduced. This modification allowed for the occasional translational read-through of a designed coat protein amber stop-codon (UAG), resulting in the production of native coat proteins along with smaller amounts of the fusion proteins. This expression occurred without interfering significantly with the systemic invasion of tomato and tobacco plants, and the virus vector was also relatively persistent.

Complementation Systems

Foreign genes or gene fragments from autonomously replicating viruses can infiltrate the plant systemically. These techniques have advantages, although most viral gene replacements disrupt key processes, and gene insertions increase genome size, which may influence packaging. Helper-dependent systems, in which foreign genes are inserted into faulty (sub)viral components that either depend on transgenically introduced viral genes or co-infection with a helper virus for key tasks, can solve some of these issues. Transgenic complementation "disarms" the virus by replacing an important gene with a foreign gene. The disarmed virus vector is seeded onto transgenic plants that express the "missing" virus gene to complement it. Transgenic complementation of viral functions has been shown to work for several virus systems, but its potential for increasing virus-mediated foreign gene expression is unknown. Caulimoviruses have been used to complement purposely faulty viral entities that can carry a foreign gene. These tests repair faulty components using wild-type CaMV or a virus

with another deletion. Because rapid recombination events regenerate wild-type genomes, these systems have failed as gene carriers.

Schematic representation of viral delivery methods

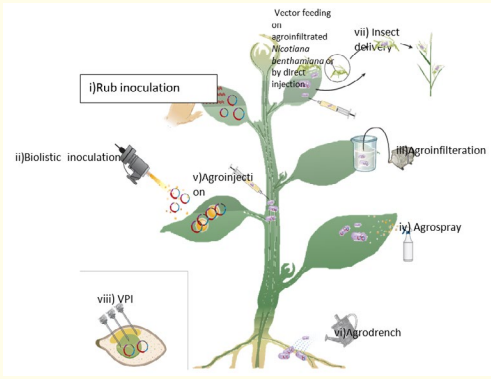

Figure 5: Plant virus vector delivery. I Mechanical rubbing fluids on plant leaves with a fine abrasive applies RNA transcripts and plasmids.(ii) A gene gun can inoculate difficult-to-inoculative virus vectors coated on gold particles. (iii) Agroinfiltration with a syringe or immersion in a bacterial slurry under vaccuum is the most popular vector delivery method. Agrobacterium can be applied by spray, injection, or drench (agrodrench). (vii) Microinjection of pure virions into an insect vector that feeds on the plant's vascular system can transmit phloem-limited viruses. (viii) The inset shows the virus vector in germinating seedlings.

Some examples of viruses used as a vector

Cauliflower mosaic virus

The Cauliflower mosaic virus (CaMV) is an isometric virus that infects many species of cruciferous plants (7 ORF and 2 RNA transcript). Viruses use their two-part promoter (35s and 19s) for this function. Chimeric viral DNA derived from the bacterial dihydrofolate reductase (dhfr) gene resulted in the production of functional DHFR in infected turnip plants.

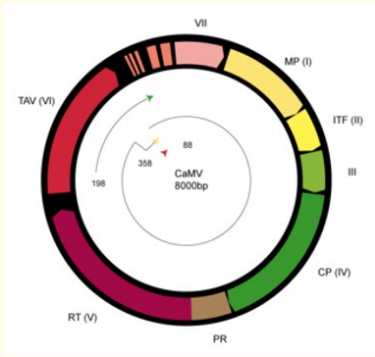

Figure 6: source: Cauliflower mosaic virus from Wikipedia.

Geminivirus

The Geminivirus vector lacks a coat protein gene, and the viral itself consists of two identical particles encaing single-stranded DNA (ssDNA) that replicate in the nucleus via a double-stranded DNA intermediate. The geminivirus family often infects a wide variety of host organisms. To put it simply, bipartite geminiviruses have a genome that is split into two parts, while monopartite geminiviruses only have one. These VIGS vector has been employed in experiments to mute single or several genes, including the meristemic gene, proliferating cell nuclear antigen (PCNA) (PCNA).

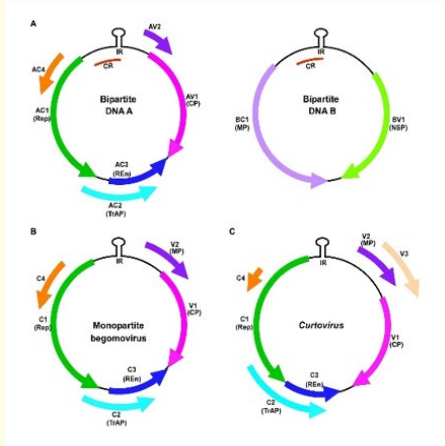

Figure 7: source: Karuppannan Veluthambi Front. Microbiol., 09 April 2021 Sec. Microbe and Virus Interactions with Plants.

Tobacco mosaic virus

The genome of TMV is made up of a single strand of RNA, which serves as mRNA. There are 4 ORF. Magnification is a method that the German biotechnology company Icon Genetics came up with for putting these recombinant virus vector modules into plants. Magnification is a method that uses agroinfiltration and the delivery of a deconstructed vector that can't spread to other plants. TMV infectious viral clones genomic models. (a) Wild-type (wt) TMV transcripts have 5cap (gold circle) and 3 tRNA-like structures. The wt genomic RNA produces four gene products, including two replicase proteins, 126 kDa and 183 kDa, which are translated via a ribosomal readthrough from an amber stop codon (). During replication, two subgenomic RNAs are produced: MP for cell-to-cell mobility and CP for coat protein. (a) An example of gene replacement for vector construction. The intact CP subgenomic promoter produces the second subgenomic RNA to translate the GFP (green fluorescent protein) coding sequence. (c) A heterologous gene-insertion model. The second subgenomic RNA is produced by a CP promoter duplication.

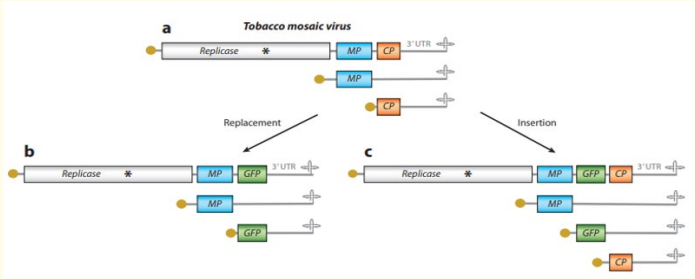

Figure 8: TMV infectious viral clones genomic models. (a) Wild-type (wt) TMV transcripts have a 5 cap (gold circle) and 3 tRNA-like structures. The wt genomic RNA produces four gene products, including two replicas proteins, 126 kDa and 183 kDa, which are translated via a ribosomal readthrough from an amber stop codon (). During replication, two subgenomic RNAs are produced: MP for cell-to-cell mobility and CP for coat protein. (a) An example of gene replacement for vector construction. The intact CP subgenomic promoter produces the second subgenomic RNA to translate the GFP (green fluorescent protein) coding sequence. (c) A heterologous gene-insertion model. The second subgenomic RNA is produced by a CP promoter duplication.

Virus-induced gene silencing

Viral-based functional genomics tools (VIGS) are used to shut down the host gene in order to study its function. The study of gene function in plants can benefit greatly from the use of virus-induced gene silencing (VIGS).

Requirements for a VIGS vector virus.

Should have low symptom severity, broad host range, methodical spread, weak silencing suppressor, and no quarantine risk.

Vigs Based Vector

a) RNA BASED VIGS based vector Dicot TMV -VIGS, PVX -VIGSTRV-VIGS (universal)Monocot -BSMV -VIGS (ßa silencing suppressor is removed) FRUIT ASLV-VIGS.bDNA-basedd VIGS VECTORGEMINIVIRUS (bc1 silencing suppressor is removed).

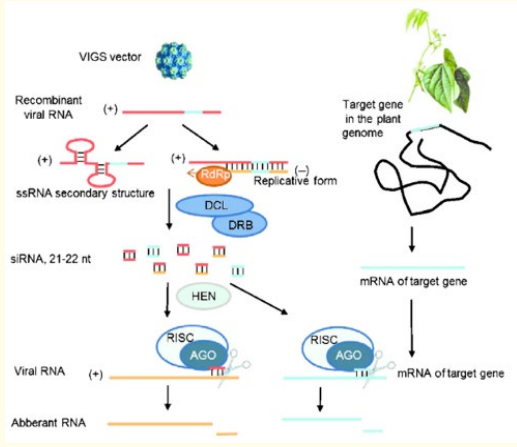

Figure 9: source Stéphanie Pfliege VIGS technology: An attractive tool for functional genomics studies in legumes.

Molecular mechanism of virus-induced gene silencing (VIGS) induced by RNA viruses in plants. Abbreviations: AGO, argonaute protein; DCL, dicer-like protein; DRB, dsRNA-binding protein; HEN, Hua enhancer protein; RdRp, RNA dependent RNA polymerase; RISC, RNA-induced silencing complex; siRNA, short-interfering RNA; ssRNA, single-stranded RNA. By the 1990s, viral vectors were being used outside of virology for protein production, but viruses were still a mystery for basic plant biology research. The discovery of posttranscriptional RNA silencing (PTGS) and the development of modern sequencing tools-first Sanger sequencing, then massively parallel high-throughput (next-generation) sequencing-led to the wider use of viral vectors outside of virology. Both technologies promoted virus-induced gene-silencing (VIGS) screens to suppress host target gene expression, advancing plant functional genomics. Modern sequencing and RNA silencing synergistically led to new viral-based tools for plant virologists and biologists. Tobacco rattle virus (TRV) and PVX vectors were used to design and test VIGS for large functional genetic screens approximately two decades ago. The diversification of the VIGS screening tool from experimental plant species like *Nicotiana benthamiana* and A. thaliana to agriculturally relevant or physiologically interesting plants was not easily predicted until high-throughput sequencing became widely available and affordable. An exponential increase in virus and plant sequences in public databases has allowed individuals working on plant species without genomic material or with only rudimentary cDNA libraries needing a lot of human effort to generate strong sequence data to strengthen screening approaches. Instead of fishing for traits like the early cDNA VIGS experiments, genomic and transcriptome data allowed reverse genetics screening of genes of interest.

CRISPR-Cas9

Edited Genes A new technique or tool based on the old idea of editing genomes, which was not feasible in plants until recently due to the complexity of gene-editing technologies, is accelerating functional genomic applications of virus vectors. This has historically been achieved with site-directed nucleases that create double-stranded breaks (DSBs) in the genomic DNA, although it is not limited to nucleases. Targeted DSBs form the basis for the implementation of the bacterial-derived clustered regularly interspaced palindromic repeats (CRISPR)-associated (Cas) gene-editing platform as a diverse toolset for functional genomics. At present, there are two classes, six types, and many more subtypes of CRISPR that are defined by the nature of the nuclease complex and the mechanism for producing and integrating the complementary target-seeking CRISPR RNAs (crRNAs). Currently, the most developed CRISPR-Cas system for biotechnology purposes is CRISPR-Cas9, whose hallmark is the simplicity of its design. There are three main genetic components for CRISPR-Cas9 functionality: (a) the target DNA; (b) an RNA programmable DNA nuclease (Cas9); and (c) a sequence-specific single-guide RNA (sgRNA; not to be confused with viral subgenomic RNA). These last two components require a mechanism for cellular delivery. Cas9 binds to the heavily structured portion of the sgRNA scaffold sequence (80 nts from the 3' end) to create a Cas9-sgRNA duplex, which upon binding becomes catalytically active. The Cas9-sgRNA duplex then localises to genomic DNA; in eukaryotes, this is achieved by tagging the originally prokaryotic Cas9 with one or more nuclear localization signals (NLSs). In the natural setting, CRISPR-Cas systems are used to combat phage infections in a manner comparable to antiviral RNA silencing in eukaryotes. From that perspective, it is intriguing to note that phages can produce anti-CRISPR components comparable to suppressors of silencing encoded by eukaryote-infecting viruses. Upon genome binding, the CRISPR-Cas9 complex initiates scanning of the DNA strands for a protospacer adjacent motif (PAM). Although different Cas9 proteins bind to varying PAM sequences, Cas9 from Streptococcus pyogenes, also referred to as spCas9, recognises a 3'-proximal NGG sequence, where N represents any nucleotide, albeit not with equal preferences. Once this PAM sequence is recognised by the Cas9-sgRNA complex, the genomic complementary region of the sgRNA (mostly 5 nts) or the programmable portion of the sgRNA that is defined by the user anneals to the genomic DNA through Watson-Crick base-pairing. Once the sgRNA and complementary genomic DNA sequence are bound, the Cas9-sgRNA-DNA complex can catalyse the formation of a DSB. Nuclear genomic DSBs are repaired by one of the two host DNA repair systems, nonhomologous end-joining (NHEJ) or homology-directed repair (HDR), to obtain gene knockouts or insertions, respectively. Details of these repair processes are of great importance and have been discussed extensively in two recent reviews. In short, NHEJ is a rather inconsistent method for DSB repair because of the generation of nucleotide insertions and deletions (indels) at the DNA break site. Owing to the imprecise nature of this type of DNA repair, some have argued that cellular repair by this mechanism coincidentally does not fit the definition of gene editing. Although this is a compelling argument, most scientists in the field use the NHEJ and subsequent downstream analysis of indels as a method to identify and quantify

the editing functionality of NHEJ-based indel production handles the editing. HDR editing events correct DSBs more precisely than NHEJ but are harder to set up. The sgRNA design targets a specific allele to replace a genomic segment. Cas9, sgRNA(s), a DNA template with homologous arms, and a replacement DNA sequence are needed to repair DNA. Due to the specificity of the repair and the likelihood of genomic replacement, HDR-based locus repairs are desirable in most gene-editing applications. Much effort has been put into making the route more efficient. Adaptive immunity and microbial CRISPR systems After phage infection, several CRISPR-associated (Cas) enzymes acquire spacers from exogenous protospacer sequences and install them into the CRISPR locus in the prokaryotic genome (step 2: spacer acquisition).Spacers between direct repeats let the CRISPR system recognise self and nonself. CRISPR arrays are noncoding RNA transcripts that are enzymatically matured by unique pathways for each CRISPR system (Step 3: crRNA biogenesis and processing). CRISPR-associated ribonucleases break the pre-crRNA transcript within repetitions in types I and III CRISPR, releasing numerous tiny crRNAs. Unidentified RNases process type III crRNA intermediates at the end to create the mature transcript. In type II CRISPR, tracrRNA hybridises with direct repeats to generate an RNA duplex that is cleaved and processed by endogenous RNase III and other unknown nucleases. Effector protein complexes recognise and degrade type I and III CRISPR systems' mature crRNAs. Type II systems use crRNA-tracrRNA hybrids and Cas9 to interfere. Multiprotein interference modules help type I and III CRISPR systems recognise targets. The cascade complex in type I CRISPR detects target DNA by loading a crRNA molecule. The cascade-bound R loop recruits Cas3 nuclease to degrade targets. Type III CRISPR crRNAs bind and cleave DNA and RNA substrates using CSM or CMR complexes. The type II system just needs Cas9 nuclease to destroy DNA matching its crRNA-tracrRNA hybrid dual guide RNA.

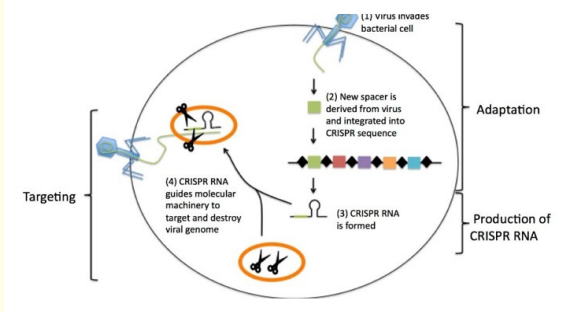

Figure 10: Source: Figureure adapted from Molecular Cell 54, April 24, 2014 [5].

Advantages of Virus-Based Systems for Transient Gene Expression

Benefits of Viral-Based Systems for Temporary Gene Expression Advantages of using autonomously replicating viruses as vehicles for the transient expression of foreign genes include the viruses' characteristically high rates of multiplication and accompanying rates of transient gene expression (Figure 1). Maximum levels of foreign gene expression from viral genomes are expected to occur rapidly upon infection, typically within the first two weeks. Moreover, many plant viruses are very contagious, meaning that they could be utilised economically for the speedy mechanical inoculation of extensive crop plantings. Furthermore, the expression of foreign genes can be promptly investigated in numerous plant hosts once an appropriate transient gene vector is established. This quality is especially helpful for preliminary studies of genes that cause observable phenotypic effects. Since this method can examine the potential consequences of mutant genes before committing to more time- and resource-intensive transgenic technologies for genetic analysis or breeding, it has the potential to speed up preliminary screening procedures for selection. Recent advances in using plant viruses as vectors for the transitory expression of foreign genes in entire plants are summarised here. Some extremely helpful reviews on the topic of plant viral gene vectors, especially DNA viruses, have been published recently. There is some duplication of information from earlier reviews in this one, but we have tried to highlight more recent findings, especially those that deal with the creation or use of RNA virus-based gene vectors. Autonomously replicating viruses are the focus of our discussion, but dealing with them has drawbacks. For example, in annual crops, crop-enhancing characters must be introduced every growing season. Additionally, introduced genes may be lost over time due to

deletion or mutation that affects expression, and there is always the risk of adverse effects on the host or interactions with other viruses.

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