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Applications of Virus Induced Gene Silencing (VIGS) in Plant Functional Genomics Studies

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Abstract

Virus induced gene silencing (VIGS) is an effective technology that exploits an antiviral defense mechanism in plants. It is a recently developed gene transcript suppression technique for characterizing the function of plant genes. VIGS is rapid, efficient and specific system for transient gene silencing. The major steps in VIGS includes; engineering viral genomes to the appropriate viral vector to incorporate fragments of host genes that are targeted to be silenced, infecting the appropriate plant hosts and silencing the target genes as part of the defense mechanism of the plant against virus infection. The VIGS vector is a recombinant virus engineered to be able to carry a piece an endogenous gene from the host. During infection with the modified vector, the host's defense reaction will be induced against the cloned host gene; a loss of function phenotype makes it possible to identify the function of the gene. The recombinant virus is introduced into plant cells through *Agrobacterium* tumefaciens mediated transient expression or *in vitro* transcribed RNA inoculation or direct DNA inoculation. The Trans gene is amplified along with the viral RNA by RNA dependent RNA polymerase generating dsRNA molecules. dsRNA is the triggering molecule of Post transcriptional gene silencing. VIGS as a reverse genetics tool for functional genomics studies presenting several advantages. Despite its great potential, many limitations remain to be overcome. In this review, the molecular mechanism in VIGS technology, its advanced application in plant functional genomics studies and the major limitation and potential future prospects were briefly discussed.

Keywords: VIGS; Plant; Functional genomics; RNA; dsRNA; Transcription

Introduction

Virus induced gene silencing (VIGS) is an effective technology that exploits an antiviral defense mechanism in plants. It provides a powerful approach for genetic and functional characterization of genes in plants [1]. The term Virus induced gene silencing was first used to describe the phenomenon of recovery from virus infection [2]. Viruses are one of the most damaging plant pathogens. Virus infected plants are subsequently resistant to infection by the same or a closely related strain of the same virus. This phenomenon is known as cross protection.

An early attempt in engineering resistance depends on the introduction of viral genetic material into the plant genome, a technique described as pathogen derived resistance [3-5]. Some plants engineered for pathogen derived resistance exhibited a recovery phenotype. They initially developed disease symptoms upon virus infection, but these symptoms were absent in newly emerging tissue [6]. The underlying molecular basis of cross protection, pathogen derived resistance and recovery was later found to be post transcriptional gene silencing [3,7].

Post transcriptional gene silencing (PTGS) is an epigenetic phenomenon that results in the sequence specific degradation of endogenous mRNAs [8]. PTGS in plants is dependent on a relatively high degree of nucleotide homology between RNA transcripts and target endogenous gene sequences [1,9,10]. It is characterized by the absence of accumulated transcript from either an endogenous gene or a stably integrated Trans gene when a sequence bearing homology to that gene or trans gene is introduced [1].

The discovery of PTGS of endogenous genes by recombinant viruses carrying a near identical sequence was made in 1995 [11]. As it allows the targeted down regulation of a particular gene through the degradation of its transcripts, the potential of VIGS as a tool for the analysis of gene function was quickly well recognized [12].

There are several evidences pointing to the evolution of PTGS as an antiviral mechanism in plants [1,8,13]. Viruses by themselves can trigger PTGS in certain plant species [8,14]. Viruses are not passive in the face of plant defense, and they have evolved proteins that can act as suppressors of PTGS [8,15]. The main triggering molecule of PTGS is double stranded RNA, dsRNA. This dsRNA is cleaved to produce small guide molecules called short interfering RNA (siRNA). The antisense strand of the siRNA associates with the RNAi silencing complex (RISC) to target homologous RNA for degradation [8,16].

A DNA fragment with a minimum of 23 nucleotides appears to be required in order for silencing to occur [17]. However, a 23-nucleotide long sequence is often not sufficient to initiate silencing and longer identical sequences must sometimes be used [1,18]. It is possible that other factors including such as the nucleotide composition of the targeting sequence [17] and the thermodynamic properties of the

siRNA and target sequence pair [19] are also important for determining the efficacy of silencing by a given targeting sequence.

VIGS is useful for high throughput functional genomics studies in plants, particularly plant species that are difficult to stably transform [1,20-23]. As a gene silencing method VIGS has several advantageous. It is easy and rapid gene silencing, do not need for stable plant transformation, partial sequence information is sufficient to silence a gene, can be used for both forward and reverse genetics and etc.

VIGS has been used in many plant species to characterize genes involved in various plant development processes disease resistance, abiotic stress tolerance, symbiosis, nematode resistance, insect resistance, and nutrient acquisition or nutrient stress [1,22,24]. For the application of VIGS technology, three major steps are involved; engineering viral genomes to include fragments of host genes that are targeted to be silenced, infecting the appropriate plant hosts, and silencing the target genes as part of the defense mechanism of the plant against virus infection [22].

The first report of VIGS was obtained silencing phytoene desaturase (PDS) gene in *Nicotiana benthamiana* [8]. PDS is a gene involved in the carotenoid biosynthesis pathway was silenced by incorporating parts of its cDNA into a hybrid viral vector composed of sequences from the *Tobacco mosaic virus* (TMV) and the tomato mosaic virus [22]. Virally delivered PDS antisense RNA resulted in inhibition of the carotenoid synthesis downstream of phytoene and this.

Literature Review

VIGS vectors

Vectors for VIGS are standard binary Ti plasmid derived vectors used for *Agrobacterium* tumefaciens mediated plant transformation in which part of a viral genome is inserted [25]. The VIGS vector is a recombinant virus engineered to be able to carry a piece an endogenous gene from the host. During infection with the modified vector, the host's defense reaction will be induced against the cloned host gene; a loss of function phenotype makes it possible to identify the function of the gene [26].

Around 37 VIGS vectors have been developed for gene function studies in dicotyledonous plants, but far fewer are available for studies with monocotyledonous plants [1,24,27-29]. For examples, tobacco rattle virus (TRV) derived vector is one of the most widely used systems in dicots because of its broad host range and ability to infect meristematic tissue [30,31]. Bean pot mottle virus (BPMV) derived vectors have been developed to induce VIGS in soybean and common bean [32]. Cabbage leaf curl virus (CLCV) derived vector has already been developed to trigger VIGS for siRNA mediated silencing in Arabidopsis [33,34].

To date only few RNA viruses and one DNA virus have been modified as a vector for VIGS in monocotyledonous plant inhibition was observed to spread systemically throughout the entire plant [8]. Reduced levels of photo protective carotenoids lead to the rapid destruction of chlorophyll by photo oxidation which subsequently resulted in a white leaf phenotype which can be easily followed visually.

Species as indicated in Table 1 [1,24] of which Barley stripe mosaic virus (BSMV) based VIGS has been applied for functional genomics in barley and wheat [21,35-38]. Brome mosaic virus (BMV) in rice, barley and maize [10]; Bamboo mosaic virus (BMV) and its satellite RNA in *Nicotiana benthamiana* and *Brachypodium distachyon* [28] and Rice tungro bacilliform virus in rice [39].

Besides, Cucumber mosaic virus (CMV) based VIGS in maize is reported [24]. BMV and BMV based vectors are frequently used for VIGS in some monocot plants. However, these vectors do not work in many other monocot species and not effective for all cultivars within a particular host species [26,38].

Foxtail mosaic virus (FoMV) is a species of the genus Potex virus and possesses a broad host range including 56 Poaceae species and at least 35 dicot species [26] Short and Davies [40]. A VIGS system based on potato virus X (PVX), the member of Potex virus, has been developed to silence target genes successfully in plants [41,42] viruses in their genomic organization [1,26]. Very recently FoMVsg vector developed that able to trigger efficient gene silencing in barley, wheat, and foxtail millet. Therefore, FoMVsg mediated VIGS could possess a great potential for functional genomics in a variety of monocot crops [1,43-50].

VIGS vectors	Monocotyledonous plant species	Reference
BSMV	*Hordeum vulgare*	[43,44]
	Triticum aestivum	[45]
	Brachypodium distachyon	[43]
	Zingiber officinale	[46]
	Costus spicatus	[46]
BMV	*Hordeum vulgare*	[47]
	Zea mays	[47,48]
	Oryza sativa	[47]
RTBV	*Oryza sativa*	[49]
CymMV	*Phalaenopsis*	[50]

Table 1: The most frequently used VIGS Vectors in monocotyledonous plant species.

Molecular mechanism of VIGS

The major steps in VIGS includes; engineering viral genomes to the appropriate viral vector to incorporate fragments of host genes that are targeted to be silenced, infecting the appropriate plant hosts and silencing the target genes as part of the defense mechanism of the plant against virus infection [22]. VIGS vectors are constructed by cloning fragment of the plant target gene with efficient siRNA. The size of the inserted fragment of target endogenous gene usually affects the efficiency of VIGS. Several VIGS vectors have the capacity to carry a fragment size between 150 and 800 bp. VIGS vectors can fail to induce gene silencing if the insert fragment is greater than 1500 bp.

Even though several studies indicated that a 23 bp insertion can to induce VIGS, fragments of 200 to 350 bp in length is mostly preferred to induce higher silencing efficiency [51] and improper sized gene fragment can induce non-target silencing, producing inappropriate phenotype. Besides, the orientation of the inserted gene fragment was another very important factor which can affect the efficiency of VIGS. The higher silencing efficiency usually induced by a reverse oriented insertion compared with that of a forward oriented insertion.

The silencing efficiency significantly enhanced if the target fragment was constructed as a hairpin structure. Selection of the target gene is important for VIGS. Different candidate fragments can be used for silencing of a specific gene. If the target gene belongs to a gene family, some sequences possibly have conserved domains between different genes in the gene family and the fragment of the target gene may have more than 23 bp which is homologous to other genes in the gene family resulting in the degradation of non-target genes and in this case, a very specific fragment needs to be selected. Mostly a fragment from UTR region is mostly appropriate and the conserved domains should be chosen to avoid functional complementation by genes from the same family. The efficiency of gene silencing may be affected by the types of the inoculation methods. The recombinant virus is most frequently introduced into plant cells through *Agrobacterium* tumefaciens mediated transient expression or *in vitro* transcribed RNA inoculation or direct DNA inoculation [52].

After the recombinant virus is introduced in to plant cells, the trans gene is amplified along with the viral RNA by either an endogenous or a viral RNA dependent RNA polymerase (RdRp) enzyme generating dsRNA molecules [39,52]. This dsRNA is the triggering molecule of PTGS. It is cleaved to produce small guide molecules called short interfering RNA (siRNA) [39]. The antisense strand of the siRNA associates with the RNAi silencing complex (RISC) to target homologous RNA for degradation [8,16,25]. Dicer like proteins cleave these viral dsRNAs into short interfering RNAs (siRNAs) duplexes which are approximately 21–24 nucleotides in length [25]. These siRNA in turn are incorporated as single strand RNA molecules into RISC (RNA)-induced.

Silencing Complex) which screens for and destroys RNAs complementary to the siRNA [25,53-63]. Interaction of viral siRNA with target RNA can lead to endonucleolytic cleavage and translational inhibition of cognate RNAs [1,22]. This leads to posttranscriptional gene silencing.

As indicated in Figures 1 and 2 the virus derived silencing signal is further amplified and spreads systemically throughout the plant. siRNAs about 21 in length are assumed to mediate the short-range transport and the RNA dependent RNA Polymerase (RDR) is required for long range transport, possibly by amplifying the silencing signal [22,25]. This systemic spread of the silencing signal occurs regardless of the successful movement of the virus particles in the plant. When VIGS is applied to a susceptible plant, the host plant's target gene mRNA is degraded in large portions of the plant [25].

Virus induced gene silencing (VIGS) is a quick and robust method to assess the function of a gene by transient post transcriptional gene silencing and does not affect the next generation [64,65]. The degree of efficiency of silencing in the live plant can be monitored through observing plant part discoloration and photo bleaching [65].

Advances in VIGS

Apart from a number of new VIGS vectors developed for several crop species, the existing VIGS vectors and technique have under gone several improvements in the recent past [1]. Many viral vectors have been modified to improve silencing efficiency. Besides, different attempts have been made to identify gene silenced tissues through a VIGS vector. For instance, a Green Fluorescent Protein (GFP) gene has been tagged to the coat protein gene of *TRV2* for easy identification of silenced tissue [36]. This will help in tracing only green fluorescent tissues that have the virus, which are expected to have silencing, and hence facilitate the use of these tissues for abiotic stress assay [36,52].

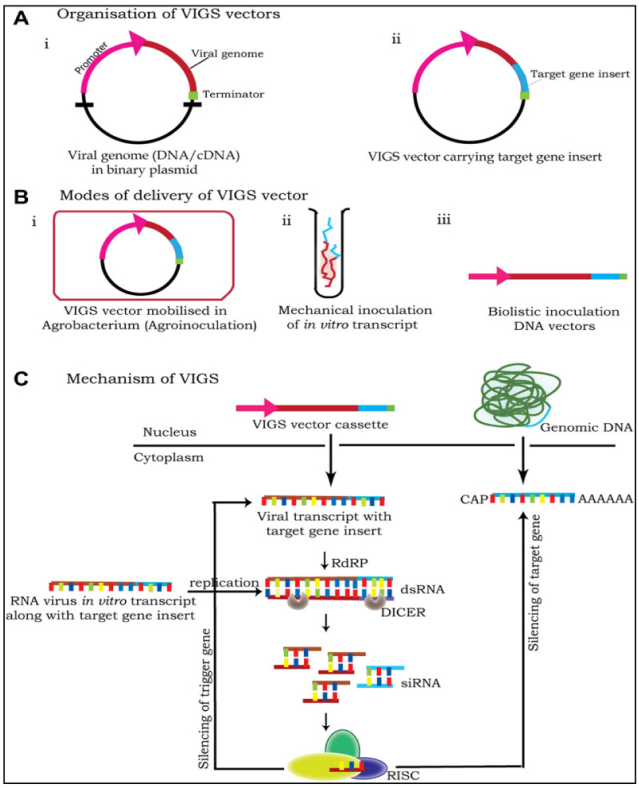

Figure 1: Major steps in VIGS [39]. (A) Organization of vectors, (B) Mode of delivery, (C) Molecular mechanism.

Some VIGS vectors have also been used to induce transcriptional gene silencing [22]. Cloning of endogenous target gene promoter into viral vector and delivery into plants results in the production of siRNAs homologous to the endogenous gene promoter. These siRNAs facilitate RNA directed DNA methylation and histone modifications, resulting in RNA mediated gene silencing [22,54]. This can help suppress the regulators of a biotic stress response. Additionally, VIGS procedure has been modified to perform silencing in different tissues. Gene silencing has been demonstrated in detached plant parts like petals [55] leaves and fruits [56,57]. This facilitates high throughput silencing and multiple stress impositions. VIGS has also been used to silence genes during tissue culture and callus development [58] which can facilitate precise stress imposition and high throughput screening.

VIGS applications in functional genomics

The first RNA virus used as silencing vectors is Tobacco mosaic virus [3]. Transcripts of recombinant virus carrying a sequence from the phytoene desaturase (PDS) gene were produced *in vitro* and inoculated onto *Nicotiana benthamiana* plants to successfully silence PDS. Investigations using VIGS has been made in the wild tobacco species *Nicotiana benthamiana* and its susceptibility to virus infection exhibited efficient gene silencing because of good infection. Subsequently, VIGS vector based on potato virus X (PVX) developed which is more stable than the TMV based vector [6]. However, PVX has a more limited host range than TMV. Both TMV and PVX based

vectors are excluded from the growing points or meristems of their hosts, which precludes effective silencing of genes in those tissues [7].

In earlier study, VIGS vector based on the tomato golden mosaic DNA virus (TGMV) was used to successfully silence a meristem gene in *Nicotiana benthamiana*. This TGMV based vector had been used to silence a non-meristematic gene as well as a foreign transgene [56]. The limitations of host range and meristem exclusion were overcome with the development of VIGS vectors based on tobacco rattle virus (TRV) [7]. TRV is able to spread more vigorously throughout the entire plant, including meristem tissue, yet the overall symptoms of infection are mild compared with other viruses. The function of a wheat starch regulator 1 (*TaRSR1*) in regulating the synthesis of grain storage starch was determined using the barley stripe mosaic virus induced gene silencing (BSMV-VIGS) method in field experiments [1]. Chlorotic stripes appeared on the wheat spikes infected with barley stripe mosaic virus induced gene silencing wheat starch regulator at 15 days after anthesis, at which time the transcription levels of the *TaRSR1* gene significantly decreased [1].

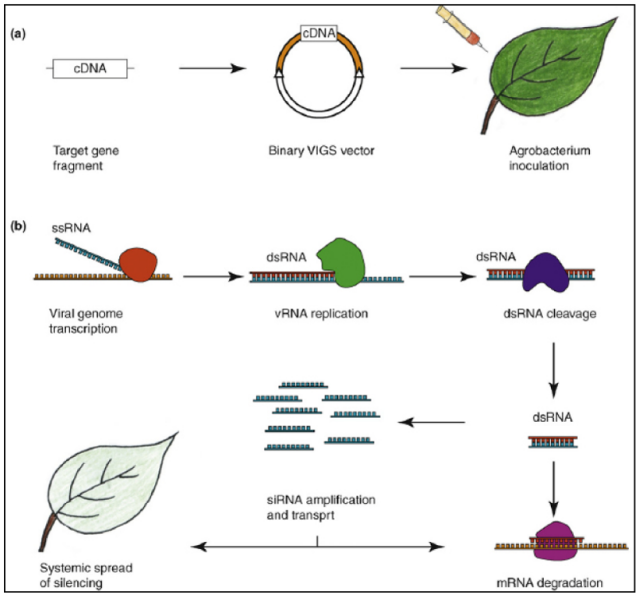

Figure 2: Over view of VIGS in plants and systemic spread of silencing signal [25].

VIGS has been successfully used to silence genes in tomato and strawberry fruits even after the fruits were detached from the plant. This method is particularly useful if a specific target gene, to be silenced in the reproductive organs, is involved in basic metabolic processes during the early stages of plant growth to avoid deformities in vegetative plant growth. Pepper huasteco yellow veins virus (PHYVV) derived vector were used to postulate the involvement of three genes *Comt*, *pAmt*, and *Kas* genes in the biosynthesis of capsaicinoids which are responsible for the pungent taste of chilli pepper fruits of Capsicum species and expressed differentially placenta tissue [59]. They have successfully produced non-pungent chilli peppers at high efficiency with these VIGS approach.

Besides, several VIGS vectors have been used to investigate gene functions under a biotic stress in different plant species. Recently, development of a wide range of VIGS vectors with high silencing efficiency has expanded the application of VIGS to several crop species for studying abiotic stress responsive genes [52]. VIGS is a valuable tool for functional validation of drought responsive genes identified from transcript profiling of plants exposed to drought stress. TRV VIGS mediated silencing of *lea4*, a gene encoding late embryo genesis abundant protein (LEA), resulted in increased susceptibility of tomato plants to drought stress. VIGS has also been used to study drought stress response in monocotyledonous crop species.

In a recent study [60], two drought stress responsive genes, *Era1* and *Sal1*, were individually silenced in wheat using the BSMV VIGS vector. *Era1* and *Sal1* silenced plants subjected to drought stress showed increased relative water content, improved water use efficiency and better vigor compared to vector inoculated plants. This suggests that down regulation of *Era1* and *Sal1* genes enhances drought tolerance in wheat by decreasing sensitivity to ABA [52].

Besides, in crop plants, various investigations discovered some abiotic stress related genes using VIGS (Table 2). The utility of VIGS in investigating salt stress tolerance in crop plants has also been demonstrated. *SlGRX1* gene silencing in tomato by a satellite DNA mβ based VIGS vector resulted in yellowing of leaves under salinity stress compared to vector control plants due to a reduction in chlorophyll content, suggesting the role of *GRX1* in salt tolerance [52,61].

Major challenges in VIGS application

Although improvements in the protocols used for VIGS in several plant species, several limitations of VIGS remain unaddressed. Mainly, VIGS requirement to initiate viral infection restricts its application in certain varieties of crop plants [22,26]. There are several viral resistance genes known in cultivated varieties of crops such as bean, cucumber, pea, pepper, potato, tomato, etc., which confer resistance against certain viruses and thus, vectors derived from those make VIGS ineffective [26,39,62].

Besides, to minimize the potential hazards and prevent unintentional escape of the VIGS vectors in to the environment, an appropriate biosafety precautions need to be practiced. Although it has been shown that siRNAs and triggers of silencing can be transferred from one organism to another, there is no report that indicates this actually occurs in VIGS [65]. A more realistic scenario is the transfer of silencing from plants to silencing competent soil organisms.

However, there is no experimental indication yet that this transfer is possible in VIGS [64]. Besides, a transfer of silencing between plants would also be undesirable. Although not ruled out yet by experiments, that a mechanical transfer of silencing from plant to plant is also possible [65].

VIGS cannot be used in some plant species because of the lack of appropriate VIGS vectors. This can be overcome in two ways. One way is to use heterologous gene sequences, wherever possible, from plant species that are recalcitrant to VIGS to silence genes in a closely related VIGS amenable species [1,22]. The other option is developing new specific vector for a desired plant species from a suitable virus that infects the same species. Besides, developing broad host range VIGS vectors will be more useful. For instance, Tobacco rattle virus (TRV) and Apple latent spherical virus (ASLV) vectors can provoke VIGS in many plant species [26].

Lack of an efficient method for virus vector delivery is another challenge in application of VIGS in plants. *Agrobacterium* mediated delivery of binary VIGS vectors is efficiently used in many dicot plants.

However, for plants that are recalcitrant to *Agrobacterium* mediated transformation, VIGS can be induced by the virus sap inoculation method, RNA transcript inoculation or DNA bombardment [22]. Uneven or localized VIGS resulting in a lack of silencing in certain tissues is mainly the result of ineffective virus movement. This can be addressed by maintaining environmental conditions favoring systemic virus movement. Furthermore, appropriate virus vectors that have the ability to spread systemically in the host plant without deleting the insert should be chosen and such vectors should not have a strong silencing suppressor.

Plant species	VIGS vectors	Abiotic stress	Silenced target gene
Wheat	BSMV	Drought	*TaEra1* (Enhanced response to abscisic acid) *TaBTF3* (Basic transpiration factor)
		High light-induced photo-inhibition	*TaPGR5* (Proton gradient regulation)
Wild emmer wheat	BSMV	Drought	*TdAtg8* (Authophagy related)
Barley	BSMV	Drought	*HvHVA1* (Abundant protein)
Soybean	BPMV	Iron deficiency	*GmRPA3* (Replication protein)
Pea	PEBV	Oxidative stress	*TRX-F*, *TRX-M* (Thioredoxin)
Tomato	TRV	Drought	*SlMPK4* (Mitogen activated proteinkinase)
Chili pepper	TRV	Salt or osmotic stress	*CaPO2* (Peroxidase)
Rose	TRV	Dehydration	*RhETR3* (Ethylene receptor)
Tomato	TYLCCNV	Deficiency	*SlFRO1* (Feric chelate reductase)

Table 2: List of some abiotic stress related genes silenced in different crop plants using VIGS.

Reporter gene expression along with the expression of VIGS vectors should be useful for visualizing the silenced tissues. VIGS vectors that produce severe symptoms in host plants should be avoided. Selection of an appropriate virus host system where viral symptoms are less obvious is important. Besides, gene silencing in many plants is affected by gene target position, insert length and orientation. For optimum VIGS, insert lengths should be in the range of 200 bp to 350 bp [22].

VIGS inherent limitations

Although several of the above-mentioned short comings of conventional VIGS have been overcome, a few of them are inherent to VIGS and remain a challenge. The presence of a virus vector can interfere with the metabolism of the plant [24,26] and affect results from some plant microbe interaction studies. Hence, apart from using appropriate controls, it is sometimes necessary to confirm VIGS results using other gene disruption approaches. Second, gene insertion in the VIGS vector can hinder virus multiplication and many viruses are known to delete the gene insert during multiplication and spread.

Most viral vectors are excluded from meristematic tissue and, therefore, gene silencing in the meristem is not possible in most instances and VIGS often results in incomplete silencing of a target gene [22]. Although this is indeed a limitation, a potential tradeoff between existences of such a limitation and need for rectifying it should be considered. Because of this characteristic, VIGS is more amenable to study gene functions associated with plant development that would be otherwise lethal and intractable in mutant plants. Moreover, genotype of a plant species can affect the performance of VIGS construct. Hence, specific standardization of a VIGS protocol is required for each genotype in some plant species.

Off target silencing of VIGS

Virus infection not only induces RNAi but also can alter the gene expression pattern of the host and induce efficient mRNA down regulation of important endogenous host genes [22]. Off target silencing is silencing of an unintended gene because of partial homology between siRNA produced by the target gene trigger and the unintended mRNA sequence.

Shut off affects a number of host genes in the nucleus, at the transcription level, by an unknown mechanism. VIGS vectors usually behave similarly to their virus of origin during virus infection; however, as they are engineered, changes in their movement, replication ability, their potential to suppress RNAi and also their effect one endogenous genes can be changed [26,63].

Off target silencing can be minimized by careful selection of an insert gene sequence by using publicly available software [22]. The software siRNA scan has a search environment with several integrated components, including a sequence similarity search to identify potential off-targets and efficiency estimation of siRNAs. This software can find a potential region that generates efficient siRNAs for the target gene with no sequence similarity to any other off target genes in the searched database. Therefore, this can be used to design VIGS constructs or siRNAs with minimal off targets.

Future prospects of VIGS application

The application of VIGS has now become more versatile. However, studies related to heritable and long duration VIGS have yet to be extended to other genes, apart from marker genes, in a wide range of plant species. The potential application of VIGS in crop improvement has yet to be realized. In light of recent advances in VIGS technology,

the prospects of using VIGS for various applications in modern plant biology are promising.

PTGS achieved by VIGS vectors can be used for both genetic engineering and molecular breeding aimed at crop improvement. Reduction or alteration of the flowering time of certain genotypes or of indeterminate cultivars, wild relatives or inbred lines can be achieved by using viral vectors [1,22].

Silencing of a negative regulator of flowering in a late flowering genotype can help to match flowering time, enabling crossing with an early flowering genotype. This can also facilitate early and uniform flowering needed for crossing in indeterminate growth genotypes and reduce hurdles related to pollination time. Progeny plants can be made virus free using methods such as heat or freeze shock [1,22,26] and the elimination of viruses can also reverse the PTGS of target genes. In the case of viruses that do not invade meristematic tissue, virus free plants can be developed by meristem tip culture.

Virus free plants can also be identified among the progeny plants because seed transmission of the virus is not always 100% effective. However, wherever possible, a non-seed transmitted VIGS vector can be used to avoid virus transmission to the next generation. Results from VIGS mediated forward genetics screens can also be used by breeders to identify the genes important for a plant process and for quick validation of putative candidate genes during map-based cloning of important traits in crop plants.

Discussion and Conclusion

Virus-induced gene silencing (VIGS) is a technology that exploits an antiviral defense mechanism in plants as a tool for plant reverse genetics. It is rapid, efficient and specific system for transient gene silencing. The VIGS technology has, to a great extent, fulfilled its promise of being a fast and efficient functional genomics tool. It has been used extensively to characterize gene functions in a wide range of plant species.

The increasing number of genes silenced in various plant species using newly designed VIGS vectors is a testimony to its usefulness. It also is useful for high throughput functional genomics studies in plants. VIGS as a reverse genetics tool for functional genomics studies presenting many advantages. With the completion of whole genome sequencing of many important crops, VIGS approach will be widely and mostly used. Despite its great potential to extensively use, many limitations remain to be overcome.

Host range of viral vectors will become wider; the VIGS assays and viral vectors for model organisms need to be well optimized. As sequence information is crucial for VIGS approach, so the whole genome sequence databases will be adding great contribution of VIGS usage. Large scale screening through VIGS based method to detect important and fascinating phenotypes should be performed. Future progress will depend on the ability of investigators to extend the technique to more plant species and to develop high throughput silencing methods in model plants and crops.

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